EVALUATION OF NITROGEN FIXING ABILITY OF SRI LANKAN RHIZOBIUM SPECIES'

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ABSTRACT

The nodulation characteristics of the principal Sri Lankan grain legumes were surveyed and 67 strains of Rhizobia were isolated from 7 legume species. The cowpea group of rhizobia was screened for nitrogen fixing activity on *Vigna unguiculata* variety MI.35 and of the 50 isolates tested 10 produced a plant yield that was not statistically better ($p=0.05$) than the uninoculated control. Four of these were strains of reputed productivity obtained from Niftal, Hawaii, and USA. Fingerprinting, based on intrinsic antibiotic resistance, was undertaken for both the cowpea group and *Rhizobium japonicum* isolates. Of the 42 isolates tested, 33 unique fingerprints were obtained for the former and for the latter 18 from the 23 isolates tested. Since the majority of *R. japonicum* strains were introduced through inoculation, it indicates that some degree of adaptation to local conditions may have occured.

INTRODUCTION

Leguminous food crops are of major significance in many farming systems in Sri Lanka and each year over 60,000 ha of cowpea (*Vigna unguiculata*), green gram (*Vigna radiata*), black gram (*Vigna munga*) and groundnut (*Arachis hypogea*) are cultivated.

The national average yields of legumes are low and with pressures on food resources there is a need to increase legume production both through expanding the area of cultivation and increasing yields. One important prerequisite for legume production is satisfactory nodulation and nitrogen

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fixation by the appropriate rhizobium species. However in Sri Lanka there are virtually no reports on the nodulation characteristics of the widely grown food legumes and on the nitrogen fixing potential of the indigenous populations of rhizobia A major objective of the study reported here was to survey the existing nodulation characteristics of the major legumes and to evaluate the nitrogen fixing abilities of the indigenous rhizobia in comparison with known productive strains. In addition the survey also included sampling of soybean (*Glycine max*) plants, although commercial varieties of this species are a relatively recent introduction.

One of the major methodological difficulties facing studies of the ecology and characteristics of rhizobia is the question of strain identification. Until recently recognition of strains has depended on morphological, serological and genetic tests (Vincent, 1970; Koonty and Faber, 1961; Schwinghammer and Dudman, 1973) but these have often proved in practice to be time consuming, imprecise and have even affected strain performance (Josey *et al*, 1979). The development of a technique based on the intrinsic antibiotic resistance of rhizobia (Josey *et al*, 1979) has offered an approach that is both simple, reliable and allows the handling of a large number of strains. It has been successfully used in the identification and characterization of rhizobia, in grouping native populations and in following the fate of strains introduced into field environments (Benyon and Josey, 1980). This technique was used in the studies reported here.

MATERIAL AND METHODS

1. Survey of the existing nodulation characteristics of currently grown food legumes and collection of strains of rhizobia. The principal legume growing areas were visited during this survey which was carried out over two growing seasons. Samples were taken from 50 sites in 14 districts in the major agro-ecological zones as defined by soil type and rainfall pattern. All isolated strains were authenticated on *Macroptilium atropurpureum* (siratro). Methods for strain isolation and characterisation followed those of Vincent (1970).

2. Strains. All strains isolated from the survey were catalogued in the following manner. A prefix of SLM was designated and strain numbers were allocated according to the host plant. These were as follows : *Arachis hypogea* 001 — 100; *Glycine max* 101 — 200; *Phaseolus vulgaris* 201 — 300;

Psophocarpus tetragonolobus 301 — 400. *Vigna mungo* 401 — 500; *Vigna radiata* 501 — 600 and *Vigna unguiculata* 601 — 700. Rhizobia strains of known productivity were obtained from the Nitrogen Fixation in Tropical Legumes Programme (Niftal), Hawaii, USA and are identified by the TAL designation.

3. Media. A nitrogen free nutrient solution (Broughton & Dilworth, 1971) was used for plant nodulation tests. Strains were isolated and maintained on yeast mannitol agar (YMA, Vincent, 1970). Yeast mannitol broth (YMB) was its liquid equivalent.

4. Testing the efficiency of rhizobial isolates on host plants. *Glycine max* Pubjab variety Pb1 and *Vigna unguiculata* MI 35 were the two standard host plants. For screening experiments with *G. max*, plants were grown in ½ L nitrogen free nutrient solution in Leonard bottle-jar assemblies (Norris, 1964). The host symbiotic efficiency in cowpea was tested using plastic pots containing sterile river sand. Plant seeds were surface sterilised, pregerminated and innoculated following the procedures described by Vincent (1970). All tests were arranged on bench tops in randomised blocks with three replications and were harvested 42 days after sowing. Plants were scored for leaf colouration and number and colouration of nodules. Plant dry weight and dry weight of nodules were recorded separately.

