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Tolerance of Soil Acidity in Symbioses of Mung Bean with Rhizobia[1]

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ABSTRACT

Variation in tolerance of soil acidity among 40 rhizobial strains was assessed in greenhouse trials in which the strains were applied as separate seed inoculants (5×10^4 cells per seed) to two cultivars of mung bean (*Vigna radiata* L.) and performance was measured by nodulation, growth, and N-yield of the host plant. The plants grew in a low N, low Ca acid subsoil, Goldridge fine sandy loam (Typic Hapludult, fine loamy, mixed, mesic), left at its natural pH of 5.0 (saturation paste) or limed with $CaCO_3$ to pH 6.3. Each pH × strain treatment was triplicated in separate pots.

Strains demonstrated a large and perhaps continuous variation in acid tolerance. A few were very sensitive: they failed to nodulate at pH 5.0. About half were moderately sensitive: nodulation and growth were significantly impaired at pH 5.0. The remainder were tolerant: like NH_4NO_3 they supported similar plant growth at both soil pH values. Some strains combined high tolerance with high effectiveness.

A strain's acid tolerance could not be predicted from the abundance or effectiveness with which it nodulated at favorable pH, or from its growth rate or acid production in conventional yeast mannitol medium.

A few strains were sensitive on one host cultivar and tolerant on the other, implying that acid tolerances of symbiotic legumes cannot be compared validly in trials with only one inoculant.

Additional index words: Aluminum, Rhizobial growth, Infection, N fixation, Tropical soils, Tropical legumes.

THIS paper describes a systematic screening for effectiveness and tolerance of soil acidity amongst 40 rhizobial strains on two cultivars of mung bean (*Vigna radiata* L., also known as green gram and *Phaseolus aureus*). Several of the strains are also effective on other agricultural legumes including cowpea (*V. unguiculata*), peanut (*Arachis hypogea*), Dolichos lablab (*Lablab purpureus*), and *V. (Phaseolus trilobus.*

Soil acidity is known to inhibit rhizobial growth, colonization of the host rhizosphere, infection, and the activity of established nodules (Munns 1976, 1977, 1978). Evidence exists that rhizobial strains, as well as legume cultivars, may vary in tolerance at some of these separate stages. Our main concern was to assess overall "symbiotic acid tolerance", as expressed by ability of the dinitrogen-dependent host to maintain growth despite acidity stress.

For realism we used an acid soil as test medium, recognizing that our rankings of tolerance of strains might depend on the particular set and intensity of soil acidity factors present. The soil is moderately acid, pH 5.0 in saturation paste, low in soluble Ca, mildly Al-toxic, and neither Mn-toxic nor Mo-deficient. Its acidity did not impair growth of mung bean plants fertilized with N or inoculated with the most tolerant rhizobia.

Plant growth experiments for screening large numbers of strains are cumbersome. An easily observable rhizobial characteristic that indicated acid tolerance with reliability would be valuable, at least for prescreening. Norris (1965, 1967) suggested that slow growth and lack of acid production on yeast mannitol medium indicated rhizobial tolerance of soil acidity. The physiological and ecological significance of this relationship has been contested (Parker, 1968), and important exceptions are now known (Munns, 1976,

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Table 1. Rhizobial strains and sources.†

Strain	Source	Strain	Source	Strain	Source
b4 = 3I6b4	1	m8 = TAL443	2	189 = TAL189 = 176A31	7
b7 = 3I6b7	1	11 = TAL11	3	281 = TAL281	3
n9 = 3I6n9	1	32H1	4	207 = TAL207	3
n10 = 3I6n10	1	68 = IQ68-5 = TAL463	5	209 = TAL209	3
n12 = 3I6n12	1	98 = TAL98 = CIAT239	6	303 = TAL303a	3
n15 = 3I6n15	1	108 = IQ108	5	305 = TAL305	3
n16 = 3I6n16	1	163 = TAL163	3	306 = TAL306	3
n22 = 3I6n22	1	169 = TAL169 = 176A22	7	420 = TAL420 = THA301	8
m2 = TAL437	2	170 = TAL170 = 176A23	7	421 = TAL421 = THA302	8
m3 = TAL438	2	171 = TAL171 = 176A27	7	423 = TAL423 = THA304	8
m4 = TAL439	2	172 = TAL172 = 176A28	7	425 = TAL425 = THA306	8
m5 = TAL440	2	173 = TAL173 = 176A30	7	756 = CB756	9
m6 = TAL441	2	174 = TAL174 = 176A32	7	921 = IQ921	5
m7 = TAL442	2				

