World Journal of Soil and Crop Sciences Research
Vol. 1, No. 1, March 2014, pp. 1 - 9
Available online at http://wjscsr.com/

Research article

Micronutrients uptake in soybean (*Glycine max L.*) as affected by *Bradyrhizobium japonicum* inoculation and phosphorus (p) supplements.

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Abstract

Field and glasshouse experiments were set up to study the effect of *B. japonicum* inoculation and P supply on the availability of micronutrients (Zn, Cu, Fe and Mn) in soybean (*G.max* L.).The treatments consisted of *B. japonicum* inoculation (with & without), phosphorus supplementation at the levels of 0, 20, 40 and 80kgP.ha^{-1}. Both treatments were replicated four times in a split plot under randomized complete block design. The following plant nutrient elements were analyzed; Zn, Cu, Fe and Mn. Both in glasshouse and field experiment, results showed that inoculation with *B. japonicum* significantly contribute the uptake of Zn, Cu, Fe and Mn in soybean plant. Similarly, phosphorus supplementation significantly enhanced the uptake of micronutrients Zn, Cu and Mn. At the highest level of P supply (80 Kg P.ha^{-1}), Zn uptake was reduced.

Key words: legumes, nutrient uptake, Zinc (Zn), Copper (Cu), Iron (Fe), Manganese (Mn)

Introduction

Soybean (*Glycine max* L.) is an important leguminous crop which has been recognized as one of the premier agricultural crops today. It is the best source of potential protein and oil which can substitute other sources of good quality protein such as milk, meat and fish, therefore has become very suitable to some areas where other protein sources are scarce or too expensive to afford particularly to poor communities in Africa (Anwar et al., 2010). From this scenario, an established technological aspect trying to improve the productivity quantity and nutritional quality such as micronutrient content is of best solution for attaining protein ratio in sustainable healthy condition for poor community in Africa. The efficiency production of *G. max* L. is inhibited by several factors; one of them is inadequate N levels in soil and lack of appropriate rhizobia strain which can fix atmospheric nitrogen in the beneficial of the legumes for best production in term of yields (Woomer et al., 1997). However, soybean (*G.max* L.) has a unique feature of symbiotically associating with specific strain of *Rhizobium* mostly *B. japonicum* and convert atmospheric nitrogen into utilizable form to the plant; some may remain in the soil as residues which can enhance other biological processes in the soil (Coskan and Dogan, 2011). Research verification propose that, *B. japonicum* inoculation of legumes such as soybean and consequent N_2 fixation process might have constructive effects on legume growth and micronutrients availability in different plant components (Rengel, 1999). The availability of certain micronutrients such as Fe and Zn in plants and perhaps other macronutrients may be influenced by certain rhizobial bacteria which enabled them to become more available to plants (Fabiano et al., 1994; Wani et al., 2008; Ndakidemi et al., 2011). To-date, most *Bradyrhizobium* inoculants have been developed and are primarily used for supplying N_2 to plants. Beneficial soil bacteria such as *B. japonicum* may significantly influence the chemistry of micronutrients in soils by enhancing or reducing micronutrient uptake by plants (Ndakidemi et al., 2011). Little is known about their effect on influencing the availability of micronutrients such as Mn, Fe, Cu, and Zn in leguminous plants such as soybean. Therefore, there is a need of establishing a possible role which could be played by legumes with *B .japonicum* inoculants on the availability of micronutrients in legumes such as soybean.

Fertility and productivity of soils in different parts of the world is rapidly declining due to nutrient harnessing without replenishment. P is an important plant nutrient for root development, crop maturity and production, crop quality and resistance to plant diseases (Dubey, 1997: Schachtman et al., 1998). Studies done by Eutropia and Ndakidemi, (2013) reveal that phosphorus enhanced growth and development of soybean. P tends to promote strong early plant growth and development of a strong root system, and enhances metabolic functions for the uptake of different nutrients by various plant organs (Leidi and Rodriguez-Navarro, 2000).This study was conducted with the objective of evaluating effects of *Bradyrhizobium japonicum* inoculation and phosphorus supplementation on the uptake of micronutrients uptake in soybean.

Materials and Method

Description of site Location

Glasshouse and field experiments were conducted at Selian Agricultural Research Institute (SARI) and Tanzania Coffee Research Institute (TaCRI) at Northern part of Tanzania. The coordinates of SARI lies at Latitude 3°21'50. 08" and Longitude 36°38'06.29"E at an elevation of 1390 masl with mean annual rainfall of 870 mm. The mean maximum temperature ranges from 22°C to 28°C whiles the mean minimum temperature ranges from 12°C to 15°C respectively. Meanwhile, TaCRI is located at Latitude 3°13'59.59"S and Longitude 37°14'54"E, at an elevation of

1268 masl with mean annual rainfall of 1200 mm. The mean maximum temperature is about 21.7°C and the mean minimum temperature is about 13.6°C with relative humidity of about 94%. Physical and chemical characteristics of the soil were shown in Table 2.

