

SHORT COMMUNICATION

Beneficial Effects of a Plant Growth-promoting Rhizobacterium on the Early Growth of Pueraria phaseoloides

A. IKRAM*

The effect of a fluorescent pseudomonad on the early growth of Pueraria phaseoloides was studied in four soils. Mean growth increases in shoot dry weights were 125% and 38% for Serdang and Holyrood series soils, respectively; but were 11% and 8% only for Rengam and Munchong series soils. The potential of employing fluorescent pseudomonads to enhance crop growth and as biological control agents against important diseases caused by soil-borne fungi is discussed.

Renewed attention on the use of bacteria in agriculture followed reports in recent years that specific strains of fluorescent pseudomonads, when incorporated into soil, or inoculated onto roots or seeds, increased growth and yield of some agricultural crops¹⁻⁵. The plant growth-promoting rhizobacteria (PGPR), usually fluorescent pseudomonads, are believed to function mainly by displacing deleterious micro-organisms (subclinical or exopathogens) in the rhizosphere, production of siderophores (iron chelators) and thereby allowing the plant to achieve more of its growth potential^{5,6}. Niche exclusion is apparently a key factor in this type of antagonism. Increased growth from PGPR inoculation have previously been reported for potato^{2,3}, sugar beet⁸, barley⁹, wheat¹⁰, rough lemon¹¹, apple¹² as well as for legumes (*Trifolium subterraneum*, *Phaseolus vulgaris*, *Arachis hypogaea*)^{13,14,15}. Legumes are commonly used as ground covers in the planting of rubber and their beneficial effects on soil fertility and on yield of the main tree crop are well-documented^{16,17}. This report presents results of a study on the effect of a strain of PGPR on growth of the common plantation legume, *Pueraria phaseoloides*.

MATERIALS AND METHODS

Soils

The soils used were collected (0-15 cm depth) from the Rubber Research Institute of Malaysia Experiment Station at Sungai Buloh. These were Serdang (Typic Paleudult), Holyrood (Typic Quartzipsamment), Rengam (Typic Paleudult) and Munchong (Typic Haplorthox) series soils, that had been described earlier⁷. The soils were potted in 8.5 cm diameter plastic pots, after air drying and sieving (< 4 mm).

The treatments were a factorial combination of PGPR inoculation (with/without) and mineral N application (with, at 20 µg/g N as KNO₃/without) in ten replications. The N was applied to overcome an unexplained glasshouse phenomenon whereby *P. phaseoloides* temporarily yellowed at five to six weeks but recovered thereafter.

Bacterial Inoculum

The fluorescent pseudomonad strain (isolate 7NSK2) was supplied by Prof W. Verstraete (Laboratory of Microbial Ecology, State University of Gent, Belgium). This bacterium

*Rubber Research Institute of Malaysia, P.O. Box 10150, 50908 Kuala Lumpur, Malaysia

was grown on King's Medium B (KB) agar¹⁸ at 26°C for 48 h. Inocula were prepared by suspending cells from the agar slants in a sterile buffer (0.1 M MgSO₄ · 7H₂O) to provide initial concentrations of *ca* 2 × 10¹⁰ colony-forming units/ml. The soils were then individually mixed with calculated amounts of the undiluted inoculum to provide *ca* 1 × 10⁸ viable cells/g soil.

Seeds of *P. phaseoloides* were surface-sterilised (concentrated H₂SO₄), pre-germinated (three days) and sown (two plants per pot) after bacterial application to soil. Control pots received similar volumes of buffer solution. All pots were placed in randomised blocks in the glasshouse and maintained at field capacity throughout the experiment. Plants were grown to six weeks before harvesting for shoot dry weights (80°C, 48 h).

RESULTS AND DISCUSSION

The results showed that growth stimulation due to PGPR inoculation were highly significant in two (Serdang and Holyrood series) of the four soils tested (Table 1). Mean growth increases in shoot dry weights were 125% and 38% for Serdang and Holyrood series soils, respectively;

but were 11% and 8% only for Rengam and Munchong series soils. Differences in growth between the N treatments were significant in Holyrood and Rengam series soils but these were due to the lesser fertility of such soils.

