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UTILIZATION OF ROCK PHOSPHATE IN ALKALINE SOILS BY PLANTS INOCULATED WITH MYCORRHIZAL FUNGI AND PHOSPHATE-SOLUBILIZING BACTERIA

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Summary—Interactions between vesicular-arbuscular (VA) mycorrhizal fungi and phosphate-solubilizing bacteria were studied in a low-phosphate alkaline soil amended with 0, 0·1% and 0·5% rock phosphate. *Endogone* (E3 and yellow vacuolate spore types) and two bacteria able to solubilize rock phosphate *in vitro* and produce plant growth regulating substances were used as inocula. Lavender (*Lavandula spica* var. vera L.) plants with mycorrhiza plus bacteria (either E3 plus bacteria or "yellow vacuolate" plus bacteria treatments) took up more total P than plants with either *Endogone* or bacteria separately at each concentration of rock phosphate. Plants not inoculated with bacteria or *Endogone* derived no benefit from the rock phosphate.

INTRODUCTION

In a previous study (Barea *et al.*, 1975) we found interactions between vesicular-arbuscular (VA) mycorrhizal fungi (*Endogone* spp.) and phosphate-dissolving bacteria in the rhizospheres of plants inoculated with these microorganisms which affected phosphate uptake and plant growth in low-phosphate soils. That study showed: (i) the inoculated bacterial population remained higher in the rhizospheres of mycorrhizal than of non-mycorrhizal plants for 30 or 45 days, so that the metabolic activities of the bacteria were probably maintained for a longer period; and (ii) plants inoculated with bacteria and *Endogone* together took up significantly more P than plants inoculated with either microorganism separately in one out of four tests. In that one positive test, but not in the other three, the bacteria enhanced mycorrhizal development.

As Sanders and Tinker (1971) and Hayman and Mosse (1972a) indicated, mycorrhizal plants take up P from the same source of readily soluble P as non-mycorrhizal plants. VA mycorrhiza does not increase phosphate uptake by dissolving complex soil phosphates (Hayman and Mosse, 1972b). Although Daft and Nicolson (1966), Murdoch *et al.* (1967) and Jackson *et al.* (1972) showed that VA mycorrhiza seemed to hydrolyse rock phosphate, these results could be due to a more efficient uptake of chemically dissociated phosphate ions by fungal hyphae. Ross and Gilliam (1973) showed that mycorrhizal plants given rock phosphate in soil grew better than non-mycorrhizal ones, but both series of plants exhibited symptoms of P-deficiency. Plants with mycorrhiza did not yield more seed than plants without.

Some bacteria can hydrolyse insoluble phosphate *in vitro* (Greaves and Webley, 1965; Barea *et al.*, 1970; Tardieux-Roche and Tardieux, 1970) including rock phosphate (Ramos *et al.*, 1966). However, it is uncertain whether bacteria with this ability, when introduced into the rhizosphere, might dissolve rock phosphate added to soil and so benefit the plant. The etiolated stems of plants inoculated with phosphate-solubilizing bacteria and growing in sand plus rock phosphate (Gerretsen, 1948) may be a hormonal effect rather than the result of improved phosphate nutrition.

Swaby and Sperber (1959) list soils where microbial solubilisation of phosphate is most likely to occur.

The present study is concerned with aspects of mycorrhizal stimulation of the bacterial population in the rhizosphere and tests the possibility that phosphate-solubilizing bacteria and *Endogone* inoculated separately or together might enhance the ability of a plant to use P from sparingly soluble rock phosphate added to soil.

MATERIALS AND METHODS

Two *Endogone* species, E3 and YV (yellow vacuolate) spore types (Mosse, 1973) provided by Dr. B. Mosse at Rothamsted, were used. These are probably synonymous with *Glomus fasciculatus* and *G. mosseae*, respectively, as described by Gerdemann and Trappe (1974). Two bacterial species, a *Pseudomonas* sp. and an *Agrobacterium* sp., which were efficient solubilizers of rock phosphate *in vitro* were tested. Inocula were prepared as described before (Barea *et al.*, 1975).

The test soil was collected from the province of Granada, Spain. It is a brown calcareous type, pH 7·6, containing 5 mg $NaHCO_3$–soluble P, 132 mg N and 45 mg K/Kg soil (for methods of analysis see Barea *et al.*, 1975). The soil was steam-sterilized at 100°C and distributed in pots (250 g/pot) after mixing with 25% sand, by volume, and with 100-mesh rock phosphate at 0, 0·1% and 0·5% (w/w) concentrations.

