

Effect of zinc solubilizing bacteria on growth promotion and zinc nutrition of rice

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Abstract

A comparison study between the isolated indigenous bacteria and chemical Zn fertilizer ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) was conducted to evaluate their potential to augment Zn nutrition of Zn responsive (NDR 359) and Zn non responsive (PD 16) varieties of rice under the green house. Three bacterial strains namely; BC, AX and AB isolated from a Zn-deficient rice-wheat field belonging to the genera *Burkholderia* and *Acinetobacter* were investigated for the growth promotion and Zn uptake in rice plants. The plant growth promotory properties such as Zn solubilization and IAA production of the isolates was checked in a previous study. These three isolates when used individually or in combination were found effective in significantly increasing the mean dry matter yield/pot (12.9%), productive tillers/plant (15.1%), number of panicles/plant (13.3%), number of grains/panicle (12.8%), grain yield (17.0%) and straw yield (12.4%) over the control and Zn fertilizer treatment, respectively. Bacterial inoculations also significantly enhanced the total Zn uptake/pot (52.5%) as well as grain methionine concentration (38.8%). Effect of bacterial treatments on the bioavailability of Zn was assessed by estimating the levels of phytic acid in grains. A reduction of nearly 38.4% in phytate: Zn ratio in grains was observed under bacterial inoculations.

Keywords: Rice, PGPR, Zn uptake, Methionine, Phytic acid

1. Introduction

India is considered to be one of the original centers of rice cultivation, covering 44 million hectares of land. The rice harvesting area in India is the largest in the world. In India, rice accounts 40% of the nation's food production and it is a staple food of around 65% total population. Rice-based production systems provide the main source of income and employment for more than 50 million households. The rice production in India was 15.57 thousand tons for the year 2011 (<http://www.irri.org>).

The main constraints in rice production in India include high rainfall/drought conditions, continuous use of traditional varieties due to non availability of seeds of improved varieties or unawareness of the farmers about them, heavy infestation of weeds/pests, low soil fertility, imbalanced use of fertilizers and many more which altogether result in low rice production. Among these factors, low soil fertility is the most important factor which not only seriously affects the rice production but reduce the quality of the rice. Chaudhary *et al.* (2007) reported Zn deficiency as a key factor in determining the rice production in several parts of India. According to Singh (2009), 48% soils in India are afflicted with Zn deficiency. In rice, Zn deficiency causes multiple symptoms that usually appear 2 to 3 weeks after transplanting (WAT) rice seedlings; leaves develop brown blotches and streaks that may fuse to cover older leaves entirely, plants remain stunted and in severe cases may die also, while those which recover show substantial delay in maturity and reduction in yield (Yoshida and Tanaka, 1969; Van Breemen and Castro, 1980; Neue and Lantin, 1994). In countries,

like India where the diet is mainly cereal based, consumption of rice grown on these low Zn soils often cause the adequate intake of Zn in young children and population. The aftermath of low Zn consumption is the impairment in growth and immune functions leading to infectious diseases such as diarrhoea and pneumonia in the developing world (Volkmar and Bremer, 1998; Graham, 2008). Brain function and development is also significantly affected by Zn deficiency, mainly in newborns (Benton, 2008). Zinc deficiency was found to be one major reason for child death worldwide, and was responsible for nearly 450,000 deaths in children less than 5 years of age (Black *et al.*, 2008; Cakmak, 2010). According to a report published in 2002 by WHO, Zn deficiency is considered as the fifth most important risk factor responsible for illness and death in the developing world. In human body, Zn plays an important role in maintaining the structure and function of large number of macromolecules and is also found responsible for controlling over 300 enzymatic reactions (Tapiero and Tew, 2003). Graham (2008) stated that Zinc deficiency is the highest priority among micronutrients for agriculture to address.

The use of chemical fertilizers and manures, to enhance soil fertility and crop productivity has often negatively affected the complex biogeochemical cycles (Perrott *et al.*, 1992; Steinshamn *et al.*, 2004). Continuous application of fertilizers as well as their low use efficiency has caused leaching and run-off of nutrients, especially N and P leading to environmental degradation (Tilman, 1998; Gyaneshwar *et al.*, 2002). On the other hand,

high cost associated with the application of Zn fertilizers in order to correct Zn deficiency places considerable burden on resource poor farmers (Wissuwa *et al.*, 2006). One of the possible ways to increase crop productivity as well as food quality without creating the environmental issues is by the use of plant growth promotory rhizobacteria (PGPR). There are several studies where PGPR were proved as good alternative of chemicals for increasing the plant growth and yield and help reduction in the use of hazardous agro-chemicals. The PGPR were capable of colonizing the rhizosphere, root surface and internal tissues in plants. The main microbial mechanisms by which PGPR improved plant growth include N-fixation, inorganic P solubilisation, siderophore production, phytohormone synthesis and by controlling plant pathogens (Lugtenberg and Kamilova, 2009). Different plant growth promoting bacteria including free living and associative such as *Azospirillum*, *Azotobacter*, *Bacillus* and *Pseudomonas* have been used in agricultural systems as biofertilizers for their beneficial effects on plant growth (Tilak *et al.* 1982). Tran Van *et al.* (2000) has reported the beneficial effects of *Burkholderia vietnamiensis* inoculation on rice plants. Recently, Sharma *et al.* (2014) have also reported the positive inoculation effect of PGPR's isolated from rice rhizosphere on growth promotion of different rice genotypes. Ogut *et al.* (2013) have observed the P solubilisation potential of *Acinetobacter* strains. They inoculated wheat plants with *Acinetobacter* sp. WR922 and found increased plant P nutrition as well as dry matter accumulation.

Most crops are found deficient in certain essential amino acids that cannot be synthesized *de novo* by humans. In non-ruminants even if one essential amino acid is present in insufficient amounts, the remaining amino acids would be broken down, leading to nitrogen loss and poor growth (Shewry, 2006). Zhu *et al.* (2007) had reported that 50% of the world's population relies on cereals or legumes as a staple diet so strategies must be developed to enhance the content of essential amino acids in order to prevent deficiency diseases in the population. Considering this fact in mind we have checked the effect of bacterial inoculations on methionine concentration in rice grains.

Phytic acid is myo-inositol 1,2,3,4,5,6-hexakisphosphate (InsP6), and is a known form of stored phosphorus (P) in most seeds and cereal grains. Approximately 70% of total phosphorus in seeds coexists with phytic acid and its content typically accounts for 1% or more of seed dry weight (Lott, 1984). Phytic acid has a strong ability to chelate multivalent metal ions, specially zinc, calcium and iron. The binding can result in very insoluble salts with poor bioavailability of minerals (Rhou and Erdman, 1995). InsP6 induced low nutrient uptake poses a major public health problem in the form of zinc and iron deficiency, in populations that live mainly on grains and legumes.