Differences in the magnitude of plant growth responses to PGPR inoculation between different soils are known in several cases, e.g. Burr *et al.*² did not get increased yields of potato planted in peat soils whereas Kloepper *et al.*³, using different PGPR strains, observed significant yield increases. In earlier studies, Rovira¹⁹ found that silt loam and soil sand types affected the plant growth-promoting activity of *Azotobacter* sp. treated on wheat and tomato. A consistent plant growth response depends greatly on successful colonisation of the rhizosphere and rhizoplane by PGPR, and these may be modified by factors such as soil type, soil moisture, plant species and cultivar, root exudates and nature of the inoculum^{2,20}. All of these factors, together with the soil factors that may possibly affect siderophore production and their Fe-chelation activity in soils may need to be resolved, suggesting an extensive evaluation programme for PGPR strains prior to their agronomic use. Although the soils differed in their physical and chemical characteristics, it is likely in the present

TABLE 1. SHOOT DRY WEIGHT OF *PUERARIA PHASEOLOIDES* INOCULATED WITH THE PGPR STRAIN 7NSK2 IN FOUR SOILS^a

Treatment	PGPR	N	Shoot dry weight (g/pot)			
			Serdang series	Holyrood series	Rengam series	Munchong series
Nil	-		101.3c	243.0b	980.3b	1 009.0a
	+		106.0c	399.9a	1 237.3 a	1 058.9a
PGPR	-		186.4b	396.2a	1 117.3 a	1 038.3a
	+		279.7a	493.3a	1 338.2 a	1 189.3a
Significance:						
PGPR			P < 0.001	P < 0.01	N.S.	N.S.
N			N.S.	P < 0.01	P < 0.01	N.S.
PGPR × N			N.S.	N.S.	N.S.	N.S.
CV(%)			9.6	7.9	3.2	4.9

^a Means of ten replicate pots, two plants per pot. Plants sampled at six weeks after sowing. Means within a soil type not followed by common letters are significantly different (P < 0.05), on a transformed (lnX) basis.

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study that the variable contents of a specific deleterious root micro-organisms in them were partly responsible for the increase in plant growth from PGPR inoculation. Iswandi *et al.*²¹ have shown that for maize and barley grown in pots, significant plant growth increases from seed inoculation with strain 7NSK2 were possible in 'active' soils with higher densities and activity of micro-organisms. Bacterisation offers exciting areas for future research since fluorescent pseudomonads, besides enhancing crop growth and yield, have been successfully used as biological control agents against important diseases caused by soil-borne fungi²². The original assumption of a fluorescent siderophore-mediated Fe deprivation in soil may not necessarily be a consistent mechanism of disease suppression; disease suppression or plant growth-promotion mediated by fluorescent pseudomonads may also be due to specific antibiotics, to competition for essential elements by microbially-produced non-fluorescent siderophores, and possibly to other unknown mechanisms²³.

