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Effects of P solubilizer bacteria and AM fungi on forage maize growth in a semi-arid region in Iran

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Applications of beneficial microbes, biofertilizer, are well known to an alternative for chemical fertilizer application in sustainability agricultural crop production. The objective of this field study was to evaluate P solubilizer (*Bacillus coagulance,* B) with or without an arbuscular mycorrhizal fungus (*Glomus mosseae,* GM) on the growth of maize (*Zea mays*) forage in a clay–loam soil. The application treatments included control (no fertilizer), chemical fertilizer, and two types of biofertilizer, arbuscular mycorrhizal fungus and P solubilizer bacterium. Growth and nutrient response of maize to the application of chemical fertilizer (CF) significantly resulted in the highest biomass, seedling height and N assimilation of plant. Growth response of maize to the application of biofertilizer containing mycorrhizal fungus and solubilizer bacteria (MB) significantly resulted in the highest biomass and seedling height compared to control, 24.4% and 6%, respectively. Mycorrhizal fungus had the more positive effect in N and P assimilation of corn, especially in leaf and stem, in compared to B, BM and control (F_0). While the AMF seemed to have an inhibiting effect on the P-solubilizing bacteria, P solubilizer bacterial inoculation had positive effect on the mycorrhizal colonization value. Mycorrhizal fungus significantly increased N-fixer bacteria of maize rhizosphere in compared to P solubilizer bacterial inoculation.

Key words: Corn, P solubilizer microorganism, mycorrhizal

Introduction

Chemical nitrogen fertilizers are used worldwide to sustain and increase the yield of crop. Despite its efficiency in promoting crop yield, it have proved to be hazardous for soil health as well as for the well being of human and animal populations (Nain *et al.,* 2009). Current trends in agriculture are focused on the reduction of the use of inorganic fertilizers, forcing the search for

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alternative ways to improve crop yield in sustainable agriculture (Smit *et al.* 2001). Plant Growth-Promoting Rhizobacteria (PGPR) play an important role in mineralization and mobilization of nutrients required for plant growth. They assumed to be an alternative to the use of chemicals (Fischer *et al.,* 2007). PGPR may benefit the host by causing plant growth promotion or biological disease control (Fischer *et al.,* 2007). PGPR activity has been reported in strains belonging to several genera, such as *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Acetobacter*, *Burkholderia* and *Bacillus* (Kloepper, 1993; Glick and Bashan, 1997).

Providing a direct physical link between the soil and plant roots, arbuscular mycorrhizal fungi (AMF) are important rhizospheric microorganisms. There is well-documented evidence that arbuscular mycorrhizal fungi (AMF) contribute to increasing availability and uptake of P and micronutrients (Krishna and Bagyaraj, 1991). The AMF symbiosis enhances plant growth and increases the plants' access to forms of N that are unavailable to non mycorrhizal plants (Barea *et al.* 1987; Azco'n-Aguilar *et al.* 1993).

Despite the vast amount of data accumulated on beneficial biofertilizer on plant growth promoting, our knowledge of this association is limited to ensure a consistent positive interaction between the bacteria, AMF and the plant in terms of yield. There is a need to conduct a series of research in a semi-arid environment to evaluate plant growth promotion co-inoculated with arbuscular mycorrhizal fungus (AMF). This research was carried out to evaluate the effects of this biofertilizer, AMF and P solubilizer bacteria**,** on the promotion of corn forage growth (*Zea mays*) under semi-arid environment condition.

Materials and methods

To evolution potential of biofertilizer, AMF and PGPR, a field experiments were conducted in a semi-arid area of Lorestan province, Koohrang (47° 39′N, 33° 31′W, and 1197 m above sea level), Iran, during the dry season. Annual rainfall of this area is 405 milliliters that is mostly occurred during winter and less in spring. The experimental design was a factorial design with three randomized complete blocks. The treatments included AMF and its combination with (MB) or without *Bacillus coagulance* (B), fertilization level (100%) and control (F_0). Fertilization level for this experiment was 250 kg ammonium-N and 50 kg P per hectare which is prevalently used for corn production of this area. The inocula of the arbuscular mycorrhizal fungi (AMF) species, *Glomus mosseae* and P solubilizer, *Bacillus coagulance,* were purchased from the Agricultural and Biotechnology Research Institute, Karaj, Iran.

