SCREENING STRAINS OF *RHIZOBIUM* FOR THE TROPICAL LEGUMES *CLITORIA TERNATEA* AND *VIGNA TRILOBATA* IN SOILS OF DIFFERENT pH

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ABSTRACT

Several strains of Rhizobium *were greenhouse-tested on clitoria* (*Clitoria ternatea*) *and phillipesara* (*Vigna trilobata*) *to determine effectiveness in a soil of pH 6.3 that was N-deficient but otherwise fertile. Selected strains were further tested on phillipesara in an acid soil, unlimed or limed to pH 6.6.*

Yield of N and visual ratings of shoot growth and color gave similar ratings of effectiveness. Variations of effectiveness did not relate to variation in nodule number, nodule weight, or rates of acetylene reduction when the acid soil was used.

In soil at pH 6.3 four strains that were highly effective on clitoria outyielded the NH_4,NO_3, control treatment. With phi'llipesara the best strains yielded 75% as much as the NH_4NO_3 control. In the acid soil, symbiotic performance of strains ranked differently than in the neutral soil. Liming the acid soil depressed plant growth.

INTRODUCTION

Phillipesara *(Vigna trilobata)* and clitoria *(Clitoria ternatea)* are potentially important irrigated forage legumes in the Sudan (Musa and Burhan *1974).* Both are high-yielding and vigorous competitors with associated weeds under rainfed conditions; and phillipesara has shown promise as a pasture legume either mixed with *Cenchrus ciliaris or* directly seeded into native grasslands (M. G. Zaroug, unpublished). Success in these roles will depend on the natural occurrence or introduction of strains of *Rhizobium* able to persist and nodulate with high effectiveness under a variety of soil conditions including acidity.

Favorable effects of calcium and increased pH on nodulation are well documented for both tropical and temperate legume species (Hutton and Andrew *1978,* Munns *1978).* Lime-induced depression of plant growth has been reported also (Kamprath *1971,* Munns and Fox *1976,* Hutton and Andrew *1978).*

This report describes tests of effectiveness of *Rhizobium* strains with phillipesara and clitoria grown in a neutral sandy soil, and performance of selected strains with phillipesara in an acid soil.

MATERIALS AND METHODS

Rhizobium strain effectiveness on clitoria and phillipesara

Eleven strains for clitoria and *13* for phillipesara were tested, with uninoculated and plus nitrogen checks. Treatments were replicated three times in a completely random design. Tujunga sand (pH *6.3* in saturated paste) collected at Davis, California from the top *30* cm, was screened, air-dried, and dispensed into pots lined with polyethylene bags at *1.7* kg of soil per pot. Each pot received 20 ppm S and *4* ppm P as K_2SO_4 and KH_2PO_4 solutions. Each pot was mixed and watered to field capacity *(18.5 %)* before being planted.

Clitoria seeds (from the Department of Range Management, Khartoum, Sudan), were scarified with concentrated H_2SO_4 *for 15* minutes and planted five per

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pot. Seeds of phillipesara were surface sterilized in H_20_2 for 6 minutes. Pure cultures of rhizobial strains were applied in suspension at 10^4 cells per seed. A week after emergence, seedlings were thinned to three per pot. The plus N controls received 200 ppm N as NH_4NO_3 solution directly after thinning. Distilled water was used to bring soil to field capacity by weight every second day.

The appearance of yellow coloration with green midribs on clitoria leaflets indicated zinc or iron deficiency. This was corrected by foliar spraying with a mixture of 0.5% $ZnS0_4$, 0.5% $FeS0_4$ and wetting agent.

Eight weeks from planting, tops were removed and oven dried at 70°C for 24 hours. Roots were washed for determination of nodule number and fresh weight.

Nodulation of phillipesara in an acid soil

Pots containing 1600 g of Goldridge fine sand (pH 4.7 in saturated paste) were used. This soil was calcium-deficient, mildly aluminium toxic but neither manganese toxic nor molybdenum deficient (Munns, *et al.* 1979). Calcium carbonate was added to half the pots at the rate of 3 g kg^{-1} of soil. Then all pots were watered to 70% of field capacity and allowed to equilibrate for one week (pH 6.6). Each pot then received 20 ppm S and 60 ppm P as K_2S0_4 and KH_2P0_4 solutions. Two days later the soils were mixed and rewatered to field capacity. Ten seeds of phillipesara were planted and inoculated in each pot, and thinned after emergence to six plants. Ammonium nitrate was added to the plus nitrogen controls in two doses, 100 ppm N directly after thinning and an equal amount ten days later. Ten strains of *Rhizobium* were assayed.