In this study, three Zn solubilising bacteria previously isolated from the rhizosphere of rice plants growing in Zn deficient soil were evaluated singly as well as in combination for growth promotion and Zn uptake of two rice varieties having varying Zn response under greenhouse conditions. To explore the effect of bacterial inoculations on Zn bioavailability,

phytic acid concentration as well as phytic acid: Zn ratio was also examined.

2. Material and Methods

2.1. Bacterial culture

Plant growth promotory bacteria used in this study were three bacterial strains that have been previously tried on wheat (Vaid *et al* 2013). Based on partial 16S rRNA sequencing, these bacterial strains were identified as belonging to the genera *Burkholderia* and *Acinetobacter*. These three bacterial cultures are submitted in Genbank (NCBI) and accession numbers are obtained. All the three bacterial isolates were checked previously for their PGPR properties such as Zn and P-solubilization as well as for siderophore and IAA production (Vaid *et al* 2013).

2.2. Experimental design and conditions

A pot experiment was carried out in the experimental area of Department of Microbiology, G. B Pant University of Agriculture & Technology, Pantnagar India. Surface (0-15cm) soil used in this experiment was a Zn deficient loam belonging to soil order mollisol which was collected from the Norman E. Borlaug Crop research centre of the university. Since the critical limit of Zn in mollisols in terms of DTPA (pH 7.3) extractable Zn has been reported as 1.24 mg Zn kg⁻¹ soil (Srivastava and Gangwar, 1990) and the content of DTPA (pH 7.3) extractable Zn was 0.42 mg Zn kg⁻¹ soil, therefore, a prefix 'deficient' was used before loam. The total Zn content of soil used in pot experiment was 245 mg Zn kg⁻¹

soil. The physico-chemical characteristics of the experimental soil are shown in (Table 1). For pot experiment, air dried soil was passed through 2.0 mm sieve before use. Plastic pots used in the experiment were filled with 4 kg soil, watered and left for 1 to 2 days for equilibration. A basal dose of 33.48 mg N as urea, 11.71 mg P and 14.82 mg K through potassium dihydrogen orthophosphate and potassium chloride kg⁻¹ soil was uniformly applied to each pot. There were total nine treatments and each treatment was replicated thrice. The treatments were: control, basal application of Zn at the rate of 2.5 mg Zn as ZnSO₄.7H₂O kg⁻¹ soil, bacterial cultures *Burkholderia* sp. SG1 (BC), *Acinetobacter* sp. SG2 (AX), *Acinetobacter* sp. SG3 (AB) and their combinations (BC+AX), (BC+AB), (AX+AB) and (BC+AX+AB). The control and basal application of 2.5mg Zn kg⁻¹ soil did not receive any bacterial treatment. The experimental design used was a factorial complete randomized design (CRD) with two factors.

Bacterial inocula for plant inoculation was prepared by growing pure cultures of Zn solubilising bacteria AX in Nutrient broth, BC in King's B medium and AB in Tryptic soy broth. The inoculated broths were placed on a rotary shaker at 100 rpm and 30 °C for 24 to 48 h. The final concentration of the bacterial suspension was enumerated by plate count and adjusted to 10⁸ Cfu/ml. The seedlings of two rice varieties PD 16 (Zn non-responsive) and NDR 359 (Zn responsive) were separately raised in the experimental soil. The roots of rice seedlings (21 days old) were thoroughly washed with tap water, surface sterilized in 2% NaOCl for 15 min and finally rinsed with sterile distilled H₂O and were inoculated by soaking them overnight in different bacterial inocula

maintained at 10^8 cfu/ml. Six seedlings were transplanted per pot (two plants per hill) and grown until maturity to get final yield. Two top dressings of urea were done at 22 and 60 d after

transplanting of rice. Before harvesting, the data on productive tillers/plant, total number of panicles/plant and number of grains/panicle were recorded at maturity.

Table 1: Some physical and chemical properties of soil used for pot experiment.

Property	Values
Texture	Loam (Mollisol)
Organic carbon (%)	1.03
Sand (%)	36
Silt (%)	40
Clay (%)	24
pH (1:2) soil:water suspension	7.0
Electrical conductance (dS m^{-1})	0.278
DTPA (pH 7.3) extr. Zn (mg kg^{-1})	0.42
DTPA (pH 7.3) extr. Cu (mg kg^{-1})	1.24
DTPA (pH 7.3) extr. Fe (mg kg^{-1})	25.3
DTPA (pH 7.3) extr. Mn (mg kg^{-1})	5.79

2.3. Harvesting of plants

All the plants in the pots were harvested close to soil surface at maturity and washed thoroughly with tap water followed by washing with 0.1 N HCl and then with distilled water. After initial air drying, plant samples were kept in paper bags, dried at 60°C in an electric oven for 48 h and weighed to determine the dry matter yield. The thousand grain weight, grain and straw yields were also recorded. After harvesting of rice, the soil from each pot was also collected to analyse the effect of different bacterial treatments on the amount of DTPA extractable Zn (Lindsay and Norvell 1978) using atomic absorption spectrophotometer (GBC Avanta M).

2.4. Zn analysis

For Zn analysis, the grains and straw samples were finely ground and 1.0 g sample was digested in 10 ml diacid mixture (HNO_3 : HClO_4 , 4:1 v/v) and diluted to 50 mL using distilled water. Zinc was analyzed by atomic absorption spectrophotometry (GBC Avanta M). Methionine and phytic acid concentration was estimated spectrophotometrically in grains by the methods of Latta and Eskin. (1980) and Horn *et al.* (1946), respectively.

2.5. Statistical analysis

The data were subjected to the analysis of variance using a factorial completely randomized

design using an STPR software designed at the Department of Mathematics, Statistics and Computer Science, CBSH, G.B. Pant University of Agriculture and Technology, Pantnagar, India. The significant differences among the treatments were calculated at 5% probability levels ($p \leq 0.05$) (Snedecor and Cochran, 1967).

3. Results

3.1. Bacterial isolates

The bacterial isolates described as Zn solubilizers were identified as strains of *Burkholderia* sp. SG1 (BC), *Acinetobacter* sp. SG2 (AX) and *Acinetobacter* sp. SG3 (AB) according to 16S rDNA sequencing. These strains were submitted to NCBI and the assigned accession numbers were: HQ844430, KC202811 and HQ844431, respectively. The Zn solubilizing capacity of BC, AX and AB in broth assay as well as on plate assay was checked in a previous study by Vaid et al. (2013).

