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A CROSS-INOCULATION STUDY ON INDIGENOUS RHIZOBIA

AND

SELECTED TREE LEGUMES

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Summary

A cross-inoculation study with indigenous rhizobia was carried out on seven host legume species from where they were originally isolated. The legumes included *Acacia albida*, *Acacia mearnsii*, *Calliandra calothyrsus*, *Leucena leucocephala*, *Prosopis juliflora* *Sesbania grandiflora* and *Sesbania sesban*. The host plant species were variably promiscuous in their rhizobial associates. Conversely rhizobia isolated from *Sesbania grandiflora* and *Sesbania sesban* were more specific with their host partners in terms of symbiotic effectiveness.

Introduction

The association between trees and rhizobia is important in that it renders **the trees self sufficient in nitrogen.** However, nitrogen self-sufficiency occurs only when the Nitrogen Fixing Tree (NFT) associates with a compatible rhizobium strain. Thus, prior knowledge of compatibility between legumes with rhizobia is an important criterion for nursery seedling inoculation exercise. This is because a particular level of host taxonomic group (species, genus, family, etc.) may either be specific or promiscuous in its rhizobial requirement. A cross- inoculation study helps to reveal the specificity or promiscuity of the host species.

Most of the work reported in the past on natural nodulation, rhizobia isolation, inoculation and selection studies in studies in Kenya have been on agriculturally important pasture and grain legumes (Ssail and Keya 1986; Ssail 1988; Karanja and Wood, 1988a; 1988b). Other than recent work by Mittienen et al (1988) on Prosopis juliflora, little is known about the host spectrum and symbiotic effectiveness of naturally occurring rhizobia with legume NFT and some indigenous rhizobia.

Materials and Methods

Eight strains of Rhizobium isolated from geographically different areas were selected for the study. Strain NUM 777 was acquired from the Nairobi Microbiological Resources Centre (MIRCEN). Each strain was characterized by streaking on plates of yeast mannitol (YEM) agar. The composition of the medium was as follows:- (g/1), mannitol, 10; yeast extract 0.4; K_2HPO_4, 0.5; $MgSO_4,7H_2O$,0.2; Nacl, 0.1 and distilled water. Bromothymol blue (BTB) was incorporated into the medium at the rate of 5ml of 0.5% alcoholic solution per litre for pH reaction of the Rhizobium strains. Streaked plates were incubated at 26 degrees for 10 days. Growth rates were described by cultural and colony size (Vincent, 1979). Fast growers achieved moderate to abundant growth with colony sizes equal or greater than 2 mm within 5 days. Slow growers achieved slight to moderate growth with colony sizes of less than 2mm after 5 days. The sources, hosts of isolation and characteristics of the strains are shown in Table 1.

Table 1: Growth Characteristics of Eight Rhizobium Strains on Yeast Extract Mannitol Agar

Rhizobium Strain	Locality	Host of Isolation	Growth Rate	Reaction on Bromothymol Blue (BTB)	Colony Morphology
18b	Magarini	S. grandiflora	Slow	Alkaline	Flat, dull, cream coloured with translucent margin differentiating into greater opacity at the centre of the colonies.
11a	Turbo (Rift Valley)	S. sesban	Fast	Acidic	Flat, shiny, white coloured with translucent margin differentiating into greater opacity at the centre of the colonies.
2c	Mbita Point (Nyanza Province)	L. leucocephala	Fast	Acidic	Domed, shiny cream coloured with translucent margin differentiating into greater opacity at the centre of the colonies.
8b	Kisumu (Nyanza Province)	L. leucocephala	Fast	Acidic	Domed, shiny cream coloured with translucent margin differentiating into greater opacity at the centre of the colonies.
15b	Katangi (Eastern Province)	P. juliflora	Fast	Acidic	Flat, shiny, white coloured and evenly opaque colonies.
16c	Mwandani (Coastal Province)	C. calothyrsus	Fast	Acidic	Domed, shiny, white coloured and evenly opaque colonies.
22	Mbita Point (Nyanza Province)	A. albida	Fast	Acidic	Domed, shiny, cream coloured with translucent margin differentiating into greater colonies at the centre of the colonies.
NUM [illegible]*	Eldoret wattle extracting factory (Rift Valley)	A. mearnsii	Fast	Neutral	Flat, dull, white colour and evenly opaque colonies