Corn was tilled planted in a north–south orientation in 3-m-wide, 0.60 cm between rows, 6 m in length. The crorn hybrid was 'cordana', a full-season corn hybrid (120 d), was planted at the rate of 120 000 seeds ha^{-1} at the irrigated site. Previous crop was wheat. Granular NH_4NO_3 (400–0–0 N–P–K) was broadcast with similar fractions being applied at planting, tillering, and reproductive stage. 150 kg granular P was broadcast prior to planting. Corn planting dates were 25 July. Yield and yield component data collected included total dry forage yield $plant^{-1}$, dry leaf and stem forage yield $plant^{-1}$, dry ear weight $plant^{-1}$, dry ear leaf weight $plant^{-1}$, and nutrient P and N content uptake. Total P were extracted by digesting shoot tissue with 3 ml concentrated H_2O_2 at 500 C, and determined by the molybdenum blue method (Page *et al.,* 1982). Total N was determined according method described by Page *et al.* (1982). Leaf chlorophyll rate was assayed by chlorophyll meter (SPAD-502 Minota Co., Ltd. Japan) from 15 samples per plant m^{-2} at grain filling stage growth. Chlorophyll rate was non-destructively measured on leaves that were fully expanded.

Microbial inocula

The mycorrhizal fungus inocula consisted of spores and hyphal root fragments from a stock culture of *G. mosseae*, and were a pure culture of *G. mosseae* isolated from Karaj (Iran). The dose of inoculum (115 spores mL^{-1} of inoculum) was 600 g m^{-2}. The soil of the field was fumigated. The mycorrhizal colonization assessment was performed with the method described by Brundrett *et al.* (1996). The root samples were washed in distilled water, stained with trypan blue, and the mycorrhizal colonization levels determined using the gridline intersect method of Giovanetti and Mosse (1980).

A bacterial culture with a density of 10^8 cells ml^{-1} was used as the inoculum which treated corn seed just before sowing. Enumeration and of N-fixing bacteria, and P bacteria from the fresh soil sample was conducted using suspension dilution techniques on agar plates with differentiating media (Wu *et al.,* 2005).

Data analysis

The analysis of variance (ANOVA) was performed using SAS software (1990). The significance of the differences between treatments was estimated using the LSD range test, and a main effect or interaction was deemed significant at $P \leq 0.05$.

Results

The mycorrhizal inoculum significantly increased the extent of AMF colonization of the root system compared to the uninoculated control treatments (Fig. 1). Plant inoculated with *Bacillus coagulance* had higher colonization value. However this value was not significantly different from plant that were no-inoculated with *Bacillus coagulance.* The present results demonstrated that the population size of the solubilizer bacteria and fixer-bacteria varied in accordance with the type of fertilization and AMF colonization in the rhizosphere. GM+B resulted in a higher level of mycorrhizal root and a larger community of P solubilizer and P solubilizer bacteria in rhizosphere.