5. Determining patterns of intrinsic antibiotic resistance of locally isolated rhizobia. The following antibiotics were used in this study, concentrations in mg / l are given in parentheses: Nalidixic Acid (2.5, 10, 15); Erythromycin (2.5, 10, 15); Rifampicin (0-25, 0.5, 2.5); Kanamycin (2.5, 10, 20); Streptomycin (2.5, 10, 20, 40); Tetracyclin (0.2, 0.5); Carbenicillin (1.0, 2.5, 5); Neomycin (1.25, 2.5, 10, 15); Choramphenicol (5.0, 10, 20); Lincomycin (1.25, 2.5, 10). Antiobiotic test plates (YMA / plus antibiotic) were innocuated with a multiple inoculator as described by Josey *et al* (1979). Plates were incubated at room temperature (26°C) for ten days and then the growth of the isolates on the antibiotic media was observed and scored based on the isolate growth on control plates: O for no growth, 1 for weak growth, 2 for medium growth and 3 for good growth. The test was repeated at weekly intervals for five weeks and each test was carried out with five replications.

RESULTS

1. Survey of the nodulation characteristics of the principal food legumes. All indigenous legumes showed effective nodulation although there was considerable difference between samples as to the degree of nodulation. However,

no conclusive evidence was obtained for such variation being attributable to the effect of different cropping histories. Satisfactory nodulation was found on legumes grown on newly cleared land in shifting cultivation systems which apparently had not been cultivated for the previous 15 years and had been prepared by firing. Two grain legumes, *P. vulgaris* and *G. max* are known to be specific in their rhizobia requirements. *P. vulgaris* has been grown in Sri Lanka for a long time as a vegetable crop and on all sites it showed profuse nodulation. However, *G. max* is a relatively recent introduction and on those sites where no inoculation had been recorded, nodulation was not observed; on 17 other sites where inoculation was reported, profuse nodulation was found. There is one location in Sri Lanka, Mathurata in the hill country, where a local variety of *G. max* had been grown for a long time prior to the introduction of the commercial varieties; most farmers in this district reported that no inoculum had been used yet effective nodulation was observed in all samples, suggesting the persistence of *R. japonicum* in this area. Details of the number of strains of rhizobia isolated and authenticated from the survey are given in Table 1. These numbers are not a reflection of the importance of the various legume species but are associated either with field difficulties in obtaining specimens from remote areas, as with *A. hypogea*, or a particular interest in recording the presence or absence of nodulation as with *G. max*.

2. *a.* **Screening of cowpea group rhizobia on V. unguiculata for nitrogen fixation activity.** *V. unguiculata* was selected as the test species for the cowpea type rhizobia isolates due to its relative importance in comparison with other grain legumes. The rhizobia isolates were tested in comparison with known productive foreign strains for efficiency of nitrogen fixation. Fig. 1 shows the mean top dry weights of *V. unguiculata* at harvest after 42 days. Uninoculated plants were used as the basis for the comparison of effectiveness. Uninoculated plants had stunted growth and yellow leaves and a top dry weight of only 0.679g. A wide range of relative effectiveness was found among the isolates. Out of the 50 inoculated treatments, 32 isolates were better than the uninoculated control at the 1% level of significance. Such plants had top dry weights of between 1.65 and 2.38g and had dark green healthy foliage. Of these 32 isolates, 30 were local strains and two were foreign, TAL235 and TAL309. The two foreign strains only ranked 27 and 29 out of the 32 effective strains and some local strains clearly performed considerably better than the best of the foreign strains. The 30

local strains were isolated from the range of legumes nodulated by the cowpea type rhizobia and these results demonstrate the non-specificiy of this group. Only ten of the 50 isolates tested produced a plant yield that was not statistically better than the uninoculated controls at the 5% level of significance. Four of these were foreign strains (TAL641, TAL176, TAL11 and TAL169) and six were local strains. Plants inoculated with these strains had stunted growth and pale yellow-green leaves.

Nodules were formed on all inoculated plants and nodule dry weights per plant ranged from 0.13 to 0.32g. The uninoculated and nitrogen controls were devoid of nodules. Nodule dry weights were positively correlated with above ground plant dry weights with a correlation coefficient of 0.77, p=0.001 (Fig. 2).

2. *b*. Screening of R. japonicum strains on G. max for nitrogen fixing activity. Fig. 3 records the mean top dry weights of plants at harvest inoculated with different isolates of *R. japonicum*. The plant top dry weights varied from 0.71g to 2.16g. The nitrogen control had a top dry weight of 1.47g while the uninoculated control had the minimum dry weight of 0.71g. Out of the 38 inoculated treatments, 23 isolates produced a plant yield that was better than the uninoculated at the 1% level of significance with top dry weights ranging from 1.45 to 2.16g. Of these 23 isolates, 8 were of local origin while 15 were foreign; the fields from which the 8 local isolates were collected had all reportedly been inoculated at the time of planting. Nine isolates of local origin did not produce a statistically significant difference ($p = 0.05$) in plant top dry weights from the uninoculated controls and the plant top dry weights of these treatments varied from 0.76g to 1.16g. Six of these 9, (SLM 107, 108, 115, 116, 119 and 120) had been isolated from the Mathurata area where no inoculum had been reported to have been used.

For all inoculated plants nodulation was recorded; no nodules were found on the uninoculated or nitrogen controls. For those strains that did not produce a plant top dry weight significantly greater than the uninoculated control, a few (up to 10) small nodules were formed. As shown in Fig. 4 the above ground plant dry weight and the nodule dry weights were positively correlated ($r=0.77$, $p=0.001$).