3.2. Effect of bacterial inoculation on Growth and yield attributing characters of rice plants

The data on the effect of different treatments on growth and yield attributing characters of both the rice varieties are presented in (Table 2). Rice variety NDR 359 (Zn responsive) showed higher total number of panicles/pot and 1000 grain weight as compared to Zn non-responsive variety (PD 16). The two varieties were found statistically similar to each other with respect to number of productive tillers/plant.

The effect of different treatments averaged over the rice varieties revealed that all the treatments significantly increased the dry

matter yield/pot over the control (Table 2). The maximum dry matter yield/pot was observed under the treatment BC+AX with an increase of 12.9 and 7.3% over the control and Zn fertilizer application, respectively. Statistically the treatment BC+AX was found *at par* with treatments AX and BC+AX+AB. As regards the number of productive tillers per plant, the best treatments observed were BC+AB and AX+AB. These treatments increased the number of productive tillers per plant by 15.1% over the control and Zn fertilizer application, respectively. Application of Zn at the rate of 2.5 mg kg⁻¹ soil as well as the bacterial treatments BC, AB and BC+AX could only maintained the number of productive tillers per plant equal to the control. All the treatments except AX+AB were found statistically ineffective in increasing the total number of panicles per pot as compared to the control. The increase recorded in the number of panicles per pot under the treatment AX+AB was 13.3 and 18.5% over the control and Zn fertilizer application, respectively. The number of grains per panicle were found maximum in plants which received BC treatment and the increase recorded was 12.8 and 7.4% over the control and Zn fertilizer application, respectively. The treatments AX, BC+AX and AX+AB were found statistically *at par* with treatment BC. With respect to 1000 grain weight, none of the bacterial treatment or application of Zn fertilizer were found to be statistically significant over the control.

The interaction effect of treatment \times variety significantly influenced all the growth and yield attributing characters except 1000 grain weight (Table 2). In PD 16, treatment BC+AX+AB increased the dry matter yield per pot maximally over the control; the magnitude of increase

recorded was 21.3 and 15.8% over the control and Zn fertilizer application, respectively. Statistically, the treatment BC+AX and BC+AX+AB were found similar to each other. In NDR 359, all the treatments except BC+AB and BC+AX+AB significantly increased the dry matter yield per pot. The maximum dry matter yield per pot was observed for plants which received the treatment AX and the increase recorded was 9.0 and 3.3% over the control and Zn fertilizer application, respectively. In PD 16, none of the treatment was found statistically significantly effective in increasing the number of productive tillers per plant. In the case of NDR 359, the treatments AX, BC+AX, BC+AB and AX+AB significantly increased the number of productive tillers per plant over the control and were found statistically similar to each other. Among these treatments, the maximum increase (30.4%) over the control and Zn fertilizer application was recorded under the treatment AX+AB. In PD 16, all the treatments were found statistically ineffective in enhancing the

total number of panicles per pot over the control. On the other hand in NDR 359, total number of panicles per pot was significantly increased under the treatments BC, BC+AB and AX+AB. However, treatment AX+AB maximally increased the total number of panicles per pot and this increase was 30.4% over the control and Zn fertilizer application, respectively. In PD 16, number of grains per panicle was found higher under treatments BC and AX+AB. Statistically, treatments BC and AX+AB were found similar to each other. Among these maximum increase in number of grains per panicle was observed under treatment BC and the increase was 13.2 and 13.0% over the control and Zn fertilizer application, respectively. In NDR 359, plants received the treatment AX produced the highest number of grains per panicle. The increase in number of grains per panicle recorded under treatment AX was 21.4 and 10.2% over the control and Zn fertilizer application, respectively. The treatment BC+AX was found statistically similar to treatment AX.

Table 2: Growth response of rice plants inoculated with Zn solubilizing bacteria.

Treatments	Dry matter yield/pot (g)			Productive tillers/plant			Total no. of panicles/pot			No. of grains/panicle			1000 grain weight (g)		
	PD16	NDR 359	Mean	PD16	NDR 359	Mean	PD16	NDR 359	Mean	PD16	NDR 359	Mean	PD16	NDR 359	Mean
Control	59.1 ^a	59.9 ^{ab}	59.5	7.67 ^a	7.67 ^a	7.67	7.33 ^{abc}	7.67 ^{bcd}	7.50	138.5 ^{abc}	129.4 ^a	133.9	25.1 ^a	26.3 ^{abcd}	25.7
2.5 mg Zn/ kg soil															
BC	64.7 ^{defg}	63.4 ^{cdef}	64.1	8.33 ^{abc}	8.00 ^{ab}	8.17	7.33 ^{abc}	8.67 ^{de}	8.00	156.8 ^c	145.3 ^{cd}	151.1	25.2 ^{ab}	26.8 ^{abcd}	26.0
AX	66.0 ^{fg}	65.3 ^{efg}	65.7	7.67 ^a	9.33 ^{cd}	8.50	7.33 ^{abc}	8.00 ^{cd}	7.67	139.9 ^{abc}	157.1 ^c	148.5	25.1 ^a	28.7 ^d	26.9
AB	64.4 ^{cdefg}	63.2 ^{bcddef}	63.8	8.67 ^{abc}	7.67 ^a	8.17	7.33 ^{abc}	7.67 ^{bcd}	7.50	140.7 ^{abc}	143.7 ^{cd}	142.2	25.6 ^{abc}	28.0 ^{cd}	26.8
BC+AX	69.8 ^{hi}	64.7 ^{defg}	67.2	7.67 ^a	9.00 ^{bcd}	8.33	6.33 ^a	8.00 ^{cd}	7.17	144.7 ^{cd}	148.4 ^{cd}	146.6	25.4 ^{ab}	28.0 ^{cd}	26.7
BC+AB	66.8 ^{gh}	62.2 ^{bcd}	64.5	8.67 ^{abc}	9.00 ^{bcd}	8.83	7.00 ^{abc}	9.33 ^{ef}	8.17	143.7 ^{cd}	137.7 ^{abc}	140.7	25.7 ^{abc}	27.7 ^{bcd}	26.7
AX+AB	61.0 ^{abc}	63.3 ^{cdef}	62.2	7.67 ^a	10.00 ^d	8.83	7.00 ^{abc}	10.00 ^f	8.50	152.3 ^{bc}	138.0 ^{abc}	145.2	25.1 ^a	26.5 ^{abcd}	25.8
BC+AX+AB	71.7 ⁱ	61.5 ^{abcd}	66.6	8.67 ^{abc}	8.6 ^{bc}	8.67	7.33 ^{abc}	7.67 ^{bcd}	7.50	142.4 ^{bcd}	131.8 ^{ab}	137.1	25.4 ^{ab}	28.7 ^d	27.0
Mean	65.0	63.0	64.01	8.07	8.56	8.31	7.07	8.30	7.69	144.2	141.6	142.9	25.3	27.6	26.4

Pair of treatments that are not significantly different from one another share the same letters checked by the Duncan’s test at 5% probability level.

