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Assessment of Organic Nitrogen Availability to Potato Plant under Affecting Promoting *Rhizobacterium* and Mycorrhizal Inoculation

Heba M.A. Khalil

Microbiology Department, Soil, Water and Environmental Institute,
Agriculture Research Center, Giza, Egypt

Abstract: The benefits of AMF and GPRB of available N and P to potato were examined in pot experiment containing clay loam soil and fertilized with organic matter. Mycorrhizal colonization of potato plants was over whelmed by bacterial inoculation. Results indicated inoculation with GPRB strains and AMF increased shoot, root and total dry weight of potato plants significantly compared with single inoculation with AMF, or *Ps. Putida*, or *B. megatherium* or *Azosp. Brasilense*. Nitrogen and phosphorus uptake were also increased in both single and dual inoculation compared with uninoculated. So synergistic effect between AMF and GPRB was observed in mycorrhizal root infection. *Ps. Putisa* inoculation significantly increased percentage of mycorrhizal infection. Dual inoculation of potato plant with *Ps. Putida* and AMF improved their growth significantly than other treatments. Generally, the dry matter accumulation, nitrogen and phosphorus uptake by shoots and roots were significantly higher with organic matter by single or dual non-inoculation than inoculated. Similarity, the nitrogen and phosphorus derived from organic matter recovered by shoots were increased by inoculation. These of soil incorporated with organic matter than in soil non-fertilized. It is worthy to mention that the inoculation of potato with *Azospirillum* seems to be promising and beneficial for enhancing potato growth and production. Furthermore, the use of plant residues may act as an ideal slow release source of N and P because of its stability through the period of experiment.

Key words: Potato plant, Arbuscular mycorrhizal fungi (AMF), Growth promoting rhizobacteria (GPRB), P-recovery, N-recovery, Organic residues

INTRODUCTION

The inter relationships between the microorganisms and host plant can play an important role in improving and altering crop productivity through the manipulation of the rhizoplane or rhizosphere microorganisms communities[8]. Recent work with *Azospirillum* spp., has shown that when the N metabolism of diazotrophic bacteria is altered, these bacteria are able to excrete N derived from N_2 fixation[7,6] found that inoculation of wheat with various strains of *Azospirillum* spp. Caused significant increases over the uninoculated controls in grain yield, N,P and K acquisition by the plant. The same author added that these increments might be attributed in process other than nitrogen fixation.

With respect to plant residue application with microbial inoculation Halsall and Gibson[13] reported that rice straw yielded the highest nitrogenase activies with *Azospirillum* than sawdust and sugarcane trash. Similarity, wheat residues maintained high populations of *Rhizobium* spp. in the soil. In most soils where microbial activity is limited by a lack of carbon, cereal stubble represented a substantial potential source of energy and the incorporation into the soil was more effective than mulching on the surface. Seed inoculation with *Azospirillum* spp. in combination with organic amendments induced stimulation of wheat growth and nitrogenase activity[18].

The interactions between functional groups of soil microflora are a key to under standing the dynamic processes that depict plant soil relationships. Among those, the effects of rhizobacteria on the development and functioning of arbuscular mycorrhizal (AM), fungi[19] are of notable interest because the latter form a living link between roots and soil[9]. A.M fungi in turn, affects the composition of bacterial communities[2] and fungi and bacteria in the mycorrhizosphere are through to evoke in concert such plant responses as resistance to stress and diseases[19,10].

The aim of the present work was to evaluate interaction between mycorrhizal fungi and some growth promoting rhizobacteria (GPRBs), on potato plant grown. The effect of GPRBs on mycorrhizal symbiosis was assessed using a potato.

Corresponding Author: Heba M.A. Khalil, Microbiology Department, Soil, Water and Environmental Institute, Agriculture Research Center, Giza, Egypt.

MATERIAL AND METHOD

The bacteria strains, *Pseudomonas putida, Bacillus megatherium, Azospirillum brasilense* were obtained from culture collection of Agric. Microbiology Dept., NRC, Giza, Egypt.

Mycorrhizal spores used in this wre mixtures of *Glumus* spp. These spores were originally extracted by a wet sieving and decanting technique[12] from rhizosphere soil of anion (grown in clay loam soil).

Soil sample, clay loam was in dried, passed through 4 mm mesh sieve. Potato (*Solanum tuberosum* cv. Diamond) was used as the test plant which was obtained Agric. Res. Centre, ARC.

A pot experiment was carried out in the greenhouse of Agric. Res. Centre, A.R.C, Giza, Egypt, Five kg of an air dried and sieved (> 4 mm) clay loam soil mixed with vermiculite and perlite 2:1:1 by volume were placed in earth pots with capacity of 5 L. Five tubers of potato were planted per pot and the seedlings were thinned to five per pot after emergence. Potato tubers were coated with peat-based inoculum of *Ps. Putida* at 10^7, *B. megatherium* 10^8 and *Azosp. Brasilense* at 10^8 before sowing. Treatments were:

1. Uninoculated control.
2. Inoculated with mycorrhiza (AMF).
3. Inoculated with *Ps. putida* (GPRB) + AMF/
4. Inoculated with *B. megatherium* + AMF.
5. Inoculated with *Azosp. brasilense* (GPRB) + AMF.
6. Inoculated with a mixture of both AMF + GPRB in ratio 10:5:1:1 respectively.

