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The Co-application of Plant Growth Promoting Rhizobacteria and Inoculation with Rhizobium Bacteria on Grain Yield and Its Components of Mungbean (*Vigna radiate* L.) in Ilam Province, Iran

Abdollah Hosseini, Abbas Maleki, Khalil Fasihi, Rahim Naseri

Abstract**—**In order to investigate the effect of Plant Growth Promoting Rhizobacteria (PGPR) and rhizobium bacteria on grain yield and some agronomic traits of mungbean (*Vigna radiate* L.), an experiment was carried out based on randomized complete block design with three replications in Malekshahi, Ilam province, Iran during 2012-2013 cropping season. Experimental treatments consisted of control treatment, inoculation with rhizobium bacteria, rhizobium bacteria and *Azotobacter*, rhizobium bacteria and *Azospirillum*, rhizobium bacteria and *Pseudomonas,* rhizobium bacteria, *Azotobacter* and *Azospirillum*, rhizobium bacteria, *Azotobacter* and *Pseudomonas*, rhizobium bacteria, *Azospirillum* and *Pseudomonas* and rhizobium bacteria, *Azotobacter*, *Azospirillum* and *Pseudomonas*. The results showed that the effect of PGPR and rhizobium bacteria were significant affect on grain and its components in mungbean plant. Grain yield significantly increased by PGPR and rhizobium bacteria, so that the maximum grain yield was obtained from rhizobium bacteria + *Azospirillum* + *Pseudomonas* with the amount of 2287 kg.ha^{-1} as compared to control treatment. Excessive application of chemical fertilizers causes environmental and economic problems. That is, the overfertilization of P and N leads to pollution due to soil erosion and runoff water, so the use of PGPR and rhizobium bacteria can be justified due to reduce input costs, increase in grain yield and environmental friendly.

Keywords**—***Azotobacter*, Mungbean, *Pseudomonas*, Rhizobium bacteria.

I. Introduction

LEGUMES are the second most important food source of protein that belongs to the leguminosae family. Mature and dried seed of legumes have high nutritional value and storage good ability [1]. Legumes increase soil fertility due to nitrogen fixing on their roots, so they added large amounts of nitrogen to the soil after harvesting [2]. Mungbean (*Vigna radiate* L.) is an important crop in Asia that its green pod uses fresh and some types of them apply as cover crops and so to produce hay [3]. Efforts to increase production per unit area and excessive and imbalance consumption of chemical fertilizers has negative environmental implications in addition to increasing costs of production and low yields. The production and release of organic acids into the soil environment is considered the principal mechanism for mineral-P solubilization in phosphate-solubilizing microorganism (PSM) [4]-[6]. Numerous authors have reported positive effects of a single inoculation with phosphate-solubilizing microorganism (PSM) on various crops [4], [7], [8] while little is known on the use of mixed cultures or co-inoculation with other rhizospheric microorganisms. Results by Zaidi and Khan [9] suggest a synergistic interaction between PSM (*Pseudomonas striata* and *Penicillium*) and *Azotobacter chroococcum*, allowing a better use of poorly soluble P sources (rock P) and enhanced dry-matter accumulation, seed yield, and P uptake by wheat plants. Interactions of PSM reportedly result in an increase of the plant-available soil P, biological N_2 fixation, an enhanced availability of some trace elements such as Fe and Zn, and the production of plant growth–promoting substances [4], [10]. Long-term studies showed that crop yield decreased due to excessive use of fertilizers due to soil loss of acidification, biological activity and soil loss and reduce the lack of use of chemical fertilizers, that lead to acidification of soil, decades of the biological activities of soil, reduce of the soil properties and decrease of the micronutrients such as Fe, Zn, Cu and Mn ultimately it has threated the health of human [11]. In this connection, the use of PGPR is a strategy for improving plant growth, plant nutrition and environmental sanitation. These microorganisms are capable to convert unusable nutrients to usable in a biological process. Bio-fertilizers contain symbiotic bacteria, mycorrhizal fungi and PGPR [12]. Kennedy et al. [13] reported that inoculation with *Azotobacter* could increase grain yield of rice plant by 0.9 kg.ha^{-1}. This inoculation with *Azotobacter* increased nitrogen accumulation by 15 kg.ha^{-1} due to biological nitrogen fixation. Also, the inoculation with *Azotobacter* in wheat plant provided 50% of the required nitrogen of plant in form urea in greenhouse conditions.

