Peanut Science (1984) 11:83-87

Studies on Competition, Persistence, and Methods of Application of a Peanut *Rhizobium* Strain, NC 92 [1]

P.T.C. Nambiar*[2], B. Srinivasa Rao[3], and V. Anjaiah[3]

ABSTRACT

A series of different field experiments were conducted at ICRISAT to assess the practical utility of a strain of *Rhizobium*, NC 92, as an inoculant for peanut, *Arachis hypogaea* L. Experiments were conducted in soil where substantial native *Rhizobium* populations exist (10^2 -10^4 cells/g dry soil), and ELISA (enzyme-linked immunosorbent assay) was used to identify NC 92 nodules. The results indicate that inoculation with strain NC 92 (a) results in 25-40% of the total nodules formed (b) mixing with other strains reduces the percent success of the inoculated strain in some experiments (c) whenever fungicides are used for seed treatment, direct application of rhizobia to the soil as liquid (by mixing the peat containing *Rhizobium* with water and pouring the resulting mixture into the furrow just before sowing) results in the formation of more nodules by the inoculated strain than the conventional seed inoculation method (d) very few NC 92 nodules are formed when the rate of inoculum is 10^4 cells/seed or less (e) the inoculated strain, NC 92 is able to survive in the soil for the following season.

Key Words: Groundnut, *Arachis hypogaea* L., ELISA, root nodules, *Rhizobium*

Peanut (*Arachis hypogaea* L.) yield has been shown to be increased by inoculating with efficient and competitive strains of *Rhizobium*, even in fields where substantial soil native *Rhizobium* populations exist and the crop is nodulated by these rhizobia (10,11). One strain of *Rhizobium* (NC 92) was found to increase the yield of peanut genotype Robut 33-1 during several seasons at ICRISAT Center (10,11) and in several other locations in India during the 1981 and the 1982 rainy seasons (1). This strain also produced higher yields when inoculated with certain other peanut genotypes: ICGS 15 and ICGS 27 at ICRISAT Center (7), cv. JL 24 at Junagadh (Gujarat, India) (8) and at ICRISAT Center (7) and cv. 28-206 at Guiring in Cameroon (2). In this paper we summarize the results of several experiments designed to assess the practical utility of this strain as an inoculant. Experiments were conducted to study (a) competitive ability of strain NC 92, introduced either as a single or mixed strain inoculum in fields with high indigenous peanut *Rhizobium* populations (10^2 to 10^4 cells/g dry soil) (b) the effect of seed fungicide treatments on nodulation (c) the effects of rate and the method of *Rhizobium* inoculation on strain competition, and (d) the persistence of the introduced strain in the soil.

Materials and Methods

Genotypes

The peanut genotypes used in these experiments were Robut 33-1 (virginia bunch), ICGS 15 (spanish bunch), PI 259747 (valencia) and J 11 (spanish bunch).

Strains of *Rhizobium*

The strains of *Rhizobium* used in these experiments were NC 92 (obtained from Professor G. H. Elkan, North Carolina State University, Raleigh, U.S.A.), 5a/70 (obtained from Dr. Rina Lobel, Volcani Center, Israel) and IC 6006, and IC 6009 (isolated from peanut nodules collected from a field at ICRISAT Center, Patancheru, near Hyderabad and from a farmer's field near Pune, respectively). Peat inoculants of these strains were prepared as described by Thompson (16). Unless otherwise mentioned the *Rhizobium* inoculum was applied as a 'liquid inoculum' (10^6 cells/seed) as described by Nambiar *et al.* (11). Direct seed inoculation was carried out as follows: five mL of 1.5% aqueous methyl cellulose was mixed with 0.2 g of peanut inoculum and coated on 100 g of seeds. The seeds were then air dried at room temperature 27±5 C) and sown within 24-30 hours after seed treatments. Uninoculated plots, nodulated by the native *Rhizobium* population, served as controls.