Experimental design

The field experiment was carried out during the long rain season while for glasshouse was conducted under controlled conditions during 2013 cropping season respectively. The glasshouse and field experimental treatments consist of 2 levels of Rhizobia (with and without rhizobia), 4 levels of P-supplementation (0, 20, 40, and 80kg $P.ha^{-1}$). The experimental design pursued a split plot design with 4 replications per treatment. The soybean seeds were seeded at a spacing of 50cm by 20cm while the plot size measured at 4m by 3m. The plant population density was 200,000 plants per hectare. The *Bradyrhizobium japonicum* strain USDA 110 (Batch number 23011302, S) were obtained from *MEA* Company Nairobi-Kenya, sold under license from the University of Nairobi. Soybean seeds (Soya 2 variety) were obtained from the breeder based at Uyole Agricultural Research Institute-Mbeya-Tanzania. Before sowing, the soybean seeds were thoroughly mixed with *B. japonicum* inoculants to supply (10^9cells/gseed), following procedures stipulated by products manufacturer. To avoid contamination, the uninoculated seeds were planted first followed with the inoculated seeds. Three seeds were planted and thinned to two plants after full plant establishment. Weeding and other agronomic practices were done manually using hoe at different growth stage of the soybean plant.

Plant Harvest and Sample preparation

Before flowering, plant samples from glasshouse and field experiments were collected for plant nutrients analysis. For field experiment, 10 plants were randomly sampled from the middle rows from each plot. The same exercise was done at glasshouse, but, all two plants from each pot were collected. Sampled plants were carefully removed out of the soil with their entire root system. The plants were oven dried at 60^0C for 48hours, ground into a fine powder (2mm sieve) for plant nutrient analysis of Zn, Cu, Fe and Mn.

Measurements of nutrients in soybean plant organs

Micronutrients (Cu, Zn, Fe and Mn) were extracted by diethylene triamine penta acetic acid (DTPA) (Lindsay and Norvell, 1978) and determined by an atomic absorption spectrophotometer.

Statistical analysis

A 2-way ANOVA was used to analyze the nutrients in soybean plant organs. The analysis was done using the STATISTICA software program 2013. Fisher's least significant difference was used to compare treatment means at p=0.05 (Steel and Torrie, 1980).

Results and Discussion

Effects of *B. japonicum* inoculation on micronutrient uptake in the whole plant of *G. max* L.

Bradyrhizobium japonicum inoculation in *G. max* L. plants significantly increased the uptake of Zn, Cu, Fe and Mn in glasshouse experiments, whereas the uptake of Cu, Fe and Mn were significantly increased and that of Zn reduced in the field experiment (Table 1).

Effects of P supplementation on micronutrient uptake in the whole plant of *G. max.* L.

P supply significantly affected the uptake of Zn, Cu and Mn in glasshouse experiment (Table 1). The highest uptake in Zn was observed by supplying 20 and 40 kg $P.ha^{-1}$, whereas, for Cu, the best uptake was recorded by supplying between 40-80 kg $P.ha^{-1}$ in the glasshouse experiment relative to the control treatment. The uptake of Mn was significantly higher by applying 80 kg $P.ha^{-1}$ in the glasshouse experiment relative to all other treatments (Table 1).

In field experiment, P supply significantly increased the uptake of Cu and Mn only. The highest uptake of Cu was recorded in treatments supplied with 40-80 kg $P.ha^{-1}$ and with Mn the best uptake was observed in plots supplied with 80 kg $P.ha^{-1}$. P supply also numerically increased the uptake of Zn and Fe in the field experiment, but the increase was not statistically significant (Table 1).

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Table 1: Effect of *B. japonicum* and P supplementation on the micronutrient uptake in the whole plant of *G.max*.L