Abdollah Hosseini is with M.Sc in Agronomy, College of Agriculture, Islamic Azad University, Ilam Science and Research Branch, Ilam, Iran (corresponding author: phone: +9183421191; fax: +98841-222-7526; aria8652116@gmail.com).
Abbas Maleki is with Department of Agronomy and Plant Breeding, College of Agriculture, Islamic Azad University, Ilam Science and Research Branch, Ilam, Iran (phone: +989188426034; maleki_abbas@ymail.com).
Khalil Fasihi is with Department of Agronomy and Plant Breeding, College of Agriculture, Islamic Azad University, Ilam Science and Research Branch, Ilam, Iran (phone: +989181410284; fax: +98841-222-7526; kh_fasihi2005@yahoo.com).
Rahim Naseri is with Young Researchers and Elite Club, Ilam Branch, Islamic Azad University, Ilam, Iran (phone: +988410134; rahim.naseri@gmail.com).

Sayadi et al. [14] stated that efficient effect of *Azospirillum* on plant growth caused by its effect on root growth at the early stage of germination. Also their indicated that the effect of inoculation with *Azospirillum* was significant on chlorophyll index, root dry weight, number of branch and pod per plant, grain per pod, 1000-grain weight, pod length and pod width and biological yield.

Hamidi and Asgharzadeh [15] found that inoculating maize seeds with *Azotobacter chrococcum*, *Azospirillum brasilense*, *A. lipoferum* and *Pseudomonas fluorescens* increased the number of leaves. Zahir et al. [16] also reported the enhancement of maize leaves number inoculated with *Azotobacter* and *P. fluorescens*. Other studies represented that the bacterial inoculation improved catalase activity and chlorophyll content, growth and the number of leaves in sunflower [17], [18]. Rohitashav-Singh et al. [19] indicated the increased number of leaves in maize as the result of bacterial inoculation. The improvement of stem length may be attributed to the effect of the applied bacterium on the plant vegetative growth. Hernandez et al. [20] represented that the bacterial inoculation significantly increased maize plant height and stem diameter. Zahir et al. [16] also reported 8.5% enhancement in maize height as the result of *Azotobacter* and *Pseudomonas fluorescence* inoculation. Tilak et al. [21] represented that the co-inoculation of *Pseudomonas putida* and *Rhizobium* strains affected growth, nodulation and enzymes activity in pigeon pea, better that the individual application.

Dardanelli et al. [22] reported that inoculation with *Azospirillum* on cowpea and alfalfa increased root growth. Therefore, the present study was carried out to determine the most suitable strain of PGPR and rhizobium bacteria to improve quality and quantity of grain yield in mungbean in Ilam province, Iran.

II. Materials and Methods

A. Site Description

This experiment was carried out in a controlled field (Malekshahi, Iran) during 2012-2013 cropping season. Field is geographically located about 330˚4'- 33˚32' latitude and 47 ˚16'-47 degree 53' E longitude with 1427 m above mean sea level. In order to analyse the soil properties before performing experiment, sampling of soil took from 0-30 cm depth of soil. Analysis of soil properties is shown in Table I.

TABLE I
Soil Properties of Experimental Field

EC (ds/m)	1.4
pH	7.1
Total nitrogen (%)	0.06
Potassium (ppm)	190
Phosphorus (ppm)	9.8
Soil texture	Loam
Clay (%)	29
Silt (%)	44
Sand (%)	27

B. Experimental Details

The experiment was carried out based on randomized complete block design with three replications. experimental treatments consisted of control treatment (without inoculation), inoculation with rhizobium bacteria, rhizobium bacteria and *Azotobacter*, rhizobium bacteria and *Azospirillum*, rhizobium bacteria and *Pseudomonas,* rhizobium bacteria, *Azotobacter* and *Azospirillum*, rhizobium bacteria, *Azotobacter* and *Pseudomonas*, rhizobium bacteria, *Azospirillum* and *Pseudomonas* and rhizobium bacteria, *Azotobacter*, *Azospirillum* and *Pseudomonas*. Each plot had 4 plant line with 50 cm row spacing and 15 cm plant density each plot had 4 meters length with width 2.5 m, plots within 2 m and distance between replications were 1 m. Research Institute for the Biology Laboratory of bacteria from the soil and water were provided. Inoculation with bacteria was performed following way: initially seed spilled into a polyethylene bag and for every 1 kg of seed 1-15 ml sticky substance was added to seeds and the seeds well shaken.

