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Solubilization of insoluble zinc compounds by *Gluconacetobacter diazotrophicus* and its influence on maize

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Zinc deficiency was created in maize seedlings in the sand culture and deficiency was rectified with the addition of insoluble zinc sources and *G. diazotrophicus*. The basic strategy behind this approach is decreasing the pH and making zinc soluble and as a consequence, the available zinc will get increased in the soil system. Since lowering of soil pH to 5 or below increases the Zn solubility. The plant growth, chlorophyll content and plant zinc content were found to be enhanced due to inoculation of *G. diazotrophicus* with insoluble zinc compounds.

Key words : *G. diazotrophicus*, Zinc solubilization, Chlorophyll content.

INTRODUCTION

Zinc plays amazing number of critical roles in all organisms. It required in small but critical concentration to allow several key plant physiological pathways to function normally. These pathways have important roles in photosynthesis, sugar formation, protein synthesis, fertility and seed production, growth regulation and defence against disease. Unlike other micronutrients deficiency, Zn deficiency is ubiquitous (Guerinot and Eide, 1999). Where zinc is deficient, morphological functions will be impaired and the health and productivity of the plants will be adversely affected, resulting in lower yields (or even crop failure) and frequently in poor quality crop products.

The whole world is shifting to an organic based sustainable agriculture and is also our duty to preserve the nature's wealth without any pollution for the future generation. So the need of the hour is to devise some technology or strategies to alleviate zinc deficiency. Heterotrophic bacterial zinc solubilization was reported in literatures (Di Simine *et al.*, 1998; Coles *et al.*, 2001; Whiting *et al.*, 2001and Fasim *et al.*, 2002) focusing mainly on the remediation aspects of zinc pollution. Recently a bacterial based approach was devised to solve these micronutrient deficiency problems (Saravanan, 2004 and Anthoni Raj, 2002).

MATERIALS AND METHODS

A pot culture experiment was conducted to study the zinc solubilization potential of *G. diazotrophicus*. Acid washed sand was used as medium for the experiment. About 2.5 kg of acid washed sand was taken in plastic pot (size: 15cm height, 15cm diameter) and sterilized at 20 lbs pressure for 2hrs each for two consecutive days. Maize seeds (Col) obtained from the Department of Millets, Tamil Nadu Agricultural University, Coimbatore were used. Watering was done with sterile distilled water. Fahraeus N-free nutrient solution without zinc source was used as the nutrient solution for maize. Initially 30 ml of *G. diazotrophicus* culture was added for each pot and every 10 days another 20ml was added. One gram fresh leaf sample taken from each replication was analyzed for total chlorophyll content following the method of Hiscox and Isrealstam (1979). Total zinc content of maize seedlings was analyzed according to Jackson, 1973. The shoot and root length of maize plant was measured. According to treatment details Zn is added in the form of insoluble sources (zinc oxide, zinc carbonate and zinc phosphate).

The treatment details of this experiment are as :

T_1 - Uninoculated control+ without Zn, T_2- $ZnCO_3$ alone, T_3 - $ZnCO_3$ + *G. diazotrophicus*, T_4 - ZnO alone, T_5 - ZnO + *G. diazotrophicus*, T_6 - $Zn_3(PO_4)_2$ alone, T_7- $Zn_3(PO_4)_2$+ *G. diazotrophicus*, T_8 - $ZnSO_4$

RESULTS AND DISCUSSION

The maize seedlings in pot culture experiments developed Zn deficiency after two months of sowing. This deficiency was corrected with the application of insoluble forms of Zn *viz.*, $ZnCO_3$, ZnO and Zn_3 $(PO_4)_2$ along with inoculation of *G. diazotrophicus*. The inoculated organism might have released the gluconic acid during its growth which in turn solubilize the insoluble zinc compounds there by making available the zinc for plants

(Srivastava and Gupta, 1996). Solubilization of insoluble Zn compounds *viz.*, ZnO, $ZnCO_3$ and Zn_3 $(PO_4)_2$ by *G. diazotrophicus* was reported (Saravanan, 2004). Microbial solubilization of micronutrients in the rhizosphere was earlier reported by Meyer and Linderman, (1986); Kucey *et al.* (1998) and Doyle *et al.* (1991).

Significant increase in the total zinc content of maize seedlings was observed due to inoculation of *G. diazotrophicus* compare to the control (Table 1). Among treatments the $ZnCO_3$ with *G. diazotrophicus* treatment recorded more Zn content. This was followed by Zn_3 (PO_4) with *G. diazotrophicus* (24.0mg/g). Marschner (1991) focused a bacterial approach to supply zinc for soybean plant and found increase in total zinc content of plant samples.

Significant variation in the chlorophyll content of maize seedlings before and after inoculation of *G. diazotrophicus* was observed (Table 2). Among the insoluble forms ZnO + *G. diazotrophicus* recorded maximum chlorophyll content on 105th days of maize growth (1.58mg/g). This was followed by $Zn_3(PO_4)_2$ with *G. diazotrophicus* (1.51mg/g). Zinc have important role in better seedling vigour, root and shoot growth and chlorophyll content in wheat plants (Grewal and Graham, 1997). In the present study also, increased chlorophyll content, seedling vigour, shoot and root growth in the *G. diazotrophicus*, inoculated treatments were observed which might have been the result of zinc nutrition.

