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***Short Communication***

# Effect of zinc solubilising bacteria on root colonization and zinc uptake in rice (*Oryza sativa*)

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The $Zn^{2+}$ ion activity in soil solution is a fundamental aspect, which determines its bioavailability to plants and microorganisms. However, the mobility of applied zinc fertilizers is too slowly to meet the plant demand at critical plant growth stages. The plant root exudates are sources of many complex compounds such as simple and complex sugars, amino acids, phenolics, proteins, flavinoids and OAs. The production of root exudates like organics acids is greatly altered by root morphology and presence of insoluble minerals in the soils (Badri and Vivanco, 2009). Many findings represent the positive interaction of zinc solubilizing bacteria (ZSBs) with plant root exudates and are mediating mineralization, solubilization, and biofortification of insoluble zinc compounds. The supporting mechanisms are acidification, chelation and exchange reaction as a function of organic acids of microbes and plant origins. Hence, application of ZSBs with an aim to alter the root morphology in presence of zinc sources can help to mineralize Zn for a longer period of time. Therefore, the present study was designed to determine the effects of ZSB and zinc fertilizers on root growth and zinc uptake in rice.

Table 1: Response of different levels of zinc oxide and ZSB (*Pantoea agglomerans*) inoculation on plant height, plant biomass, root length and root volume, Zn uptake in straw and root of rice

| Treatment | Plant height (cm) | Spad value | Shoot dry biomass (gm/pot) | Root length (cm) | Root volume ($cm^3$) | Zn uptake in Straw (µg/kg) | Zn uptake in root (µg/kg) |
|---|---|---|---|---|---|---|---|
| Control | 69.48±1.3 $^{e**}$ | 29.1±0.5 $^{cde}$ | 11.7 ± 1.5 $^{e}$ | 24.5 ± 0.5 $^{e}$ | 0.35± 1.5 $^{e}$ | 324.1± 1.2 $^{e}$ | 120.8± 0.9 $^{e}$ |
| ZSB | 71.47± 2.0 $^{cd}$ | 29.9±0.8 $^{cde}$ | 12.9 ± 0.9 $^{d}$ | 26.8 ± 0.9 $^{d}$ | 0.43± 1.8 $^{d}$ | 387.0± 1.0 $^{d}$ | 171.5± 1.0 $^{d}$ |
| 20 mg Zn* + ZSB | 72.17± 1.8 $^{bc}$ | 28.8±1.1 $^{bcde}$ | 13.6 ± 1.8 $^{c}$ | 28.3 ± 1.1 $^{bc}$ | 0.47± 2.1 $^{c}$ | 616.1± 0.9 $^{c}$ | 292.4± 0.9 $^{c}$ |
| 40 mg Zn + ZSB | 73.43± 1.4 $^{a}$ | 30.9±0.6 $^{a}$ | 15.4 ± 0.6 $^{a}$ | 33.0 ± 0.7 $^{a}$ | 0.56± 1.6 $^{a}$ | 851.6± 1.4 $^{a}$ | 429.1± 1.1 $^{a}$ |
| 60 mg Zn + ZSB | 72.51± 2.1 $^{ab}$ | 29.6±1.1 $^{ab}$ | 14.8 ± 0.9 $^{b}$ | 27.2 ± 0.4 $^{b}$ | 0.53± 0.9 $^{ab}$ | 775.5± 1.2 $^{b}$ | 395.2± 0.8 $^{b}$ |

**Zn- ZnO, ZSB- Pantoea agglomerans, **The experimental data are the average of four replicates ± SD. Within a column, Means with different letters in the same column differ significantly at P ≤ 0.05 by post-hoc Tukey HSD Test*

A pot experiment was conducted using non-sterilized soil samples (10 kg $pot^{-1}$) fertilized with different doses of zinc oxide. The physico-chemical properties of initial soil had pH- 7.3, organic carbon -8.1 g $kg^{-1}$, available N - 88.2 mg $kg^{-1}$, available P - 16.5 mg $kg^{-1}$, and available K- 71.8 mg $kg^{-1}$ respectively. The DTPA-Zn in initial soil was 0.6 mg $kg^{-1}$. The experiment consisted of five treatments with four replication : 1) control (omission of ZSB and ZnO), 2) ZSB inoculation (*Pantoea agglomerans*), 3) 20 mg Zn 10 $kg^{-1}$ soil + ZSB, 4) 40 mg Zn 10 $kg^{-1}$ soil +ZSB and 5) 60 mg Zn 10 $kg^{-1}$ soil +ZSB. The rice seedlings were harvested after 45 days .The root samples were thoroughly washed with tap water, followed by dipped in calgon solution for 30 mints, afterwards fresh root weight were recorded and kept in oven for drying at $60C^0$ till the constant weight observed. The root samples were digested in diacid ($HNO_3$:$HCLO_4$-9:4) determination using atomic absorption spectroscopy (AAS). One-way ANOVA was applied to test the significance of treatments related to greenhouse experiment and comparisons between means were analyzed using post-hoc Tukey's HSD Test at P≤ 0.05 level using social science SPSS software (ver. 16.0 for windows).