* Source: The Nairobi Rhizobium MIRCEN

Modified Leonard Jar (Fig. 1) assemblies with vermiculite as the medium were used to grow seedlings. The composition of nutrient solution for assemblies was as described by Somasegaran and Hoben (1985) as follows (UM) : $CaCl_2..H_2O$,1000; KH_2PO_4.500; $C_6H_5O_7Fe.\ H_2O$ 10, $MgSO_4.7H_2O$,250; KH_2SO_4,250: $MnSO_4.H_2O$,1; H_3BO_3, 2; $ZnSO_4.7H_2O$.0.5;$CuSO_4.5H_2O$, 0.2; $COSO_4$.01 0.1;Na_2MoO_2 2H0, 0.1 and 0.5% KNO_3 (w/v) was added to the nutrient solution in the plus nitrogen (+N) control assemblies with nutrient solution and vermiculite medium were autoclaved as 121°C for 1 hour.

Figure 1

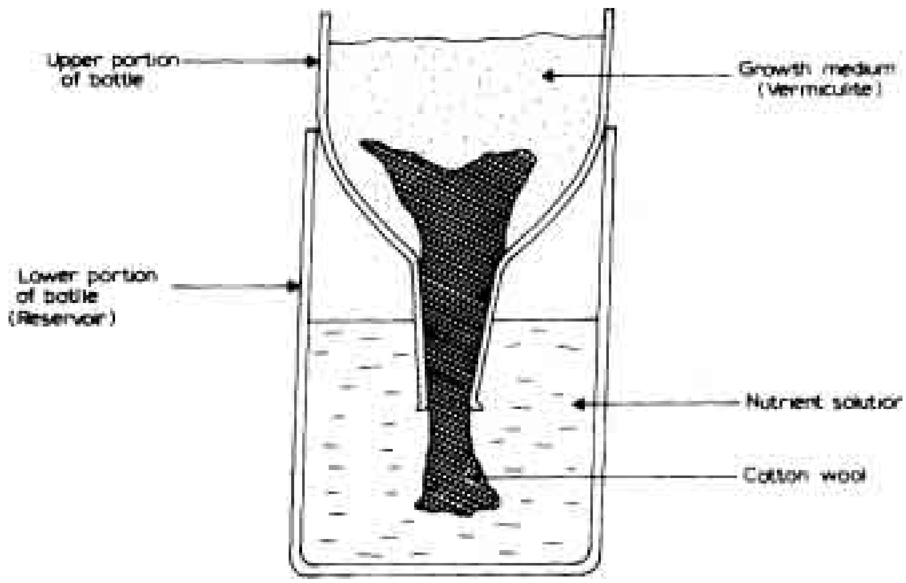

FIG. 1. MODIFED LEONARD JAR

The NFT selected for the experiment were: Acacia albida, A. mearnsii, Leucaena leucocephala, (2 proenances), Calliandra calothyrsus, Sesbania sesban, S. grandiflora and Prosopis juliflora.

Seeds of these species were pre-treated with concentrated sulphuric acid, washed in sterile distilled water until all traces of the acid were removed, and then asetically placed onto O.75% (w/v) water agar plates and incubated at 28° for 72 hours. The pre-germinated seedlings of similar size and radicle length were grown per assembly and inoculated by dispensing 1m1 of a fully grown Rhizobium culture around their roots. Plants were then thinned to one per assembly after one week. Each Rhizobium strain was used to inoculate all the seven host tree species. Thus there were 56 host strain combinations.

Uninoculated plus nitrogen (+N) and minus nitrogen (-N) were included for comparison for each test legume. Each **combination treatment was replicated 4** times and randomly grouped according to test legume species. The plants were then grown in the glass house for a period of ten weeks. Replenishment of water and nutrient solution was done once every fortnight.

Infectiveness* of each host-strain combination was described by visual growth ratings. An effective association (E) produced nodulated dark-green plants; partially effective association (PE) produced nodulated plants with light green colour and restricted growth; ineffective* association (e) produced nodulated plants which are pale green and stunted; and plants which were not nodulated were designated non-ineffective* (ni). The shoot dry weights of the various tree species were determined after oven-drying at 70° for 48 hours. Analysis of variance was carried out on mean dry weight within a test legume species.

