Effectiveness of Rhizobia Forming Nodules on Texas Grown Peanuts[1]

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ABSTRACT

The effectiveness of the symbiotic association of rhizobia with peanuts grown in four production areas of Texas was investigated. This study was undertaken because farmers indicated that they were obtaining yield increases from fertilizing peanuts with N. The location and abundance of nodules on peanut roots indicated that numbers of rhizobia in 15 of 17 fields sampled were adequate for good to fair nodulation. However, nodules sampled from only one of the four production areas consistently contained leghaemoglobin. Twenty-one strains of rhizobia were isolated from peanut nodules from the four areas. Greenhouse trials revealed that only a few strains were highly effective. Results of these studies suggest that peanuts grown in Texas would respond to inoculation with highly effective rhizobia. Field tests are needed to verify the potential benefit from inoculation.

Efficient peanut (*Arachis hypogaea* L.) production is dependent on the effectiveness (N-fixing ability) of the association between the plant and bacteria (rhizobia) that nodulate it. The rhizobia transform atmospheric nitrogen into a form plants can use. Fertilization of peanuts with 31 to 358 kg. of nitrogen per ha did not produce as great a nut yield as plants inoculated with highly effective rhizobia (6, 7, 8). Peanuts grown in Texas generally have not responded to fertilization with nitrogen applied at 28 and 56 kg/ha (unpublished data of W. B. Anderson and J. S. Newman, Texas A&M University).

Inoculation of peanuts with commercial inoculants has not been a common practice since seed treatments for controlling fungal diseases were initiated (2). The seed treatments were not compatible with rhizobia and inoculation procedures requiring extra labor were required to achieve good inoculation (2, 7).

Strains of rhizobia nodulating peanuts have been shown to vary in effectiveness (1, 5). Effective strains generally nodulated both tap and lateral roots, whereas ineffective strains primarily nodulated the lateral roots (1, 5). Tap root nodulation was considered desirable because it indicated early infection and nitrogen fixation (11). The internal color of effective nodules was red due to the presence of leghaemoglobin which has been positively correlated with the quantity of nitrogen being fixed (4). Ineffective nodules were pale pink or green and contained little or no leghaemoglobin.

The study reported in this paper was undertaken to ascertain the effectiveness of rhizobia formed nodules on peanuts produced in Texas. Effectiveness of the plants was assessed qualitatively using the criteria of nodule location and color. The effectiveness of individual rhizobial isolates was measured by plant growth tests.

Materials and Methods

Spanish peanut root samples were collected from 17 fields located in North Central, East Central, South Central, and Central Texas. Approximately 10 root samples were taken from each field and rated qualitatively according to extent of tap root nodulation. The samples were taken from plants having well developed but still immature pods. The roots were carefully dug and washed to reveal the nodules. A sample having an average of

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five or more firm nodules on the tap root was rated as good, two to four as fair, and one or zero nodules as poor.

The percentage of effective nodules per sample was estimated by visual inspection of cross-sectioned nodules for nodule color. Pink or red nodules were categorized as effective, pale or green nodules were categorized as ineffective, and soft or rotting nodules were disregarded.

Attempts were made to isolate rhizobia from two apparently effective (red) nodules from each sample using standard isolation procedures (3). Isolated rhizobia were cultured on agar slants made from the same medium that was used for isolations.

Spanish peanuts, Starr variety, were grown in 1 liter plastic pots containing a sterile mixture of 1 part perlite, 1 part exploded vermiculite, and 1 part washed quartz sand. The plants were inoculated upon emergence with a 10 day old agar slant culture of rhizobia. Two containers with two plants each were used to test the effectiveness of each isolate. For inoculation, the rhizobia were scraped from the slants and suspended in 10 ml of 0.85% NaCl. Five ml of inoculum containing approximately 14 million cells per ml was placed, by pipette, 2 cm below the surface of the planting mixture near roots of 1 week old plants. Subirrigation was used to replenish water and avoid contamination of roots by airborne rhizobia falling on the surface of the planting mixture. A constant water table of 3 cm was maintained in each pot by the addition of nitrogen free nutrient solution (9). Plants were grown in the greenhouse for 6 weeks, at temperatures maintained between 20 and 32C. Plant tops were clipped off, dried in a forced draft oven at 110°C and weighed for dry matter production.

