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Role of Plant Growth-promoting Rhizobacteria in Influencing the Early Growth of Pueraria phaseoloides – *Strain and Soil Factors*

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The common plantation legume Pueraria phaseoloides *was used to evaluate growth stimulation by the plant growth-promoting rhizobacteria (PGPR) strain 7NSK2 in 20 soils and by strains TL3, 34-13, 1-102 and 1-4E1 in six soils, in glasshouse pot experiments. The PGPR strains showed strong interaction with soils in affecting shoot dry weight (DW) yields after five weeks growth. Strain 7NSK2 increased shoot DW significantly in 12 of the 20 soils tested but significantly depressed yields in one soil. In another experiment, growth responses to PGPR inoculation occurred only in three of the six soils tested. Relating features of the soil (physical, chemical and biological) to shoot DW responses from inoculation in a multiple regression only showed total soil phosphorus (P) to be the most significant variable, followed by lesser significant relationships with total manganese (Mn), exchangeable calcium (Ca) and aluminium (Al). This could arise from indirect rather than direct causal relationships since the variables accounted for only 50% of the variance. The results suggest that other variables were either not measured or unknown, and that may include control of deleterious rhizobacteria or some undiagnosed minor pathogens in the rhizosphere. The findings are discussed in relation to the need to test selected PGPR strains over a range of field soils and sites prior to their agronomic use as beneficial crop inoculants.*

There have been many reports that specific strains of plant growth-promoting rhizobacteria (PGPR), mainly fluorescent pseudomonads, promote plant growth by suppressing non-specific deleterious rhizobacteria (DRB) in the rhizosphere ('niche exclusion'), or to control plant diseases caused by soil borne pathogens by one or more of a variety of metabolites that include antibiotics, siderophores and bacterial hydrocyanic acid (HCN)[1-3]. Aggressive competition for sites and nutrients in the rhizosphere allow introduced PGPR to pre-empt establishment of pathogens on roots or to reduce the amount of substrate available to pathogens and thereby confers a certain capacity of suppressiveness to these beneficial organisms. Such a control mechanism, although not exclusive, often caused large increases in growth and yield of some agricultural crops[4-7].

Knowledge on the mechanisms of plant growth-promotion or biological control of plant pathogens largely derived from *in-vitro* studies may not be reliably expressed in natural soil conditions since the micro-organisms or the mechanisms involved are influenced by different biotic and abiotic factors. Thus the

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variability of the soil factors may sometimes explain the effectiveness or ineffectiveness of these micro-organisms, if they are appreciably affected by changes in soil parameters[8-10].

Identifying the nature of site conduciveness should help promote use of PGPR as crop inoculants. This paper presents results to evaluate the potential of PGPR to stimulate early growth of *Pueraria phaseoloides*, a primary cover legume grown in the interrows of immature rubber, in several local soils, and to relate growth responses of the legume to features of the soil by correlation and multiple regression analysis.

MATERIALS AND METHODS

Soils

Topsoils (0–15 cm) under native vegetation were collected from various sites in Malaysia and filled into 8.5 cm (10 cm height) plastic pots after air drying and sieving (<4 mm). The weight of soils potted per cup ranged from 273–424 g. These infertile soils represented a range of physical, chemical and biotic properties *(Table 1)*. The methods of soil analyses were as described by RRIM[11]. Total bacteria was determined by the pour plate method[12], total fungi on Martin's medium[13]; cellulolytic bacteria as described by Voets[14];

TABLE 1. RANGE OF PROPERTIES AMONG THE DIFFERENT SOILS USED *(EXPERIMENT 1)*

Properties	Range
Soil Factors	
Physical	
Coarse sand, %	5.5 – 93.3
Fine sand, %	4.3 – 50.3
Silt, %	0.1 – 41.4
Clay, %	1.9 – 72.6
Chemical	
pH	4.1 – 7.0
Org. C, %	0.78 – 4.00
Total N, %	0.08 – 0.28
Total P, p.p.m.	42 – 617
Avail-P, p.p.m.	5 – 14
Exch. K, m.e.%	0.04 – 0.79
Exch. Ca, m.e.%	0.24 – 16.63
Exch. Mg, m.e.%	0.06 – 3.87
Total Mn, p.p.m.	11 – 5598
Exch. Al, p.p.m.	25 – 451
Free Fe, %	0.03 – 10.72
Microbial Factors	
Total bacteria, $X10^7$/g soil	8.0 – 811.5
Total fungi, $X10^3$/g soil	0.73 – 306.5
Soil respiration, mg CO_2-C/kg soil	2.43 – 4.47
Nitrifiers, $X10^3$ MPN/g soil	2.85 – 196.66
Cellulolytic bacteria, $X10^4$/g soil	4.0 – 76.5

soil respiration by the method of Jenkinson and Powlson[15] and nitrifiers by the most-probable-number (MPN) method[16]. The soils in the top half of the pots were mixed with Christmas Island rock phosphate (CIRP; 17.64% P, 7.34% citrate-sol P) corresponding to the recommended field rate of 720 kg/ha[17] for legume cover establishment.