† Sources: 1. USDA, Beltsville, Md. 2. Univ. of Philippines, Los Banos, via Univ. Hawaii NifTAL Project, Paia, Hawaii. 3. NifTAL isolates. 4. Nitragin Co. Inc., Milwaukee, Wis. 5. Iraq Ministry of Agriculture (via NifTAL). 6. Centro Internacional de Agricultura Tropical, Cali, Colombia (via NifTAL). 7. Nitragin Co., Milwaukee, Wis. (via NifTAL). 8. Thai Ministry of Agriculture, Bangkok (via NifTAL). 9. CSIRO Division of Tropical Crops and Pastures, Brisbane, Australia. Note: Inclusion of a strain does not imply that its supplier recommends it for use on mung, or any other species.

Table 2. Soil properties—cation analyses.

	Exchangeable me/kg				Saturation extract me/liter					
	Ca	Mg	Na	Al	Ca	Mg	K	Na	Mn	Al
pH 5.0 unamended	20	10	4	6	0.1	0.06	0.2	0.3	<0.5	0.002
pH 6.3 limed	26	10	4	1	0.3	0.12	0.2	0.3	<0.5	<0.001

1978); but nobody, to our knowledge, has tested the relationship of either growth rate or acid production to acid tolerance in a group of rhizobia associated with the one host. We took the opportunity to do so.

MATERIALS AND METHODS

Seed of 'Berken' mung bean was supplied by Mr. C. L. Tucker, Department of Agronomy, University of California, Davis; and V2184 by the Asian Vegetable Research and Development Center by way of the University of Hawaii NifTAL Project, Paia. Immediately before planting, seeds were submerged in 30% hydrogen peroxide for 5 min and washed in three changes of sterile water.

Rhizobial cultures are listed in Table 1. All except those from USDA had recently been tested for effectiveness on Berken by us or by Dr. Victor Reyes of NifTAL. For inoculation, each seed received about 5×10^4 viable cells, freshly suspended from a yeast mannitol slant, applied in 3 ml water to the seed immediately before it was covered with soil.

The soil was B-horizon material (100 to 150 cm) from a Goldridge loamy fine sand (Typic Hapludult, fine loamy, mixed, mesic) developed in fine argillaceous sandstone at Sebastopol, Calif. Saturation paste pH was 5.0, organic C 0.1%, basal fertilizer 5 mmol KH_2PO_4, 2 mmol K_2SO_4, 5 mg Zn, 0.1 mg Mo/kg soil. Limed treatments received 0.7 g $CaCO_3$/kg soil, mixed and watered 3 weeks before planting. Soil pH was checked at several points in each of several pots just before planting to make sure mixing and reaction had been adequate. Analytical data on treated soils are in Table 2. Nitrogen fertilized controls, uninoculated, received 5 mmol NH_4NO_3/kg soil, applied in two dressings, 1 week and 2 weeks after seedling emergence.

Two separate trials were done. Each included zero and NH_4NO_3 controls, and rhizobial strains 756, 11, 98, 173, and 209. The second trial included the USDA strains, and the series "m", "IQ", and 420-425. The first trial included the others. In each trial, two plants of each cultivar were planted in each pot containing 1.5 kg soil. Both trials had three replicate pots of each treatment, arranged in randomized blocks.

Greenhouse temperatures varied between 28 (day) and 22 C (night). Pots were watered with distilled water, to 20% soil water content, at intervals of 3 days at first but with increasing frequency up to daily toward the end of each trial. Color and relative size of shoots were observed daily. At 35 days in the first trial, and 28 days in the second, plants were washed out of the soil, bagged in plastic, and refrigerated until examined for nodule number, size, color, and distribution. Shoots were then cut off and dried at 75 C for weighing and in some cases, microKjeldahl determination of N.