Field Experiments

All experiments were carried out during three growing seasons in Alfisol fields at ICRISAT Center, Patancheru, near Hyderabad. The plants were grown in 5 m rows, 75 cm apart, with plant to plant spacing of 15 cm. The details of individual experiments are given below.

1. Competition of single and mixed strains with the native *Rhizoibum* population.

Three experiments were conducted to study the success of NC 92. In the first experiment, conducted in the 1981 rainy season, NC 92 was inoculated as a single strain (10^6 cells/seed) and in a mixture with two other strains, 5a/70 and IC 6006 (0.33×10^6 cells/seed/strain). In a second experiment, conducted in the 1982 rainy season, NC 92 was applied as single and in combination with either 5a/70 (0.5×10^6 cells/seed/strain) or IC 6009 (0.5×10^6 cells/seed/strain). These treatments were repeated in the third experiment conducted in the 1982-83 post rainy season. During the 1981 rainy season, three genotypes, Robut 33-1, ICGS 15, and J 11 were tested, in a split-plot design with four replications using *Rhizobium* strains as main-plots and genotypes as sub-plots. The crop was sown on June 16, 1981. During the 1982 rainy season and the 1982-83 post rainy season two genotypes, Robut 33-1

[1]Submitted as Journal Article No. 445, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru P. O. Andhra Pradesh 502 324, India.

[2]Microbiologist, [3]Research Associates Groundnut Improvement Program, ICRISAT.

and ICGS 15, were tested. A factorial design was used with five replications during the 1982 rainy season. The crop was sown on June 17, 1982. In 1982-83 a split-plot design with genotypes as the main-plots and strains as sub-plots with five replications was used. The crop was sown on November 26, 1982.

2. Effects of different methods of *Rhizobium* inoculation and fungicide treatments on nodulation.

During the 1982-83 post rainy season the effects of different methods of *Rhizobium* inoculation and of different fungicide seed treatments were investigated. The seeds were inoculated either by directly coating the *Rhizobium* (10^8 cells/seed) on the seed (seed inoculation) or by applying the peat inoculum mixed in water (liquid inoculation) as described by Nambiar *et al.* (11). Five fungicide treatments, untreated seeds and seeds treated with Captan, Thiram, Dithane, or Bavistin (3g/kg) were tested. The experimental design was a split-plot with fungicide as the main-plots and *Rhizobium* inoculation methods as sub-plots. The treatments were replicated four times. The genotype used was Robut 33-1, and the strain of *Rhizoibum* was NC 92. The crop was sown on November 27, 1982. The plants were sampled 60 and 116 days after sowing.

3. Effect of different rates of inoculum on competition

Two experiments were carried out to study the effect of inoculum rate on the success of the inoculant strain in nodule formation. (a) During the 1982 rainy season, genotype Robut 33-1, was inoculated with strain NC 92 at 10^2, 10^4, and 10^6 cells/seed. A randomized block design with six replications was used. The crop was sown on June 16, 1982. (b) During the 1982-83 post rainy season two genotypes, Robut 33-1 and ICGS 15, were inoculated at 10^4, 10^6, and 10^8 cells/seed. A split-plot design with genotypes as main-plot and inoculum rates as sub-plot with five replications was used. The crop was sown on November 27, 1982.

4. Persistence of inoculant strain over seasons

An experiment to study the persistence of the inoculated strain was also conducted. During the 1982 rainy season four genotypes, Robut 33-1, J 11, PI 259747, and ICGS 15, were sown with and without inoculum (NC 92) in a randomized block design with five replications. During the 1982/83 post rainy season plots were subdivided into two, one part was re-inoculated with strain NC 92 and other left as an uninoculated control. The genotypes sown were the same as grown in the previous season, (split-plot design, genotypes as main-plot and *Rhizoibum* inoculation treatment as sub-plot). The plants were sampled at 72 and 116 days after sowing and nodules were typed by ELISA.