Initially Gdiazotrophicus inoculated treatments showed better performance than the uninoculated treatments. Root length and shoot length were significantly influenced by insoluble zinc sources and *G. diazotrophicus* all the treatments when compared to control (Table 3). After inoculation of *G. diazotrophicus* there was gradual increase in plant root and shoot length compare to control. Among the treatments, ZnO with showed better performance than other treatments.

Among the zinc sources ZnO performed better than $ZnCO_3$ and Zn_3 $(PO_4)_2$. This may be due to partial solubilization of ZnO than the $ZnCO_3$ and Zn_3 $(PO_4)_2$. However, the solubilization of the three zinc sources by

Table 1 : Influence of insoluble Zn sources and *G.diazotrophicus* inoculation on the total zinc content of maize seedlings grown on sand culture

Treatments	Plant Zinc content (mg/g)					
	30th day	45th day	60th day	75th day	90th day	105th day
$ZnSO_4$	19.0	22.0	23.0	24.0	24.5	26.0
$ZnCO_3$ alone	13.0	14.5	15.0	15.0	16.0	16.8
$ZnCO_3$+*G. diazotrophicus*	14.0	15.0	18.0	22.0	23.0	24.5
ZnO alone	13.5	14.5	14.7	16.0	16.3	16.8
ZnO+ *G. diazotrophicus*	14.5	15.0	16.5	19.0	22.0	23.5
$Zn_3(PO_4)_2$ alone	13.5	13.5	15.0	15.0	16.0	16.8
$Zn_3(PO_4)_2$ + *G. diazotrophicus*	14.0	14.0	16.0	19.5	23.5	24.5
Control	15.0	15.5	16.0	16.2	16.5	17.0
S.E.±	1.613	2.191	2.278	2.946	3.420	3.756
C.D. (P=0.05)	3.421	4.646	4.829	6.246	7.252	7.964

Table 2 : Influence of insoluble Zn sources and *G.diazotrophicus* inoculation on the total chlorophyll content of maize seedlings grown on sand culture

Treatments	Total chlorophyll content (mg/g of fresh wt)						
	15th day	30th day	45th day	60th day	75th day	90th day	105th day
$ZnSO_4$	1.61	1.63	1.67	1. 71	1.76	1.79	1.81
$ZnCO_3$ alone	1.02	1.10	1.13	1.17	1.13	1.12	1.15
ZnCO3+G. *diazotrophicus*	1.08	1.13	1.14	1.16	1.34	1.45	1.47
ZnO alone	1.04	1.06	1.12	1.14	1.17	1.19	1.17
ZnO+ *G. diazotrophicus*	1.05	1.08	1.14	1.18	1.29	1.46	1.58
$Zn_3(PO_4)_2$ alone	1.07	1.12	1.14	1.16	1.18	1.19	1.17
$Zn_3(PO_4)_2$+ *G. diazotrophicus*	1.09	1.13	1.16	1.17	1.31	1.47	1.51
Control	1.06	1.07	1.09	1.14	1.18	1.17	1.15
S.E.±	0.05	0.05	0.06	0.06	0.06	0.06	0.06
C.D. (P=0.05)	0.11	0.11	0.11	0.12	0.12	0.13	0.13

Table 3 : Influence of insoluble Zn sources and *G. diazotrophicus* inoculation on the shoot and root growth of maize seedlings grown on sand culture

Treatments	15 DAS		30 DAS		45 DAS		60 DAS		75 DAS		90 DAS		105 DAS	
	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)
$ZnSO_4$	8.0	24.0	10.0	30.0	125	33.5	13.7	37.0	15.8	39.0	16.9	41.8	18.0	43.0
$ZnCO_3$ alone	5.0	14.0	6.0	19.0	7.8	21.0	8.9	23.6	9.6	27.8	9.9	29.8	10.1	32.0
$ZnCO_3$+ *G. diazotrophicus*	7.0	19.0	8.0	24.0	9.2	26.0	9.6	28.0	9.8	30.6	10.8	34.8	11.0	36.9
ZnO alone	6.0	18.0	7.0	22.0	7.8	24.0	8.2	27:0	8.6	29.7	9.2	30.2	9.8	31.0
ZnO+ *G.diazotrophicus*	5.0	20.0	8.0	26.0	9.6	28.7	9.8	30.6	10.6	30.4	11.6	34.6	11.9	38.0
$Zn_3(PO_4)_2$ alone	4.0	19	6.8	21.0	7.2	24.5	7.8	27.0	8.2	28.9	8.9	30.4	9.4	32.0
$Zn_3(PO_4)_2$ + *G. diazotrophicus*	7.0	21.0	8.0	24.0	9.2	26.0	9.8	28.6	10.4	31.0	11.2	34.8	11.6	37.0
Control	6.0	18.6	7.0	23.0	7.8	25.6	8.5	29.0	8.8	30.5	9.2	31.8	9.8	32.6
S.E.±	0.28	0.88	0.35	1.10	0.42	1.21	0.45	1.33	0.49	1.43	0.52	1.56	0.54	1.66
C.D. (P=0.05)	0.58	1.81	0.72	2.25	0.86	2.48	0.92	2.73	0.99	2.93	1.06	3.20	1.10	3.39

DAS -Days after sowing

G. diazotrophicus was evident from the light of above discussions as the experiment was carried out in sterile sand culture under controlled condition.

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