Chemical fertilizer had the positive effect on yield and components yield as expected (Table 1). Biofertilizer affected the plant growth of corn. The control plants showed poor growth, which may be attributed to nutrient deficiency, e.g. the lack of available N/P in the unfertilized soil. In addition, sterilization of the soil killed the native microflora which assisted plant growth and nutrient uptake. The inoculation of corn with bacteria *Bacillus coagulance,* AMF *Glomus mosseae* and GM+B significantly ($P<0.05$) increased the dry forage weights from 41% to 43% as compared to the control (Table 1). The most effective shoot and root dry weight fertilizer was CF which generated 97.5% increase in dry forage over the control (Table 1). Bifofertilizer contain GM and B increased seed weight per ear (26.4% to 12.7%) as compared to the control. Individual icoculation of GM and B more increased dry ear weight with compared to GM+B (Table 1). The maximum yield of dry ear weight was obtained with the treatment of CF. GM, B and GM+B had the similar effect on dry ear weight. The maximum yield of dry stem and leaf weight was obtained with the treatment of CF. GM, B and GM+B had the similar effect on dry stem and leaf weight. Plant inoculated with biofertilizer, GM, B and GM+B, achieved the greatest height from 6% to 10% when compared to control treatment. CF significantly increased 48% in height as compared to the control. Inoculation corn with GM and B significantly ($P<0.05$) increased chlrophyll of leaf (96%- 98%) as compared to the control (Table 1). CF Had the most effective increasing in chlorophyll, 218% as compared to the control.

Results showed that GM seemed to be more effective than B to improve plant nutrient uptake N concentration in plants (Fig. 3 and 4). N concentration in shoot and ear was higher in plant inoculated with GM compared to B or control. Plants under CF treatments achieved the greatest height of N concentration in plants (Fig. 3 and 4). GM seemed to be more effective on leaf and stem P content than B while B had more effective on ear P content (Fig. 5).

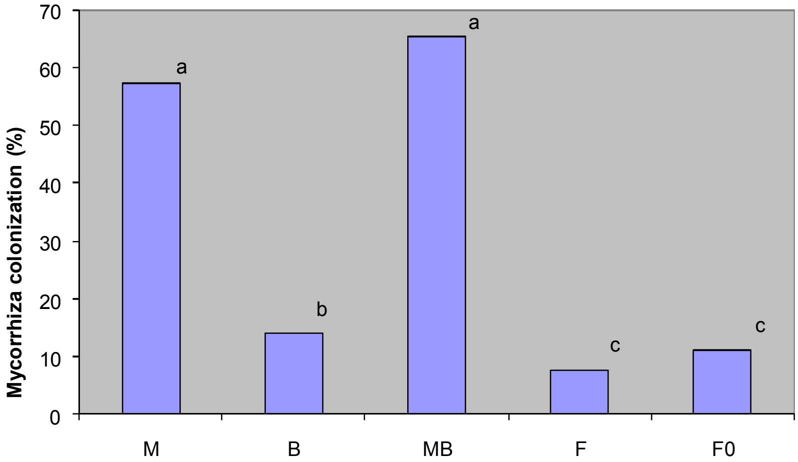

Fig. 1. Effect of chemical fertilizer (F), no-fertilizer (F0) and biofertilizer, *Bacillus coagulance* (B) and *G. mosseae* (M), on mycorrhiza colonization value.

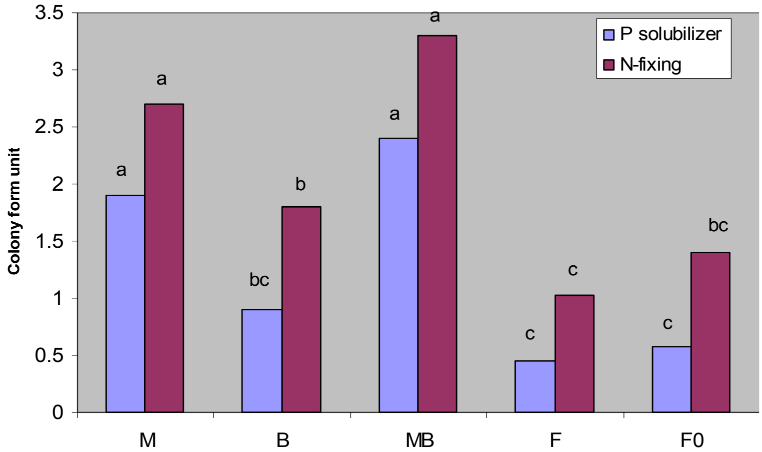

Fig. 2. Effect of chemical fertilizer (F), no-fertilizer (F0) and biofertilizer, *Bacillus coagulance* (B) and *G. mosseae* (M) on population size of introduced P solubilizers bacteria (10^6 CFU g^{-1} dry soil) and indegenus N-fixing (10^4 CFU g^{-1} dry soil) in the rhizosphere of maize after 100 days of growth.