Sampling procedure

In all experiments the nodules were typed by ELISA (enzyme-linked immounosorbent assay) (9). Five plants were randomly selected from each plot. The age of the crop at sampling is given in the tables. The roots were washed immediately with water and then Teepol B-300 (liquid detergent, National Chemical Industries, Bombay, India; 0.05% in physiological saline) followed by four successive washings with tap water. The nodules were separated manually dried at 60 C for 48 hours, and then stored at room temperature for typing by ELISA. Forty-eight nodules were subsampled from each plot for ELISA typing.

ELISA Procedure

Chemicals and Materials

Unless otherwise mentioned all chemicals and reagents were obtained from Sigma Chemicals, St. Louis, U.S.A. Polystyrene Microtitre plate was obtained from Dynatech Laboratories Inc., Switzerland.

Antisera against strains of rhizobia were raised as described by Bergersen (3). Gamma globulins were purified by sodium sulphate precipitation (17). The procedure for direct ELISA was as described by Kishinevsky and Bar-Joseph (9).

Enumeration of soil *Rhizobium* populations

Soil samples were collected by using a soil sampling tube, i.d. 1.9 cm, (Soil Test Inc., Evanston, Illinois, U.S.A.) at 0-15 cm depth. Soil *Rhizobium* populations were determined by the plant infection technique using siratro (*Macroptilium atropurpureum)* as the test plant as described by Brockwell (4).

Statistical analysis

The results were analyzed using conventional techniques. Wherever necessary, the data were transformed to the arcsine or square root variable for the analysis of variance (15). All results are tested for significance at the 5% level of probability.

Results and Discussion

The antisera raised against strains of rhizobia 5a/70, IC 6006, and IC 6009 cross reacted with native *Rhizobium* populations as indicated by large numbers of positive reactions in the nodules of plants collected from the uninoculated control plots. Hence, it was not possible to type nodules for the presence of these strains. However, in many of the experiments only a few nodules collected from the uninoculated plots showed a positive ELISA reaction with strain NC 92 antiserum. Thus, ELISA was used to type the percentage of the nodules formed by this strain, and these results are described below. The native *Rhizobum* populations, as enumerated by the plant infection technique varied from 3.2 x 10^3 to 8.7 x 10^3 cells/g dry soil depending on the site.

1. Competition of single and mixed strains with the native *Rhizobium* population.

Results of three field experiments conducted to study the competition of strain NC 92 inoculated as a single strain or in mixture, are given in Tables 1, 2, and 3. Strain NC 92 used alone as the inoculant formed 14 to 30% of the total number of nodules on genotype Robut 33-1, and 21 to 36% on the genotype ICGS 15. However, there was some variation in the data from year to year, probably due to differences in the soil *Rhizobium* populations in different fields, and/or seasonal differences. During the 1981 rainy season, the strain NC 92 formed 30% of the total nodules (140 NC 92 nodules/plant) on genotype Robut 33-1. Pod yield data from this experiment were reported elsewhere (11). Yields of both of these genotypes were increased when inoculated with strain NC 92. Inoculation of genotype Robut 33-1 with strain NC 92 produced 17.5% higher yields (2350 kg/ha uninoculated vs 2760 kg/ha inoculated, SE±187.8). and that of genotype ICGS 15 produced 21.3% higher yields (1970 kg/ha uninoculated vs 2390 kg/ha inoculated, SE ±187.8). Though the strain

Table 1. Effect of mixing with other strains on the success of *Rhizobium* strain NC 92 in producing nodules on three peanut genotypes, 70 days after sowing, rainy season 1981.

Inoculation Treatment	Nodules/plant and percentage formed by NC 92							
	ICGS 15		J 11		Robut 33-1		Mean	
	Total (no.)	NC 92 (%)[a]	Total (no.)	NC 92 (%)[a]	Total (no.)	NC 92 (%)[a]	Total (no.)	NC 92 (%)[a]
Uninoculated	514	6(2)[b]	350	7(4)[b]	420	2(1)[b]	430	5(2)[b]
NC 92	520	30(25)	390	33(30)	460	33(30)	460	32(28)
NC 92+5a/70 +IC 6006	500	25(18)	320	14(6)	470	22(15)	430	21(13)
Mean	510	27(21)	354	24(18)	450	28(22)		
SE			±10.4	±1.1			±21.6	±1.3

The standard errors for comparing genotypes within a inoculation treatment = ±2.3 (% NC 92 nodules) and ±37.3 (total nodules), and for comparing inoculation treatment within the same or different genotype = ±2.0 (% NC 92 nodules) and ±32.2 (total nodules).

a - Data presented after arcsine transformation; orginal means are given in parentheses.

b - Values excluded for calculating SE.

