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Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizal fungi on the utilization of Bayovar rock phosphate by alfalfa plants using a sand-vermiculite medium

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Abstract

Phosphate-solubilizing bacteria (PSB) exhibited a high efficiency to improve plant growth and nutrition in the presence of Bayovar rock phosphate when sand-vermiculite was used as a culture medium. Treatments with dual inoculum (PSB plus mycorrhiza) significantly ($P \leqslant 0.05$) increased alfalfa growth. Bacteria-microbial fungi interactions resulted in a greater utilization of the rock phosphate added to the rooting medium. Although Bayovar rock phosphate *per se* can be considered an inert substrate because it did not stimulate plant growth, metabolites released by PSB were able to transform the rock into available forms which could be utilized by alfalfa plants. *Glomus fasciculatum* was the most efficient mycorrhizal endophyte under the experimental conditions employed.

Introduction

Simultaneous dual inoculation of vesicular-arbuscular (VA) mycorrhiza and phosphate-solubilizing bacteria (PSB) has been shown to stimulate plant growth more than inoculation of either microorganism alone in certain situations when the soil is phosphorus deficient (Barea *et al.*, 1975). The combined action of bacteria solubilizing rock phosphate and mycorrhiza which enable the plant roots to rapidly absorb solubilized P might enhance the ability of plants to utilize the phosphorus which is present in a sparingly soluble natural phosphate. Nevertheless the utilization of insoluble P sources is only occasionally proved (Barea *et al.*, 1983). One possible explanation for the fact that rock phosphate dissolution is not systematically encountered could be that most studies have been conducted with alkaline soil, rich in Ca^{++}, in which newly solubilized P can be easily fixed before plant roots have had a chance to absorb it. A substrate without any P-adsorbing capacity was therefore selected for the present study.

In addition to the possible VAM and PSB cooperation in phosphorus uptake by plants the interaction between microorganisms could also be seen as the result of the bacterial ability to stimulate plant growth (Lynch, 1976; Rovira, 1965), VA fungi development and mycorrhizal formation (Azcón *et al.*, 1978; Azcón-Aguilar and Barea, 1985). These facts have been attributed to bacterial metabolites like plant growth-promoting substances (Strzelczyk and Pokojska, 1984).

The objective of this report was to evaluate the efficiency of three mycorrhizal fungi and of a phosphate-solubilizing bacterium in making rock phosphate P available to alfalfa plants which grow on a sand-vermiculite substrate. Interactions between inoculants were also examined.

Materials and methods

Alfalfa was grown during six weeks in 300 g of a sand vermiculite (1:1 v/v) medium which had been previously washed and sterilized by autoclaving.

Plants were treated twice weekly with the nutrient solution previously described (Hepper and O'Shea, 1984) (50 ml/pot, week). This mineral solution devoid of nitrogen and enriched with $CaCl_2$ ($0.115\,g\,l^{-1}$) has been found to give high levels of infection in the presence of *Glomus mosseae*. The pH was adjusted to 6.8–7. Plants were inoculated with 3 different endomycorrhizal fungus species: *Glomus mosseae* (Nicol. dx Gerd) Gerd & Trappe, *Glomus fasciculatum* (Taxter sensu Gerd) Gerd. and Trappe and *Glomus* sp. (unidentified form of Glomus) by placing into the planting hole 2 g per pot of soil from a stock culture rich in spores and infected root fragments.

The phosphate-solubilizing bacterial inoculum assayed was isolated from local soil and cultivated in our laboratory. It is capable of dissolving rock phosphate *in vitro*. Bacteria were grown in a rotatory shaker at 150 ppm at 28°C for 14 days in 250-ml flasks containing 50 ml of a nutrient broth/soil extract ($8\,g\,l^{-1}$) added with L-tryptophan ($0.2\,g\,l^{-1}$, filter sterilized Millipore, 0.2-um pore size (Strzelczyk and Pokojska-Burdziej, 1984). Two ml (1 ml/plant) were added per pot after three days of plant growth. The rock phosphate assayed was from Bayovar in Perú with 31.7% P_2O_5 content (Olsen *et al.*, 1954). The quantity used was 0.45 g per pot.