3. Intrinsic antibiotic resistance of the local rhizobia isolates. a. Fingerprint of the cowpea group rhizobia. As shown in Table 2, variation in the intrinsic antibiotic resistance of the cowpea group rhizobia isolates was

found, suggesting a range of strains. Thirty three different fingerprints were obtained from the 42 isolates tested. Eight combinations of isolates proved to have similar fingerprints; SLM601 and 602. SLM501 and 502, SLM604 and 605. SLM 606 and 404, SLM03 and 609, SLM615, 405 and 616. SLM617 and 407 and SLM301 and 621. Isolates SLM601 and 602 were both isolated from the same site and plant species which would explain the similarity in their fingerprint. This also applies for the strain pair SLM501 and 502. Strain pairs SLM03 and 609, SLM617 and 407 were isolated from different host plants but from the same sites. However, the remaining four combinations of isolates had no such point of common origin and the possibility that isolates of rhizobia from different geographical regions could produce similar fingerprints, or that these were in fact the same strain would require further examination using a larger series of antibiotic fingerprinting (Antoun H. *et al*, 1982). There were no differences in nitrogen fixing ability as indicated by plant top dry weight among the combination or strain pairs that produced similar fingerprints.

b. **Fingerprints of the R. japonicum isolates.** The fingerprints of the *R. japonicum* isolates are summarised in Table 3 and show that 18 different fingerprints were detected, indicating the heterogeneity of the population. Strain combinations SLM102 and 103, SLM105 and 106, SLM 110 and 109, SLM117 and 118 and SLM119 and 120 produced similar fingerprints to each other. However, the strains of each combination were all isolated from the same site. Since the source of strains SLM101, 102, 103, 014,105, 106, 109, 110, 111, 112 and 113 is probably through inoculation, in comparison with the Mathurata strains, it suggests, that adaption of the introduced strains to local conditions may have occured. Typing of the inoculum and subsequent monitoring of soil *R. japonicum* populations would clarify this point.

DISCUSSION

From the field survey conducted on the nodulation of the main food legumes, the nodulation of *V. unguiculata, V, radiata, V. munga, A. hypogea, P. vulgaris* and *P. tetragonolubus* was observed to be profuse in many locations suggesting that effective rhizobia strains of the correct species grouping were present in the soil. The comparison of the nitrogen fixing ability of the local isolates with known productive strains in pot trials suggested that the introduction of new strains of cowpea type rhizobia would be unlikely to confer any nitrogen fixing advantage and emphasises the importance of fully evaluating introduced strains. However it should be noted that the rhizobia isolates were screened against only one variety of cowpea and it is known (Bergersen, 1977) that there are important varietal differences in relation to specific rhizobia strains and their nitrogen fixing ability. Future work should compare different plant varieties.

The survey of the nodulation of *G. max* indicated the importance of using inoculum on this species in Sri Lanka. The characteristics of the Mathurata strains require further examination. However, the use of the fingerprinting technique suggests that local adaptation of the introduced *R. japonicum* may be taking place and monitoring of the persistence of introduced *R. japonicum* strains and their nitrogen fixing ability would be valuable. While the results of the fingerprinting technique using intrinsic antibiotic resistance were in a few cases ambiguous, the technique did allow the characterisation relatively easily of a large number of strains. Again further monitoring on the reliability and persistence of intrinsic antibiotic resistance as a diagnositic feature of a particular strain is needed.

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Table 1. Authenticated strains of rhizobia

Isolated from:	*No.*	*Strain No.*
A. hypogea	4	01—4
C. max	23	101—123
P. vulgaris	2	201—202
P. tetragonolobus	2	301—302
V. mungo	7	401—407
V. radiata	8	501—508
V. unguiculata	21	601—621

Table 2. Antibiotic resistance patterns of cowpea group *Rhizobium* isolated in Sri Lanka

(Antibiotic Concentration in mg/l)

