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Full Length Article

Inoculation of Zinc-Solubilizing Bacteria with different Zinc Sources and Rates for Improved Growth and Zinc Uptake in Rice

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Abstract

An experiment was conducted in growth chamber using sand culture technique to determine the effect of zinc-solubilizing bacteria (ZSB) inoculation with different zinc sources and zinc rates on growth and zinc uptake of rice. The treatments were consisted of non-inoculated control, two bacterial isolates (*Acinetobacter* sp. and *Serratia* sp.), two zinc sources (zinc sulfate and zinc oxide) with three rates (0, 0.2 and 0.4 mg L^{-1}). The experiment was arranged in a factorial complete randomized (CRD) with three replications. Results revealed significant difference ($p<0.05$) among treatments on plant height, plant biomass, and leaf area index (LAI). The treatments also affected zinc concentration and its uptake. Plants inoculated with *Acinetobacter* sp. showed higher plant growth, zinc concentration and zinc uptake compared to non-inoculation and *Serratia* sp. Zinc sulfate at 0.2 mg L^{-1} was also recorded for high plant growth and zinc concentration. It can be concluded that *Acinetobacter* sp. and zinc sulfate at 0.2 mg L^{-1} have good potential in alleviating zinc deficiency in rice and important in solubilizing the insoluble zinc in soil for improved growth of rice.

Keywords: Zinc-solubilizing bacteria; Rice; Growth; Zinc; Uptake

Introduction

Zinc (Zn) is one of the important nutrients in rice cultivation. However, the yield of rice has been observed to reduce as zinc is present in the insoluble form that makes it unavailable to the plant. Two types of zinc fertilizers are being used with different solubilization properties. Zinc sulfate is highly water soluble compared to zinc oxide. But zinc sulfate also can reverse back to insoluble form after 7 days of application during rice plant growth (Rehman *et al.*, 2012; Hafeez *et al.*, 2013). Zinc solubilization can be influenced by several factors including through activity of the zinc solubilizing bacteria. The bacteria solubilize Zn through several mechanisms, which include excretion of metabolites such as organic acids, proton extrusion, or production of chelating agents (Fasim *et al*., 2002). This solubilization property is important in nutrient cycling especially during planting. Goteti *et al*. (2013) found that gluconic acids produced in by the bacteria helped in solubilization of Zn salts and increased growth of maize. In another study the bacteria, *Pseudomonas* sp. produced 2-ketogluconic acid during Zn solubilization, which increased growth of tomato and reduce root knot nematode disease (Saravanan *et al*., 2007). Fasim *et al*. (2002) also found the same solubilization trend in different Zn salts. Bacteria isolated from rice roots can solubilize insoluble zinc and also have ability to proliferate rice root cell wall (Maheshwari *et al*., 2013). Rice plants inoculated with suitable combination of Zn solubilizing bacterial strains were found having higher plant biomass because of higher zinc uptake occurred during plant growth (Vaid *et al*., 2014). ZSB helps in acquiring Zn from Zn deficient soil as compared to non-inoculated plants. Thus, rhizospheric microorganisms play a pivotal role in the enhancement of crop production by the solubilizing unavailable form of metals into available form. Information on the influence of ZSB especially *Acinetobacter* sp. at different zinc rate and sources are still minimal (Gandhi and Muralidharan, 2016). Hence, the present study was undertaken to determine the effect of ZSB inoculation with different zinc sources and zinc rates on growth and zinc uptake in rice.

Materials and Methods

Studies were conducted at Soil Microbiology Lab, University Putra Malaysia, Serdang, Selangor. Rice seeds were surface sterilized following the method modified from Amin *et al*. (2004). Rice seeds were dehusked and shaked in

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70% ethanol for 5 min. The ethanol was then discarded and the seeds were agitated in 3% hypochlorite solution and rinsed 3 time with sterilized distilled water. The seeds were then rinsed with 2% sodium thiosulphate solution to neutralize chloramine residue on the seeds. Surface seeds were grown in petri dishes lined with wet filter paper.

Preparation of Sand Culture and Growing of Rice Seedlings

River sand was sieved through 2.0 mm sieve and soaked overnight with 0.1 N HCl. The sand was washed several times with distilled water until pH 6.0. The acid washed sand was air dried and sterilized using autoclave at 15 psi at 121ºC for 15 min in a plastic container. Few holes were made on the top of the sand plastic bag with same distance using hot stainless steel loop (Naher *et al*., 2009) Germinated rice seeds were then transferred into each hole in the plastic container. Prior to transplanting rice seedlings, the sand was filled up with 200 mL of sterilized Zn free Hoagland nutrient solution (Hoagland and Amon, 1950). The zinc sources used were zinc sulfate ($ZnSO_4$) as soluble and zinc oxide (ZnO) as insoluble source. The zinc rates used were 0, 0.2 and 0.4 mg L^{-1}. Every alternate day, the plants were watered with 200 mL water and once a week with nutrient solution for five times during 40 days growing period. The rice seedlings in the container were grown in growth chamber with 12 h light dark cycle with constant room temperature.