3.3. Effect of bacterial inoculation on Grain and straw yield of rice plants

Rice variety PD 16 showed higher grain yield while straw yield was found higher for NDR 359 (Table 3).

The effect of different treatments averaged over the rice varieties revealed that all the treatments significantly increased the grain and straw yield per pot (Table 3). Grain yield

was found maximum under the treatment AX which was 17.0 and 5.8% higher over the control and Zn fertilizer application, respectively. Statistically, the treatments AX and BC were found similar to each other. On the other hand, the maximum straw yield was observed under the treatment BC+AX+AB and the increase recorded was 12.4 and 4.8% over the control and Zn fertilizer application, respectively.

Table 3: Effect of different treatments on grain and straw yields of two rice varieties.

Treatments	Grain yield (g pot ⁻¹)			Straw Yield (g pot ⁻¹)		
	PD 16	NDR 359	Mean	PD 16	NDR 359	Mean
Control	19.4 ^b	18.3 ^a	18.8	41.0 ^a	41.3 ^{ab}	41.2
2.5 mg Zn/kg soil	20.2 ^{de}	21.4 ^{fg}	20.8	46.6 ^h	41.8 ^{abcd}	44.2
BC	22.2 ^h	21.7 ^{gh}	21.9	42.5 ^{def}	41.7 ^{abcd}	42.1
AX	20.1 ^{cde}	23.8 ⁱ	22.0	41.7 ^{abcd}	41.6 ^{abcd}	41.7
AB	21.1 ^f	21.9 ^{gh}	21.5	43.3 ^{cf}	41.6 ^{abcd}	42.4
BC+AX	20.4 ^c	22.7 ⁱ	21.6	49.2 ⁱ	42.0 ^{abcd}	45.6
BC+AB	21.5 ^{fg}	19.8 ^{bcd}	20.6	45.3 ^g	42.4 ^{cde}	43.8
AX+AB	19.6 ^{bc}	21.0 ^f	20.3	43.4 ^f	42.3 ^{bcd}	42.9
BC+AX+AB	20.5 ^c	20.2 ^{cde}	20.3	51.2 ^j	41.4 ^{abc}	46.3
Mean	20.5	21.2	20.9	44.9	41.8	43.4

Pair of treatments that are not significantly different from one another share the same letters checked by the Duncan's test at 5% probability level.

The interaction effect of treatment × variety significantly influenced the grain and straw yield of both of the rice varieties (Table 3). In PD 16, all the treatments except AX+AB significantly enhanced the grain yield per pot

and highest value (22.2g) was observed under the treatment BC. The increase in grain yield recorded under treatment BC was 14.4 and 9.9% over the control and Zn fertilizer application, respectively. In NDR 359, plants receiving the

treatment AX showed the maximum grain yield per pot with percent increase of 30.1 and 11.2 over the control and Zn fertilizer application, respectively. As regards the straw yield, in PD 16 all the treatments except AX were found effective in enhancing the straw yield per pot. Among all the treatments, the best treatment observed was BC+AX+AB which increased the straw yield per pot by 24.9 and 9.9% over the control and Zn fertilizer application respectively. While in NDR 359, the increase in straw yield per pot was observed only under the treatments BC+AB and AX+AB and the rest all treatments only maintained the straw yield per pot equal to control. Application of BC+AB increased the straw yield per pot by 2.7 and 1.4% over the control and Zn fertilizer application, respectively. The treatments BC+AB and AX+AB were also found statistically similar to each other.

3.4. Effect of bacterial inoculation on Zn concentration in grains

The mean Zn concentration in grains of two rice varieties did not differ significantly (Table 4). The performance of different treatments averaged over the rice varieties showed that most of the treatments significantly increased the Zn concentration in grains (Table 4). The highest Zn concentration in grains was observed in plants received the treatment BC+AX and the increase recorded was 56.9 and 41.6% over the control and Zn fertilizer application, respectively. The interaction effect of treatment \times variety significantly influenced the Zn concentration in grains (Table 4). In PD 16 and NDR 359, the highest Zn concentration in grains was recorded under the treatment BC+AX. In PD 16, the percent increment in grain Zn concentration

observed under BC+AX was 50.5 and 34.2 over the control and Zn fertilizer application, respectively. While in NDR 359, the increase in grain Zn concentration recorded under BC+AX was 66.7 and 49.5% over the control and Zn fertilizer application, respectively. In NDR 359, the treatments AX+AB and BC+AX+AB were found statistically *at par* to treatment BC+AX.

3.5. Effect of bacterial inoculation on Zn concentration in straw

The statistical analysis revealed that Zn concentration in straw of NDR 359 was significantly higher as compared to that of PD 16 (Table 4).

The performance of different treatments averaged over the rice varieties showed that all the treatments significantly increased Zn concentration in straw (Table 4). Among all the treatments, the best treatment was BC+AB which increased the straw Zn concentration by 42.7 and 14.4% over the control and Zn fertilizer application, respectively.

The interaction effect of treatment \times variety significantly influenced the Zn concentration in straw (Table 4). In PD 16, Zn concentration was the highest in straw of plants receiving BC+AB treatment. The increase recorded under BC+AB was 55.2 and 9.1% over the control and Zn fertilizer application, respectively. On the other hand in NDR 359, treatment BC was adjudged as the best treatment which increased the Zn concentration in straw by 36.0 and 25.9% over the control and Zn fertilizer application, respectively. Statistically, the values obtained under treatments BC+AX and BC+AB were found *at par* with the value obtained under treatment BC.

Table 4: Effect of different treatments on Zn concentration in grain and straw of two rice varieties.

