Peanut Science (1980) 7, 95-97

Nodulation and Nitrogenase Activity of Peanuts Inoculated with Single Strain Isolates of *Rhizobium*[1]

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ABSTRACT

Nodulation and nitrogenase activity (μM C_2H_4/plant/hr) for 48 diverse peanut (*Arachis hypogaea* L.) genotypes were determined in a field site where the soil supported high populations of endemic rhizobia. These same rhizobia and peanut genotypes had previously been evaluated in a greenhouse study.

Both host genotypes and rhizobial strains significantly influenced nodulation and nitrogenase activity. Roots of Virginia-type host plants were better nodulated and exhibited higher nitrogenase activity than genotypes of the fastigiate type. Florigiant, the predominant cultivar in the Virginia-North Carolina area, produced the most nodules and had the greatest nitrogenase activity.

Variation in nodulation and nitrogenase activity for the single strain isolates in the presence of naturally occurring field populations indicated that the strains were able to compete for nodule sites. Strains both less and more effective than the naturally occurring rhizobial population were observed.

Nitrogenase activity of the strains was correlated with previous greenhouse results suggesting that greenhouse evaluation of rhizobial strains for peanuts is useful as a preliminary screen before evaluation in the field.

Key Words: *Arachis hypogaea* L., nitrogen fixation, nodulation, nitrogenase activity.

Peanuts (*Arachis hypogaea* L.) will produce higher yields of seeds with greater protein if inoculated with proper nitrogen-fixing bacteria. Peanuts, a member of the so-called "cowpea cross-inoculation group," are nodulated by bacteria from a large group of diverse legumes (3, 5, 9, 22). However, not all rhizobial strains are equally effective in fixing nitrogen in symbiosis with peanuts (2, 3, 7, 12, 23, 24).

Several researchers have suggested that efficient strains of rhizobia should be identified and used to inoculate peanut fields (4, 12, 13, 16, 17, 20, 23). Unfortunately, effective strains are not always able to survive in and colonize the soil due to lack of adaptation to climatic or edaphic conditions or to antagonism from other soil organisms (13, 17, 19).

Significant yield responses to inoculation with effective strains of rhizobia have been generally restricted to tests conducted under controlled conditions and field tests on "new" peanut land on which the crop has never grown (4, 7, 11, 17, 18, 19). Burton (4) and Date (8) have suggested that effective strains be identified through evaluation in plant tests conducted in separate phases. The first recommended stage is greenhouse testing followed by evaluation in the field.

Previously, we reported the results of a greenhouse study designed to select strains of *Rhizobium* useful for peanuts and to estimate potential variation in the nitrogen-fixing ability of these strains in symbiosis with diverse peanut germplasm (24).

The objectives of the current study were to evaluate previously greenhouse-tested strains of *Rhizobium* for nodulation and nitrogenase activity in a field previously planted to peanuts and to compare the nitrogen-fixing ability of these strains in the field with the earlier obtained greenhouse results.

Materials and Methods

The experiment was planted at the Upper Coastal Plain Research Station at Rocky Mount, NC, on May 15-16, 1977 in a field where peanuts had previously been grown. Forty-eight diverse peanut genotypes and 10 inoculant treatments were arranged in a split-plot design. The peanut genotypes were whole plots while the subplots consisted of nine *Rhizobium* strains and an uninoculated control. Whole plots were replicated twice. Subplots consisted of two 15-plant rows spaced 90.4 cm apart with 25.4 cm between plants within a row.

Nine strains previously used in a greenhouse study were selected for field testing. The source and origin of the nine strains listed in Table 1 are described in the previous paper (24). Stationary phase cells of the nine strains described, grown on buffered growth medium as described by Cole and Elkan (6) at 28 C, were used for field inoculation. One liter of culture was diluted wtih 6 L of water and applied directly to the seed with a stainless steel tank sprayer. Cross-contamination during inoculation was prevented by thoroughly rinsing the tank with commercial bleach between rhizobial treatments. The seeds were covered with soil immediately after inoculation. Adequate moisture was provided by rainfall during the growing season, so the plots were not irrigated.

The experiment was divided into five smaller tests for evaluation of nitrogenase activity using the acetylene reduction methodology (14) as described for peanuts by Scheeweis *et al.* (21). Whole plots were grouped for sampling based on the maturity of the peanut cultivars planted within the plots. The cultivar 'Florigiant', the predominant cultivar in North Carolina, was sampled as a check at each sampling date. The five subtests were sampled on 8/15, 8/19, 8/24, 9/12, and 9/20, respectively. Two plants were sampled from each subplot; the two roots were handled as a unit.

All plots were dug on 10/19. Nodulation was then rated by a

[1]Paper No. 6282 of the Journal Series of the North Crolina Agricultural Research Service, Raleigh, NC 27650. This work was partially supported by a grant under A.I.D. PASA AG/TAG 610-9-76 (USDA-SEA-CR-701-15-24).

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single observer using a subjective scale ranging from 1 (little nodulation) to 5 (heavy nodulation). Nodulation scores were analyzed using the standard procedures for a split-plot design. Nitrogenase activity data from the five sampling dates were combined in a single analysis of variance to assess the effects of the strains over all host genotypes. The genotype sum of squares was partitioned into two portions: that arising from variation among tests (sampling dates) and that from variation among host genotypes within tests. Data on Florigiant were deleted for all but one date so that the tests involving early maturing fastigiate genotypes would not be affected by inclusion of a later Virginia type. Florigiant was included in the test with cultivars which mature at approximately the same time.

The strain means for nitrogenase activity over all 48 genotypes in the field were correlated with strain means obtained in greenhouse tests using two peanut genotypes (25).