These treatment were arranged in completely randomized blocks with three replicates. The plants were harvested at maturity stage (120 days after emergence) and the dry matter of shoot and roots were recorded separately. The plant components were analyzed for nitrogen concentration, accumulation and uptake. Total N was determined by micro-kjeldahl method[5].

The nitrogen and phosphorus derived from organic fertilizer were estimated as described by Galal and Thabet[11]. Nitrogen and phosphorus uptake were calculated as described by Heijden and Kuyper[14].

RESULTS AND DISCUSSION

Micorrhizal Colonization: Inoculation with mycorrhizal fungi increases AMF colonization of roots (Table 1). This indicates that propageus of the native AM fungi was insufficient. Mycorrhizal colonization was enhanced by bacterial inoculation (Table 1). Mycorrhizal colonization of potato plant was over whelmed by bacterial inoculation. *Azospirillum brasilense* exercised lowest effect on mycorrhizal colonization of potato. While inoculation with *Ps. putida* enhanced mycorrhizal colonization. GPRB inoculation stimulated mycorrhizal colonization of potato grown in clay loam soil but had a similar effect on roots of potato plants.

A similar strain effect has been observed previously for early stages of root colonization by mycorrhizal fungi[3]. There are several possible mechanisms for the stimulatory effects. Bacteria might exudates some biologically active molecules that directly or indirectly affect the mycorrhizal fungi. Fluorescent pseudomonades (Like *Ps. putida*) produce numerous metabolites, including plant growth regulators such as auxins, gibberellins, and ethylene, biotin, nicotinic acid and pantothenic acid which effect the growth of plants and microorganisms in soil[3]. The production of physiologically active concentrations of indole-3-acetic acid and some other auxin molecules has been reported in *Pseudomonas* and *Azospirillum*[20,3]. On the other hand, bacteria might affect root cell walls, thereby increasing susceptibility of plant tissue to fungal penetration[26] *Azos. brasilense* produces pectolytic enzymes *in vivo* which soften root cell walls in the soil[24]. *Klebiella* sp. Might produce a volatile substance which stimulates hyphal extension[26].

Table (2) indicates the accumulation of high dry matter and N in shoot and roots of inoculated potato under organic matter. Single inoculation with AMF or *Ps. putida, Azosp. brasilence*, *B. megatherium* had a significant positive general effect on dry matter and N uptake by shoot and roots as compared to the uninoculated control. On the other hand organic matter significantly increased shoot and roots dry weight of potato plant (Table 2).

Simulative effects of AMF and GPRB inoculation on potato were more prominent in the soil whereas the dual effect of AMF and GPRB on potato plants was observed. In general, growth of mycorrhizal potato plants was worse under all GPRB strains. Mycorrhizal potato inoculated with *Ps. putida* or *Azosp. brasilence* produced more root dry weight relative to other treatments. *Azospirillum* potato relation results in higher dry matter accumulation and N-uptake than those recorded with AMF or *Ps. putida*. The *Azosp. brasilence* induced relative increase accumulated for dry matter and N uptake by shoot for (52% and 65%) higher than AMF or *Ps. putida*. Low relative increments of 17% and 44% were estimated for roots. Shoot dry matter and N yields indicated the superiority of dual inoculation over the individual inoculation with organic matter residue incorporated into the soil. Superiority of dual inoculation was more pronounced with the addition of the organic matter residue, when N uptake was considered. In this respect, dual inoculation had increased the shoot dry matter and N accumulation by about 92% (about four fold) over the control, respectively.

Table 1: Mycorrhizal colonization of potato plants grown in clay loam soil and inoculated with GPRB.

Treatments	AMF colonization
AMF	65
AMF + *Pseudomonas putida*	78
AMF + *Bacillus megatherium*	71
AMF + *Azospirillum brasilense*	58
AMF + PS + B + *Azosp.*	68
Uninoculated	15

Table 2: Effect of AMF and GPRB inoculation on plant growth and nitrogen yields of potato plants fertilized with organic matter.