Results and Discussion

Peanuts collected from 15 of the 17 fields sampled, in four production areas of Texas, were nodulated on the upper portion of their tap roots (Table 1) indicating that rhizobia were abundant in the sampled fields (11). Peanut plants from only 2 of the fields were poorly nodulated as evidenced by few or no nodules on the tap roots.

Although most plants were nodulated, many nodules appeared to be ineffective as indicated by interior color (Table 1). Nodules on peanuts from production area 4 did appear to be effective. Generally 90% or more of the nodules on the plants from this area had red contents. Of the remaining 13 fields, only field 2 in area 1 contained peanuts with primarily effectively nodules. It appears that peanuts grown in areas 1, 2, and 3 would benefit more from inoculation than peanuts grown in area 4.

Only 21 isolates from approximately 70 nodules were obtained for plant growth tests. The presence of fast growing bacteria in nodules made isolation of rhizobia from many nodules impossible. Others have reported on bacterial contaminants in peanut nodules (10).

Effectiveness tests on the individual isolates of *Rhizobium* revealed extreme variation in effectiveness (Table 2). Many of the isolates were relatively poor in comparison to the best isolate in stimulating dry matter production of peanuts even though all of the isolates were taken from nodules containing visible amounts of leghaemoglobin. The effectiveness tests verified the qualitative data based on nodules color (Table 1) which indicated that many of the nodules were formed by relatively ineffective rhizobia. Isolates St-2, T-1, and Su-1 appear to be very effective in stimulating plant growth. The 21 isolates tested for effectiveness might be arbitrarily categorized into 4 effectiveness groups: 3 high, 5 medium, 5 low, and 8 nearly ineffective. The 3 highly effective strains will be used in field studies on increasing efficiency of peanut production by inoculation.

Table 1. Nodulation characteristics of field grown peanuts.*

Area	Field	Nodulation Rating	% Red Nodules
1	1	fair	20
1	2	fair	80
1	3	good	20
2	1	poor	15
2	2	good	10
3	1	good	10
3	2	good	15
3	3	fair	10
3	4	fair	10
3	5	good	0
3	6	good	20
3	7	poor	20
3	8	fair	20
4	1	good	95
4	2	good	90
4	3	good	95
4	4	good	100

*Area 1, 2, 3, and 4, respectively, refer to North Central, East Central, South Central and Central Texas. Nodulation rating was based on nodulation of tap root. The interior of firm nodules were examined for nodule color.

Table 2. Effect of Rhizobium isolates on production of peanuts tops.

Isolate Origin Area*	Field	Isolate Name	Dry Weight+
			g/pot
3	6	St-2	3.9a
4	4	T-1	3.8ab
3	7	Su-1	3.7abc
3	6	St-1	3.3bc
4	3	E-1	3.3bc
3	8	Sm-1	3.3bc
3	2	F-1	3.2c
2	2	P-1	3.0cd
3	4	S-1	2.7de
2	2	P-3	2.5e
3	1	R-2	2.5e
2	1	A-2	2.4f
3	4	S-2	2.4f
3	5	Se-1	1.8dg
4	1	J-1	1.7dgh
4	4	T-2	1.7gh
4	2	M-1	1.4ghi
2	2	P-2	1.3hi
4	2	M-2	1.3hi
2	2	P-4	1.3hi
3	1	R-1	1.0ij
-	-	Uninoculated	0.8j

* See footnote of Table 1.

+ Numbers having a letter in common were not significantly different at the 0.05 level by Duncan's new multiple range test.

Application of low levels of nitrogen fertilizer to peanuts grown in Texas generally has not increased yields. Nevertheless, there may still be a potential for increasing the yields of peanuts by inoculation with highly effective rhizobia. Other workers have reported that effectively nodulated peanuts yielded more than those fertilized with

nitrogen (6, 7, 8). The results of this paper strongly suggest that the rhizobia nodulating peanuts in Texas are not highly effective in fixing nitrogen for peanut production.

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