Then 0.5 g bacteria added for every 1 kg of seed and the mixture was stirred well. Bacteria-treated seeds flattened on clean foil in shadow and dried. Planting was done August 7, 2013 and the first irrigation was performed immediately.

C. Crop Sampling and Calculation

Harvesting was performed on 5th October 2013. Sampling to investigate the different traits was done from two middle lines after excluding the marginal effect. Studied traits consisted of number of pod per plant, grain per pod, 1000-grain weight, grain yield and biological yield.

D. Statistical Analysis

After collecting the necessary data for statistical analysis of sampled data included analysis of MSTATC, SAS and Excel programs were used to sort the data and draw the shape. Mean comparison was also conducted with Duncan's Multiple Range Test (DMRT).

III. Results and Discussion

A. The Number of Pod per Plant

Results of variance analysis showed that effect of PGPR and rhizobium bacteria were significant effect on the number of pod per plant (Table III). The results showed that inoculation with *Pseudomonas* + Rhizobium + *Azospirillum* had a positive effect on the number of pod per plant, so that it obtained by 23.83 pod with *Pseudomonas* + Rhizobium + *Azospirillum* which increase to 32% as compared with control treatment (Fig. 1). In general *Azotobacter* followed by nitrogen fertilizer increased seed yield and yield components by positive influence on macro elements absorption such as N, P and K, micro elements such as Zn and Fe improving water distributing in plant, developing nitrate reductase activity and finally increased the plant hormones which play animportant role in plant growth [24], [25]. Sayadi et al. [14] stated that *Azospirillum* significantly increased the number of pod per plant in mungbean. Since the number of pod per plant is one of the factors related to grain yield, therefore any factor that increases yield, it also affect on this trait. Possibly on of the reasons increase in the number of pod per plant was mainly due to increase in the number of branch by inoculation with bio-fertilizer.

B. The Number of Grain per Pod

The results showed that PGPR and rhizobium bacteria had a positive significant effect on the number of grain per pod, so that the maximum the number of grain per pod was obtained from inoculation with *Azospirillum* + Rhizobium + *Pseudomonas* treatments by 10.96 grain that the number of grain per plant increased by 15% as compared with control treatment (Fig. 2).

TABLE II
ANALYSIS OF VARIANCE FOR PLANT GROWTH PROMOTING RHIZOBACTERIA AND INOCULATION WITH RHIZOBIUM BACTERIA ON GRAIN YIELD AND IMPORTANT AGRONOMIC TRAITS OF MUNGBEAN

		MS				
S.O.V	d.f	number of pods per plant	number of grain per pod	1000-grain weight	Grain yield	biological yield
Replication	2	4.127 ^{ns}	0.097 ^{ns}	6.960 ^{ns}	82692.7 ^{ns}	593549 ^{ns}
PGPR × Rhizobium bacteria	8	10.683 **	1.439 **	45.198 **	422314.1 **	1598779 **
Residual	16	0.438	0.077	9.606	23152.5	155829
CV (%)	df	3.08	2.72	5	8.3	10.22

ns, * and ** Non Significant and significant at 5% and 1% probability levels, respectively.

Shokuh Far et al. [25] reported that the PGPR and rhizobium bacteria significantly increased the number of grain per pod of soybean plant. The sink capacity of plant determines by the number of grain per plant. More grain per pod makes more assimilates store in grain, so grain yield increased [26].

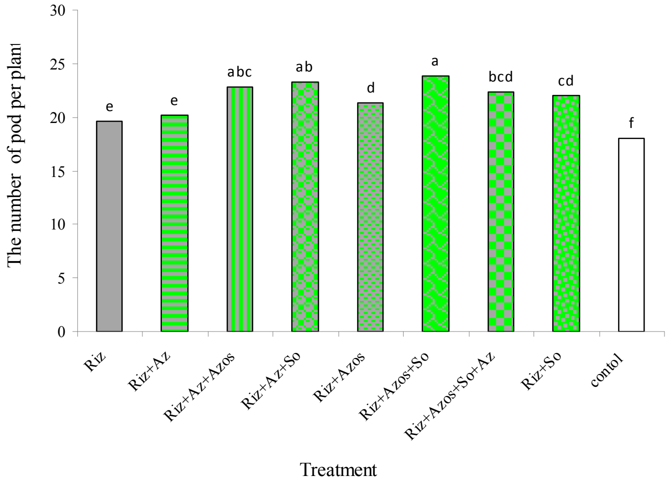

Fig. 1 Effect of PGPR and rhizobium bacteria on the number of pod per plant in mungbean