Plants were harvested eight weeks from planting. Roots were washed and blotted for acetylene reduction assays (by incubation for one hour in 0.1 atmosphere acetylene in air). Then nodule number and fresh weight were determined. Kjeldahl nitrogen analyses were done on shoot material (McKenzie and Wallace 1954).

RESULTS

Effectiveness of rhizobia on clitoria

There were large differences between strains (Table 1) . Some strains were very effective. Four of them (TAL 173, 29B2, TAL 200 and TAL 305) exceeded the nitrogen-treated plants in dry matter production but the difference was significant ($P < 0.05$) for strain TAL 173 only. Some strains were poorly effective.

Nitrogen content also varied between the different treatments. Five strains (173, 29B2, 305, 200 and 169) gave nitrogen yields not significantly ($P < 0.05$) less than the nitrogen-treated plants. Nitrogen yield, plant color and dry weight yield gave similar rankings of strain effectiveness. Effectiveness bore little relationship to nodule number, weight or distribution.

Effectiveness of rhizobia on phillipesara

As for clitoria there were large differences in nitrogen content and dry matter yield (Table 2). Effectiveness ranked similarly according to plant dry matter, nitrogen content or color. By contrast with clitoria, nodule fresh weight correlated with nitrogen content significantly ($r = 0.70$, $P < 0.05$) ; and plant yield correlated significantly with both nodule fresh weight ($r = 0.73$, $P < 0.05$) and nodule number ($r = 0.67$, $P < 0.05$). Nitrogen yield of the plus N plants exceeded that from any of the inoculants (Table 2).

Ability of selected rhizobia to nodulate phillipesara in an acid soil

All strains except CB1024 (Fig. 1) nodulated phillipesara in acid Goldridge soil, some better than others. As in Tujunga soil, no strain gave plant yields as high as the N-control treatment. One strain, CB1024, failed completely. Liming the Goldridge soil reduced nodulation, and also reduced growth in most treatments including

TABLE 1

Response of Clitoria *to inoculation with different rhizobia*

Treatment	Shoot color*	Nodule no. plant^{-1}	Nodule fresh weight (g plant^{-1})	Shoot dry weight (g. plant^{-1})	N content (mg plant^{-1})
NH_4NO_3	G	0	0	1.48	67
TAL173 (176A30)θ	G	15	0.42	1.74	65
29B2$\triangle$	G	30	0.42	1.54	59
TAL305^{II}	G	17	0.51	1.53	56
TAL200 (150C6)θ	G	19	0.47	1.57	55
TAL169 (176A22)θ	G	20	0.42	1.47	55
Clitoria isolate^{III}	G	19	0.46	1.36	55
CB756^{IV}	G	14	0.46	1.41	50
TAL174 (176A32)θ	G	15	0.47	1.40	44
CB1024^{IV}	YG	23	0.41	1.28	42
29B3$\triangle$	Y	32	0.53	1.16	32
TAL303^{II}	Y	9	0.24	1.07	28
Check	Y	0	0	1.03	17
L.S.D. (p=0.05)†		4.7	0.07	0.22	13
(p=0.01)		6.3	0.09	0.28	17

† Zero values not included in analyses of variance.
* G=green, YG=yellow-green, Y=yellow.
θ From Nitragin Co., Milwaukee *via* NifTAL Project, Hawaii.
$\triangle$ From Nitragin Co.
II From NifTAL.
III From Sudan Ministry of Agriculture, Khartoum.
IV From CSIRO, Brisbane, Australia.

TABLE 2

Response of Vigna trilobata *to inoculation with different rhizobia*

Treatment	Shoot color*	Nodule no. plant $^{-1}$	Nodule fresh weight (g plant^{-1}	Shoot dry weight (g plant^{-1})	N content (mg plant^{-1})
NH_4NO_3	G	0	0	1.27	42
CB1024θ	G	47	0.52	0.95	30
TAL11△	G	35	0.52	0.93	29
TAL163△	G	35	0.49	0.99	27
TAL207△	YG	27	0.33	0.89	25
CB756θ	G	35	0.49	0.86	24
TAL189 (176A31)II	G	32	0.41	0.81	23
TAL98 (CIAT239)III	YG	41	0.47	0.90	23
TAL170 (176A23)II	YG	32	0.43	0.79	22
TAL173 (176A30)II	YG	35	0.43	0.92	22
TAL169 (176A22)II	YG	31	0.30	0.78	20
TAL303△	Y	22	0.31	0.78	20
TAL174 (176A32)II	Y	39	0.44	0.90	20
TAL172 (176A28)II	Y	26	0.33	0.69	17
Check	Y	0	0	0.66	10
L.S.D. (p=0.05)†		11.7	0.11	0.19	10
(p=0.01)		15.7	0.14	0.26	13

† Zero values not included in analysis of variance
* G=green, YG=yellow-green, Y=yellow
θ From CSIRO, Brisbane, Australia
△ From NifTAL
II From Nitragin Co., Milwaukee *via* NifTAL Project, Hawaii.
III From CIAT *via* NifTAL Project, Hawaii

the N-control treatment. There was no significant effect of treatment on ethylene formed.