Bacteria

There were two separate experiments. In *Experiment 1,* the fluorescent pseudomonad strain 7NSK2 *(Pseudomonas aeruginosa)* supplied by W. Verstraete (Laboratory of Microbial Ecology, State University of Ghent, Belgium) was evaluated for its growth stimulation of *P. phaseoloides* in 20 soils. This strain produced the fluorescent siderophore pyoverdin under iron limiting conditions and in stress situations[18]. In *Experiment 2,* only six soils were used to evaluate four additional widely-known PGPR strains. The soils were chosen on the basis of plant responsiveness to 7NSK2 in *Experiment 1.* The strains used were TL3 *(Pseudomonas fluorescens)* supplied by T.J. Burr (Dept. of Plant Pathology, Cornell University, NY), 1-102 (*Serratia* sp.) and 34-13 *(P. fluorescens)* from Esso Ag Biologicals (Esso Chemical Canada, Saskatchewan) and the unknown isolate 1-4E1 from A.J. Caesar (Dept. of Plant Pathology, University of California, Berkeley, USA).

All strains were cultured on King's Medium B (KB)[19] for 72 h at 28°C, and inocula prepared by suspending cells in a sterile buffer (0.1*M* $MgSO_4.7H_2O$). In *Experiment 1,* the soils were individually mixed with calculated amounts of the inocula to provide *ca.*1×10^8 viable cells/g soil, while in *Experiment 2,* inoculation was as 2.0 ml applied to the base of seedlings to provide ca.1.5×10^{12} viable cells/pot.

Plant Growth Conditions

Seeds of *P. phaseoloides* sown (4 plants/pot, *Experiment 1;* 2 plants/pot, *Experiment 2*) were surface-sterilised (conc. H_2SO_4) and pre-germinated (3 days) earlier. The pots were placed in randomised blocks in the glasshouse (10 replicates in *Experiment 1;* 5 replicates in *Experiment 2*) and watered daily to field capacity throughout the duration of the experiments. Nitrogen (N), at 20 μg/g N as KNO_3 was applied once at the beginning of the experiment. *Bradyrhizobium* strain RRIM 968 was applied one week after sowing to ensure nodulation. Average maximum and minimum glasshouse temperatures during the period was 35.6°C and 23.6°C respectively (38.3°C and 25.5°C, *Experiment 2*). Maximum photosynthetic photon flux density varied from 298–453 W/m^2 from 1000–1400 h.

Harvest and Measurements

At harvest (6 weeks after sowing in *Experiment 1,* 5 weeks in *Experiment 2*), shoots were cut at ground level, oven-dried (80°C, 48 h) and weighed. In *Experiment 1,* nutrient contents were determined on the bulked shoot samples. The data were subjected to analyses of variance and the treatment means tested for significance using appropriate values for least significant differences.

RESULTS

In both experiments, the effect of PGPR, soils or their interactions on shoot DW yields were highly significant ($P<0.01$) *(Tables 2* and *4).* In *Experiment 1,* PGPR inoculation significantly increased shoot weights in 12 of the 20 soils used, but depressed yields in one *(Table 2).* The range of increase relative to the uninoculated controls was 16%–63%. The overall yield increase averaged over 20 soils was 23% (PGPR=0.633g; Nil=0.514g;

TABLE 2 EFFECT OF A PLANT GROWTH-PROMOTING RHIZOBACTERIUM 7NSK2 *ON GROWTH OF P PHASEOLOIDES* IN 20 SOILS[a]

Soil series	Taxonomy[b]	Shoot DW, g/pot PGPR	Nil	Change from control (%)
Marang	Typic Paleudult	0 729	0 608	20 P<0 05
Bungor	Typic Paleudult	0 623	0 467	33 P<0 05
Rasau	Typic Quartzsipsamment	0 763	0 649	18 NS
Apek	Typic Paleudult	0 540	0 390	38 P<0 05
Lanchang	Typic Paleudult	0 952	1 105	-14 P<0 05
Prang	Tropeptic Haplorthox	1 007	0 717	41 P<0 05
Rengam	Typic Paleudult	0 724	0 523	39 P<0 05
Kaki Bukit	Typic Paleudult	1 115	0 942	18 P<0 05
Durian	Orthoxic Tropudult	0 142	0 205	-31 NS
Segamat	Tropeptic Haplorthox	0 708	0 436	63 P<0 05
Beserah	Tropeptic Haplorthox	0 595	0 403	48 P<0 05
Batu Anam	Oxic Dystropept	0 409	0 330	24 NS
Harimau	Typic Paleudult	0 482	0 416	16 NS
Tai Tak	Typic Paleudult	0 676	0 476	42 P<0 05
Bt Temiang	Orthoxic Tropudult	0 168	0 136	23 NS
Ulu Tiram	Orthoxic Tropudult	0 414	0 422	-2 NS
Langkawi	Typic Hapludox	0 649	0 530	22 P<0 05
Pohoi	Oxic Dystropept	0 450	0 324	39 P<0 05
Jambu	Typic Troporthod	0 305	0 229	33 NS
Serdang	Typic Paleudult	1 210	0 968	25 P<0 05