Treatments	Zn concentration (mg kg ⁻¹)					
	Grain			Straw		
	PD 16	NDR 359	Mean	PD 16	NDR 359	Mean
Control	10.7 ^{abcd}	9.6 ^a	10.2	20.1 ^a	21.1 ^{ab}	20.6
2.5 mg Zn/kg soil	12.0 ^{cde}	10.7 ^{abcd}	11.3	28.6 ⁱ	22.8 ^{bcd}	25.7
BC	11.3 ^{abcde}	10.3 ^{abc}	10.8	22.8 ^{bcd}	28.7 ⁱ	25.7
AX	11.7 ^{bcde}	10.1 ^{ab}	10.9	23.8 ^{cdef}	21.8 ^{abc}	22.8
AB	12.3 ^{de}	11.4 ^{bcde}	11.8	27.0 ^{ghi}	23.2 ^{bcd}	25.1
BC+AX	16.1 ^f	16.0 ^f	16.0	27.3 ^{ghi}	27.0 ^{ghi}	27.2
BC+AB	12.1 ^{de}	12.6 ^c	12.3	31.2 ^j	27.6 ^{hi}	29.4
AX+AB	12.0 ^{cde}	15.1 ^f	13.5	25.8 ^{efgh}	23.5 ^{bcde}	24.6
BC+AX+AB	12.7 ^e	15.9 ^f	14.3	26.3 ^{fghi}	24.9 ^{defg}	25.6
Mean	12.3	12.4	12.3	25.9	24.5	25.2

Pair of treatments that are not significantly different from one another share the same letters checked by the Duncan's test at 5% probability level.

3.6. Effect of bacterial inoculation on Zn uptake in grains

Statistical analysis revealed that Zn uptake in grains of both the rice varieties was similar (Table 5).

The effect of different treatments averaged over the rice varieties revealed that all the treatments significantly increased Zn uptake in grains and it was the highest under the treatment BC+AX (Table 5). The increase observed under this treatment was 79.9 and

47.2% over the control and Zn fertilizer application, respectively.

The interaction effect of treatment × variety significantly influenced the Zn uptake in grains per pot (Table 5). In PD 16 and NDR 359, the maximum Zn uptake in grains per pot was recorded under the treatment BC+AX. In PD 16, the magnitude of increase recorded under BC+AX was 57.6 and 35.8% over the control and Zn fertilizer application, respectively. However, this increase in NDR 359 was 106.4 and 59.2% over the control and Zn fertilizer application, respectively.

Table 5: Effect of different treatments on Zn uptake in grain and straw of two rice varieties.

Treatments	Zn uptake ($\mu\text{g pot}^{-1}$)					
	Grain			Straw		
	PD16	NDR359	Mean	PD16	NDR359	Mean
Control	207.8 ^b	175.7 ^a	191.8	824.7 ^a	869.7 ^{ab}	847.2
2.5 mg Zn/kg soil						
BC	241.1 ^{bcd}	227.9 ^{bcd}	234.5	1332.7 ^f	952.7 ^{bc}	1142.7
AX	249.1 ^{cd}	222.6 ^{bc}	235.8	970.7 ^{bc}	1195.0 ^e	1082.8
AB	234.1 ^{bcd}	241.3 ^{bcd}	237.7	994.3 ^{bc}	905.7 ^{abc}	950.0
BC+AX	259.4 ^d	249.1 ^{cd}	254.2	1168.7 ^c	963.3 ^{bc}	1066.0
BC+AB	327.4 ^e	362.8 ^f	345.1	1345.7 ^f	1133.3 ^{de}	1239.5
AX+AB	259.2 ^d	248.4 ^{cd}	253.8	1412.0 ^f	1172.0 ^e	1292.0
BC+AX+AB	234.7 ^{bcd}	317.5 ^e	276.1	1119.3 ^{de}	992.3 ^{bc}	1055.8
Mean	260.4 ^d	319.9 ^e	290.1	1345.7 ^f	1030.7 ^{cd}	1188.2
Mean	252.6	262.8	257.7	1168.2	1023.9	1094.6

Pair of treatments that are not significantly different from one another share the same letters checked by the Duncan's test at 5% probability level.

3.7. Effect of bacterial inoculation on Zn uptake in straw

Zinc uptake in straw per pot was more in NDR 359 as compared to PD 16 variety (Table 5).

The effect of different treatments averaged over the rice varieties significantly influenced Zn uptake in straw (Table 5). All the treatments effectively enhanced Zn uptake in straw per pot over the control. The treatment which increased Zn uptake maximally was BC+AB and the magnitude of increase was 52.5 and 13.1%

over the control and Zn fertilizer application, respectively. Statistically treatment BC+AX was found *at par* with treatment BC+AB.

The interaction between treatment and varieties significantly influenced the Zn uptake in straw (Table 5). In PD 16, all the treatments significantly increased Zn uptake in straw per pot over the control. The maximum Zn uptake in straw per pot was recorded under the treatment BC+AB with an increase of 71.2% over the control. Application of 2.5mg Zn kg⁻¹ soil, BC+AX and BC+AX+AB were

found statistically *at par* with the treatment BC+AB. In NDR 359, Zn uptake in straw per pot was the highest under the treatment BC. The increase recorded under treatment BC was 37.4 and 25.4% over the control and Zn fertilizer application, respectively. The treatments BC, BC+AX and BC+AB were found statistically similar to each other.

3.8. Effect of bacterial inoculation on total Zn uptake

Total Zn uptake/pot was found to be statistically similar for both rice varieties (Table 6). The performance of different treatments averaged over the rice varieties indicated that total Zn uptake per pot was significantly increased under all the treatments over the control (Table 6). The maximum total Zn uptake/pot occurred in plants receiving the treatment BC+AX which

was 52.5 and 15.0% higher over the control and Zn fertilizer application, respectively. Statistically, the treatment BC+AB was found *at par* with treatment BC+AX.

The interaction effect of treatment \times variety significantly influenced the total Zn uptake/pot (Table 6). In PD 16, all the treatments enhanced total Zn uptake per pot over the control. The highest total Zn uptake was recorded under the treatment BC+AX. The increase recorded under this treatment was 62.0% over the control. The values of total Zn uptake/pot under treatments 2.5mg Zn kg⁻¹ soil, BC+AB and BC+AX+AB were found statistically similar to treatment BC+AX. On the other hand, in NDR 359, the treatment BC+AX increased total Zn uptake by 43.1% over the control. Total Zn uptake values observed under treatments BC and BC+AB were statistically *at par* with the value obtained under treatment BC+AX.

Table 6: Effect of different treatments on total Zn uptake of two rice varieties.