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REFERENCES

1. KLOEPPER, J.W. AND SCHROTH, M.N. (1978) Plant Growth-promoting Rhizobacteria on Radishes. *Proc. Int. Conf. Plant Pathog. Bact.* 4th. Angers, France, 879.
2. BURR, T.J., SCHROTH, M.N. AND SUSLOW, T. (1978) Increased Potato Yields by Treatment of Seedpieces with Specific Strains of *Pseudomonas fluorescens* and *P. putida*. *Phytopath.*, **68**, 1377.
3. KLOEPPER, J.W., SCHROTH, M.N. AND MILLER, T.D. (1980) Effect of Rhizosphere Colonization by Plant Growth-promoting Rhizobacteria on Potato Plant Development and Yield. *Phytopath.*, **70**, 1078.
4. OLSEN, M.W. AND MISAGHI, I.J. (1984) Responses of Guayule (*Parthenium argentatum*) Seedlings to Plant Growth Promoting Fluorescent Pseudomonads. *Pl. Soil*, **77**, 97.
5. ELLIOTT, L.F. AND LYNCH, J.M. (1985) Plant Growth-inhibitory Pseudomonads Colonizing Winter Wheat (*Triticum aestivum* L.) Roots. *Pl. Soil*, **84**, 57.
6. SUSLOW, T. (1982) Role of Root-colonizing Bacteria in Plant Growth. *Phytopathogenic Prokaryotes*. (Mount, M.S. and Lacy, G.H. ed.) Vol. 1. p. 187. London: Academic Press.
7. IKRAM, A., MAHMUD, A.W. AND NAPI, D. (1987) Effects of P-fertilization and Inoculation by Two Vesicular-arbuscular Mycorrhizal Fungi on Growth and Nodulation of *Calopogonium caeruleum*. *Pl. Soil*, **104**, 195.
8. SUSLOW, T.V. AND SCHROTH, M.N. (1982) Rhizobacteria on Sugar Beets: Effects of Seed Application and Root Colonization on Yield. *Phytopath.*, **72**, 199.
9. LYNCH, J.M. AND CLARK, S.J. (1984) Effects of Microbial Colonization of Barley (*Hordeum vulgare* L.) Roots on Seedling Growth. *J. appl. Bact.*, **56**, 47.
10. BROWN, M.E. (1972) Plant Growth Substances Produced by Micro-organisms of Soil and Rhizosphere. *J. appl. Bact.*, **35**, 443.
11. GARDNER, J.M., CHANDLER, J.L. AND FELDMAN, A.W. (1984) Growth Promotion and Inhibition by Antibiotic-producing Fluorescent Pseudomonads on Citrus Roots. *Pl. Soil*, **77**, 103.
12. CAESAR, A.J. AND BURR, T.J. (1987) Growth Promotion of Apple Seedlings and Rootstocks by Specific Strains of Bacteria. *Phytopath.*, **77**, 1583.
13. MEYER, J.R. AND LINDERMAN, R.G. (1986) Response of Subterranean Clover to Dual Inoculation with vesicular-arbuscular Mycorrhizal Fungi and a Plant Growth-promoting Bacterium, *Pseudomonas putida*. *Soil Biol. Biochem.*, **18**, 185.
14. GRIMES, H.D. AND MOUNT, M.S. (1984) Influence of *Pseudomonas putida* on Nodulation of *Phaseolus vulgaris*. *Soil Biol. Biochem.*, **16**, 27.
15. SAVITHIRY, S. AND GNANAMANICKAM, S.S. (1987) Bacterization of Peanut with *Pseudomonas fluorescens* for Biological Control of *Rhizoctonia solani* and for Enhanced Yield. *Pl. Soil*, **102**, 11.
16. PUSHPARAJAH, E. AND TAN, K.H. (1976) Legumes in the Nitrogen Economy of Rubber Cultivation. *Soil Microbiology and Plant Nutrition* (Broughton, W.J., John, C.K., Rajarao, J.C. and Lim, B., ed.), p. 413. University of Malaya Press.

17. BROUGHTON, W.J. (1977) Effect of Various Covers on Soil Fertility under *Hevea brasiliensis* Muell. Arg. and on Growth of the Tree. *Agro-Ecosystems*, **3**, 147.
18. KING, E.O., WARD, M.K. AND RANEY, D.E. (1954) Two Simple Media for the Demonstration of Pyocyanin and Fluorescin. *J. Lab. Clin. Med.*, **44**, 301.
19. ROVIRA, A.D. (1963) Microbial Inoculation of Plants. I. Establishment of Free Living Nitrogen-fixing Bacteria in the Rhizosphere and their Effects on Maize, Tomato and Wheat. *Pl. Soil*, **19**, 304.
20. HOWIE, W.J. AND ECHANDI, E. (1983) Rhizobacteria: Influence of Cultivar and Soil Type on Plant Growth and Yield of Potato. *Soil Biol. Biochem.*, **15**, 127.
21. ISWANDI, A., BOSSIER, P., VANDERNABEELE, J. AND VERSTRAETE, W. (1987) Relation between Soil Microbial Activity and the Effect of Seed Inoculation with the Rhizopseudomonad Strain 7NSK2 on Plant Growth. *Biol. Fertil. Soils*, **3**, 147.
22. WHIPPS, J.M. AND LYNCH, J.M. (1986) The Influence of the Rhizosphere on Crop Productivity. *Advances in Microbiol Ecology (Marshall, K.C., ed.)*, Vol. 9. Plenum.
23. MISAGHI, I.J., OLSEN, M.W., COTTY, P.J. AND DONNDELINGER, C.R. (1988) Fluorescent Siderophore-mediated Iron Deprivation — a Contingent Biological Control Mechanism. *Soil Biol. Biochem.*, **20**, 573.