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Four-week-old seedlings of lavender (*Lavandula spica* L.) were inoculated using a small pad (about 2 mm^3) of *Endogone* inoculum or by dipping seedling roots in the two bacterial cultures combined at the time of inoculation. The *Endogone* inoculum consisted of spores, hyphae and infected root fragments obtained by wet-sieving rhizosphere soil from a plant infected with the E3 or YV spore types.

There were six inoculation treatments plus two types of controls: C_1 = Control (autoclaved bacteria); C_2 = Washings of *Endogone* inoculum (to provide the bacteria associated with the inoculum); B = Bacteria: E3 = *Endogone* E3 type: YV = *Endogone* YV type: B-E3 = Bacteria − E3, and B − YV = Bacteria − YV.

Plants were grown in open pots for 5 months in a glasshouse at 19–25°C, watered on sand trays and fed with nutrient solution (Hewitt, 1952) lacking phosphate.

In the course of the experiment, rhizosphere soil was sampled at 15-day intervals in bacteria-inoculated pots as described by Brown *et al.* (1962). Total and phosphate-solubilizing bacteria were counted on a medium containing 0·02% rock phosphate. Bacterial numbers were related to 1 g dry rhizosphere soil.

At harvest, *Endogone* infection was assessed microscopically by staining as described by Phillips and Hayman (1970). Dry weights of roots and shoots were recorded and plant tissues analysed for P as before (Barea *et al.*, 1975).

RESULTS

Table 1 gives dry weights of shoots, roots and root/shoot ratios for the different treatments. Least significant differences (LSD at 5%) between results due to

Table 1. Dry weights and root/shoot ratios of lavender plants given different inoculation treatments and three concentrations of rock phosphate

Inoculation treatment	% Rock phosphate added	Dry weight/3 plants (mg) Shoots	Roots	Root/shoot ratio
C_1=Dead bacteria	0	180	110	0.61
	0.1	180	110	0.61
	0.5	186	110	0.59
LSD (5%)		NSD	NSD	
C_2=Endogone washing	0	180	110	0.61
	0.1	170	112	0.65
	0.5	180	118	0.65
LSD (5%)		NSD	NSD	
B=Bacteria	0	200	200	1.00
	0.1	250	185	0.74
	0.5	380	300	0.79
LSD (5%)		40	45	
E3=Endogone	0	320	125	0.39
	0.1	480	210	0.43
	0.5	490	210	0.43
LSD (5%)		67	39	
E3 + B	0	580	300	0.51
	0.1	640	310	0.48
	0.5	870	340	0.39
LSD (5%)		60	45	
YV=Endogone	0	1550	450	0.29
	0.1	1710	420	0.25
	0.5	1950	440	0.22
LSD (5%)		153	59	
YV + B	0	1670	500	0.30
	0.1	1500	450	0.30
	0.5	1800	460	0.25
LSD (5%)		170	61	

NSD = No Significant Difference.

Table 2. Significance levels at which effects of treatments on plant dry weights differ

Inoculation treatments compared	% Rock phosphate added (Shoots) 0	0.1	0.5	(Roots) 0	0.1	0.5
B vs C_1	NS	***	***	***	***	***
E3 vs C_2	***	***	***	NS	***	***
E3 vs B	***	***	***	***	NS	***
B + E3 vs B	***	***	***	***	***	NS
B + E3 vs E3	***	***	***	***	***	***
YV vs C_2	***	***	***	***	***	***
YV vs B	***	***	***	***	***	***
B + YV vs B	***	***	***	***	***	***
B + YV vs YV	NS	NS	NS	NS	NS	NS

C_2 = Endogone washing; B = bacteria, E3 and YV = Endogone

*** Significant values for P = 0.001

NS = not significant at P = 0.05

effects of rock phosphate concentrations in soil for each inoculation treatment are also shown.

Increased additions of rock phosphate did not increase growth of control plants. With the inoculated plants, in contrast, shoot dry weights clearly increased with additions of rock phosphate in the B, E3 and B-E3 treatments, although root dry weights were less affected.

In Table 2 the effects of inoculation treatments for each phosphate concentration are statistically compared.

The results in Tables 1–2 clearly show that the combined inoculum of *Endogone* E3 plus bacteria significantly increased plant growth above that achieved with either separately. The combined inoculum of *Endogone* YV plus bacteria did not significantly increase plant growth above YV inoculation alone. YV-inoculated plants grew best of all, regardless of whether bacteria or rock phosphate were added. Thus the YV inoculum was much more affective than the E3 inoculum in this soil and presumably brought the lavender plants onto the plateau of a phosphate response curve.

Table 3 shows P content (% of dry matter) expressed as % P in plant shoots and roots. Least significant differences (LSD at 5%) due to the amount of rock phosphate added are indicated.