All treatments without bacteria and VAM fungus received autoclaved bacteria and washed endomycorrhizal inocula. *Rhizobium meliloti*, strain 102F28 grown in Allen medium (Allen, 1957) was used in all treatments. There were seven inoculation treatments plus one control treatment without any inoculation. P-solubilizing bacteria and each of the three mycorrhizal fungi were assayed as single inocula and in dual fungus-bacteria combinations.

The experiment was conducted in a greenhouse maintained at 20–22°C at daytime and 12–17°C at nighttime and 16/8 h light/dark photoperiod. The relative humidity was 70–90%. There was five replicates per treatment and two plants per pot.

At harvest VMA infection was assessed in the root systems by the stain method (Phillips and Hayman, 1970). The percentage of total root length infected was calculated with the gridline intersect plate technique (Giovannetti and Mosse, 1980). Dry weights of roots and shoots were recorded and the shoot tissue analyzed for N, P, K, Ca and Mg concentrations.

Differences between treatments were determined using the statistical DUNCAN'S Multiple range test and the significance of differences among treatments was tested at the 5% probability level.

Results

The sand-vermiculite mixture used in this experiment as a rooting medium proved to be a reliable substrate for mycorrhizal inoculum performance, with the exception of the *Glomus* sp. strain. Treatments using the *Glomus* sp. strain as well as those that were not inoculated remained without VA infection. *G. fasciculatum* and *G. mosseae* showed a low potential of infectivity ranging between 9% and and 13% of root colonization (Table 3). Such a percentage of fungal infection was not related to their effect since *G. mosseae* did not increase alfalfa dry weight and plants infected with *G. fasciculatum* widely stimulated their growth (Table 1).

Phosphate-solubilizing bacteria inoculation enhanced alfalfa growth ($P \leqslant 0.05$) only in plants colonized by *G. mosseae* fungus when the medium was lacked of rock-phosphate. In the presence of Bayovar rock-phosphate, PBS increased alfalfa dry weight in all of the treatments. Bayovar rock phosphate *per se* did not increase plant growth in either mycorrhizal or non-mycorrhizal plants.

Dual PSB and mycorrhizal fungi inoculation

Table 1. Effect of rock phosphate addition on the shoot dry weight ($mg\,pot^{-1}$) of alfalfa plants not inoculated and inoculated with mycorrhizal fungi and phosphate solubilizing bacteria (PSB)

Mycorrhizal treatment	Bacterial treatment	
	−PSB	+PSB
Minus *rock phosphate*		
Non inoculated	62 a	72 a
Glomus fasciculatum	152 bc	205 cd
Glomus mosseae	85 a	152 bc
Glomus sp.	72 a	72 a
Plus *rock phosphate*		
Non inoculated	110 ab	175 c
Glomus fasciculatum	198 cd	308 f
Glomus mosseae	132 abc	238 d
Glomus sp.	112 ab	178 c

Means (five replicates) not followed by a common letter differ significantly ($P = 0.05$) from each other according to Duncan's multiple-range test.

Table 2. Effect of rock phosphate addition on the root dry weight (mg pot^{-1}) of alfalfa plants not inoculated and inoculated with mycorrhizal fungi and phosphate-solubilizing bacteria (PSB)

Mycorrhizal treatment	Bacterial treatment	
	−PSB	+PSB
Minus *rock phosphate*		
Non inoculated	86.2 a	116.6 ab
Glomus fasciculatum	193.0 d	250.1 f
Glomus mosseae	92.2 ab	165.7 c
Glomus sp.	119.5 ab	137.5 bc
Plus *rock phosphate*		
Non inoculated	132.0 bc	224.0 fd
Glomus fasciculatum	221.9 d	308.0 s
Glomus mosseae	125.4 b	233.0 f
Glomus sp.	134.4 bc	201.1 df

Means (five replicates) not followed by a common letter differ significantly ($P = 0.05$) from each other according to Duncan's Multiple-range test.

stimulate alfalfa dry weight more than either organism alone. Such an effect was especially relevant ($P \leqslant 0.05$) in the PSB.-*G. mosseae* association.

Alfalfa plants reached the maximum yield in the presence of rock phosphate plus PSB and mycorrhizal colonization.