[a]Means of 10 replicate pots, 2 plants/pot

[b]Soil Management Support Services (1985) *Keys to Soil Taxonomy* Technical Monograph No 6, Cornell University, Ithaca, NY

TABLE 3 MEAN SHOOT NUTRIENT CONCENTRATION OF *P PHASEOLOIDES* INOCULATED WITH PGPR STRAIN 7NSK2 IN 20 SOILS[a]

Treatment	N(%)	P(%)	K(%)	Mg(%)	Ca(%)	Cu(p p m)	Zn(p p m)	Mn(p p m)	Fe(p p m)
PGPR	2 59	0 196	2 47	0 401	0 781	7 98	66 1	358 3	0 024
Nil	2 69	0 204	2 50	0 388	0 684	9 17	65 4	362 6	0 024
Significance									
PGPR	P<0 05	NS	NS	P<0 01	P<0 01	P<0 01	NS	NS	NS
Soil	P<0 01	P<0 01	P<0 001	P<0 001	P<0 001	P<0 01	P<0 01	P<0 01	P<0 01
PGPR x Soil	P<0 05	P<0 05	P<0 01	P<0 001	NS	P<0 01	P<0 05	NS	NS

[a]Data averaged over all 20 soils

P<0 05) Increases or decreases in root DWs were however not significant (data not shown) For most nutrients, the concentrations in shoots of PGPR-inoculated and uninoculated plants when averaged over all soils were generally not different, except for N and copper (Cu), which were significantly higher in uninoculated plants, and magnesium (Mg) and Ca, which were significantly higher only in PGPR-inoculated plants *(Table 3)*

In *Experiment 2*, the strains showed variable effectiveness depending on the soils used *(Table 4)*. Growth responses to PGPR inoculation only occurred in three of the six soils tested. In Bungor series soil, all five strains were effective, with growth increases ranging from 78%–166%. In Rengam series soil, three (1-4E1, 1-102, TL3) gave bigger responses but only one strain (1-4E1) was effective in Tai Tak series soil. Strains 7NSK2, 1-4E1, 1-102 and TL3 performed better than the controls ($P<0.05$) when averaged over all soils.

Since responses in the different soils have not been fully investigated, attempts were made to further explore the relationships between the growth-promotion phenomena to features of the soil (physical, chemical and biotic) by correlation and multiple regression. Using mean values for each soil, simple correlation coefficients were calculated between every possible pair of variables listed in *Table 1*, and the variables taken in turn to look for the best fit. To disentangle and measure the effect of the different independent variables on the features of growth of plants with and without PGPR, the more important independent variables likely to determine yields were chosen, and ranked in order of their importance. *Table 5* shows details of the multiple regressions of taking plants grown with and without PGPR as dependent variables. The most significant variable for either PGPR or uninoculated plants was total P since plant

TABLE 4. SHOOT DRY WEIGHT (G/POT) OF *P. PHASEOLOIDES* DUE TO PGPR INOCULATION IN SIX SOILS[a]

PGPR strain	Soil series					
	Bungor	Beserah	Tai Tak	Segamat	Serdang	Rengam
7NSK2	0.905 a-d	0.938 a-c	0.426 h-n	0.591 e-j	0.152 p-s	0.220 n-s
1-4E1	0.952 a-c	1.078 a	0.723 b-g	0.429 h-n	0.156 p-s	0.436 h-m
34-13	0.645 d-h	0.646 e-i	0.266 m-q	0.333 k-p	0.047 rs	0.062 rs
1-102	0.744 b-f	1.019 ab	0.475 g-l	0.568 e-k	0.112 q-s	0.242 n-r
TL3	0.964 ab	0.679 c-h	0.388 i-o	0.336 l-p	0.258 m-q	0.256 m-q
Control	0.363 j-p	0.792 a-e	0.330 l-p	0.507 f-l	0.169 o-s	0.043 s