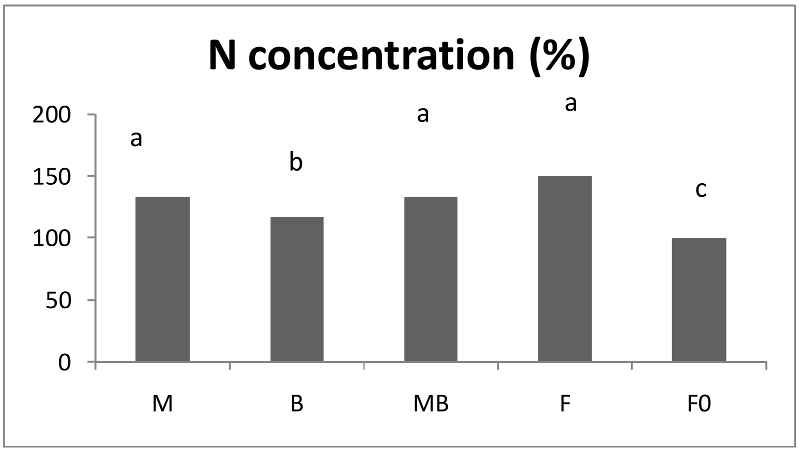

Fig. 3. Effect of chemical fertilizer (F), no-fertilizer (F0) and biofertilizer, *Bacillus coagulance* (B) and *G. mosseae* (M), on N content of corn leaf and stem. N content in control treatment was 0.6% (100%).

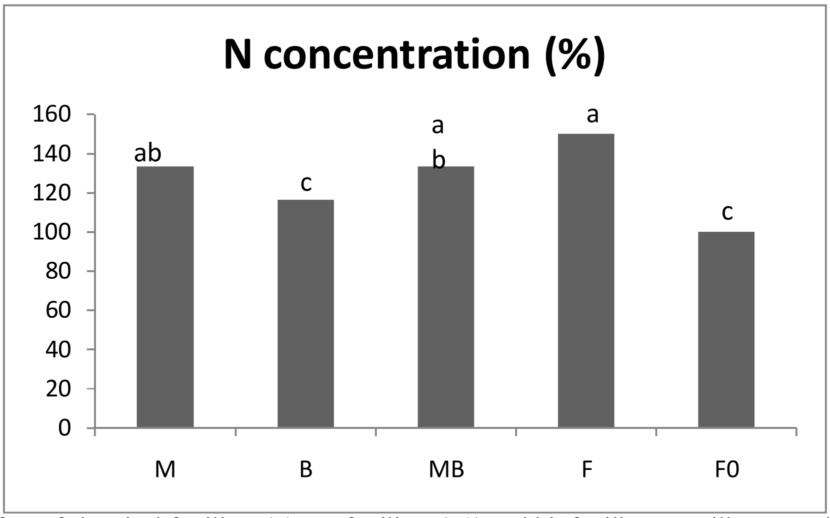

Fig. 4. Effect of chemical fertilizer (F), no-fertilizer (F0) and biofertilizer, Bacillus coagulance (B) and G. mosseae (M), on N content of ear. N content in control treatment was 0.62% (100%).