The addition of 0·5% rock phosphate did not significantly affect the % P in uninoculated plants or in plants inoculated separately with either bacteria or *Endogone* YV. In plants with these inocula combined, the % P increased with rock phosphate added, but this increase was not significant at the 5% level. 0·5% rock phosphate also had a barely significant negative effect on the P concentration in plants inoculated with E3 *Endogone*—this is presumably a growth-dilution effect. Table 3 also shows the total P taken up by plants. This was calculated by multiplying mean values of % P by mean values of dry weight.

In Table 4 the effects of inoculation treatments are statistically compared for each concentration of rock phosphate. The bacteria markedly increased the P content of non-mycorrhizal plants and of plants mycorrhizal with YV but not with E3 *Endogone*.

Table 5 shows that inoculation with *Endogone*, especially YV, favoured the early maintenance of phosphate-solubilizing bacteria. After 2 months of plant

Table 3. P content (% P) and total P taken up (mg) by lavender plants given different inoculation treatment and three concentration of rock phosphate

Inoculation treatment	% Rock phosphate added	Shoots % P	Roots % P	Total P (mg) taken up per 3 plants
C_1 = Dead bacteria	0	0.060	0.072	0.187
	0.1	0.060	0.075	0.190
	0.5	0.061	0.076	0.196
LSD (5 %)		NSD	NSD	NSD
C_2 = Endogone washing	0	0.061	0.071	0.187
	0.1	0.060	0.079	0.189
	0.5	0.062	0.080	0.204
LSD (5 %)		NSD	NSD	NSD
B = Bacteria	0	0.120	0.128	0.496
	0.1	0.121	0.130	0.560
	0.5	0.121	0.131	0.852
LSD (5 %)		NSD	NSD	0.098
E3 = Endogone	0	0.160	0.170	0.724
	0.1	0.156	0.174	1.113
	0.5	0.125	0.135	0.895
LSD (5 %)		0.022	0.024	0.165
E3 + B	0	0.178	0.170	1.542
	0.1	0.162	0.166	1.650
	0.5	0.140	0.142	1.701
LSD (5 %)		0.026	0.024	0.108
YV = Endogone	0	0.148	0.106	2.771
	0.1	0.134	0.107	2.740
	0.5	0.127	0.122	3.035
LSD (5 %)		0.021	0.017	0.186
YV + B	0	0.188	0.148	3.879
	0.1	0.210	0.166	3.897
	0.5	0.214	0.162	4.597
LSD (5 %)		0.030	0.021	0.241

NSD = No Significant Difference

growth, this effect disappeared, and the number of inoculated-bacteria declined in all treatments in the usual way. Calculation of total bacteria suggests no stimulation of bacterial numbers by *Endogone* after 8 weeks.

Control plants and those inoculated only with bacteria remained non-mycorrhizal. The development of VA infection in *Endogone*-inoculated pots ranged between the following values (% of the feeder root system infected): E3-inoculated pots: 25–45%; Bacteria plus E3-inoculated pots: 35–60%; YV-inoculated pots: 60–85%; Bacteria plus YV-inoculated pots: 60–80%.

DISCUSSION

The improved phosphate uptake and growth of inoculated over uninoculated plants where rock phosphate was added to the soil must have resulted from

Table 4. Significance levels at which effects of treatment on P concentration (expressed as % P) in plants differ

Inoculation treatments compared	% Rock phosphate added (Shoots) 0	0.1	0.5	(Roots) 0	0.1	0.5
B vs C_1	***	***	***	***	***	***
E3 vs C_2	***	***	***	***	***	***
E3 vs B	***	***	NS	***	***	NS
B + E3 vs B	***	***	*	***	***	NS
B + E3 vs E3	NS	NS	NS	NS	NS	NS
YV vs C_2	***	***	***	***	***	***
YV vs B	**	NS	NS	*	*	NS
B + YV vs B	***	***	***	*	***	**
B + YV vs YV	***	***	***	***	***	***

Significant levels at which treatments differ:
* P = 0.05; ** P = 0.01; *** P = 0.001.
Other conventions as in Table 2.