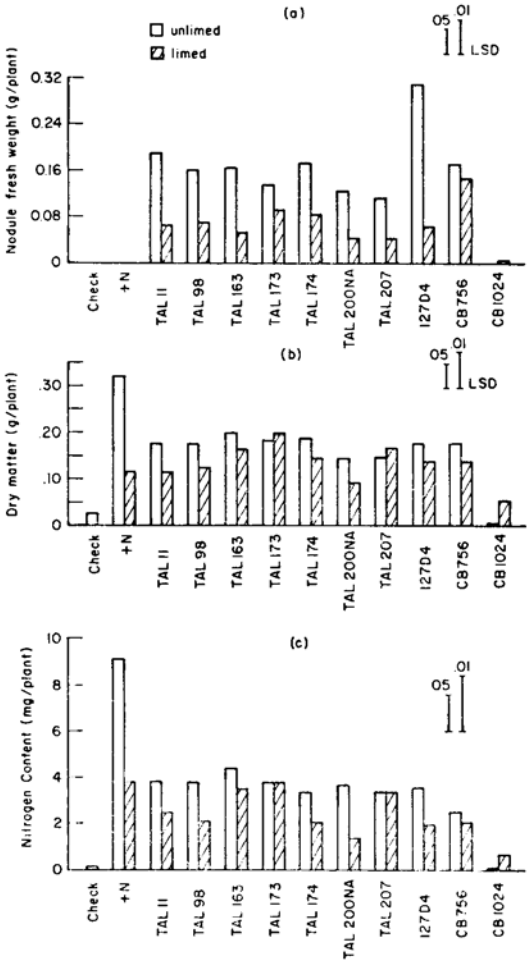

FIGURE 1

Influence of liming an acid (pH 4.7) soil on (a) nodule fresh weight, (b) dry matter and (c) nitrogen content of phillipesara (*Vigna trilobata*) inoculated with different strains of *Rhizobium*.

DISCUSSION

Effectiveness on clitoria

Measurements of nodule number, nodule fresh weight or weight of individual nodules did not indicate effectiveness of symbiosis in clitoria. Small nodules were produced by both highly effective (29B2) and ineffective (29B3) strains; and strain 29B3 though ineffective had the largest nodule mass. Discrepancy between nodule mass and effectiveness has been reported for soybean and subterranean clover (Gibson 1977) and mung (Munns *et al.* 1979). Correlations between nodule number or mass and plant dry matter yield are evidently species-dependent.

Effectiveness on phillipesara

Phillipesara grew normally in the acid soil with N supplied, but not when it was symbiotically dependent. The rhizobial strains differed in response to low soil pH. In

particular strain CB1024, one of the most effective in neutral soil, failed completely in the acid Goldridge soil as expected from its known acid-sensitivity (Keyser and Munns 1979). Some strains, particularly TAL 163, combined a degree of acid tolerance with high effectiveness in association with phillipesara.

Strain 127D4, which nodulated abundantly in the absence of lime, nevertheless produced a low plant N content, perhaps because of impaired nodule activity. This effect has been noted elsewhere, along with the more usually encountered depression of nodule formation (Munns 1978).

Decline in nodulation in the unlimed Goldridge soil when compared to Tujunga sand might be explained by the effect of low pH and aluminium toxicity on *Rhizobium* survival and growth (Keyser and Munns 1979) and the possible interference by acidity, aluminium and low calcium with one of the steps later than the colonization of the rhizosphere (Munns et al. 1979). The sparse nodulation when Goldridge soil was limed was more likely to result from curtailment of shoot growth and the observed reduction in root mass.

Reduction in growth and nodulation as a result of high rates of lime has been observed for several legume species (Kamprath 1971, Munns and Fox 1976, Hutton and Andrew 1978). In this case it was not accompanied by any symptoms of nutrient deficiency or toxicity. The negative response to liming in this trial does not imply that phillipesara is in general intolerant of high soil pH. In Sudan it has been grown satisfactorily on alkaline heavy clay soils (Zaroug, unpublished).

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