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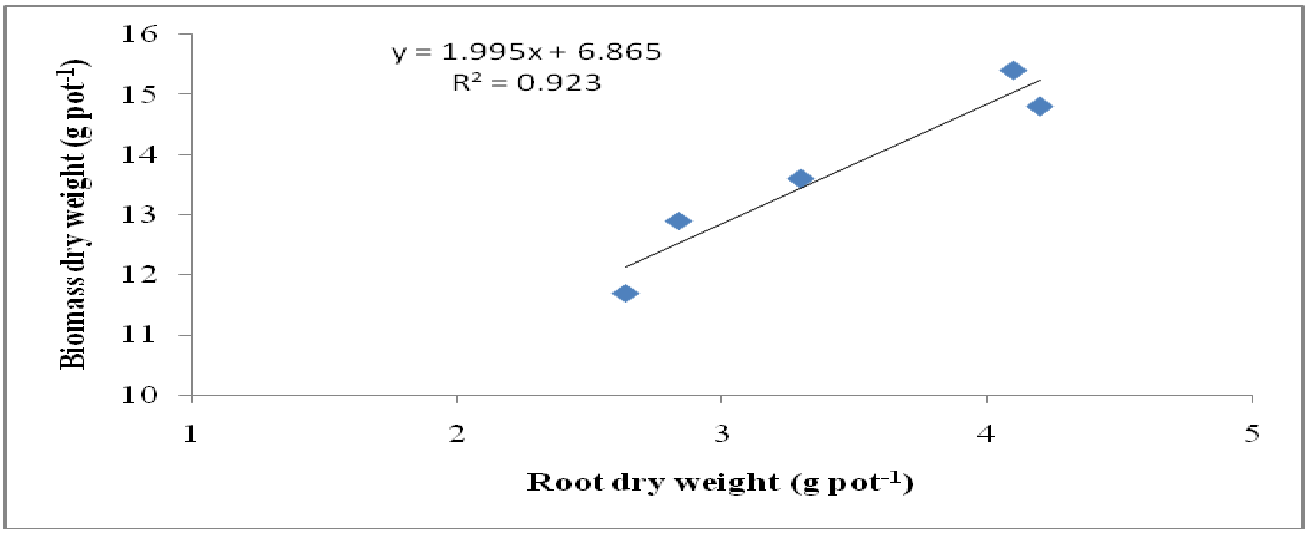


Figure 1: The relationship between shoot and root dry weight of rice seedlings grown in different soil Zn treatments with zinc fertilizer and ZSB inoculation

The combined appliation of ZSB and zinc oxide significantly improved the plant height, and biomass production. The plant treated ZSB +40 mg Zn produced significanly maximum height (73.43 cm) which was 5.7% higher than control. The plant height ranged from 69.48 to 73.43 cm. The cholorophyll content mesaerured with the help of spad meter was maximum in treatment of ZSB +40 mg Zn, while rest of treatments were showed non-significant difference with each other. The biomass production was significantly different with each other and ranged from 11.7 to 15.4 g $pot^{-1}$. The percent increase in biomass yield due to Zn application and ZSB among the treatments varied from 10.26 to 31.62 % over control (table 1). Similar positive effect of ZSB and Zn fertilizers was reported by Dinesh *et al.* (2018). In the treatment of ZSB + 60 mg Zn, the biomass yield was reduced which may be ascribed to higher Zn content hinders the bacterial efficiency, hence declination in biomass was observed. A simple correlation between the biomass dry matter and root dry biomass (g $pot^{-1}$) was established and found positive (Figure 1) with value of (r=0.96**). Gandhi and Muralidharan (2016) reported that plant growth parameters and rice yield attributes was increased over control, when combined with ZSB (*Acinetobacter* sp.) and insoluble Zn sources; zinc oxide and zinc carbonate under pot experiments. The increase in biomass may be due to greater enzyme activities, significant drop in rhizosphere pH and Zn redistribution among native zinc pools which helped in increased zinc availability for crop acquisition under Zn deficient soil.

Among the treated plants, the microbial inoculation positively affect the root length, and subsequently increased the root volume. The maximum root length was observed in treatments of $T_4$ (ZSB+40 mg Zn) with a value of 33.0 cm, however the next maximum root length was observed in ZSB +60 mg Zn which was at par with T4. The increases in root lenght due to Zn application and ZSB among the treatments were in order of 9.5% < 15.5% < 35.6% <12.2 % over control (table 1). Similar finding were reported by Gontia-Mishra *et al.* (2017). This increase in root growth might be attributed to qualitative alterations of root exudates composition due to the degradation of exudates and the release of microbial metabolites that play a central role in the soil zinc cycle through transformation of soil zinc.

Zinc uptake in straw and root was gradually increased up to of ZSB+40 mg Zn which varied from 324.1 to 851.6 and 120.8 to 429.1 µg $kg^{-1}$ in shoot and root respectively (table 1). The positive effect of ZSB was found when combined with Zn fertilizer as compared to alone microbial inoculation. Similar increases in zinc acquisition with the inoculation of zinc solubilizing bacteria have been reported by Mäder *et al.* (2010). The increased in soil Zn as a result of Zn solubilization by ZSB might be result of increase in soil Zn upon bacterial inoculations which promotes root growth proliferation.

From the results, in may be concluded that isolated bacterial strain is able to proliferate root system and the inoculation of *Pantoea agglomerans* with 40 mg Zn produced the best result for root development and zinc acquisition from deficient soil.

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