	*Nali**			*Ery**			*Rif**			*Kan**				*Strep**				*Lin**			*Tetra**		*Carb**			*Neo**				*Chlo**		
Isolate	*2.5*	*10*	*15*	*2.5*	*10*	*15*	*.25*	*.5*	*2.5*	*2.5*	*10*	*20*		*2.5*	*10*	*20*	*40*	*1.25*	*2.5*	*10*	*.2*	*.5*	*1.2*	*.5*	*5*	*1.25*	*2.5*	*10*	*15*	*5*	*10*	*20*
SLM601	2	2	1	2	1	1	2	2	2	2	1	1		2	1	0	0	1	2	2	2	2	2	2	2	1	2	2	2	2	2	1
SLM602	2	2	1	2	1	1	2	2	2	2	1	1		2	1	0	0	1	2	2	2	2	2	2	2	1	2	2	2	2	2	1
SLM603	1	1	1	1	0	0	1	1	0	1	1	0		1	1	0	0	1	1	1	2	1	1	1	1	1	1	1	1	1	2	1
SLM401	2	2	2	2	1	1	2	2	2	2	1	1		2	1	0	0	2	2	2	3	2	2	2	2	2	2	2	2	2	1	1
SLM501	3	2	1	2	2	1	2	2	2	3	3	2		1	1	0	0	3	3	2	3	2	2	2	2	3	2	2	1	3	2	1
SLM502	3	2	1	2	2	1	2	2	2	3	3	2		1	1	0	0	3	3	2	3	2	2	2	2	3	2	2	1	3	2	1
SLM01	3	2	2	2	2	1	2	2	2	2	1	0		1	0	0	0	3	3	2	3	3	3	3	2	3	3	2	2	3	2	2
SLM604	1	1	1	1	0	0	1	1	0	1	0	0		0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SLM402	2	2	2	2	1	1	2	2	2	3	2	1		2	1	1	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
SLM503	2	2	1	2	1	1	2	2	2	3	1	1		1	1	0	0	2	2	2	2	2	2	2	2	2	2	1	1	2	1	1
SLM605	1	1	1	1	0	0	1	1	0	1	0	0		0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SLM403	2	1	1	1	1	1	2	1	1	2	1	1		1	1	0	0	1	1	1	1	1	2	2	1	2	1	1	1	1	1	1
SLM606	3	3	2	2	2	1	3	2	2	3	3	2		1	1	0	0	3	3	2	3	2	3	3	3	2	2	2	2	2	2	2
SLM404	3	3	2	2	2	1	3	2	2	3	3	2		1	1	0	0	3	3	2	3	2	3	3	3	2	2	2	2	2	2	2
SLM02	2	1	1	2	1	1	2	1	1	2	1	0		1	1	0	0	2	2	1	1	1	2	2	1	2	2	1	1	2	2	2
SLM608	2	1	1	1	0	0	1	1	0	1	0	0		0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SLM03	3	3	3	3	2	1	3	3	1	3	3	3		1	0	0	0	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2
SLM609	3	3	3	3	2	1	3	3	1	3	3	3		1	0	0	0	2	3	3	3	3	3	3	3	3	3	3	3	3	2	2
SLM610	3	2	2	3	2	1	3	3	3	2	0	0		1	0	0	0	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2
SLM611	2	2	1	2	2	1	2	2	2	2	1	0		2	1	0	0	2	2	2	2	2	2	2	2	3	2	2	2	2	2	1

0 = no growth
1 = weak growth
2 = medium growth
3 = good growth

*
Nali = Nalidixic Acid
Ery = Erythromycin
Rif = Rifampicin
Kan = Kanamycin
Strep = Streptomycin
Lin = Lincomycin
Tetra—Tetracyclin
Carb = Carbenicillin
Neo = Neomycin
Chlo = Chloramphenicol

SLM504	2	2	1	2	1	1	2	1	1	2	1	0	2	2	1	0	2	2	1	1	2	1	1	2	2	2	2	2	2	2	2
SLM505	3	3	3	3	1	1	3	3	2	3	3	3	1	0	0	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2
SLM506	1	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SLM612	3	2	1	3	2	1	3	3	3	3	1	0	2	2	1	0	3	3	3	2	2	3	3	3	3	2	2	2	3	2	2
SLM201	3	2	1	3	2	1	3	3	3	3	1	0	2	1	0	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2
SLM613	3	2	1	3	2	1	3	3	3	3	1	0	2	2	1	0	3	3	3	3	2	2	3	3	3	2	2	2	3	3	2
SLM202	2	2	1	2	2	1	2	2	2	2	1	0	2	1	1	0	2	2	2	2	2	2	2	1	2	2	2	1	2	2	1
SLM615	2	2	1	1	0	0	1	1	0	1	0	0	1	0	0	0	1	1	1	2	2	2	2	1	2	2	1	1	2	1	1
SLM405	2	2	1	1	0	0	1	1	0	1	0	0	1	0	0	0	1	1	1	2	2	2	2	1	2	2	1	1	2	1	1
SLM616	2	2	1	1	0	0	1	1	0	1	0	0	1	0	0	0	1	1	1	2	2	2	2	1	2	2	1	1	2	1	1
SLM406	3	2	1	1	0	0	3	2	0	1	0	0	2	0	0	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2
SLM617	2	1	1	2	1	1	2	1	1	2	1	0	1	1	1	0	3	3	3	2	2	1	2	1	2	2	1	1	2	1	1
SLM407	2	1	1	2	1	1	2	1	1	2	1	0	1	1	1	0	3	3	3	2	2	1	2	1	2	2	1	1	2	1	1
SLM618	2	1	1	2	1	1	2	2	1	2	1	0	2	2	1	0	2	2	2	2	2	2	2	2	2	2	2	1	2	2	1
SLM619	3	3	3	2	1	3	3	3	3	2	1	0	2	1	0	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
SLM04	2	1	1	1	0	1	1	1	1	3	1	1	1	1	0	0	2	2	2	2	2	2	2	2	2	2	2	1	2	1	1
SLM620	1	1	1	1	0	0	1	1	1	1	1	0	1	0	0	0	2	2	2	2	1	1	1	1	2	1	1	1	1	1	1
SLM507	3	1	1	1	0	0	2	2	2	1	1	0	1	0	0	0	3	2	2	2	2	2	2	1	2	2	1	1	2	2	1
SLM301	3	3	2	2	1	1	3	3	2	2	2	2	2	1	0	0	0	3	3	3	3	3	3	3	3	3	3	2	2	3	2	1
SLM621	3	3	2	2	1	1	3	3	2	2	2	2	2	1	0	0	0	3	3	3	3	3	3	3	3	3	3	2	2	3	2	1
SLM302	2	1	0	1	0	0	1	0	0	1	0	0	1	0	0	0	1	1	1	1	1	1	1	2	2	1	1	1	1	1	0
SLM508	2	2	2	2	1	1	2	2	2	2	2	2	2	2	1	1	0	2	2	2	2	2	2	2	2	2	2	1	2	2	2