Preparation of Inoculums

Two ZSB species, *Acinetobacter* sp and *Serratia* sp. isolated from Malaysian rice soil were grown for 48–72 h in Nutrient Broth. The bacterial cells were harvested by centrifugation at 13500 rpm for 10 min in Eppendorf tube and washed using phosphate buffer saline. Approximately 10 x 10^4 mL^{-1} live bacterial cells were used to inoculate plants in each planting unit. The population was confirmed by cell enumeration in drop plate on nutrient agar (Somasegaran and Hoben, 1985).

Determination of Leaf Area Index (LAI)

The leaf area of rice was determined at 40 days after transplanting. Plant leaves were taken to the lab, washed, placed into plastic bag containing distilled water to ensure its turgidity level. Then leaf area was measured using a leaf area meter model LI-3100 Area meter, LI-COR. Inc. Lincoln, Nebraska, USA and the LAI was calculated by dividing leaf area of whole plant with pot surface area.

Zinc Tissue Analysis

After each harvest plant samples were carefully washed to remove all soil particles and dried in oven at 70°C for 5 days and the dried plants were finely ground. Exact amount of 100 mg leaf tissue was placed into 100 mL centrifuge tube containing10 mL nitric acid (HNO_3) and perchloric acid ($HClO_4$) at 9: 4 ratio. The tubes were placed on a digestion block and digested at 300◦C until the plant material turned colorless. The extract was transferred into 100 mL volumetric flask and the volume made up to 100 mL with distilled water. Zn concentration was determined using atomic absorption spectrophotometer (AAS) (Perkin-Elmer model 400). Plant Zn uptake was calculated by multiplying Zn concentration with plant dry weight.

Statistical Analysis

All data were statistically analyzed using the SAS Software program (Version 9.4; SAS institute, 2013) and treatment means were compared using Tukey's HSD test at 5% level.

Results

Effect of ZSB Inoculation on Plant Biomass

The results showed that application of ZSB inoculation, different zinc sources and rates affected plant biomass. High (114.7 mg) biomass was recorded for *Acinetobacter* sp. at 0.2 mg L^{-1} Zn sulfate (Table 1) and not significantly different to 0.4 mg L^{-1} of zinc sulfate. Zn sulfate showed higher plant biomass than zinc oxide. Interaction of ZSB, Zn source and Zn rates resulted in significant influence on plant biomass (Table 2).

Effect of ZSB Inoculation on Plant Height and LAI

ZSB inoculation, different zinc sources and rates significantly affected plant height and LAI of rice (Table 1). Highest plant height (23.6 cm) and (1.01 cm^2) LAI were recorded for *Acinetobacter* sp. and *Serratia* sp. with Zn sulfate treatments. Zn sulfate treatments showed higher plant height and LAI than zinc oxide treatments. The interaction effects between ZSB, zinc source and rate were found had significant influence on rice plant height and LAI (Table 2).

Effect of ZSB Inoculation on Zinc Concentration

Application of ZSB inoculation, zinc sources and rates significantly affected concentration of zinc in rice plant. Higher (47.12 mg kg^{-1}) zinc concentration was recorded for *Acinetobacter* sp. inoculation at 0.2 mg L^{-1} Zn sulfate. It was not significantly different for the same inoculation at 0.4 mg L^{-1} (Table 1). Zinc oxide treatments and *Serratia* sp. showed lower response towards zinc concentration. The interaction effects of ZSB, zinc source and rates had

Table 1: Effect of ZSB inoculation with different zinc sources and rates on plant biomass, plant height, leaf area index, zinc concentration and zinc uptake of rice plant

Bacterial inoculation	Zn source	Zn rate (mg L^{-1})	Plant biomass (mg)	Plant height (cm)	Leaf area index (cm^2)	Zinc concentration (mg kg^{-1})	Zn uptake (µg)
Non-inoculated	Zn oxide	0	48.33b	5.90bc	0.12a	12.50bc	0.61bc
		0.2	54.67b	6.80abc	0.10a	13.50b	0.68b
		0.4	50.67b	8.00a	0.20a	10.80c	0.59c
	Zn sulfate	0	46.70b	5.30c	0.14a	12.50bc	0.58bc
		0.2	75.30a	8.30a	0.19a	13.50b	0.92b
		0.4	68.30a	7.40ab	0.17a	16.80a	1.27a
Acinetobacter sp.	Zn oxide	0	75.00b	7.80d	0.14c	20.00cd	1.49c
		0.2	108.70a	18.50b	0.17c	26.80b	2.84b
		0.4	105.70a	14.40c	0.01d	23.80bc	2.60b
	Zn sulfate	0	76.30b	7.20d	0.14c	17.20d	1.31c
		0.2	114.70a	23.60a	1.01a	47.12a	4.80a
		0.4	106.70a	16.80bc	0.59b	45.00a	5.41a
Serratia sp.	Zn oxide	0	72.70b	7.80d	0.08b	16.80d	1.22c
		0.2	87.30a	18.50b	0.03b	22.00bc	1.87b
		0.4	85.00a	14.40c	0.08b	23.20b	2.02b
	Zn sulfate	0	71.00b	7.20d	0.07b	17.30cd	1.23c
		0.2	91.30a	23.60a	0.10ab	24.70ab	2.25ab
		0.4	91.00a	16.80bc	0.23a	28.30a	2.59a