Treatments	Total Zn uptake ($\mu\text{g pot}^{-1}$)		
	PD16	NDR359	Mean
Control	1032.7 ^a	1045.7 ^a	1039.2
2.5 mg Zn/kg soil	1574.3 ^{fg}	1180.7 ^b	1377.5
BC	1220.0 ^{bc}	1417.3 ^{dc}	1318.7
AX	1228.3 ^{bc}	1146.7 ^{ab}	1187.5
AB	1427.7 ^{de}	1212.7 ^{bc}	1320.2
BC+AX	1673.0 ^e	1496.0 ^{ef}	1584.5
BC+AB	1671.3 ^e	1420.7 ^{de}	1546.0
AX+AB	1354.0 ^d	1309.7 ^{cd}	1331.8
BC+AX+AB	1605.7 ^{fg}	1350.3 ^d	1478.0
Mean	1420.8	1286.6	1353.7

Pair of treatments that are not significantly different from one another share the same letters checked by the Duncan's test at 5% probability level.

3.9. Effect of bacterial inoculation on methionine concentration in grains

Relatively higher methionine concentration was recorded in grains of NDR 359 as compared to PD 16 variety (Table 7).

The performance of different treatments averaged over the rice varieties indicated that all the treatments except BC significantly increased the methionine concentration in rice grains (Table 7). The highest methionine concentration was observed under the treatment BC+AX+AB and the magnitude of increase was 38.8 and 22.4% over the control and Zn fertilizer application, respectively.

The interaction effect of treatment × variety significantly influenced the methionine concentration in grains (Table 7). In PD 16, methionine concentration in the grains was significantly increased under all the treatments except under treatment BC. Among all the treatments, the maximum methionine concentration in grains was recorded under the treatment BC+AX+AB which was 37.6 and 14.2% over the control and Zn fertilizer application, respectively. In NDR 359 also, the treatment BC+AX+AB maximally increased the concentration of methionine in the grains and the percent increase recorded was 40.7 and 29.4% over the control and Zn fertilizer application, respectively.

Table 7: Effect of different treatments on methionine and phytic acid concentration in grains of two rice varieties.

Treatments	Methionine concentration (g kg ⁻¹)			Phytic acid concentration (g kg ⁻¹)		
	PD16	NDR359	Mean	PD16	NDR359	Mean
Control	1.17 ^a	1.50 ^c	1.34	11.77 ^{ab}	17.73 ^h	14.75
2.5 mg Zn/kg soil						
BC	1.41 ^d	1.63 ^g	1.52	11.67 ^a	17.81 ^h	14.74
AX	1.20 ^{ab}	1.51 ^e	1.36	12.03 ^{bc}	17.66 ^h	14.85
AB	1.34 ^c	1.74 ^h	1.54	11.90 ^{ab}	17.76 ^h	14.83
BC+AX	1.46 ^{de}	1.50 ^e	1.48	12.59 ^{de}	17.80 ^h	15.20
BC+AB	1.26 ^b	1.52 ^e	1.39	11.79 ^{ab}	17.29 ^g	14.54
AX+AB	1.49 ^c	1.95 ⁱ	1.72	12.28 ^{cd}	17.60 ^{gh}	14.94
BC+AX+AB	1.53 ^{ef}	1.59 ^{fg}	1.56	12.96 ^f	17.85 ^h	15.40
BC+AX+AB	1.61 ^g	2.11 ^j	1.86	12.75 ^{ef}	17.85 ^h	15.30
Mean	1.39	1.67	1.53	12.19	17.71	14.9

Pair of treatments that are not significantly different from one another share the same letters checked by the Duncan’s test at 5% probability level.

3.10. Effect of bacterial inoculation on phytic acid concentration in grains

The concentration of phytic acid in rice grains was significantly higher in NDR 359 as compared to PD 16 (Table 7).

The performance of different treatments averaged over the rice varieties indicated that none of the treatment was effective in significantly reducing the levels of phytic acid in rice grains (Table 7). However treatments 2.5mg Zn kg⁻¹ soil and BC+AX could maintain the level of phytic acid in grains as recorded under the control. On the other hand, increased phytic acid concentration was recorded in grains of plants received AB, AX+AB and BC+AX+AB treatments. Rest all treatments could maintain the phytic acid level in grains similar to that of control.

The interaction effect of treatment × variety significantly influenced the concentration of phytic acid in grains (Table 7). In PD 16, all the treatments were ineffective in reducing the concentration of phytic acid in grains significantly. On the other hand, significantly higher phytic acid concentration was observed under the treatments AB, BC+AB, AX+AB and BC+AX+AB over the control. In NDR 359, only treatment BC+AX significantly reduced the concentration of phytic acid in grains and the reduction observed was 2.5% as compared to control. Rest all treatments maintained the concentration of phytic acid in grains statistically similar to that of control.

3.11. Effect of bacterial inoculation on Phytate to Zn ratio in rice grains

In general, phytate: Zn ratio was lower in grains of PD 16 variety as compared to NDR 359 (Figure 1). The effect of different treatments

averaged over rice varieties revealed that all the treatments except BC and AX significantly reduced the ratio of phytate: Zn in rice grains. A reduction of 38.4% was observed in this ratio under the bacterial inoculation BC+AX as compared to control.

The interaction effect of treatments × variety significantly influenced the phytic acid: Zn ratio in rice grains. In PD 16 and NDR 359, phytate: Zn ratio was maximally reduced under the same treatment BC+AX; the magnitude of reduction was 33.3% in PD 16 while in NDR 359 it was 41.1% as compared to control.

3.12. Effect of bacterial treatments on DTPA extractable Zn in soil after rice harvest

In general, DTPA extractable Zn in soil after crop harvest was found higher under PD 16 variety as compared to NDR 359 variety (Table 8).

The effect of different treatments averaged over the rice varieties revealed significantly higher content of DTPA extractable Zn under treatments 2.5 mg Zn/kg soil, AX and BC+AX+AB over the control.

The interaction effect of treatments × variety also significantly influenced the amount of DTPA extractable Zn in soil. In the case of PD 16 variety, the highest DTPA extractable Zn was observed under the treatment AX and the increase was 31% over the control. The DTPA extractable Zn in soil after crop harvest under 2.5 mg Zn/kg soil and BC+AX+AB treatments were statistically similar to the values recorded under treatment AX. In the case of NDR 359, the DTPA extractable Zn in soil was the highest under the treatments BC, AB and BC+AX+AB, the increase in DTPA extractable Zn in soil under these treatments was 10.84% compared to that of control pots.

Table 8: Effect of bacterial treatments on DTPA extractable Zn in soil after rice harvest.