Table 3. Antibiotic resistance patterns of *Rhizobium japonicum* isolated in Sri Lanka

(Antibiotic concentration in mg/l)

	*Nali**			*Fry**			*Rif**			*Kan**				*Strep**				*Lin**			*Tetra**		*Carb**			*Neo**				*Chlo**		
Isolate	*2.5*	*10*	*15*	*2.5*	*10*	*15*	*.25*	*.5*	*2.5*	*2.5*	*10*	*20*		*2.5*	*10*	*20*	*40*	*1.25*	*2.5*	*10*	*.2*	*.5*	*1.2*	*.5*	*3*	*1.25*	*2.5*	*10*	*15*	*5*	*10*	*20*
SLM101	1	1	0	1	1	0	2	1	1	2	1	0		2	1	1	0	2	2	1	1	1	2	2	2	2	2	1	1	1	1	1
SLM102	2	2	1	1	1	1	1	1	1	2	2	1		1	0	0	0	2	2	1	1	1	2	2	1	2	2	2	2	2	1	1
SLM103	2	2	1	1	1	1	1	1	1	2	2	1		1	0	0	0	2	2	1	1	1	2	2	1	2	2	2	2	2	1	1
SLM104	2	1	0	2	2	1	2	2	0	2	1	0		1	0	0	0	2	2	2	1	1	2	2	2	2	2	2	2	2	1	1
SLM105	2	1	0	1	1	1	2	1	0	1	1	0		1	0	0	0	2	1	1	1	1	2	2	2	2	2	1	1	1	1	1
SLM106	2	1	0	1	1	1	2	1	0	1	1	0		1	0	0	0	2	1	1	1	1	2	2	2	2	2	1	1	1	1	1
SLM107	1	1	0	1	1	1	1	1	0	1	0	0		1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SLM108	3	3	0	3	2	1	3	3	0	3	1	1		3	0	0	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2
SLM109	3	3	0	2	1	1	2	1	0	2	1	0		2	0	0	0	2	2	2	1	1	2	2	2	2	2	2	2	2	2	1
SLM110	3	3	0	2	1	1	2	1	0	2	1	0		2	0	0	0	2	2	2	1	1	2	2	2	2	2	2	2	2	2	1
SLM111	1	1	1	1	1	1	1	1	1	1	0	0		1	1	0	0	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1
SLM112	1	1	1	1	1	1	3	3	2	1	0	0		2	1	1	0	2	2	2	1	1	2	2	2	2	2	2	2	2	1	1
SLM113	1	0	0	1	1	1	1	1	0	1	0	0		1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SLM114	2	2	0	2	1	1	2	2	0	3	2	0		2	0	0	0	3	3	3	3	3	3	3	3	3	3	1	3	2	1	1
SLM115	1	1	0	1	1	1	1	1	0	1	0	0		1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SLM116	3	2	0	2	1	1	3	2	0	3	2	1		3	0	0	0	3	2	2	3	3	3	3	3	3	3	3	3	3	3	2
SLM117	3	3	0	3	2	2	3	3	0	3	2	1		3	0	0	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
SLM118	3	3	0	3	2	2	3	3	0	3	2	1		3	0	0	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
SLM119	3	3	0	2	2	1	3	3	0	3	2	1		3	0	0	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
SLM120	3	3	0	2	2	1	3	3	0	3	2	1		3	0	0	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
SLM121	3	2	0	2	2	1	3	3	0	3	2	1		3	0	0	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
SLM122	2	2	1	2	2	1	3	2	0	2	0	0		0	0	0	0	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2
SLM123	3	2	0	2	1	1	3	2	0	2	1	1		1	0	0	0	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2

0 = no growth
1 = weak growth
2 = medium growth
3 = good growth
*Nali = Nalidixic acid
Ery = Erythromycin
Rif = Rifampicin
Kan = Kanamycin
Strep = Streptomycin
Lin = Lincomycin
Tetra = Tetracyclin
Carb = Carbenicillin
Neo = Neomycin
Chlo = Chloramphenicol