Table 3. Rhizobial performance classification according to host variety.

A. Abundant nodulation at pH 6.3 (≥ 25 nodules/plant)
B. Effective at pH 6.3 (yield significantly > nil control, P = 99%)
C. Highly effective at pH 6.3 (yield not significantly < +N, P = 99%)
D. Nodulation tolerant to acidity (reduction of nodule no. not significant, P = 95%)
E. Plant growth tolerant to acidity (reduction plant yield not significant, P = 99%)

Performance classification	A	B	C	D	E
On both varieties	23	27	14	13	8
On "Berken" only	3	1	4	3	4
On "V2184" only	0	5	4	1	4
On neither variety	14	7	18	19	11
Total no. of strains classed	40	40	40	26†	27‡
X^2§	35	–	6.3	25	3.2
(% probability)	(99)	–	(99)	(99)	(95)

† Abundantly nodulating strains. ‡ Strains effective on both hosts.
§ Null hypothesis = no association between performance on "Berken" and performance on "V2184."

Rhizobial growth characteristics were observed in yeast mannitol broth medium (Vincent, 1970). As an index of growth rate we observed the time lapse between inoculation at 10^4 cells/ml of medium until development of turbidity, 10^7 cells/ml; and as an index of acid production, the pH of the medium (initial value 6.9) in the newly turbid culture.

Analysis of variance was performed on plant yield and percent N data without transformation. Error statistics given are least significant differences (99% probability) for comparisons of closely similar means of three replicates. Nodule counts were transformed for analysis, using the transform log (1 + nodule number per plant). Mean nodule counts greater than four per plant were significantly different from each other (95% probability) if they differed by a factor of about 2. Curve-fitting was done by a Hewlett-Packard 9801 routine that compares best fitting linear, polynomial, and power functions. For testing associations between rhizobial properties such as abundance of nodulation, effectiveness, etc., numbers of strains in different classes of each performance aspect (Table 3) were tallied into contingency tables and analyzed by chi-squared procedure with Yates' correction where appropriate.

RESULTS AND DISCUSSION

Plant Growth, Nodulation, and N Fixation

Plants became yellow during the week following emergence, but then became green again between the 8th and 11th day if N-fertilized or effectively nodu-