Results and Discussion

Both the host genotype and the strain treatments influenced the effectiveness of the symbiotic association (Table 1). The peanut genotypes were significantly different (.01 level of probability) for both nodulation and nitrogenase activity. Of the 48 diverse host genotypes which represented both adapted and unadapted members of the two subspecific groups of cultivated peanuts, the Virginia types (ssp. *hypogaea* var. *hypogaea*) were generally better nodulated and had higher nitrogenase activity than the fastigiate types. While this difference could have arisen from interactions between cultivars and sampling dates, similar results have been reported from studies in North Carolina (25) and other areas (1, 10, 11). There was considerable variability in nodulation and nitrogenase activity among the Virginia types. Florigiant was most heavily nodulated and had the highest nitrogenase activity. The nodulation and nitrogenase activity of Florigiant was almost twice that of cultivars of the Spanish (fastigiate) types. Nitrogen fixation of Spanish types could probably be increased by selection of segregates from Virginia by Spanish crosses while improving the nitrogen-fixing ability of Virginia types may be possible through recurrent selection among progenies of Virginia x Virginia crosses.

Table 1. Mean squares from the analysis of variance of nitrogenase activity and nodulation rating.

Source	df	Mean square: Nitrogenase activity (μM C_2H_4/plant hr)	Mean square: Nodulation rating
Total	959	--	--
Block	1	1694.90	5.7042
Host genotype	47	2951.30**	17.5871**
Test	4	17225.58**	--
Genotype in test	43	1623.46*	--
Error (a)	47	823.98	2.9978
Strain	9	789.14**	0.9069*
Strain x host genotype	423	113.49	0.4001
Strain x test	36	117.88	--
Strain x genotype in test	387	113.09	--
Error (b)	432	294.30	0.3782

*,**Denote significance at the .05 and .01 P-levels, respectively.

The rhizobial strains also significantly influenced nodulation (.05 level of probability) and nitrogenase activity (.01 level of probability). When averaged over the 48 host genotypes, the greatest nodulation was produced by strain 176A34 (Table 2). Strains 176A22 and 3G4b4 also produced significantly more nodules than the endemic strains (control). The greatest nitrogenase activity, however, occurred for strain 3G4b20. Strains 176A34, 3G4b5 and 32H1 also had significantly higher nitrogenase activity than the naturally occurring strains. Conversely, strains 3G4b4 and 32Z3 had slightly but insignificantly lower nitrogenase activity compared to the endemic strains.

No significant host genotype x strain interaction was detected in the field study (Table 1) although some of these strains exhibited host specificity for either a Virginia or Spanish genotype in greenhouse evaluation (23).

The nitrogenase activity for the rhizobial strains when applied to Florigiant was determined at five sampling dates (Table 3). Both date and strain main effects were significant ($P \leq .05$) while date-by-strain interaction was not. All plots of Florigiant including the control were heavily nodulated. Six of the nine strains, however, had slightly higher mean nitrogenase activity than the naturally occurring strains (Control), although only strain 3G4b21 was significantly better than the control. These data indicate that some of the strains were able to successfully compete for infection sites and were more effective than the naturally occurring strains. The seasonal profile of enzyme activity was similar to that reported by Hardy and Havelka (15) who observed a peak in activity during the period of reproductive growth.

It appears that strain 42B2 successfully competed for infection sites but produced less effective nodules than the naturally occurring strains. This strain was also ineffective in greenhouse evaluations (24).

Table 2. Mean nodulation rating and nitrogenase activity for strains of *Rhizobium* and an uninoculated control for field-grown peanuts.

Strain	Nodulation rating[a]	Nitrogenase activity (μM C_2H_4/plant/hr)
3G4b20	2.81	39.1
176A34	3.10	38.7
176A22	3.07	35.1
3G4b5	3.06	35.8
3G4b4	3.07	31.0
3G4b21	2.98	32.8
42B2	3.06	33.8
32H1	2.93	35.7
32Z3	3.04	31.0
Control	2.89	32.5
LSD (.05)	0.17	2.99

[a]Rated with 1 = little and 5 = heavy nodulation.

Table 3. Nitrogenase activity (μM C/H/plant/hr) for strains of *Rhizobium* and an uninoculated control for peanuts of cv. Florigiant for five sampling dates.

Strain	Sampling date 8/15	8/19	8/24	9/12	9/20	Mean
3G4b20	49	70	64	69	28	56.0
176A34	45	50	61	49	20	45.0
176A22	52	60	68	65	30	55.0
3G4b5	49	52	74	61	32	53.6
3G4b4	58	50	73	48	30	51.8
3G4b21	55	71	69	74	33	60.4
42B2	44	47	45	49	18	40.6
32H1	38	50	71	66	39	52.8
32Z3	37	56	56	62	26	47.4
Control	48	51	54	64	26	48.6
Mean	47.4	55.6	63.6	60.6	28.3	51.1
LSD (.05) sampling date = 8.29						
LSD (.05) strain = 11.76						

Nitrogenase activity measured in the greenhouse for these same strains in symbiosis with a Spanish and Virginia cultivar was found to be significantly correlated (r = 0.73*) with mean nitrogenase activity in the field study. This indicates, as suggested by Date (8), that screening of rhizobial strains in the greenhouse may be effective as a preliminary evaluation of rhizobial strain performance in the field.

Unfortunately, neither fruit yield nor plant weight was measured in this study. Future field evaluations of the effectiveness of rhizobial strains in symbiosis with Virginia and Spanish genotypes will include these traits. Nevertheless, this study clearly indicates that the testing of rhizobial strains for peanuts using greenhouse and field studies can identify strains able to compete with endemic strains and fix nitrogen more effectively than the composite strains in the soil.

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Accepted September 30, 1980