	Glasshouse				Fields			
Treatments	Zn(mg.plant^{-1})	Cu(mg.plant^{-1})	Fe(mg.plant^{-1})	Mn(mg.plant^{-1})	Zn(mg.plant^{-1})	Cu(mg.plant^{-1})	Fe(mg.plant^{-1})	Mn(mg.plant^{-1})
B.japonicum								
-R	268.26±19.80b	63.15±3.04b	576.68±17.79b	143.23±7.54b	888.53±21.27a	582.85±85.74b	612.10±24.19b	495.74±26.45b
+R	398.73±12.32a	77.41±3.83a	736.44±17.63a	172.21±8.97a	523.03±29.77b	884.52±152.57a	701.02±25.05a	644.31±22.58a
Phosphorus (kg.Pha^{-1})								
0	321.85±28.39ab	56.89±3.68c	650.79±28.17a	113.90±5.18d	690.02±98.79a	387.21±168.24b	650.05±45.62a	509.48±33.24b
20	358.87±33.82a	68.16±4.74bc	678.74±45.40a	153.20±8.86c	743.35±80.78a	397.82±130.41b	654.16±40.96a	583.01±58.02ab
40	362.81±39.37a	74.85±5.80ab	681.13±46.20a	172.79±8.85b	716.80±69.84a	1079.26±139.83 a	626.75±33.81a	563.67±27.09ab
80	290.45±27.62b	81.23±3.99a	615.57±30.71a	190.99±7.54a	672.96±57.02a	1070±279.22a	695.27±32.93a	623.92±46.52a
2-way NOVA(F-Statistic)								
B.j	32.69***	12.94**	47.44***	25.51***	102.42***	5.14*	6.15*	19.92***
P	2.24ns	6.88**	1.74ns	33.15***	0.73ns	8.76***	0.63ns	2.04ns
*B.j**P	0.20ns	0.38ns	1.92ns	0.54ns	1.53ns	0.53ns	0.82ns	0.88ns

-R: Without *Bradyrhizobium*: +R: With *Bradyrhizobium*: Values presented are means ± SE. *, *** =significant at P≤0.05, P≤0.001 respectively, ns = not significant. Means followed by similar letter in a given column are not significantly difference from each other at *p*=0.05. *B. j=Bradyrhizobium japonicum*, p=phosphorus

Table 2: Chemical & Physical characteristics of the soil

Parameter name	Units	Values
Particle size	<2µm	29
	2 – 20µm	20
	20 – 50µm	11.4
	50 - 2000µm	39.6
pH (1:2.5)	H_2O	6.4
Organic Carbon (KCL)	%	1.96
Total N	%	0.197
Available P (Bray-1)	mg/Kg	5.1
Exchangeable bases	Ca	2.69
	Mg	1.42
	K	0.94
	Na	0.11

Discussion

Plants absorb nutrients only from the liquid solution phase of the soil, but the problem with micro-elements is their limited solubility and therefore their limited presence in the solution. *Bradyrhizobium japonicum* supply to *G.max* significantly affected the uptake of some micronutrients in both glasshouse and field experiments respectively (Table 1). The significant effects observed in Zn, Cu, Fe and Mn in the whole plant of the *G.max* with *B. japonicum* inoculation is an added benefit leave alone significance role of symbiotic association of N_2 fixation into utilizable forms in legumes. Studies conducted by Ndakidemi et al. (2011) reported that rhizobial strain significantly increases the uptake of Zn, Mn, and Cu in different plant organs of common bean. The positive results of *B. japonicum* on the uptake of Zn, Cu, Fe and Mn in soybean plant is not known, but could be possibly positive modifications of rhizosphere environment (Bambara and Ndakidemi, 2010) hence improving plant growth, dry matter accumulation and hence the uptake of nutrients.

In the present study, supplying P showed some significant positive effects in the uptake of Zn, Cu, and Mn in glasshouse and/or field experiments respectively (Table 1). Phosphorus plays important role in root growth in plant (Shahid et al., 2009). The positive effects observed in the study could be due to the active root growth which enables the plant to look for more water and nutrients in soil solution, which brings about nutritional management of the plant. However, the uptake of Zn was reduced at highest P supply (80 $KgP.ha^{-1}$). It has been reported that soil and plant factors tend to be altered when high doses of phosphorus applied in the growth medium and thus induce Zn deficiency (Robson and Pitman, 1983). Studies by Singh et al. (1988) point out that increasing P supply decreased Zn concentration in *P.vulgaris*. The highest P level which induce reduced Zn uptake in the measured plant organs might be caused by the known phenomenon of higher P content in the growth medium affecting the physiological availability of Zn by lowering its mobility in the tissue (Cakmak and Marschner, 1987).

Conclusion

B. japonicum and phosphorus supplementation had positive impacts in increasing the uptake of Cu, Zn, Fe and Mn in the soybean plant. However, supplying highest P level in this study (80 kg $P.ha^{-1}$) resulted into reduced Zn uptake.

Acknowledgement

This study was supported by Tanzania Commission for Science and Technology (COSTECH) and Nelson Mandela African Institution of Science and Technology (NM-AIST) - Tanzania. The Tanzania Coffee Research Institute and Selian Agricultural Research Institute are acknowledged for providing the study sites.

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