Soil Rhizobium population at sowing was 3.78×10^3 cells/g dry soil.

Table 2. Effect of mixing with other strains on the success of *Rhizobium* strain NC 92 in producing nodules on two peanut genotypes, 70 days after sowing, rainy season 1982.

Inoculation Treatment	Nodules/plant and percentage formed by NC 92							
	Total no. of nodules				% of nodules formed by NC 92[a]			
	Robut 33-1	ICGS 15	Mean	SE	Robut 33-1	ICGS 15	Mean	SE
Uninoculated	210	200	205		2(1)[b]	4(1)[b]	3(1)[b]	
NC 92	200	210	205		29(25)	37(36)	33(30)	
NC 92+5a/70	220	220	220	±10	27(20)	26(20)	26(20)	±2.1
NC 92+IC6009	210	230	220		32(29)	38(39)	35(34)	
SE		±14				±3.0		
Mean	210	210			29(25)	34(32)		
SE		±7.1				±1.7		

a - Data presented after arcsine transformation; original means are given in parentheses.

b - Values excluded for calculating SE.

Soil Rhizobium population at sowing was 8.72×10^3 cells/g.

Table 3. Effect of mixing with other strains on the success of *Rhizobium* strain NC 92 in producing nodules on two peanut genotypes, 70 days after sowing, post rainy season 1982-83.

Inoculation Treatment	Nodules/plant and percentage formed by NC 92							
	Total no. of nodules				% of nodules formed by NC 92[a]			
	Robut 33-1	ICGS 15	Mean	SE	Robut 33-1	ICGS 15	Mean	SE
Uninoculated	67	91	79		7(3)[b]	13(8)[b]	10(6)[b]	
NC 92	73	88	81		20(14)	26(21)	23(18)	
NC 92+5a/70	70	90	80	±5.5	20(12)	21(15)	20(13)	±2.2
NC 92+IC6009	74	91	83		15(7)	19(12)	17(9)	
Mean	71	90			18(11)	22(16)		
SE		±5.5				±1.7		

The standard errors for comparing units of inoculation treatment within a given genotype = ±7.8 (total nodules) and±3.04 (% NC 92 nodules), and for comparing genotypes for the same or different levels of inoculation treatment = ±8.7 (total nodules) and ±3.05 (% NC 92 nodules).

a - Data presented after arcsine transformation; original means are given in parentheses.

b - Values excluded for calculating SE.

Soil Rhizobium population at sowing was 4.78×10^3 cells/g dry soil.

NC 92 formed more or less the same percentage of nodules on J 11, the inoculation did not increase the yield of this genotype (1950 kg/ha uninoculated vs 1870 kg/ha inoculated, SE ± 187.8) (11).

During the 1982 rainy season and the 1982/83 post rainy season, strain NC 92 formed a greater percentage of nodules on genotype ICGS 15 (36% and 21% respectively) than on genotype Robut 33-1 (25% and 14% respectively), whereas during the 1981 rainy season genotype ICGS 15 had fewer NC 92 nodules (25% of NC 92 nodules on genotype ICGS 15 compared to 30% on genotype Robut 33-1). The reasons for these observations are not clear, though it can be suggested that difference in native rhizobia and host genotype X strain interaction could influence *Rhizobium* competition (20).