Treatments	Shoot			Root		
	DM g/pot	N.conc. (%)	N. accum. mg/pot	DM g/pot	N.conc. (%)	N. accum. mg/pot
Uninoculated	0.95	0.35	4.5	0.45	0.12	1.2
AMF	1.50	0.45	5.7	0.50	0.15	1.8
Ps. putida	1.15	0.40	5.4	0.52	0.13	1.7
B. megatherium	1.20	0.42	6.0	0.70	0.13	1.6
Azosp. brasilense	1.75	0.51	7.9	0.75	0.16	2.2
AMF + PS	1.80	0.45	6.2	0.68	0.14	2.1
AMF + B.	2.20	0.49	6.5	0.70	0.16	2.65
AMF + *Azosp.*	2.50	0.58	7.5	0.80	0.17	2.5

DM : Dry matter N accum. = N accumulation
N. conc. = N. concentration

N and P Derived from Organic Matter Resistance: Amounts of N derived from organic matter by shoot and roots were presented in Table (3). Shoots gained more N from organic matter as compared to with roots. Amounts of N and P derived from organic matter were significantly increased by inoculation. Dual inoculation by *Azosp.* and AMF or *Azosp. brasilense* and *Ps. putida* was the best treatment followed by *Azosp. brasilense* alone. In this regard, the dual inoculation was still superior over individual inoculation: Lower amounts of N and P derived from organic matter residues and uptake by shoot and roots were recorded (Table 3) these portions were also affected by inoculation.

The promotion effect of *Azosp. brasilense* either alone or in combination with AMF or *Ps. putida* is the most interesting reaction released from the present study. The populations of these microorganisms are influenced by groups of abiotic, and biotic factors[25]. Also, this, AMF or bacteria can occupy another endophytic niches inside different cereal crops as we well discuss later and via different mechanisms can benefits these cereal hosts. Some of the responsible mechanisms plant growth promoting (PGP) and N_2 fixation have been approved through N determined followed in the present study. These finding lead as to focus on the synergistic effect of both bacteria and AMF on each other and gave as the opportunity to suggest further research in this area on field scale with different cereal crops and different soil types. Noteworthy, some positive finding with dual inocula of *Azospirillum* and *Pskeudomonas* were previously described by author under field conditions[10]. They showed that these bacteria could produce auxin, and gibberellins representing two major closes of plant growth regulators. In this respect, Antoun[1] explained that the beneficial effects of *Azorhizobium* are related not only to its N_2-fixing proficiency but also to the ability of producing antibacterial and antifungal compounds, growth regulators and siderophores, Salch *et al*.[22] confirmed the promotion of maize growth by *Azorhizobium caulmodans* inoculation.

Nitrogen and phosphore in shoots: N and P uptake in potato shoots were stimulated mainly by *Azosp.* and AMF inoculation. AMF inoculation with all GPRB strains significantly increased N and P uptake. The increase in N uptake was superior in combinations with AMF, GARB and *Azosp.* . Also the increase in P uptake was superior in combination with AMF, GPRB and *B. putida*.

Table 3: Nitrogen and phosphore derived from fertilizer organic matter recovery as affected by inoculation with AMF and GPRB.

Treatments	N-derived organic matter		P. derived organic matter	
	(%)	Mg/pot^{-1}	(%)	Mg/pot^{-1}
Uninoculated	43.50	1.05	3.52	0.45
AMF	43.50	1.50	4.75	0.62
Ps. putida	20.45	1.25	3.75	0.65
B. megatherium	23.15	1.45	5.25	0.85
Azosp. brasilense	28.25	1.75	4.15	0.73
AMF + PS	21.34	1.80	6.22	0.82
AMF + B	15.25	1.85	6.75	1.25
AMF + *Azosp*	20.16	1.95	6.52	0.75

Table 4: N and P uptake (mg/plant^{-1}) in shoots of potato plants inoculated with AMF and GPRB

Treatments	N-uptake	P-uptake
Uninoculated	50.75	1.25
AMF	80.75	1.75
Ps. putida	65.25	2.15
B. megatherium	75.21	1.95
Azosp. brasilense	62.82	0.95
AMF + PS	112.25	3.95
AMF + B.	135.45	3.75
AMF + Azosp.	132.15	2.25

Attia and Awad[2] found that, inoculation with AMF, *Ps. putida* and *Azsp.* increased N and P uptake in shoots of tomato plants. No synergistic effect between AMF and GPRB was observed in AMF root infection. Ravnskov and Jakobsen[21] found that, dual inoculation with *G. indraradices* and *Ps. fluorescens* and not lead to a synergistic effect on P uptake by plants. However, a synergistic effect of dual inoculation with a multi strain mix of different species of AMF and *Ps. putida* was observed on P concentration in plants by Attia and Awad[2]. These dissimilar results might be related to the use of different *Pseudomonas* species. Will and Sylvia[26] found that there was no consistent evidence for a synergistic effect of dual inoculation with *Klebsiella* sp. and AMF on sea oat growth. Bagyaraj and Menge[4] reported that there is a synergistic or additive beneficial effects on tomato plants grown in sterilized as well as in unsterilized soil, when plants were inoculated with both *Glomus* spp. and *Azotobacter* sp.

Our results further indicate a synergistic interaction between AMF and certain strains of GPRB on N and P available from organic matter and the plant growth has been no longer lost and thus, the use of combined inoculation in horticultural and field crops to maximize the contribution of plant growth is treatment. The synergistic effect on plant growth has been accepted. The study of such combinations under field conditions will be the aim of future research.

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