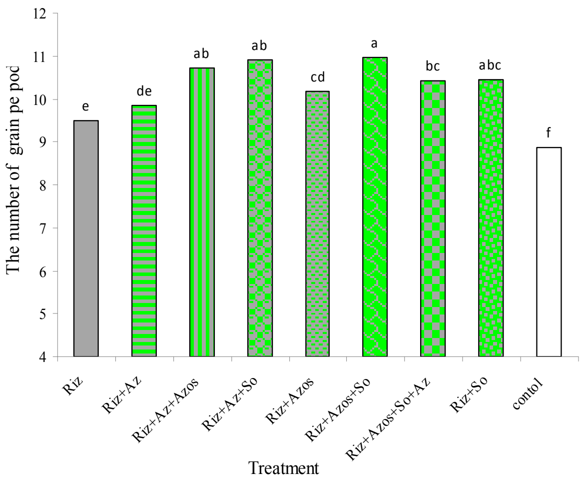

Fig. 2 Effect of PGPR and rhizobium bacteria on number of grain per pod in mungbean

C. 1000-Grain Weight

The results showed that there was significant on 1000-grain weight by rhizobium bacteria and PGPR, so that the highest 1000-grain weight with mean of 65.8 g was recorded by rhizobium bacteria and PGPR that increased by 21% as compared to control treatment (Fig. 3). Probably rhizobium bacteria and PGPR significantly affect on 1000-grain weight by providing N for host plant.

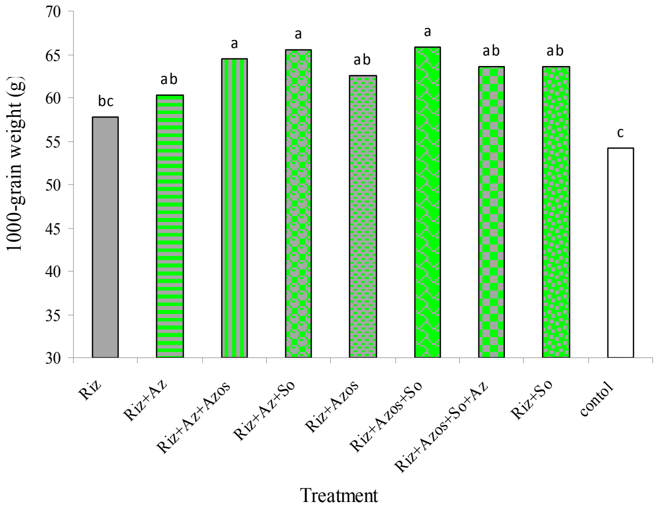

Fig. 3 Effect of PGPR and rhizobium bacteria on 1000-grain weight in mungbean

Kato [27], Kumari and Valarmathi [28] stated that grains with higher weight have the grain filling rate. Bio-fertilizers improved photosynthesis maybe by increasing water and nutrients absorption leading to produce more assimilate and improving plant growth, thus 1000-grain weight increased as compared to non-inoculation treatment [23].

D. Grain Yield

The results showed that yield significantly affected by rhizobium bacteria and PGPR (Table III), so that the highest yield was obtained from Rhizobium + *Pseudomonas* + *Azospirillum* by 2287 kg.ha^{-1} that increased by 98% as compared to control treatment (Fig. 4). The mechanisms by which PGPRs promote plant growth are not fully understood, but are thought to include: the ability to produce phytohormons, a symbiotic N_2 fixation. These results are agreement with those obtained by Naseri and Mirzaei [23], Naseri et al. [24] Singh et al. [29]. They believed that the highest production of grain yield belonged to inoculation with *Azotobacter* in safflower, rapeseed and wheat which under normal conditions, respectively. Zaidi and Khan [30] suggest a synergistic interaction between PSM (*Pseudomonas striata* and *Penicillium*) and *Azotobacter chroococcum*, allowing a better use of poorly soluble P sources. The obtained results are in agreement with those of Zahir et al. [16] who represented that the co-inoculation of *Azotobacter* and *Pseudomonas* increased maize grain yield by 19.8%.