Commercial inoculants sometimes contain two or more strains of *Rhizobium* in equal proportion, in order to safeguard against the failure of a single strain. Mixing other strains of *Rhizobium* with strain NC 92 inoculum reduced the percentage of nodules formed by strain NC 92 in few experiments. During the 1982 rainy season mixing of strains NC 92 and 5a/70 in equal proportions (0.5 x 10^6 cells/strain/seed), decreased the percentage of nodules formed on ICGS 15 by strain NC 92 (from 36% to 20%). However, mixing with strain IC 6009 did not reduce the percentage of nodules formed by strain NC 92 during the 1982 rainy season, but did so during the 1982/83 post rainy season (from 21% to 12%). These differences may be due to seasonal differences in soil temperature, moisture, and soil *Rhizobium* populations. Roughley *et al.* (13) suggested that soil temperature could influence competition among *Rhizobium japonicum* strains. However, it is difficult to conclude from these experiments on the competitiveness of strain NC 92 when inoculated in a mixture of other strains.

2. Effects of inoculum application method and fungicides on nodulation.

When sampled 60 days after sowing, strain NC 92, coated on the seed or applied as liquid below the seed, formed 20% and 27% of the total nodules respectively on genotype Robut 33-1 (Table 4). However, when the seeds coated with *Rhizobium* were treated with fungicides, the percentage of nodules formed by NC 92 was considerably reduced (between 4% and 9%). Fungicide toxicity to *Rhizobium* coated on the seed has been reported earlier (5). In contrast, where the fungicide and *Rhizobium* were separated in the liquid inoculation method, the decrease in the success of the inoculant strain was not as considerable as that observed in the seed inoculation treatment. During the later stage, 116 days after sowing, a higher percentage of strain NC 92 nodules were observed in the liquid inoculated plants (10 to 24%) than in the seed inoculated plants (2-8%, Table 5). This is probably due to a better spread of *Rhizobium* when inoculated as liquid. *Rhizobium* coated to the seed with the sticker has less mobility, and most of the bacteria may be brought out of the soil along with the cotyledons when the seed germinates. This may be the reason for the decrease in the percentage of NC 92 nodules at 116 days after sowing (8%) in the seed inoculation treatment, compared to 22% NC 92 nodules

Table 4. Effect of different methods of *Rhizobium* inoculation on percentage nodules formed by strain NC 92, 60 days after sowing, post rainy season 1982-83.

Fungicide treatment	% NC 92 nodules[a] Method of Rhizobium inoculation		
	Liquid	Seed	Uninoculated
Untreated	30(27)	22(20)	4(2)
Captan	28(23)	7(4)	3(1)
Thiram	25(18)	6(4)	7(2)
Dithane	19(10)	14(9)	7(3)
Bavistin	24(16)	14(8)	10(3)
Mean	25(19)	13(9)	6(2)
SE		±2.6	

The standard error for comparing Rhizobium inoculation mean within a fungicide treatment mean = ±5.5 and for comparing fungicide treatment within the same or different Rhizobium inoculation treatment mean = ±5.3

a - Data presented after arcsine transformation; orginal means are given in parentheses.

Soil Rhizobium population at sowing was 4.78×10^3 cells/g dry soil.

Table 5. Effect of different methods of *Rhizobium* inoculation on percentage nodules formed by strain NC 92, 116 days after sowing, post rainy season 1982-83.

Fungicide Treatment	% NC 92 nodules[a] Method of Rhizobium inoculation		
	Liquid	Seed	Uninoculated
Untreated	27(24)	11(8)	15(7)
Captan	19(10)	7(2)	11(7)
Thiram	19(11)	12(8)	12(7)
Dithane	22(15)	6(4)	7(2)
Bavistin	21(13)	12(5)	9(4)
Mean	22(15)	10(5)	11(5)
SE		±2.5	

The standard error for comparing Rhizobium inoculation mean within a fungicide treatment mean = ±5.4 and for comparing fungicide treatment within the same or different Rhizobium inoculation treatment mean = ±5.0.

a - Data presented after arcsine transformation; original means are given in parentheses.

Soil Rhizobium population at sowing was 4.78×10^3 cells/g dry soil.

at 60 days after sowing in the same treatment. The success of inoculating rhizobia on peanut as a 'liquid', as determined by peanut yield responses, has been reported earlier (10,11,14). There were few NC 92 nodules on the uninoculated plants at 116 days after sowing, probably due to cross contamination.