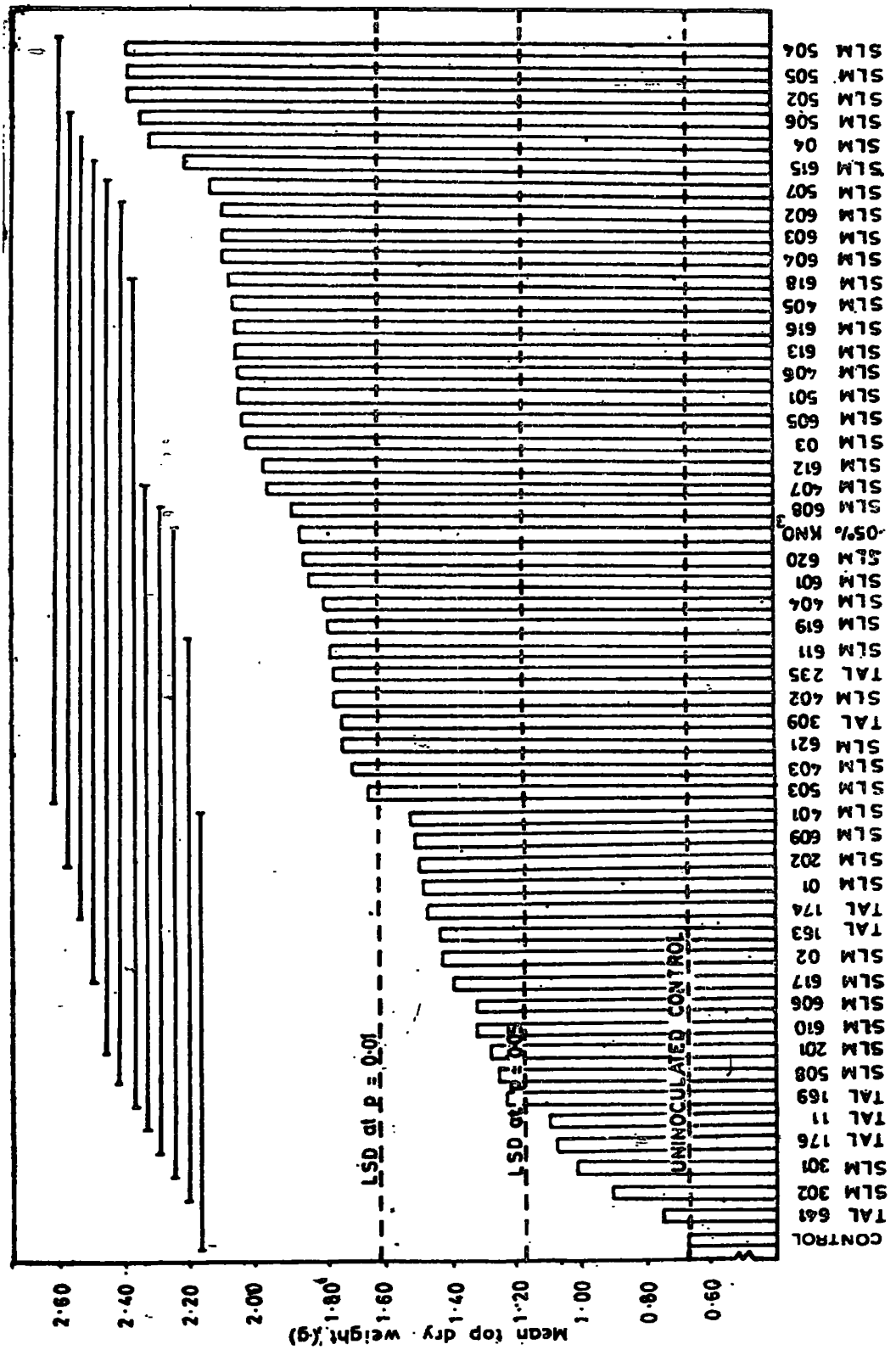

Figure 1. Effect of different cowpea type rhizobia on the top dry weight of *V. unguiculata* (variety MI 35). Lines at the top indicate Duncan's multiple range test at p=05.