Table 5. Numbers of phosphate-solubilizing bacteria in the rhizosphere of lavender as effected by *Endogone* treatment, % rock phosphate added and sampling time

	Number of bacteria (x10^6)/g dry rhizosphere soil (Weeks after inoculation) 2		4		8		12		14		16	
1.-	Ps	%	Ps	%	Ps	%	Ps	%	Ps	%	Ps	%
B	14	15	2.5	10	2	9	1.5	9	1.7	9	1.8	9
E3 + B	30**	20	5**	9	3.6*	8	1	5	1.2	4	0.2	6
YV + B	52***	50	15.5***25		10***	17	1	10	1.2	6	0.2	5
2.-												
B	17	16	6	13	4.5	8	2	13	1.5	6	0.5	5
E3 + B	31*	22	10.5*	12	8*	15	1.5	12	1	10	0.5	5
YV + B	50**	50	15**	19	10.5**	20	3	14	2	12	1	15
3.-												
B	15	15	3.5	12	2.5	7	1.5	10	0.5	6	0.5	5
E3 + B	37**	30	19***	7	10**	9	1.5	10	1	7	0.5	7
YV + B	41**	49	17.5***14		7**	11	2	12	1.5	10	1	10

1.- No rock phosphate added
2.- 0.1 % rock phosphate added
3.- 0.5 % rock phosphate added
Ps = Number of phosphate-solubilizing bacteria (x 10^6)
% = Per cent of Ps in the total number of bacteria

Significant effect of Endogone on bacterial establishment at P = 0.05 (*); P = 0.01 (**) and P = 0.001 (***).

the microbial inocula in some way increasing the accessibility to the plant of this sparingly soluble form of phosphate.

This effect did not appear to be directly attributable to phosphate solubilization because the percentage of P in the plants did not increase with increasing concentrations of rock phosphate (except in one case, Table 3). Uninoculated plants did not derive any benefit from the rock phosphate.

If it is accepted that mycorrhizal plants can take up more phosphate than non-mycorrhizal plants from the same readily soluble P source, chemical dissociation of phosphate ions from rock phosphate could account for the increased growth of those plants due to rock phosphate additions (see Introduction). If the bacteria took up this dissociated phosphate, however, they would be in competition with the roots because they cannot transfer phosphate into the root as a mycorrhizal fungus can. Since the bacteria enhanced plant growth, any competition must have been more than compensated for by other effects on the plant. One effect was on root growth. When bacteria were inoculated alone, the root/shoot ratios of the lavender plants were larger than those for the controls. These bacteria are now known to produce growth substances of the auxin, gibberellin and cytokinin types. Although the relative activities of these hormones in the rhizospheres of the plants in the present experiment were not assessed a hormonal effect could explain the increased root/shoot ratio in bacteria-inoculated pots. This increased root growth could account for the increased uptake of total P. The mycorrhizal lavender plants had smaller R/S ratios than the controls, agreeing with Hayman and Mosse (1971.

Dry weights of plants inoculated only with bacteria increased steadily with rock phosphate concentration, although the percentage of P was the same irrespective of the rock phosphate concentration. Two possible explanations are: (i) the bacteria might affect plant growth by the plant hormones that they synthesise. Such substances could influence the early stages of plant growth (Brown, 1974) so that more actively growing roots were able to explore more soil and more zones where phosphate ions were chemically liberated from the rock phosphate particles. This effect should be greatest with the largest addition of rock

phosphate: and (ii) some solubilization of phosphate by the bacteria cannot be excluded, especially as the bacteria produce acids and rock phosphate dissolves in acid but not in alkaline soils.

The improvement of the E3 *Endogone* effect by the bacteria probably includes a pH effect on the fungus because these bacteria create microhabitats with a lower pH, which are more suitable for the E3 spore type of *Endogone*, as suggested before (Barea *et al.*, 1975). In fact, E3 *Endogone* infection was improved by the bacteria. The YV type of *Endogone* is not favoured by a lowered pH, yet the bacteria strongly influenced its activity. One month before harvest, plants with YV plus bacteria were about 50% taller than plants with YV only; during the last month of assay plants with YV alone grew rapidly and at harvest shoot and root dry weights were similar for YV-inoculated plants, with or without bacteria. Nevertheless, plants took up more phosphate in the YV plus bacteria treatment. This suggests the possibility of some solubilization of rock phosphate by the bacteria, but not enough to inhibit the VA infection. Small amounts of phosphate hydrolysed by the bacteria from rock phosphate could enter the soil solution together with the chemically dissociated ions and be taken up by mycorrhizal plants. Alternatively, these extra phosphate ions could come from dead bacteria since more bacteria grow and subsequently die around the mycorrhizal than non-mycorrhizal roots as there are more present in the earlier but not later stages of plant growth and, as previously found (Barea *et al.*, 1975), *Endogone* enhances early establishment of phosphate-solubilizing bacteria introduced in the root zone.

Work is in progress, using ^{32}P, to try and establish directly if bacteria really hydrolyse some phosphate ions from rock phosphate under the conditions employed in the present study. Whatever the mechanisms involved, this study may have practical significance for alkaline soils because of the general insolubility of rock phosphate fertilizer in such soils in contrast to its availability to plants in acid soils.

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