3. Effect of different rates of inoculum on competition.

The effect of different rates of inoculum on the total nodule number and on the percentage of nodules formed by strain NC 92 are shown in Tables 6 and 7. The differences in the total number of nodules formed in different inoculum treatment were not significant during the 1982 experiment ($P<.05$). However, during the 1982-83 post rainy season there was a decrease in total nodule number at 10^6 cells/seed than at 10^4 or 10^8 cells/seed. It is difficult to explain this observation. The recommended inoculum rate differs from legume to legume depending on the seed size and on the native *Rhizobium* populations (18,19). In general, 10^3 to 10^5 cells/seed are recommended. Peanuts grown under glasshouse conditions need larger numbers of rhizobia (10^6 to 10^8 cells/seed) for maximum nodulation and nitrogen fixation (12). With a background *Rhizobium* population of 10^2 to 10^4 cells/gm soil, higher rates of inoculum may be required for field inoculants. At 10^2 to 10^6 cells inoculated per seed in a field containing 3.83×10^3 rhizobia/gm dry soil, very few nodules were formed (up to 14%) by the inoculated strain, NC 92 (Table 6). Hence, a minimum of 10^6 cells/seeds are necessary for inoculating this peanut genotype (Robut 33-1) with strain NC 92. Higher inoculum rates are desirable if economically feasible. The differences in total nodules/plant during the post rainy season and the rainy season are probably due to the late nodule initation and development during the post rainy season (6).

4. Persistence of the inoculum strain over two seasons.

The percentage of nodules formed by strain NC 92 increased with re-inoculation in a second season (Table 8). When sampled at 72 days after sowing during the second season there were 34% NC 92 nodules on genotype Robut 33-1 when inoculated for two subsequent seasons, in contrast to 22% when inoculated in either one of the seasons alone. For genotype PI 259747 subsequent inoculations with strain NC 92 increased the percentage of NC 92 nodules up to 53%. The advantage of the earlier season's inoculation was more evident during the later stages of plant growth (Table 9). When sampled at 116 days after sowing, during the second season, the mean percentage of NC 92 nodules in reinoculated plots increased up to 54%. This indicates a good distribution of strain NC 92 in the soil from the degenerated nodules of the previous season, so that with progressive root growth, this strain continues to form more nodules. Total nodule number/plant from these experiments (Table 10) indicate that nodules continue to form 72 days after sowing, and more NC 92 nodules (54%) are formed during 72-116 days after sowing on plants inoculated during two seasons than plants

Table 6. Effect of *Rhizobium* numbers in the inoculum on nodulation on genotype Robut 33-1, 85 days after sowing, rainy season 1982.

Rhizobium number/seed	Total number of nodules	% NC 92 nodules[a]
Uninoculated	433	0[b]
10^2	462	0[b]
10^4	418	2(6)
10^6	458	4(14)
SE	±27.4	±0.4

a - Data presented after square root transformation; original means are given in parentheses.

b - Values excluded for calculating SE.

Soil Rhizobium population at sowing was 3.83×10^3 cells/g dry soil.

Table 7. Effect of *Rhizobium* numbers in the inoculum on nodulation on genotypes Robut 33-1, and ICGS 15, 72 days after sowing, post rainy season 1982-83.

Rhizobium number/seed	Nodules/plant and percentage formed by NC 92							
	Total no. of nodules				% of nodules formed by NC 92[a]			
	Robut 33-1	ICGS 15	Mean	SE	Robut 33-1	ICGS 15	Mean	SE
Uninoculated	70	87	79		3(1)[b]	7(3)[b]	5(2)[b]	
10^4	72	119	96		18(11)	20(17)	19(14)	
10^6	57	96	76	±4.6	26(20)	29(25)	28(22)	±3.6
10^8	67	113	90		37(37)	28(24)	32(30)	
Mean	65	109			27(23)	26(22)		
SE	±6.5				±2.3			

The standard errors for comparing inoculation treatment means within a genotype are ±6.4 (total nodules), and ±4.7 (NC 92 nodules) and for comparing genotype for the same or different levels of Rhizobium treatment are ±8.6 (total nodules), and ±5.1 (% NC 92 nodules).

a - Data presented after arcsine transformation; original means are given in parentheses.

b - Values excluded for calculating SE.