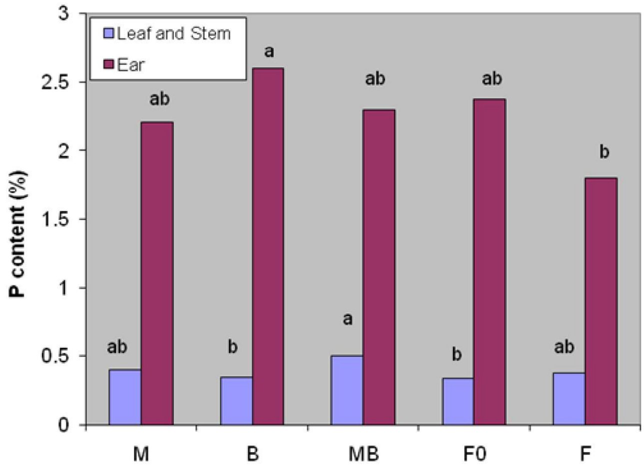

Fig. 5. Effect of chemical fertilizer (F), no-fertilizer (F0) and biofertilizer, Bacillus coagulance (B) and G. mosseae (M), on P content of corn ear and leaf and stem.

Table 1. Effect of fertilizer and biofertilizer on corn yields and yield components, Plant yield, seed weight per ear, ear weight, and stem and leaf weight for control treatment were 216.9, 56.1, 87, 13.9, 129.9 g, respectively, which is present as 100%. Plant height in control treatment was 203 cm (100%).

Treatments	forage yield Plant^{-1}	Seed weight ear^{-1}	ear weight Plant^{-1}	Ear leaf weight Plant^{-1}	Stem and leaf weight Plant^{-1}	Chlorophyll	Height (cm)
G. mosseae(GM)	162.6 b	126.4a	132.6b	192.4ab	182.5ab	198b	110.3b
Bacillus coagulance (B)	141.6 b	127.7ab	118.3b	115.1ab	157.2b	169.7bc	110b
GM + B	143.8b	103.3b	116.4b	143.3ab	162.2b	196.2b	106.1b
Ferilizer	197.5a	161.9a	184a	155.7a	206.5a	318.5a	148.5a
Control	100c	100b	100c	100b	100c	100 c	100c

Values within one column followed by the same liner letter are not significantly ($P \leq 0.05$) different.

Discussion

Stimulation of different crops by rhizobacterial inoculation has also been demonstrated by other studies both in laboratory and field trials. For example, it was reported that wheat yield increased up to 30% with *Azotobacter* inoculation and up to 43% with *Bacillus* inoculants (Kloepper *et al.,* 1989); and a 10–20% yield increase in the same crop was reported in field trials using a combination of *B. megaterium* and *A. chroococcum* (Brown, 1974). Strains of *Pseudomonas* have increased root and shoot elongation in canola, lettuce and tomato (Hall *et al.,* 1996; Glick *et al.,* 1997). Dually inoculated plants with *G. mosseae* with or without *Bacillus coagulance* showed expectedly higher N and P concentrations in the plant tissue. The fungi assisted the host to assimilate the maximum total N and P and resulted in a higher biomass. The inoculation with *G. mosseae* had a more stimulating effect on the assimilation of N than *B. megaterium*. However, *B. megaterium* performed better than control in stimulating N uptake and plant growth. *B. megaterium* when combined with AMF inoculation had the better in stimulating N uptake and plant growth. Barea *et al.* (1987) and Azco'n-Aguilar *et al.* (1993) reported that the AMF symbiosis enhances plant growth and increases the plants' access to forms of N that are unavailable to non mycorrhizal plants.

Wu *et al.* (2005) reported that the arbuscular mycorrhizal fungi (AMF) had a higher root infection rate in the presence of bacterial inoculation. It has been noted that the rhizobacteria can act as mycorrhization helper bacteria, which improve the ability of mycorrhizal fungi to colonize plant roots (Fitter and Garbaye, 1994; Wu *et al.,* 2005). However, the mechanisms by which these bacteria stimulate AM colonization are still poorly understood. Specialized bacterial activities such as the production of vitamins, amino acids, and hormones may be involved in these interactions (Barea *et al.,* 1997). The presence of rhizobacterial inoculation might have assisted in the germination of a large number of spores thus leading to a higher a infection percentage (Tandon and Prakash, 1998).

