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THE EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) AND SPRAY OF MICRONUTIRNT ON TWO SOYBEAN (*Glycine max*) CULTIVARS

Mohammadali Esmaeili, Ayoub Heidarzade and Samira Ramezani
Department of Agronomy, Sari Agricultural Sciences and Natural Resources University, Sari, Iran
E-Mail: esmaeili33@gmail.com

ABSTRACT

In soybean (*Glycine max*) the efficiency of biological fixation of nitrogen will depends on many factors which related to the plant and soil bacteria symbiosis, and to the environmental limiting factors. Low soil fertility and limited availability of macro and micro nutrients are the most important constraints. Therefore, this study was carried out to investigate the agronomically response of two soybean cultivars (033 and Elite) to foliar application of micronutrients and PGPR inoculation (such as Azotobacter and Azospirillum) under field condition in Sari Agricultural Sciences and Natural Resources University in northern Iran. The experiment was randomized in a split-split plot arrangement based on complete block design with three replications. Main plot was included two soybean cultivars (033 and Elit cultivars). Subplots consist of inoculation and non-inoculation treatment by Plant Growth Promoting Rhizobacteria (Commercial biofertilizer consist of Azotobacter and Azosprillium). Foliar application of micronutrients Iron, Zink and Manganese individually and in combination with each other (in 8 levels) was considered as the sub-sub plot. The biological yield of two studied cultivars was affected and significant in 1% probability level. The biofertilizer inoculation individually didn't show any significant effect among all studied traits but its interaction by cultivar was significant for biological yield and seed oil content at 1% probability level. Between all treatments only foliar application of micronutrients showed acceptable significantly differences for all studied traits. Overall, it can be concluded that with adequate supplies of the micronutrients and biofertilizer (contaning *azotobacter*) will have favorable effects on soybean quality and growth in similar condition.

Keywords: azotobacter, azosprillium, oil content, protein content.

INTRODUCTION

In soybean, environmental acceptable sources of N requirements are biological atmospheric nitrogen fixation (BNF) which will be possible by the symbiotic association between the plant and bacteria (*bradyrhizobium* genus). The efficiency of this biological fixation process will depends on many factors which related to the plant and soil bacteria symbiosis, and to the environmental limiting factors. Low soil fertility and limited availability of macro and micro nutrients are the most important constraints. Soybean production is reduced due to deficiency of micronutrients especially Fe, Mn and Zn (Khudsar *et al*., 2008; Caliskan *et al*., 2008). The use of microorganisms with the aim of enhancing nutrients availability for plants is an important tools and necessary for agriculture. High performance in agronomically yield of crops in response to PGPR inoculation have been several time reported during the past decades, (Zhang *et al*., 1996; Amara and Dahdoh, 1997; Chanway, 1998; Pan *et al*., 1999; Bin *et al.*, 2000; Gupta *et al.*, 2000; Asghar *et al*., 2002; Gray and Smith, 2005; Silva *et al*., 2006; Figueiredo *et al*., 2008). But it just in a few studies the interaction of micronutrient spray and using PGPR inoculation was investigated.

Also, periviose studies (Davis, 1983; Fox and Guerinot, 1998; Kobraee *et al*., 2013) have shown that the application of essential micronutrients such as Zink, Iron and Magnesium could improve the yield and yield components of crops. Foliar fertilization of nutrients due to the quick translocation of these nutrients to different parts of the plant is better method than the soil application (Neumann, 1982). Also Ghasemian *et al*. (2010) showed that, micronutrient spray can enhance soybean resistance to environmental stress.

Meanwhile role of micronutrients should not be ignored. Iron, zinc and manganese have several important roles in the plant, including protein synthesis, photosynthesis, chlorophyll synthesis, carbohydrate transport and metabolism, growth hormones regulation (auxin) pollen and flower formation (Hafeez *et al*., 2013); functioning as an activator or cofactor of at least 35 enzymes. Manganese is part of the structure of an important antioxidant (superoxide dismutase) that protects plant cells by deactivating free radicals, which can destroy plant tissue (Diedrick *et al*., 2010); Chlorophyll development and function, energy transfer within the plant, constituent of certain enzymes and proteins, plant respiration and metabolism, nitrogen fixation. Therefore, this study was carried out to investigate the agronomically response of two soybean cultivars (033 and Elite) to foliar application of micronutrients and PGPR inoculation (such as Azotobacter and Azospirillum) under field condition in Sari Agricultural Sciences and Natural Resources University in northern Iran.