Treatments	Available Zn (mg kg ⁻¹ soil)		
	PD16	NDR359	Mean
Control	0.85 ^{ab}	0.83 ^a	0.84
2.5 mg Zn/kg soil	1.30 ^f	0.88 ^{abc}	1.09
BC	1.10 ^d	0.92 ^c	1.01
AX	1.31 ^f	0.87 ^{abc}	1.09
AB	1.16 ^{dc}	0.92 ^c	1.04
BC+AX	1.20 ^e	0.89 ^{bc}	1.05
BC+AB	1.12 ^d	0.87 ^{abc}	1.00
AX+AB	1.20 ^e	0.88 ^{abc}	1.04
BC+AX+AB	1.26 ^f	0.92 ^c	1.09
Mean	1.17	0.89	1.03

Pair of treatments that are not significantly different from one another share the same letters checked by the Duncan’s test at 5% probability level.

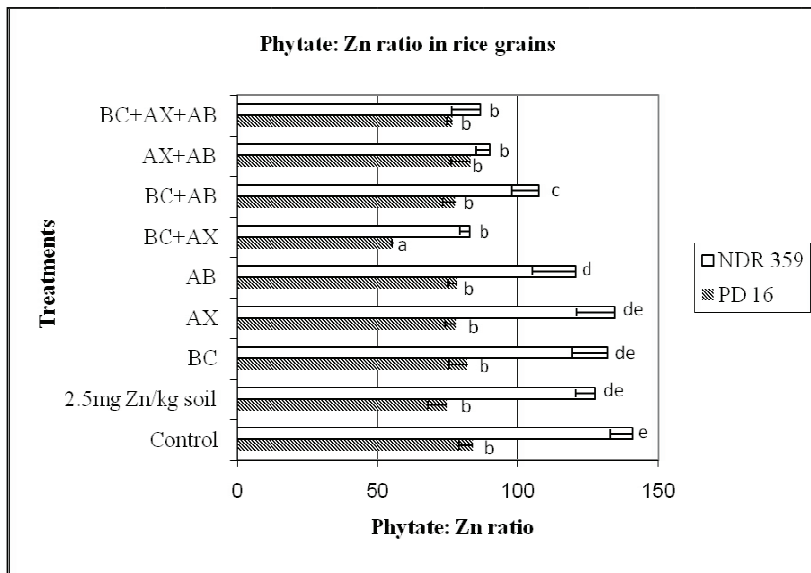


Figure 1. Effect of bacterial treatments on Phytate: Zn ratio in rice grains. Horizontal bars indicate LSD at ≤ 0.05

4. Discussion

Pot culture screening experiments are very much essential for analyzing the effects of microbial bioinoculants on various plant growth parameters as well as on nutrient uptake before evaluating their efficacy under field conditions. In the present study, the effect of *Burkholderia* sp. SG1 (BC), *Acinetobacter* sp. SG2 (AX) and *Acinetobacter* sp. SG3 (AB) isolated from Zn deficient rice field on growth parameters and Zn nutrition of rice plants was examined in a pot culture experiment. In previous study, these three bacteria were characterized for Zn solubilization, siderophore, gluconic acid and IAA production (Vaid et al., 2013). Several workers have reported the beneficial effects of different strains of *Burkholderia*, *Acinetobacter*, *Bacillus*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Beijerinckia*, *Erwinia*, *Flavobacterium*, *Rhizobium* and *Serratia* and also identified them as prominent PGPR's (rodriguez and Fraga, 1999; Sturz and Norwak, 2000; Sahin et al., 2004; Malviya et al., 2012).

Bacterial strains BC, AX and AB when used individually as well as in combination with each other improved growth and Zn uptake of plants significantly over the control as well as over Zn fertilizer treatment, respectively. The present experiment revealed that co-inoculation of rice seedlings (PD16 and NDR 359) with isolated *Burkholderia* and *Acinetobacter* strains significantly increased the dry matter yield pot⁻¹, number of productive tillers plant⁻¹, number of panicles pot⁻¹ and number of grains panicle⁻¹. In the present study, we found 15.1% increase in mean number of productive tillers per plant under bacterial treatments BC+AB and AX+AB

over the control. Similar increase was reported earlier by Tran Van et al. (2000) who observed 13% more tillering in rice plants when inoculated with *Burkholderia vietnamiensis* TVV75. The increment recorded due to bacterial inoculations in mean dry matter yield pot⁻¹ and mean number of grains panicle⁻¹ was 12.9 and 12.8% respectively, over the control. These findings were concurrent with the results obtained by Tran Van et al. (2000) who recorded 11% more grains panicle⁻¹ in rice plants inoculated with *Burkholderia vietnamiensis* TVV75 as compared to the non-inoculated ones. The results also showed significant increase in grain and straw yields due to bacterial inoculations. In experiments of Tariq et al. (2007), an increase of 65% in grain yield was observed due to inoculation with plant root associated PGPR in rice. Similar results were obtained by Minaxi et al. (2013) in wheat. They reported a remarkable 92.8% increase in seed yield when wheat plants were inoculated with a combination of Arbuscular mycorrhizal fungi and phosphate solubilizing bacteria.

The increase recorded in growth and yield attributes of two rice varieties could be attributed to higher mobilization of Zn by Zn solubilizing bacteria like *Burkholderia* sp. SG1, *Acinetobacter* sp. SG2 and *Acinetobacter* sp. SG3. These three bacterial cultures were isolated from Zn deficient rice field to ensure that they were already acclimatized to the conditions of rice field and this might be the reason for their good performance. Mader et al. (2011) also stated an increase of 23% in rice yield obtained by Jha et al. (2009) upon rice inoculation with a diazotrophic (N₂-fixing) *Pseudomonas* sp. strain. From this statement, Mader et al. (2011) concluded that the increase obtained by Jha et al. (2009) was due to the appropriate selection