[a]Means of 5 replicate pots, 2 plants/pot, after 5 weeks growth. Means within a column not followed by common letters are significantly different ($P<0.05$), on an ln (X+1) transformed basis

TABLE 5. IMPORTANT INDEPENDENT VARIABLES IN THE MULTIPLE REGRESSIONS OF SHOOT WEIGHTS DUE TO PGPR INOCULATION, LISTED IN ORDER OF STATISTICAL SIGNIFICANCE

PGPR			Nil		
	(b)			(b)	
Total P	0.616	P<0.001	Total P	0 690	P<0.01
Exch. Al	-0.403	P<0.05	Free Fe	-0.182	P<0.05
Exch. Ca	-0.156	P<0.10	Exch. Mg	-0.301	P<0.10
Silt	0.010	P<0.05	Silt	0.006	
Total Mn	0.279	P<0.05	Fine sand	0.004	
Fine sand	0.007	P<0.10			
Total bacteria	0.051				

(b) = regression coefficient

growth depended on P which is ranked second only to N in terms of growth limitation to legumes. Plants with PGPR showed a significant positive relationship to total Mn but a negative relationship with exchangeable Ca and Al. On the other hand, uninoculated plants were negatively correlated to exchangeable Mg and free iron (Fe). This could arise from indirect rather than direct causal relationships, for *e.g.* the generally greater growth of PGPR-inoculated plants could account for much greater levels of Mn accumulated in shoots since Mn becomes highly available at lower pH. Mn shows properties of the cations Mg and Ca, and zinc (Zn) and Fe in chemical behaviour, and participates in cation competition during plant uptake. A positive relationship for PGPR plants with total Mn may explain a negative relationship with Ca, Al or even Mg and Fe. Some variables *e.g.* fine sand, silt and total bacteria that were not statistically significant individually were included since they contributed to the best-fit multiple regression.

DISCUSSION

It is apparent that there is no clear separation of growth promotion and biological control induced by PGPR inoculants[3]. The mechanism of indirect growth promotion by biological control dominates the literature but direct growth promotion from rhizobacteria producing metabolites and inducing alterations in plant physiology has been recorded under gnotobiotic conditions[20]. It had already been shown in a previous experiment that the PGPR strain 7NSK2 caused growth increases in *P. phaseoloides* in four soils[21]. PGPR inoculation also stimulated early growth of *P. phaseoloides* in the present study although the major source of variation in the magnitude of responses appear related to PGPR activity per site. Differences in plant growth responses to PGPR between different soils are already known, and consistent plant growth response depends greatly on successful PGPR colonisation of the rhizosphere, which may be modified by soil type, moisture, plant species and cultivar, root exudates and nature of inoculum[4,10]. This explains why inoculation under natural soil conditions often gave rise to inconsistent results.

The study revealed that 7NSK2 was variable in effectiveness in the same soils over different occasions. In the earlier study[21], 7NSK2 on two Ultisols caused a 125% increase in shoot yields over the uninoculated controls in Serdang series but a non-significant increase (11%) in Rengam series soil. In this study, responses in both soils were significant (25% and 39% increases in Serdang and Rengam series soils, respectively) in *Experiment 1* but not in *Experiment 2*. With the exception of Bungor series soil, neither was 7NSK2 effective in Beserah, Tai Tak and Segamat soils in the second experiment. Whether this could also in part be due to the changed chemical and biotic properties of soils held in storage in addition to the factors listed above is not presently clear. This demonstrates the unpredictability of using biocontrol agents in trying to enhance plant growth. It also appeared that 7NSK2 is not effective in the sandy Entisols *e.g.* in Rasau *(Experiment 1)* and in Holyrood series soils of the previous study[21].

In *Experiment 1*, the soil variables measured did not provide a conclusive evidence of causal relationships for the PGPR effect on DW production by plants, but merely showed a relationship between the soil factors and plant growth in general. In the multiple regressions, using shoot DW of PGPR and uninoculated plants as dependent variables, only 50% of the variation of the dependent variables was accounted for by the regressions, suggesting that other factors causing variation were either not measured or unknown. The most significant

variable for DW of both treated plants was total P but this was expected. The lesser significant variables could also probably arise from indirect rather than direct causal relationships. Thus the growth responses reported could simply have resulted from control of DRB or some undiagnosed minor pathogens in the rhizosphere. As with most bacterisation studies, two major problems delaying the commercial use of many of these biological control agents are the variable results obtained in different soil types, and the inadequate survival of strains on seeds prior to planting[22].

The repeatability of strain performance in causing growth promotion is vital in an evaluation programme covering a range of field soils and sites, so as to understand the limits of effectiveness. Another challenge in addressing use of PGPR in agriculture is the need to develop an acceptable delivery system for applying inoculum to seed[23, 24].

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