In the same way as did the shoots, the roots of alfalfa plants widely increased ($P \leqslant 0.05$) when they were infected by *G. fasciculatum*. PSB also stimulated alfalfa roots dry weight either in the two mycorrhizae treatments in which rock phosphate was absent or in those treatments that had rock phosphate (Table 2).

The fact that plant growth responded better to the inoculation with *G. fasciculatum* than to the inoculation with *G. mosseae* (Table 1) can be related to the larger number of roots colonized by the former fungus (Tables 2 and 3). The synergistic bacteria-fungus interaction manifested itself in a larger value than that of the total length of infected roots (Tables 2 and 3). Root colonization levels by fungi and their effects on plant growth were enhanced by simultaneous inoculation with PSB.

Table 3. Effect of phosphate-solubilizing bacteria (PSB) and rock phosphate on the percentage of VAM root infection

Mycorrhizal treatment	Bacterial treatment	
	−PSB	+PSB
Minus *rock phosphate*		
Non inoculated	0	0
Glomus fasciculatum	10.5	12.8
Glomus mosseae	9.5	11.7
Glomus sp.	0	0
Plus *rock phosphate*		
Non inoculated	0	0
Glomus fasciculatum	13.1	11.9
Glomus mosseae	8.7	10.9
Glomus sp.	0	0

Because of the differences in vegetative production among treatments, nutrient data are presented both on the basis of concentration (percentage of dry matter, Table 4) and as total accumulation (the product of dry matter and concentration, Table 5). According to the results presented in Table 4 a nutrient concentration effect occurred under the P deficient conditions in the non-inoculated plants. The nutrients concentration in the plants were influenced by both the addition of rock phosphate and microbial treatments. Mycorrhizal fungi, phosphate solubilizing bacteria and combined inocula increased P and N shoot concentration with respect to the control plants when the medium lacked rock phosphate. Separate inoculation of each microorganism increased N concentration more than dual inoculation.

Addition of rock phosphate greatly enhanced P and N concentration. The positive effect of microbial treatments and rock-phosphate addition was noticeable in the quantities of N, P, K, Ca and Mg absorbed by alfalfa with respect to the control plants (Table 5).

Discussion

Mycorrhization by *G. mosseae* and *G. fasciculatum* was well established in alfalfa plants grown in sterilized sand-vermiculite as a rooting medium. The substrate allowed standardization of conditions to test rock phosphate solubilization by PSB and also eliminated most specifically soil-borne effects. Although it has been described that in a non-adsorbing medium the VAM fungi provide little advantage to the host, significant plant growth responses to VAM colonization specially with *G. fasciculatum* were found.

Under the experimental conditions used *Glomus* sp. was not infective, *G. mosseae* was infective but not very effective as single inoculum and *G. fasciculatum* was the most efficient microsymbiont for

Table 4. Effect of rock phosphate, mycorrhizal inoculation and phosphate-solubilizing bacteria on nutrient concentration in the alfalfa plants (% dry matter)

Non-mycorrhizal Bacterial treatment			*Glomus fasciculatum* Bacterial treatment		*Glomus mosseae* Bacterial treatment		*Glomus* sp. Bacterial treatment	
	−PSB	+PSB	−PSB	+PSB	−PSB	+PSB	−PSB	+PSB
	Minus *rock phosphate*							
N	1.48	2.73	2.32	2.24	2.59	2.13	2.18	1.94
P	0.11	0.14	0.13	0.15	0.16	0.12	0.12	0.12
K	2.81	3.12	2.96	2.98	2.73	2.89	3.62	3.01
Ca	1.26	1.16	1.82	1.29	1.69	1.59	1.46	1.33
Mg	1.00	1.05	0.82	0.71	1.06	1.02	0.96	0.98
	Plus *rock phosphate*							
N	4.16	3.85	3.24	3.16	2.82	3.83	2.45	2.53
P	0.34	0.35	0.30	0.33	0.30	0.38	0.30	0.32
K	2.96	3.08	3.03	2.71	2.35	2.51	2.61	2.34
Ca	1.62	1.31	1.55	1.82	1.68	1.87	1.90	1.87
Mg	0.80	0.84	0.59	0.67	0.68	0.65	0.80	0.77

plant growth. It is known that mycorrhizal fungi have to be selected according to environmental conditions (Fitter, 1985).