Results and Discussion

The results of the cross inoculation test are given in Tables 2 and 3. The *Rhizobium* strains exhibited a high degree of infectiveness with the test plants. All the stains formed nodules on their own host plant species of isolation. Strains isolated from *C. calothyrsus*, *L. leucocephala* and *P. juliflora* nodulated all the hosts whereas those from *S. grandiflora*, *S. sesban*, *A. albida* and *A. mearnsii* variably nodulated five host plant species each.

Symbiotic effectiveness as rated by visual appearance of test plants gave the following own host association*: effective symbiosis in S. grandiflora, S. sesban, C. calothyrus and A. albida; partially effective symbioses in L. leucocephala and A. mearnsii, and an ineffective association in P. jul iflora

The response to inoculation in terms of shoot dry weights on own host and cross-inoculation associated gave similar trend as visual ratings. Own host associations of strains 11a, 18b, and 22 gave shoot dry weights that were higher than the uninoculated -N controls (Table 3). Shoot dry weight of cross-inoculation associations of strains 15b on L. leucocephala, A. albida and C. calothyrsus indicate some potential in biological nitrogen fixation with the

* see glossary of terms for definition in Appendix I

indigenous rhizobia although the amount of N supplied in the +N control may not have been optimal. The apparent agreement of visual ratings and shoot dry weight results point to the accuracy and precision of data obtained from the two parameters used.

Table 2: Effectiveness rating of host-strain combination 10 weeks after inoculation

		Test legume						
Rhizobium strain	Original host species	S. grandiflora	S. sesban	L. leucocephala	P. juliflora	C. calothyrsus	A. albida	A. mearnsii
16b	S. grandiflora	E	e	PE	ni	ni	e	PE
11a	S. sesban	e	E	e	ni	ni	e	e
2c	L. leucocephala	e	e	PE	e	e	PE	PE
6b	"	e	e	PE	e	e	PE	PE
15b	P. juliflora	e	e	E	e	E	PE	PE
16c	C. calothyrsus	e	e	E	PE	E	e	e
22	A. albida	e	e	e	ni	ni	E	PE
NUM 777	A. mearnsii	e	e	ni	e	ni	E	PE

Ratings were as follows:

E - Effective
PE - Partially effective
e - ineffective
ni - non-ineffective

Table 3: Shoot dry weight Milligram (Mg) of host-strain combination 10 weeks after inoculation

		Test legume						
Rhizobium strain	Original host species	_S. grandiflora_	_S. sesban_	_L. leucocephala_	_P. juliflora_	_C. calothyrsus_	_A. albida_	_A. mearnsii_
18b	_S. grandiflora_	403	23	93	56	89	208	55
11a	_S. sesban_	55	1014	77	60	64	162	12
2c	_L. leucocephala_	50	19	165	56	64	215	32
6b	"	44	18	227	59	84	189	74
15b	_P. juliflora_	100	48	346	47	216	288	37
16c	_C. calothyrsus_	66	31	433	79	139	190	17
22	_A. albida_	46	28	91	61	91	340	26
NUM 777	_A. mearnsii_	82	20	69	44	72	494	41
Control +N	-	297	168	92	96	73	303	25
Control without N	-	40	15	76	57	65	133	15

Conclusion

This study has shown that Rhizobium stains isolated from the NFT were variably ineffective. On the basic of **beneficial symbiosis S. grandiflora and** S. sesban were specific in their rhizobial requirement. Other plant host species were less specific.

Results from this study indicate that it is feasible to develop broad spectrum Rhizobium inoculant for use in inoculating several leguminous tree species seedlings instead of specific inoculants that would only be used to inoculate single tree species. However, further studies are required to evaluate these strains in actual nursery soils before producing such broad spectrum inoculants.

Acknowledgements

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Appendix I

Glossary of Terms

Cross-inoculation association: an association between a host plant species and a Rhizobium strain originally isolated from host plant of different species or taxonomic group.

Effectiveness: a measure of nitrogen fixation in a functional plant Rhizobium symbiosis as evaluated by dry weight yield, nitrogen content, nitrogenase activity, etc.

Ineffectiveness: a non-functional plant - Rhizobium association, where formed nodules fix little or no nitrogen.

Ineffectiveness: invasion of root and/or stem of host plant species by Rhizobium culminating in nodule formation.

Non-effectiveness inability of Rhizobium to cause nodule formation on plant root and/or stem.

Own host association: An association in which a Rhizobium strain cause nodule formation on the same host plant from which it was originally isolated.

Symbiosis: living together of different species or organisms where at least one benefits from the other.