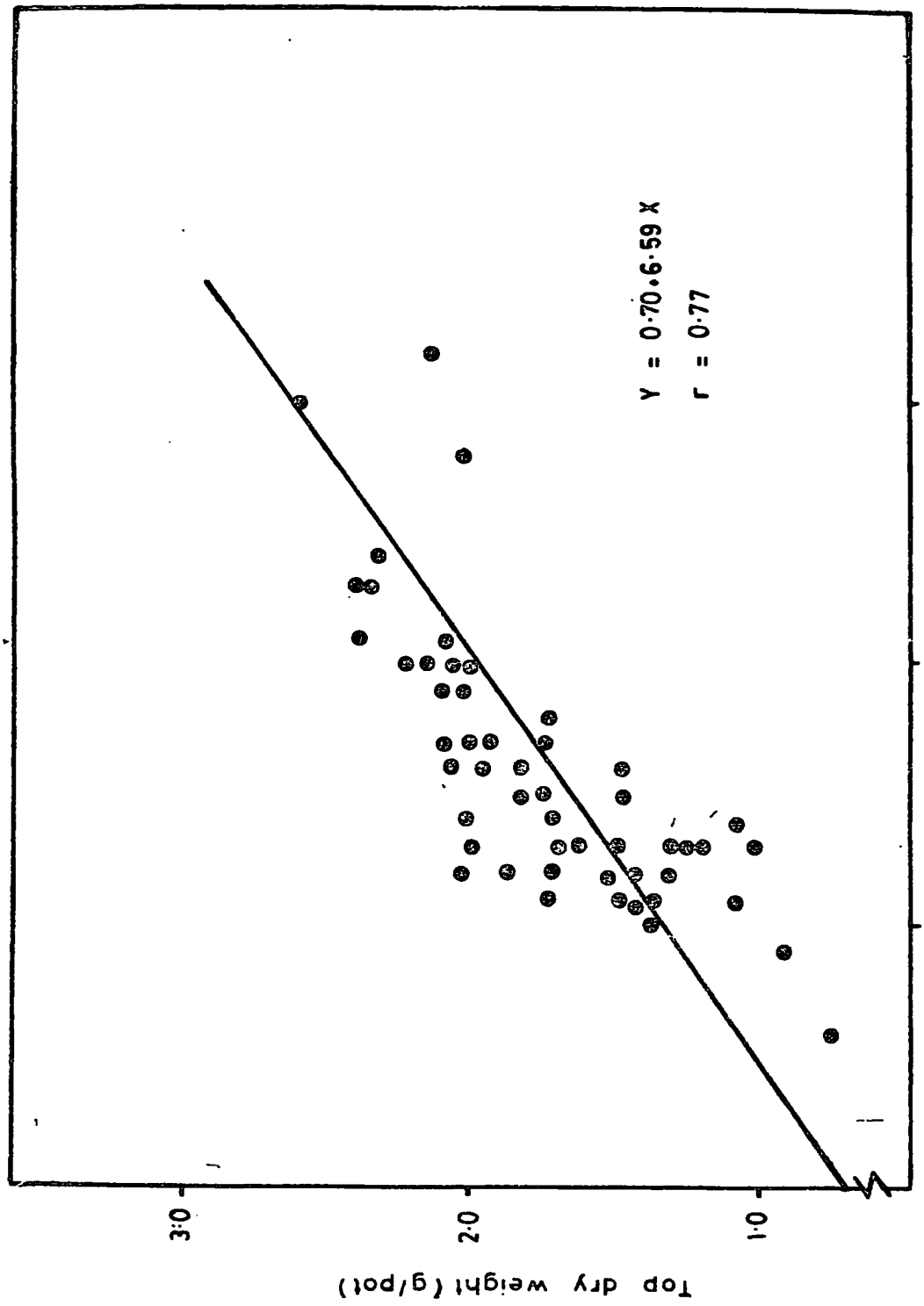

Figure 2. Correlation between nodule dry weight and top dry weight of *V. unguiculata*