Results from this study clearly show that Bayovar rock phosphate was transformed into available forms in the presence of PSB and then used up by alfalfa plants (Table 1). That mycorrhizas do not possess any ability to solubilize unavailable forms of phosphate has been previously reported (Mosse, 1973).

Although in some treatments root growth was stimulated and H^+ extrusion by alfalfa roots, resulting from an alkaline uptake pattern, might have contributed to solubilization of any rock phosphate, the overall results in this study suggest that phosphate solubilization was mainly due to bacterial activity. Whatever, the utilization of Bayovar rock phosphate was by alfalfa plants, it only took place in the presence of selected bacteria which were efficient in mobilizing P. These results agree with those previously observed (Azcón *et al.*, 1976; Banik and Dey, 1982). The single action of PSB or *G. mosseae* in the medium without rock phosphate was not sufficient to raise yield of alfalfa. However the combined action of both microorganisms was effective to this respect (Table 1). This result suggests the involvement of a microbial interaction (Azcón-Aguilar *et al.*, 1986). The simplest interpretation of this fact is that mycorrhizal endophyte could be stimulated in quantity, efficiency and longevity by metabolic products released from the bacteria. Besides root exudation and plasticity might change by PSB inoculation which could also affect VAM development. The production of plant hormones by PSB could account for by these effects (Barea *et al.*, 1976). In fact, *G. mosseae* spores growth *in vitro* has been stimulated by PSB bacteria (unpublished results).

In the present study root weight and percentage of root colonization (Tables 2 and 3) by VAM endophytes were improved by dual inoculation (Barea and Azcón-Aguilar, 1982).

Mycorrhizal fungi and PSB enhanced root growth which, in turn, is better equipped to make use of potentially available quantities of other nutrients. This resulted in a total nutrients taken up by plants increased (Table 5). Furthermore, a simulation of P and Ca uptake also can be expected to have a positive effect on symbiotic N_2 fixation.

Inoculated treatments did not have such a high nutrients concentration because of a dilution effect associated with growth (Table 4).

Although *Glomus* sp. did not infect alfalfa roots the inoculation with this fungus altered the content of N, P, K, Ca and Mg in relation to the nutrients content in the control plants (Table 4). This can be expected since as any other natural soil microorganism, *Glomus* sp. can release some active substances to the medium as well as to modify certain physical conditions.

According to the results it can be stated that PSB, under specific conditions, mobilizes unavail-

Table 5. Effect of rock phosphate, mycorrhizal inoculation and phosphate-solubilizing bacteria on nutrient content in the alfalfa plants

Non-mycorrhizal Bacterial treatment			*Glomus fasciculatum* Bacterial treatment		*Glomus mosseae* Bacterial treatment		*Glomus* sp. Bacterial treatment	
	−PSB	+PSB	−PSB	+PSB	−PSB	+PSB	−PSB	+PSB
	Minus *rock phosphate*							
N	0.91	1.96	3.52	4.59	2.20	3.24	1.57	1.39
P	0.07	0.10	0.19	0.31	0.13	0.18	0.08	0.08
K	1.74	2.24	4.49	6.11	2.32	4.39	2.60	2.16
Ca	0.78	0.83	2.76	2.64	1.43	2.42	1.05	0.96
Mg	0.62	0.75	1.24	1.45	0.9	1.55	0.69	0.70
	Plus *rock phosphate*							
N	4.57	6.73	6.41	9.73	3.72	9.11	2.74	4.50
P	0.37	0.61	0.59	1.01	0.39	0.90	0.33	0.57
K	3.25	5.39	6.00	8.85	3.10	5.97	2.92	4.16
Ca	1.78	2.29	3.07	5.60	2.22	4.45	2.13	3.33
Mg	0.88	1.47	1.17	2.06	0.90	1.54	0.89	1.37

able forms of soil and fertilizer P and mycorrhizal fungus, adequately selected, provide soluble nutrients to plants which improved plant nutrition and growth. A more thorough understanding of interactions between soil microorganisms is needed for an optimal utilization of these interactions with respect to growth and development of plants.

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