This experiment was conducted at Sari Agriculture Sciences and Natural Resources, University in north of Iran during 2013 cropping season. The experiment was randomized in a split-split plot arrangement based on complete block design with three replications. Main plot was included two soybean cultivars (033 and Elit cultivars). Subplots consist of inoculation and non-inoculation treatment by Plant Growth Promoting

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Rhizobacteria (Commercial biofertilizer consist of Azotobacter and Azosprillium). Foliar application of micronutrients Iron, Zink and Manganese individually and in combination with each other (in 8 levels) was considered as the sub-sub plot.

Soybean planting

In order to enhance the seedling emergence rate and better stand, pre wet planting system was used. After emergence at V_2 stage thinning operation was performed until the seedlings optimum density (50 Pl/m^2) was obtained. According to the soybean water requirements the drip irrigation method was used. The gap filling operation was done after 1 week of thinning. For yield and yield components the soybean plants were harvested at full maturity stage.

Protein content of soybean seeds based on total nitrogen content

Nitrogen content of soybean seeds was measured by Total Kjeldahl Nitrogen (TKN) method (Isaac and Johnson, 1976), (Kjeltec Auto1030 Analyzer, Foss Tecator AB, Hoganas, Sweden). For nitrogen determination the pure seeds of each cultivar were dried (Fan Azma Gostar, 24060, Iran) for 72 h. Total reduced nitrogen was determined by using a micro Kjeldahl procedure with sulfuric acid, digestion catalyst and conversion of organic nitrogen to ammonium form according to the Total Kjeldahl Nitrogen (TKN) method. Nitrogen content is then multiplied by a factor to arrive at protein content. The average nitrogen (N) content of proteins that found by the above method led to use of the calculation N × convert factor (5.71) (King-Brink and Sebranek, 1993).

Total seed oil content

Total seed oil content determined by using the Soksele device, so the pure seeds of each treatment were dried and weighed before insert into the device. The chloroform was used as solvent, it is a popular solvent seed oil extraction, particularly for lipids of intermediate polarity and when mixed with methanol it becomes a general extraction solvent. So the dried and powdered seed samples were inserted into the soksele device and the extraction was completed by evaporating the solvent.

Statistical analysis

Data were analyzed by General Linear Model (GLM) in SAS, and treatment means were compared by least significant differences (LSD) test at the $P \leq 0.05$ level.

RESULTS

According to analysis of variance (Table-1), the biological yield of two studied cultivars was affected and significant in 1% probability level. The biofertilizer inoculation individually didn't show any significant effect among all studied traits but its interaction by cultivar (C×B) was significant for biological yield and seed oil content at 1% probability level. Between all treatments only foliar application of micronutrients showed acceptable significantly differences for all studied traits (Table-1).

Table-1. Analysis of variance of two soybean cultivars for studied traits.

S.O.V	df	Mean square			
		Biological yield	Total seed yield	Seed protein content	Seed oil content
R	2	6226648^{ns}	610265.4*	82.68^{ns}	17.79^{ns}
Cultivar (A)	1	22748456**	483652.0^{ns}	46.24^{ns}	42.66^{ns}
Error (a)	2	72576	926615.9	13.84	8.79
Biofert. (B)	1	4477680^{ns}	5254517^{ns}	16.16^{ns}	0.66^{ns}
A×B	1	242529089**	1787276.6^{ns}	65.24^{ns}	165.37**
Error (b)	4	1879255	927325.1	5.07	2.85
Foliar App. (F)	7	233306669**	1527211.7*	109.46**	181.01**
A×F	7	128447933**	875449.1^{ns}	87.05**	10.07^{ns}
B×F	7	114250454**	606431.0^{ns}	21.41^{ns}	11.21^{ns}
A×B×F	7	86824625**	809509.8^{ns}	87.87*	30.97^{ns}
Error	56	2856367	536919.9	31.25	16.56
CV (%)		6.27	19.16	14.18	11.30
*, ** and ns; significantly at 5 and 1 % probability levels, not significant, respectively.					

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Biological yield

The highest improvement on biological yield of 033 cultivar with 28.838 ton/ha was obtain when Zn+Mn micronutrient and inoculation treatment were applied and the lowest amount of biological yield was recorded when Zn+Fe+Mn was used in non-inoculation treatment (with 10.950 ton/ha) (Figure-1 A).