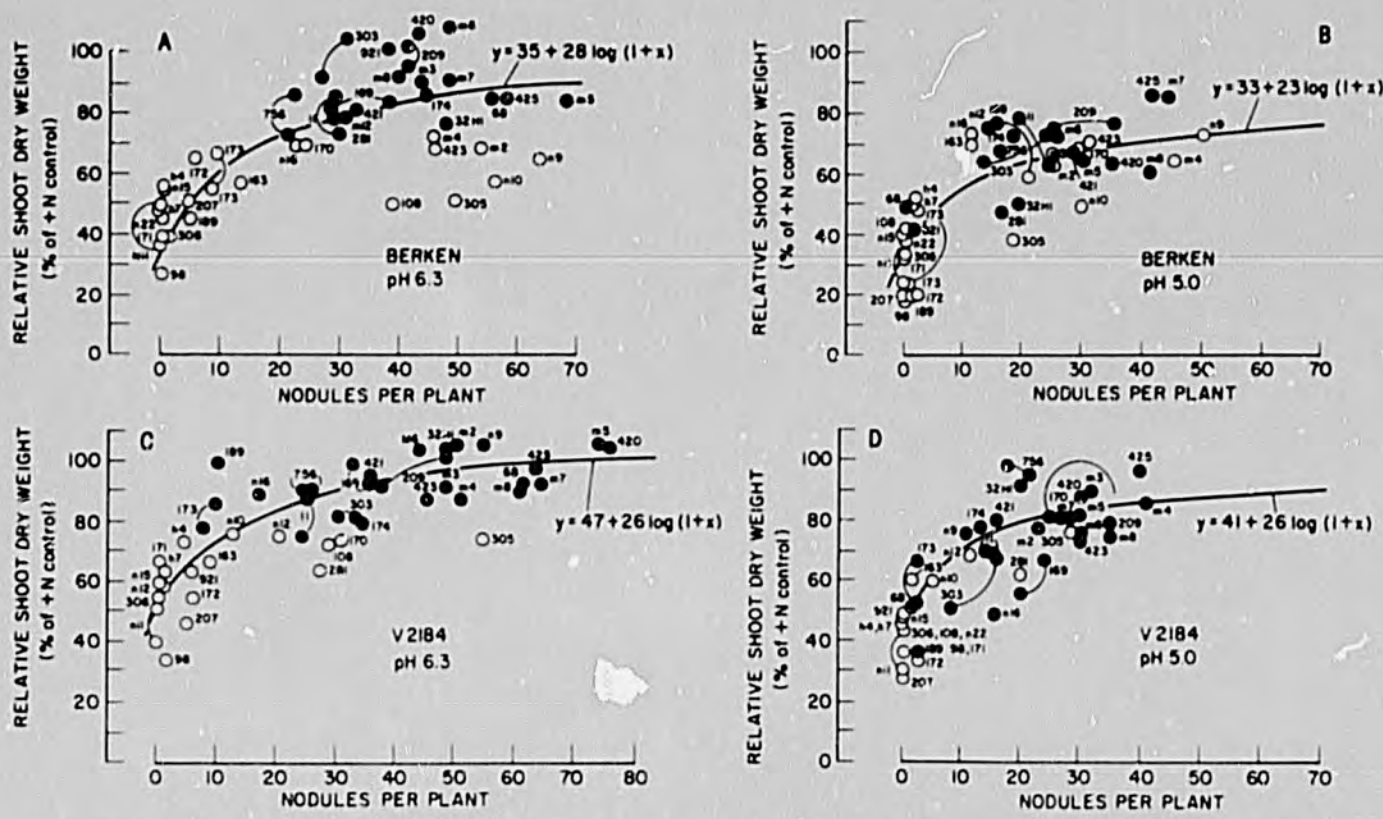

Fig. 1. Relationship between host-plant growth and nodule number, plotted separately for each host cultivar at each soil pH. Average nodule size varied negligibly with any experimental treatment. Neither soil pH nor host cultivar significantly affected dry weight of NH_4NO_3 fertilized plants (+N controls). For Berken, the slope of the fitted function relating yield to log nodule number was significantly lowered at pH 5.0 (probability 97%). Solid circles indicate associations rated highly effective at pH 6.3. Points connected by short, curved lines represent data for strains included in both greenhouse trials.

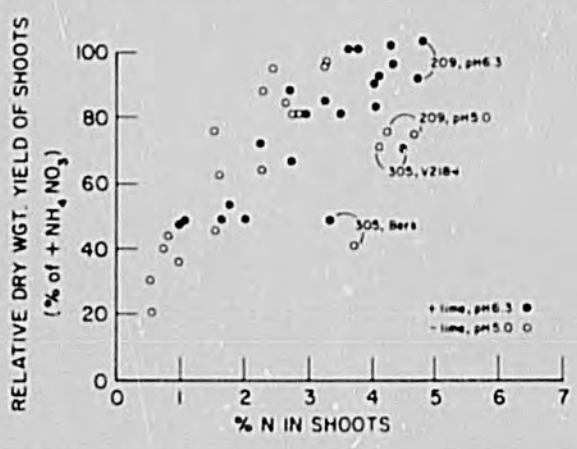

Fig. 2. Plant yield in relation to N concentration in shoots.

lated. At harvest, N-fertilized controls had shoot dry weights of 750 mg/plant in the first trial and 500 mg/plant in the second, with no significant difference due to cultivar or soil pH. Strains included in both trials behaved consistently, except for two strains on Berken at pH 5.0. Conversion of yield data to relative yields (percent of the appropriate +N control) made the data of both trials consistent enough to plot on the same graphs for ease of comparison.

Growth of the plant was related to nodule abundance as affected by rhizobial strain and soil pH (Fig. 1). Nodule number in this case is a satisfactory index of nodule mass per plant, because mean nodule size did not vary with host cultivar, rhizobial strain, or soil pH. Mung bean may be unusual in that sparsity of nodules is not compensated by nodule enlargement (Masefield, 1954) and pink coloration is retained regardless of effectiveness. All nodules were pink or red, and nearly always clustered on the oldest parts of the root system, indicating prompt infection.