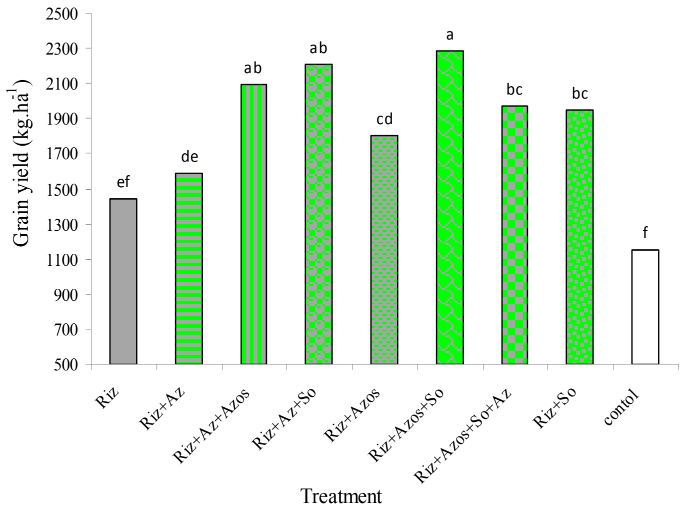

Fig. 4 Effect of PGPR and rhizobium bacteria on grain yield in mungbean

E. Biological yield

The results showed that biological yield was significantly affected by rhizobium bacteria and PGPR (Table III). The highest biological yield with the mean of 4733 $kg.ha^{-1}$ was recorded inoculation with rhizobium + *Azospirillum* + *Pseudomonas* that significantly increased as compared to control treatment (Fig. 5). These findings are supported by the past studies of Zahir et al. [16] who found that inoculating maize seeds with *Azotobacter* and *Pseudomonas* increased the shoot dry weight. Zahir et al. [31] suggested that *Azotobacter* and rhizobium bacteria are able to induce the production of IAA, GA_3 thereby improving growth of plants. Phosphate-solubilizing bacteria can produce both organic acids that can improve the chemical availability of P and growth-promoting substances, which can stimulate plant growth [32]. Hasanzadeh et al. [33] also found that bacterial inoculation increased dry matter accumulation in wheat and sorghum, also, their found that bacterial inoculation increased dry matter accumulation in wheat and sorghum. Intensive use of chemical fertilizers and other chemicals has produced environmental problems and increased production costs. Recent studies indicated that using PGPR and inoculation with rhizobium bacteria also improving soil physiological structure and also increase organic matters content, nitrogen and phosphorous available to coexistent plant. Of course, before it is recommended to massive production and widely application it is necessary to implement and replicate this study in different regions.

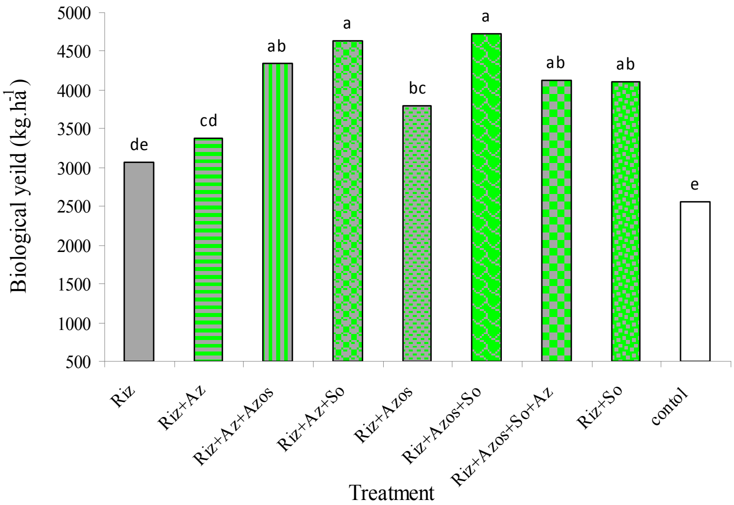

Fig. 5 Effect of PGPR and rhizobium bacteria on biological yield in mungbean

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