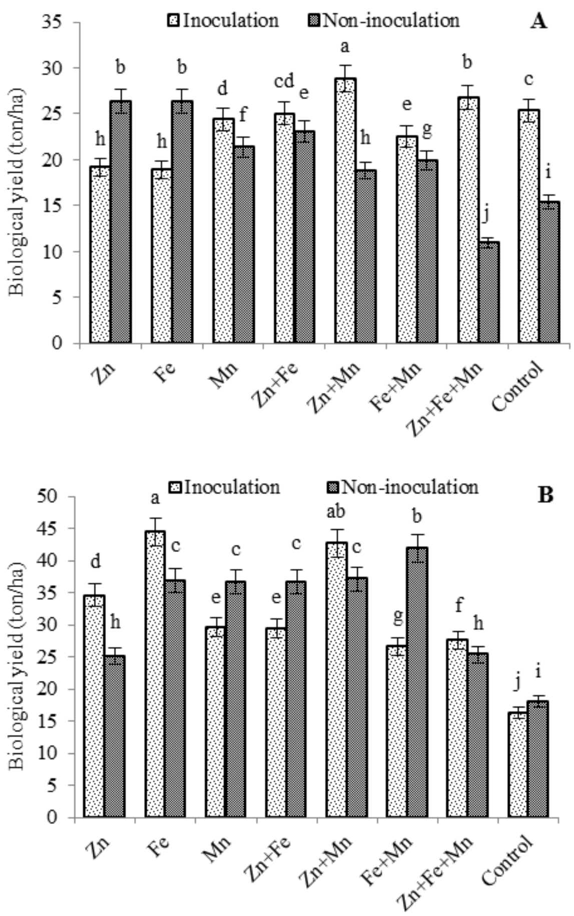


Figure-1. Response of two soybean cultivars biological yield (A, 033 and B, Elit) to micronutrient application and PGPR inoculation interaction.

In term of the other cultivar (Elit) the highest amount of biological yield was obtain for both Fe and Zn+Mn micronutrient application under inoculation treatment (with 44.456 and 42.662 ton/ha, respectively). The minimum biological yield was related to control for both inoculation and non-inoculation treatment (Figure-1 B).

Seed yield

According to the mean comparison (Figure-2), all applied micronutrients had significantly positive effect on total seed yield of studied cultivars compare to control. So the maximum seed yield was recorded in foliar application of Zn+Mn micronutrient with 37% increase compare to control (3133 kg/ha). Also in this respect, no significantly differences were observed between Zn, Fe and Zn+Mn foliar application (Figure-2). These results are partially in agreement with those of Kobraee *et al.* (2013); Mehasen and Saeed (2005); Kandil *et al.*, (2012) and Seadh and Abido (2013).

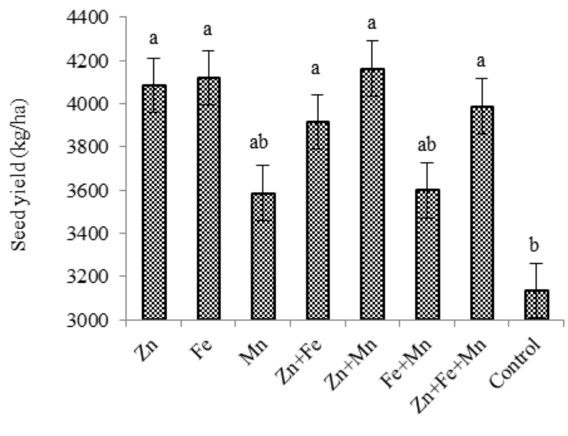
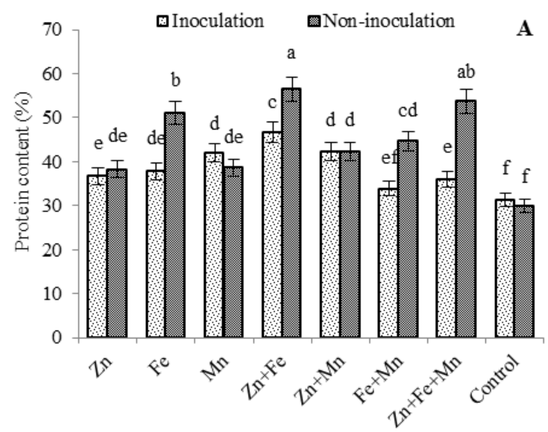


Figure-2. Response of total seed yield to micronutrient application.

Seed protein content (%)

The protein content in 033 cultivar increased by micronutrients application compare to control (Figure-3A). So the highest amount of protein content (with 56.46%) was related to Zn+Fe micronutrient application under non-inoculation treatment. Also the lowest amount was belonging to control in both inoculation and non-inoculation (with 31 and 29 %, respectively).

The results of mean comparison for Elit cultivar (Figure-3B) indicated that, all levels of micronutrient application had significantly positive effects on protein contents compare to control with the exception of Fe+Mn application in non-inoculation treatment. Also the maximum effect was obtained when Mn micronutrient was applied under inoculation treatment (with 45.83%).