Values sharing same letters differ non-significantly (P>0.05)

Table 2: Analysis of Variance (ANOVA) mean square values for the effects of ZSB inoculation , zinc source, zinc rate and their interaction on plant biomass, plant height, leaf area index, zinc concentration and zinc uptake of rice plant

Treatment	Mean square value/Significance level				
	Plant biomass	Plant height	Leaf area index	Zn concentration	Zn uptake
ZSB	5146.57***	181.73***	0.20***	840.13***	15.93***
Zn source	378.98***	17.94**	0.50***	468.25***	6.81***
ZSB x Zn source	93.12**	5.54*	0.27***	164.92***	2.25***
Zn rate	2813.41***	230.55***	0.11***	463.31***	9.74***
ZSB x Zn rate	211.69***	50.04***	0.11***	123.75***	2.50***
Zn rate x Zn source	164.76***	39.20***	0.13***	179.30***	2.42***
ZSB x Zn rate x Zn source	70.96**	11.23**	0.08***	67.62***	15.93***

Significance levels are *= 0.05, **= 0.01, ***=0.001 respectively at $p < 0.05$ level of significance

shown significant influence on rice zinc concentration (Table 2).

Effect of ZSB Inoculation on Zinc Uptake

The study showed that uptake of zinc in rice was affected by the application of ZSB inoculation, zinc sources and zinc rates High (4.80 µg) zinc uptake was recorded for *Acinetobacter* sp. inoculation at 0.4 mg L^{-1} of zinc sulfate which was not significantly different from 0.2 mg L^{-1} (Table 1). Comparatively, *Acinetobacter* sp. treatments were found higher in zinc uptake compared to *Serratia* sp. While, zinc sulfate treatments showed higher zinc uptake than zinc oxide. The interaction between treatments also showed significant influence on zinc uptake (Table 2).

Discussion

Inoculation of rice with ZSB showed highest plant growth at 0.2 mg L^{-1} Zn sulfate compared to other treatments. Zinc sulfate is highly soluble in water compared to zinc oxide and it became fully available to plant in nutrient solution except for no zinc treatment (Iqbal *et al*., 2010). Increment of biomass also might be due to zinc uptake. Zinc nutrition consisted of zinc concentration and zinc uptake which helped for plant growth (Vaid *et al*., 2014). Zinc solubilizing bacteria strains also significantly increased Zn concentration compared to non-inoculated treatment at 0.2 mg L^{-1} Zn sulfate. Increase in plant nutrient uptake could be attributed to the efficiency of these bacteria to mobilize Zn from of zinc sulfate in nutrient solution (Vaid *et al*., 2014). Hence, the Zn mobilized by these bacteria is sufficient for rice plant nutrition (Graham *et al*., 1981; Gao *et al*., 2006; Coolong *et al*., 2014). Sarathambal *et al*. (2010), found that Zn carbonate and zinc oxide tagged with ^{65}Zn. $^{65}ZnCO_3$ and ^{65}ZnO, was effectively solubilized and the content of Zn by the maize plants was also more in *Glucanobacter diazotrop*hicus inoculated treatments compared to the non-inoculated treatments due to comparatively inoculated organism better for solubilization of zinc in deficient soil. In the present study, it is obvious that existence of ZSB (*Acinetobacter* sp.) caused zinc concentration and uptake increased in rice plant. However, when the rate of plant growth exceeds the uptake of a particular nutrient, the concentration of that nutrient in the tissue decreases or become diluted which observed to rice plant at 0.4 mg L^{-1} zinc sulfate (Ahmed *et al*., 2012; Ghasemi-Fasei, 2012).

This is in agreement with Malik *et al*. (2011), which showed that the length of roots and shoots, the fresh and dry matter production decreased with increasing zinc levels for red amaranth rice from India. At 400 ppm Zn, all plants died possibly due to toxic effect of Zn. Zn concentration and uptake by plants also influenced by toxicity level and zinc deficiency in soil. Thus, through ZSB inoculation, information on the suitable rate and type zinc sources to be used would give advantages to farmers, where their cost can be reduced and less chemical needed valuable for future research.

Conclusion

The study showed that zinc solubilizing bacteria isolated from rice rhizosphere was able to solubilize the different forms of zinc. Rice plant inoculated with *Acinetobacter* sp. at 0.2 mg L^{-1} Zn sulfate showed high plant biomass, plant height, LAI, zinc concentration and zinc uptake. Thus, ZSB isolate with zinc sulfate at 0.2 mg L^{-1} have the potential to be applied to soil for efficient uptake of Zn in rice cultivation.

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