Dry weight yield of plants reflected N-fixation adequately for the purpose of these experiments. It related to percent N in the plant (Fig. 2) as well as to nodulation. Unnodulated or ineffectively nodulated plants contained less than 1% N, and yielded only 2 or 3 mg N/plant, comparable to the N content of the seed. By comparison, the N content of the most effectively nodulated plants was about 35 mg/plant in the first trial, 25 in the second.

The most abundantly nodulated plants may have had nodules with low specific (per nodule) activity. In particular, three abundantly nodulating strains, n10, 168, and 305, were ineffective on Berken (Fig. 1) and for 305 this held despite high percent N in the plant (Fig. 2), suggesting marked energetic inefficiency or some other rhizobial inhibition of plant growth.

Rhizobial Variation in Acid Tolerance

The data show a large and perhaps continuous range of rhizobial tolerance to soil acidity (Fig. 1, 3), unrelated to variation in strains effectiveness measured

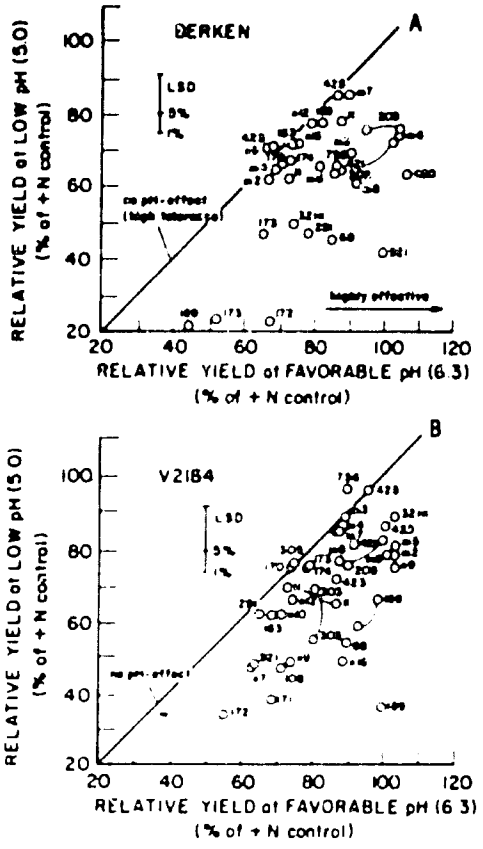

Fig. 3. Comparisons of plant yield at pH 5.0 with yield at pH 6.3, for different rhizobial strains, demonstrating lack of relationships between symbiotic tolerance and effectiveness measured at favorable pH. Data for ineffective strains omitted.

by plant performance under favorable conditions at pH 6.3 (Fig. 3). Selection for tolerance should be possible, and need entail no sacrifice of performance potential. Some of the best strains were highly tolerant, and two strains, 425 and m7, combined tolerance with high effectiveness on both hosts (Fig. 3).

A collection of candidate rhizobia can be severely reduced by a single screening for high performance under acid stress. Less stringent performance criteria might be advisable. Successful field application requires several important rhizobial characteristics, including host range versatility, longevity in inoculant preparations and soil, competitiveness, and effectiveness maintained during maturation of the plant. In a wider context of testing, strains 425 and m7 might prove inferior to slightly less effective or less tolerant strains such as 174, 209, 420, 421, 756, m3, m5, and m6.

Sparsity of nodules, the main cause of symbiotic failure at low pH, was probably not simply due to poor rhizobial growth in most cases. The rhizobia have been tested for ability to grow in acid defined media containing Al (Keyser and Munns, 1979) and except for 68, 108, and 921 should have had no difficulty growing in the Goldridge soil at pH 5.0. Further in the same soil adjusted to pH 4.6, some of the strains nodulated cowpea reasonably abundantly (strains 189, 171, and 173 of the group acid-sensitive on mung, but not 98). Perhaps there is an acid-sensitive step later than colonization of the rhizosphere in mung bean as in alfalfa (*Medicago sativa*) (Munns, 1968) and pea (*Pisum sativum*) (Lie, 1969).