Soil Rhizobium population at sowing was 3.78×10^3 cells/g dry soil.

Table 8. Persistence of inoculum strain NC 92 over two seasons (% nodules formed by strain NC 92)[a], 72 days after sowing, post rainy season 1982-83.

Treatment		Genotype					
Rainy Season 1982	Postrainy Season 1982-83	Robut 33-1	J 11	PI 259747	ICGS 15	Mean	SE
Uninoculated	Uninoculated	10(7)[b]	9(5)	5(3)	12(6)	9(5)	
Uninoculated	Inoculated	27(22)	30(29)	33(30)	32(29)	31(27)	±2.5
Inoculated	Uninoculated	26(22)	28(23)	37(37)	23(19)	28(25)	
Inoculated	Inoculated	35(34)	39(40)	47(53)	36(37)	39(41)	

The standard errors for comparing inoculation treatment means within a genotype = ±4.9 and for comparing genotype for the same or different level of inoculation treatment = ±5.1.

a - Data presented after arcsine transformation; orginal means are given in parentheses.

b - Values excluded for calculating SE.

Table 9. Persistence of inoculum strain NC 92 over two seasons (% nodules formed by strain NC 92)[a], 116 days after sowing, post rainy season 1982-83.

Treatment		Genotype					
Rainy Season 1982	Postrainy Season 1982-83	Robut 33-1	J 11	PI 259747	ICGS 15	Mean	SE
Uninoculated	Uninoculated	7(6)[b]	2(2)	28(21)	6(5)	11(8)	
Uninoculated	Inoculated	30(27)	34(33)	26(24)	18(15)	27(25)	±5.4
Inoculated	Uninoculated	37(35)	37(28)	55(42)	38(26)	42(32)	
Inoculated	Inoculated	63(51)	93(57)	69(57)	74(50)	75(54)	

The standard errors for comparing inoculation treatment means within a genotype = ±10.3 and for comparing genotype for the same or different level of inoculation treatment = ±10.8.

a - Data presented after arcsine transformation; orginal means are given in parentheses.

b - Values excluded for calculating SE.

Table 10. Nodulation status (total number of nodules/plant) at 72 and 116 days after sowing, post rainy season 1982-83.

Treatment		Genotype					
Rainy Season 1982	Postrainy Season 1982-83	Robut 33-1	J 11	PI 259747	ICGS 15	Mean	SE
Uninoculated	Uninoculated	63[a](119)	89(116)	108(127)	99(138)	90(125)	
Uninoculated	Inoculated	71(113)	91(118)	84(109)	95(155)	85(124)	±7.5(8.3)
Inoculated	Uninoculated	80(115)	108(137)	72(131)	138(151)	100(134)	
Inoculated	Inoculated	104(131)	75(157)	59(119)	130(162)	92(142)	

The standard errors for comparing inoculation treatment means within a genotype = ±14.9(16.6) and for comparing genotype for the same or different level of inoculation treatment = ±18.8(20.1).

a - Data for 72 days after sowing; Data for 116 days after sowing and SE are given in parentheses.

inoculated during either season only (25-32% NC 92 nodules). This suggests that one may not have to inoculate the field with strain NC 92 continuously. Experiments are in progress to assess how many seasons one has to inoculate to get this strain established in the soil so as to avoid re-inoculation.

Acknowledgment

We thank R. Rajeshwari and N. Bharathan (Groundnut Pathology, ICRISAT) for their help in conducting ELISA and A. Vishwanath and M. Subhash Rao for their assistance during the course of these experiments.