In conclusion result showed that biofetilizer, AMF and P solubilizer bacteria, had positive effect on corn grow and P and N content. Dual inoculation with AMF and P solubilizer seemed to be the most effective treatment combination to improve corn nutrient uptake. However further result of this field study indicated that biofertilizer can be a partly alternative for chemical fertilizer. Integrated biofertilizer and other fertilizers from organic and non-organic source may have a better effect on corn growth.

References

Azco´n-Aguilar, C., Alba, C., Montilla, M., Barea, J.M. (1993). Isotopic (15N) evidence of the use of less available N forms by VA mycorrhizas. Symbiosis 15: 39–48.

Barea, J.M., Azco´n –Aguilar, C., Azco´n, R. (1987). Vesicular–arbuscular mycorrhiza improve both symbiotic N2 fixation and N uptake from soil as assessed with a 15N technique under field conditions. New Phytologist 106: 717–725.

Brown, M.E., (1974). Seed and root bacterization. Annu. Rev. Phytopathol. 12: 181– 197.

Brundrett, M.N., Bougher, B., Dell, T., Grove, N., Malajczuk, M. (1996). Working with mycorrhiza in forestry and agricultural. Australian centre for international agricultural reaserch monograph 32, Canberra, 347pp.

Fischer, S.E., Fischer, S.I., Magris, S., and Mori, G.B. (2007) Isolation and characterization of bacteria from the rhizosphere of wheat World J Microbiol Biotechnol 23: 895–903.

Fitter, A.H., Garbaye, J., (1994). Interactions between mycorrhizal fungi and other soil organisms. Plant Soil 159: 123– 132.

Giovanetti HW, Mosse B (1980) An evaluation techniques for measuring vesicular-arbescular mycorrhiza infection in roots. New phytologist. 84: 489-500.

Glick, B.R., Bashan, Y. (1997) Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens. Biotechnol Adv 15: 353–378.

Glick, B.R., Changping, L., Sibdas, G., Dumbroff, E.B., (1997). Early development of canola seedlings in the presence of the plant growth-promoting rhizobacterium Pseudomonas putida GR12-2. Soil Biol. Biochem. 29: 1233– 1239.

Hall, J.A., Pierson, D., Ghosh, S., Glick, B.R., (1996). Root elongation in various agronomic crops by the plant growth promoting rhizobacteria Pseudomonas putida GR12-2. Isr. J. Plant Sci. 44: 37– 42.

Kloepper, J.W. (1993) Plant growth-promoting rhizobacteria as biological control agents. In: Metting FB Jr (ed) Soil microbial ecology-applications in agricultural and environmental management. Marcel Dekker, Inc., New York, pp 255–274, ISBN: 0824787374

Kloepper, J.W., Liftshitz, K., Zablotowicz, R.M., (1989). Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol. 7: 39– 43.

Krishna, K.R., Bagyaraj, D.J., (1991). Role of vesicular arbuscular mycorrhiza in the uptake of micronutrient by groundnut plants. Curr. Res. 20: 173–175.

Nain, L., Rana, A., Joshi, M., Jadhav, S.D., Kumar D., Shivay, Y.S., Paul, S., Prasanna, R. (2009). Evaluation of synergistic effects of bacterial ,and cyanobacterial strains as biofertilizers for wheat. Plant Soil.

Page, A.L., Miller, R.H., Keeney, D.R., (1982). Methods of Soil Analysis: Part 2. Chemical and Microbiological Properties, 2nd edition. Agronomy, vol. 9. ASA, SSSA Publishing, Madison, WI, p. 1159.

Smit, E., Leeflang, P., Gommans, S., Van den Broek, J., Van Mil, S., Wernars, K. (2001). Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. Appl Environ Microbiol 67: 2284–2291.

Tandon, V., Prakash, A., (1998). Influence of soil inoculation with VA mycorrhiza and phosphorus solubilising microorganisms on growth and phosphorus uptake in Sesamum indicum. Int. J. Trop. Agric. 16 (1–4): 201– 209.

Wu, S.C., Cao, Z.H., Li, Z.G., Cheung, K.C. and Wong, M.H. (2005). Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma 125> 155–166.

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