Acid Tolerance in Relation to Other Rhizobial Strain Characteristics

The hypothesis that acid production and fast growth in yeast mannitol indicates acid sensitivity gains little support from our data. In view of the wide acceptance of this hypothesis (most recently Sanchez, 1977), we tested all possible relationships. The rhizobia varied greatly in growth rate and acid production, time to turbidity ranging from 4 to 8 days, and final pH of the medium ranging from 5.8 to 7.4. Despite this, the only significant regression of the above properties with any index of symbiotic acid sensitivity based on plant yield or nodulation, was a power function regression of final pH with relative yield decline. Acid producers tended to be the more sensitive, as proposed by Norris (1965, 1967); but the coefficient R^2 was only 0.15.

Contingency analyses failed to establish relationship between a strain's acid production or growth rate and its symbiotic acid sensitivity. All but four of the strains could be classified clearly into a group of 18 strains that took less than 7 days to achieve turbidity and lowered the pH, and another group of 18 that took 7 or more days and did not lower the pH. Each group contained 12 strains that induced abundant nodulation (more than 25 nodules per plant at pH 6.3), of which seven strains gave a significant decline in nodule number due to soil acidity amongst the fast growing acid producers and six strains amongst the slow growing group. The 18 slow growers included 13 highly effective strains, the 18 fast growers included 10. Of these, five of the 13 slow growers and four of the 10 fast growers showed a significant decline in plant yield due to acidity. None of these differences between slow and fast growers is significant. Finally, soil acidity caused almost the same average percent decline in yield of plants inoculated with highly effective rhizobia, whether they were fast growing acid producers (38% decline) or slow growers (36% decline).

In short, rhizobial acid production in yeast mannitol may be some sort of indicator of acid tolerance for wide comparisons of symbiotic associations involving different host species and genera (e.g. Munns, 1977, 1978); but in selecting strains of rhizobia for a given host species, mung bean at least, acid production is a uselessly imprecise indicator of sensitivity.

A strain's acid tolerance did not relate to its ability to nodulate abundantly or effectively at pH 6.3. In relation to earlier evidence (e.g., Munns, 1965) of rhizobial variation in acid tolerance for nodulation, Burton (1972) appropriately commented that apparently more tolerant strains may simply be those with the greater general virulence. In the present trials, however, acidity significantly reduced nodule number with similar frequency in abundantly nodulating associations (in 22 out of 45) as in sparsely nodulating associations (in 17 out of 25). Acidity depressed growth of plants with precisely the same frequency in abundantly and sparsely nodulating associations.

Host Variety

At low pH, yield of Berken declined disproportionately to decline in nodule number (Fig. 1). This agrees with other evidence that soil acidity can impair nodule function over and above its influence on nodule formation, the effect varying between legumes (Munns, 1977, 1978).

In most respects, the two hosts behaved alike. Nodulation of both was abundant, effective, and acid-tolerant with a similar number of strains; and performance of a strain on one host tended to match its performance on the other (Table 3). The exceptions, however, are important. Existence of strains with different tolerances on different host cultivars complicates the business of defining tolerant rhizobia and legumes.

Efforts are being made to select legumes tolerant of soil acidity factors (Wright, 1977; Sanchez, 1976; Munns, 1978). Some of this work, done with N-fertilized plants, suffers from uncertainty whether symbiotic tolerance correlates with the normally greater tolerance of the N-fertilized host. Tests with symbiotic legumes should be more appropriate, but the results may lack generality if the test includes only one inoculant treatment, whether it be a mixture of strains or a single defined strain. With mung bean, for example, a comparison of cultivars nodulated by strain 420, or m8, or the much-used 756, would identify Berken as more acid-sensitive than cv. V2184; but a comparison using strain 169 would lead to the opposite, equally wrong conclusion. Properly, comparisons of host plant tolerance should include at least two or three effective rhizobial strains, applied as separate inoculant treatments to allow expression of interactions involving the rhizobial component of the system. Likewise, of course, symbiotic tolerances of rhizobia should be tested on more than one host.

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