Literature Cited

1. AICORPO (All India Co-ordinated Research Project on Oilseeds). 1983. Annual Progress Report, Kharif 1982-83, Rajendranagar, Hyderabad, India. Vol. 2:241-248.
2. Annon. (1983). Peanut Research Program, Institute of Agronomic Research, Centre of Maroua, United Republic of Cameroon. Annual Report 1983. pp. 51-52.
3. Bergersen, F. J. 1961. The growth of *Rhizobium* in synthetic media. Aust. J. Biol. Science 14:349-360.
4. Brockwell, J. 1980. Experiments with crop and pasture legumes - Principles and practice. *In* Bergersen, F. J. (Ed.) Methods for Evaluating Biological Nitrogen Fixation. John Wiley and Sons, New York. pp. 417-488.
5. Curley, R. L., and J. C. Burton, 1975. Compatibility of *Rhizobium japonicum* with chemical seed protectants. Agron. J. 67:807-808.
6. ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) 1981. Annual Report 1979-80. Patancheru, A.P. India: ICRISAT. pp. 159.
7. ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) 1983. Annual Report 1982. Patancheru, A.P. India: ICRISAT. pp. 200.
8. Joshi, P. K., and J. H. Kulkarni, 1983. Response of groundnut to inoculation with single and mixed *Rhizobium* strains. Indian J. Agric. Sci. 53:376-378.
9. Kishinevsky, B., and M. Bar-Joseph, 1978. *Rhizobium* strain identification in *Arachis hypogaea* nodules by enzyme-linked immunosorbent assay (ELISA). Can. J. Microbiol. 24:1537-1543.
10. Nambiar, P. T. C., P. J. Dart, B. Srinivasa Rao, and H. N. Ravishankar, 1982. Response of groundnut (*Arachis hypogaea*) to inoculation. *In* Graham, P. H. and Harris S. C. (Eds.) Proceedings of an International Workshop on BNF Technology for Tropical Agriculture, Centro Internacional de Agricultura Tropical. Cali, Colombia. pp. 241-248.
11. Nambiar, P. T. C., P. J. Dart, B. Srinivasa Rao, and H. N. Ravishankar, 1984. Response of groundnut (*Arachis hypogaea* L.) to *Rhizobium* inoculation. Oléagineux 39:149-155.
12. Nambiar, P. T. C., H. N. Ravishankar, and P. J. Dart, 1983. Effect of *Rhizobium* numbers on nodulation and dinitrogen fixation in groundnut. Expl. Agric. 19:243-250.
13. Roughley, R. J., E. S. P. Bromfield, E. L. Pulver, and J. M. Day, 1980. Competition between species of *Rhizobium* for nodulation of *Glycine Max*. Soil Biol. Biochem. 12:467-470.
14. Schiffmann, J., and Y. Alper, 1968. Inoculation of peanuts by application of *Rhizobium* suspension into the planting furrows. Expl. Agric. 4:219-226.
15. Steel, R. G. D., and J. H. Torrie, 1980. Principles and Procedures of Statistics. A Biometrical Approach. Tokyo, Japan. pp 236.
16. Thompson, J. A. 1980. Production and quality control of legume inoculants. *In* Bergersen, F. J. (Ed.) Methods of Evaluating Biological Nitrogen Fixation. John Wiley and Sons, New York. pp 489-533.
17. Van Weemen, B. K., and A. H. W. M. Schuurs, 1971. Immunoassay using antigen-enzyme conjugates. FEBS Lett. 15:232-236.
18. Vincent, J. M. 1970. Manual for the Practical Study of Root-nodule Bacteria. Black well Scientific Publication, Oxford. pp 128.
19. Weaver, R. W., and L. R. Frederick, 1974. Effect of inoculum rate on competitive nodulation of *Glycine max* L. Merrill. II. Field studies. Agron. J. 66:233-236.
20. Wynne, J. C., G. H. Elkan, T. G. Isleib, and T. J. Schneeweis, 1983. Effect of host plant, *Rhizobium* strain and host x strain interaction on symbiotic variability in peanut. Peanut Sci. 10:110-114.

Accepted October 25, 1984