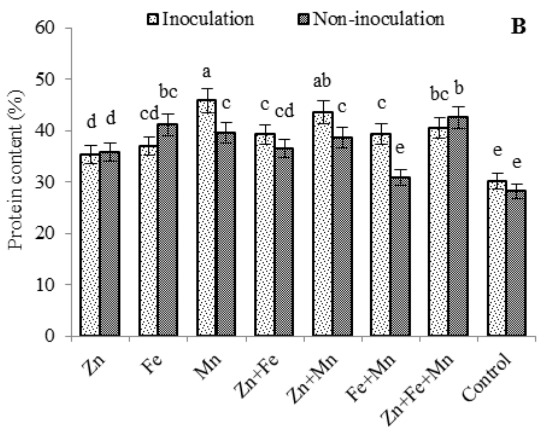


Figure-3. Response of two soybean cultivars (A, 033 and B, Elit) protein content to micronutrient application and PGPR inoculation interaction.

Seed oil content

According to the mean comparison (Figure-4) all studied treatment showed significantly effects on seed oil content compare to control. Although, there was no significantly difference between the majority of micronutrient treatment except control, but the greatest amount of seed oil content was related to Zn+Fe spray (with 40% oil content).

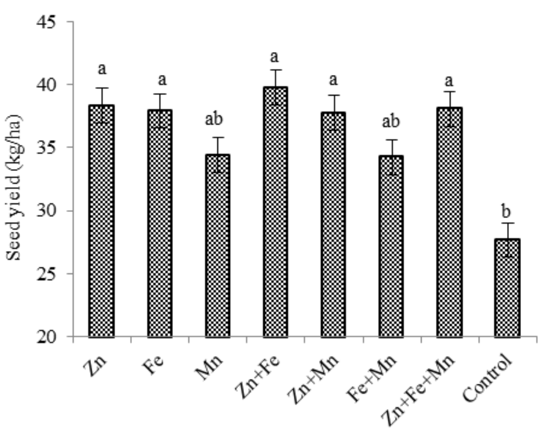


Figure-4. Response of seed oil content to micronutrient application.

DISCUSSIONS

Overall, essential micronutrients such as Zink, Iron and Manganese could improve the yield and yield components of each plant; many studies were suggested these benefit relationship between plant and micronutrients applying (Diedrick *et al.*, 2010; Kobraee *et al.*, 2013; Salwa *et al.*, 2011). But it just in a few studies the interaction of micronutrient spray and using PGPR inoculation was investigated. In addition, uptake and distribution of micronutrients into different parts of plant affected by genotypic characteristic (Heitholt *et al.*, 2002). In our present study soybean Elit cultivar showed better response to these interactions than the 033 cultivar, so that the highest seed protein content was related to Mn micronutrient spray in inoculated Elit cultivar by PGPR. Manganese functions in nitrate reduction where it acts as an activator for the enzymes nitrite reductase and hydroxylamine reductase. The preference of ammonia over nitrate as a nitrogen source by manganese deficient cells supports the above mentioned role of manganese in protein synthesis. *Aotobacter* and *azosprilium* as plant growth promoting rhisobacteria having many key roles in plant critical tasks. Some of these important mechanisms, which can be promote plant growth, were consist: increasing mineral nutrient solubilization and nitrogen fixation, making nutrients available for the plant; improving plant stress tolerance to drought, salinity, and metal toxicity; and production of phytohormones such as indole-3-acetic acid (IAA) (Gupta *et al.*, 2000). PGPR, in combination with efficient rhizobia, could improve the growth and nitrogen fixation by inducing the occupancy of introduced rhizobia in the nodules of the legume (Tilak *et al.*, 2006). According to Saravana-Kumar and Samiyappan (2007), Bradyrhizobium promoted the nodulation and growth of legumes in combination with active ACC deaminase containing PGPR. ACC-deaminase activity in PGPR plays an important role in the host nodulation response (Remans *et al.*, 2007).

CONCLUSIONS

Micronutrients such as Iron, Zinc and Manganese and PGPRs are and will be the potential tools for realizing of sustainable agriculture goals and trend for the future. Therefore, it will be necessary to know and clear explanation of the other bacterial traits effects for different environmental conditions and plants. In this experiment, the micronutrient using as a useful method in combination by PGPR inoculation were investigated and the results indicated the benefit interactions between them. Also, some Antagonistic effects between studied micronutrient were observed. Finally, it can be concluded that with adequate supplies of the micronutrients and biofertilizer (contaning *azotobacter*) will have favorable effects on soybean quality and growth in similar condition.

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