of bioinoculants. Our bacterial isolates were also found positive for IAA production, a phytohormone responsible for increasing the length of the root hairs which could promote better absorption of nutrients from the soil. According to Volkmar and Bremer (1998) rapid establishment of roots including elongation of primary roots or proliferation of lateral/adventitious roots is advantageous for young seedlings as it increases their ability to explore the soil to easily obtain water and nutrients. Nayak *et al.* (1986) reported that *Azospirillum* inoculation can increase height and number of tillers in rice plants. They mentioned that PGPR treatment increases chlorophyll content in plants and thereby enhances the rate of photosynthesis leading to improved overall plant health. Similar observations were reported by Mohite (2013) who showed that inoculation of wheat seedlings with rhizosphere soil isolates significantly increased the plant height, root length and chlorophyll content over the control. In our study we found bacterial inoculations more effective in enhancing the Zn concentration and its uptake in grains and straw of both of the rice varieties as compared to the application of 2.5 mg Zn kg⁻¹ soil. The poor performance of soil application of 2.5 mg Zn as zinc sulphate could be due to the tendency of Zn fertilizers to form insoluble complexes in the soil. According to Fageria *et al.* (1988) and Johnson-Beebout *et al.* (2009), P and Zn fertilizers can rapidly form insoluble complexes in the soil and remained unavailable for plant uptake unless genotypes with an improved capability to access these soil-bound nutrients are developed. Rose *et al.* (2012) also mentioned that P and Zn fertilizers are not always adequate to overcome the crop production constraints. They pointed out that

fertilizers are costly inputs and their use limits the profitability of rice farming due to high-input or low-input systems. Zhu *et al.* (2007) stated that micronutrient fertilizers must be applied regularly and are, therefore, costly as well as potentially damaging to the environment. We have also observed that the DTPA extractable Zn content after rice harvest was significantly increased in soil under different bacterial treatments. The possible reason for this increase was the Zn solubilizing capabilities of the bacteria used in this experiment. After rice harvesting, the amount of DTPA extractable Zn was more in soil in which the plants of PD 16 variety were growing as compared to the soil in which plants of NDR 359 variety were growing. This might be due to the combined Zn solubilizing efforts of Zn solubilizing bacteria and the effect of plants of PD 16 variety on soil. We hypothesized that the exudates of PD16 variety were containing some organic acids and also in greater amounts that might be helpful in effecting higher solubilization of insoluble Zn present in the soil. This statement is in agreement with the statement of Widodo *et al.* (2010) who reported that Zn deficiency-tolerant rice line RIL46 acquired Zn more efficiently as compared to non-tolerant maternal line IR74 under field conditions. According to them this was due to increased root exudation of deoxymugineic acid (DMA) or low molecular weight organic acids and due to increased root production.

The results showed that due to bacterial inoculation Zn concentration in grains and straw was significantly increased both in PD 16 and NDR 359. The increase observed in mean Zn concentration in rice grains due to inoculation treatment was 56.9 and 49.5% over the control and Zn fertilizer application, respectively. This

is in accordance with the findings of Tariq *et al.* (2007) who reported 157 and 9.7% increase in Zn concentration in grains over the control and Chelated Zn-EDTA treatment respectively. Mader *et al.* (2011) also reported a substantial increase in Mn and Zn concentration in rice grains due to application of combination of natural mycorrhiza consortia and fluorescent *Pseudomonas* strain R62 + R81. Our results showed that all the bacterial inoculations caused significant increase in total Zn uptake per pot compared to non inoculated plants. This might be due to solubilization of insoluble soil Zn by production of gluconic acid by these isolates. Simine *et al.* (1998) reported the production of gluconic and 2 ketogluconic acid by strain of *Pseudomonas fluorescens* and found it responsible for Zn solubilization in broth assay. The overall increase in plant growth and nutrient uptake was the result of partly Zn solubilization and IAA production by the isolates. Fageria *et al.* (2002) found that the application of 120 mg Zn kg⁻¹ soil significantly improved the root growth of upland rice and wheat. Initiation and development of lateral roots, increase in root weight and nutrient uptake by PGPR inoculation were reported by Rolf *et al.* (1997) and Canbolat *et al.* (2006). In a green house experiment, Malik *et al.* (2002) observed an increase of 32-81% in rice yield due to *Azospirillum* inoculation. Nayak *et al.* (1986) reported that *Azospirillum* inoculation can increase height and number of tillers in rice plants. They mentioned that PGPR treatment increases chlorophyll content in plants and thereby enhances the rate of photosynthesis leading to improved overall plant health. Amino acids, such as methionine, histidine and organic acids (e.g., citrate) are known to have a positive effect on zinc absorption and have been used

for zinc supplements (Lonnerdal, 2000). We analyzed methionine concentration in rice grains of both varieties and observed significant increase under different bacterial inoculations over the control. This could be due to better expression of RNA polymerase which needs Zn for its proper functioning. This fact is supported by the statement of Price (1962) who reported a sharp decline in RNA and number of ribosomes in Zn deficient cells. Marschner (1995) and Cakmak *et al.* (2010) also reported a positive correlation between grain Zn concentration and protein synthesis. Rice is an important staple food in Asian countries and in rural areas it is also a major source of micronutrients but unfortunately, the bioavailability of minerals, e.g. zinc from rice, is low because it is present as an insoluble complex with phytic acid (Liang *et al.*, 2008). Phytic acid is actually the stored form of P in seeds or cereal grains. It has been well-documented that adequate Zn nutrition is important in controlling the uptake of P by roots (Marschner, 1995). Increasing the availability of P in the growth medium can induce Zn deficiency in plants by altering soil and plant factors (Robson and Pitman, 1983), but little is known about specific mechanisms. Huang *et al.* (2000) observed that Zn deficiency (low tissue Zn concentrations) caused an increase in the expression of P transporter genes in barley roots. They also reported that enhancing P uptake efficiency may cause a decrease in plant uptake of Zn, leading to low Zn concentrations (densities) in food. According to Gianquinto *et al.* (2000) applications of large amounts of fertilizer P to soils that are low in available Zn can depress tissue Zn concentration or may even induce Zn deficiency. From the above statement we can conclude that Zn solubilizing bacteria

solubilized Zn in soil & made it available to the plants and as a result there will be no overexpression of P transporter genes in rice plants and hence no over accumulation of P in grains occurred in the present study and this might be the reason behind obtaining reduced phytate: Zn ratio under different bacterial treatments. In the present study, bacterial treatment BC+AX produced maximum reduction in phytate: Zn ratio in grains of both of the rice varieties.

5. Conclusion

Thus, use of bacterial consortium such as *Burkholderia* sp. and *Acinetobacter* sp. can effectively increase the growth attributes and yield of rice. Rice plants inoculated with suitable combination of Zn solubilizing bacterial strains were found more efficient in acquiring Zn from Zn deficient soil as compared to non-inoculated plants. The findings indicated a possibility of reducing the cost incurred on Zn fertilizers in rice cultivation however, the verification of their effectiveness in augmenting Zn nutrition of rice under field condition is needed. Further validation of results under field conditions is in process. The present investigation also indicated the necessity of investigating the reasons of bacterial strain specificity in rice varieties of varying Zn responsiveness.

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