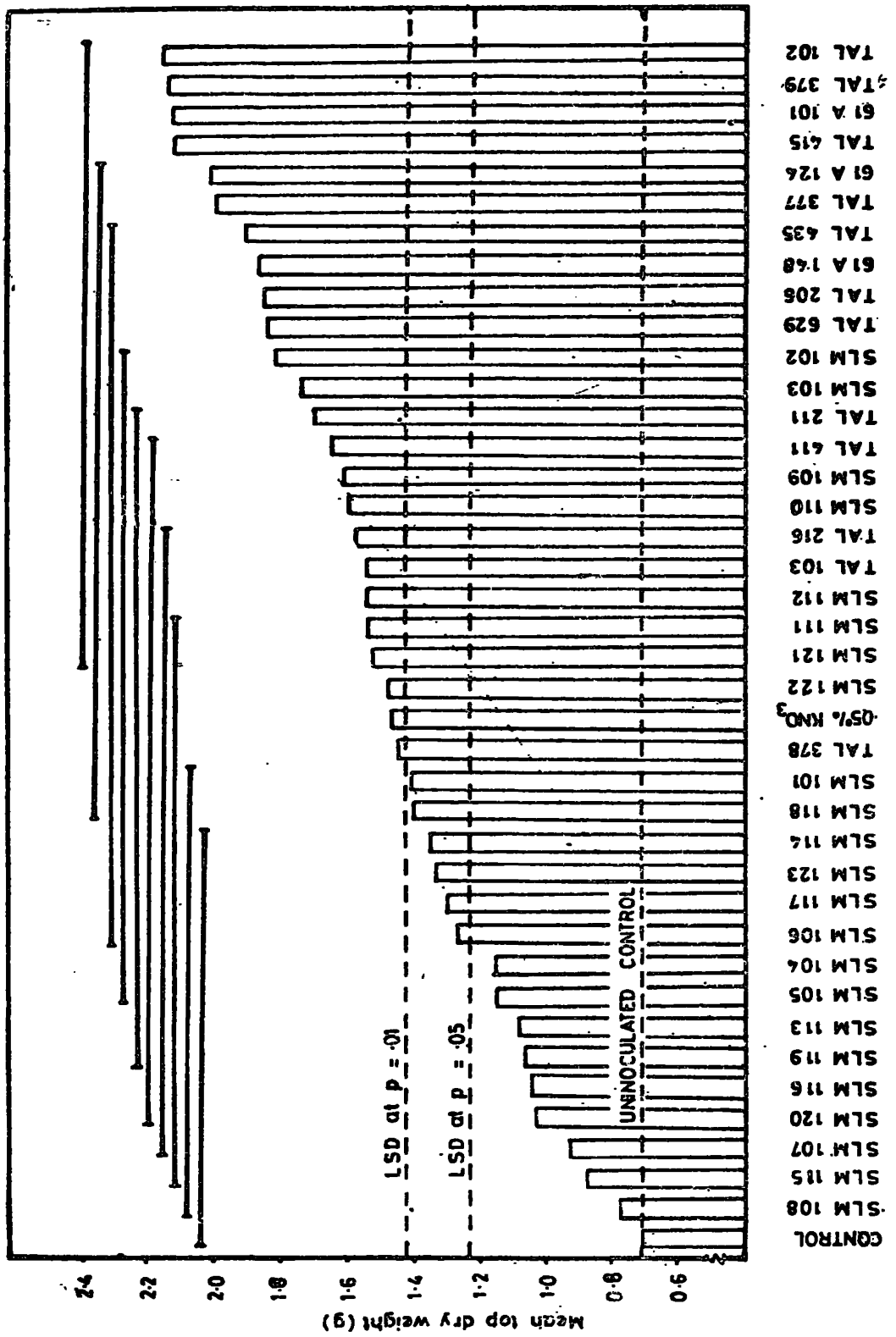

Figure 3. Effect of different *B. japonicum* isolates on the top dry weight of *G. max*. Lines at the top indicate Duncan's multiple range test at p=05

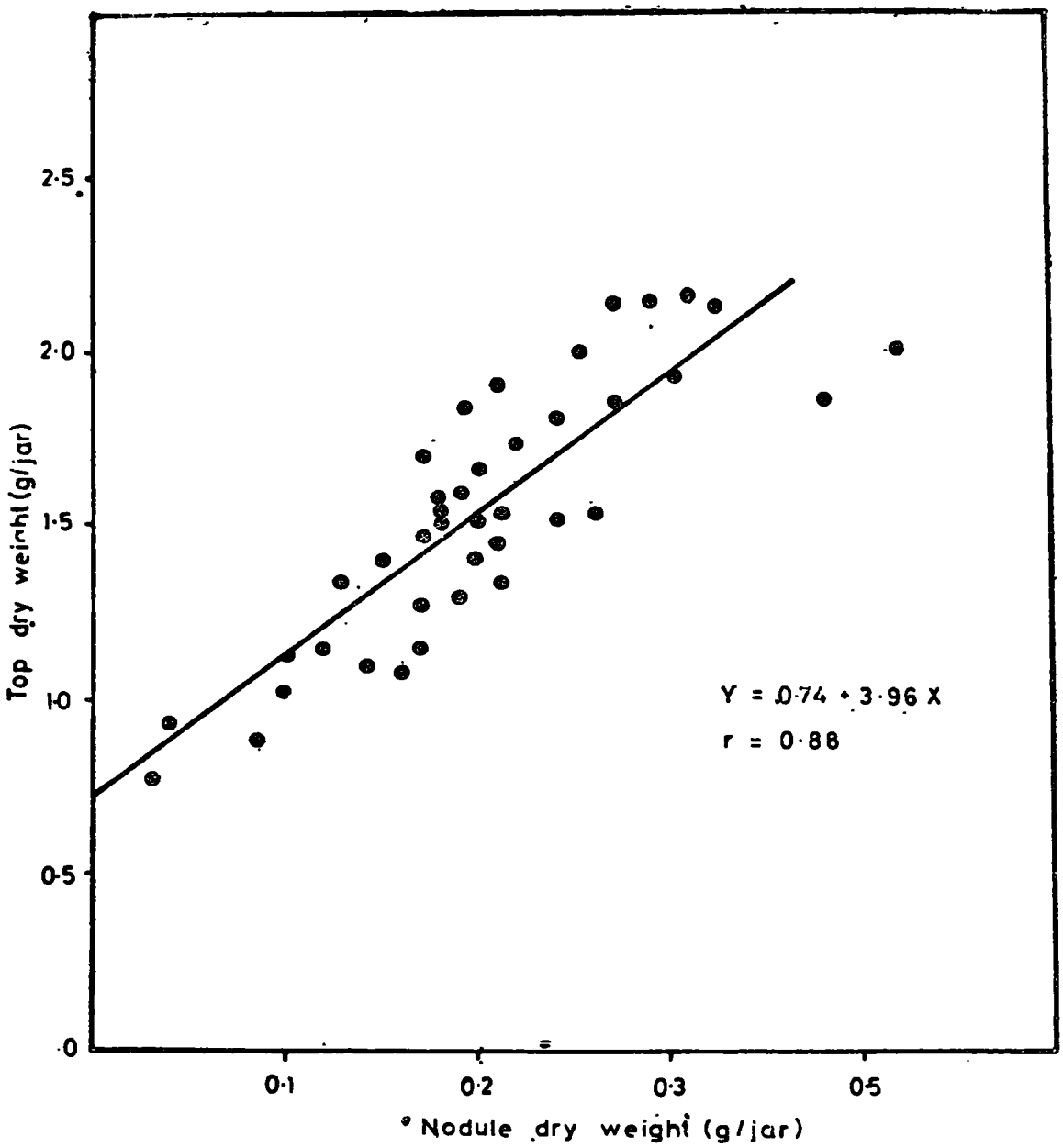

Figure 4. Correlation between nodule dry weight and top dry weight of *G. max*