RESPONSE OF GROUNDNUT (*ARACHIS HYPOGAEA* L.) TO RHIZOBIUM INOCULATION

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Technical Report

ICRISAT

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SUMMARY

* The pod yield of cv Robut 33-1, released in Andhra Pradesh as Kadiri 3, was significantly increased when inoculated with the Rhizobium strain NC 92 at the ICRISAT Center, over several rainy as well as postrainy seasons, and at Dharwar in Karnataka State during the 1981 rainy season.

* These responses were obtained in soils where groundnut had been grown earlier, and the uninoculated plants had 200-600 nodules per plant 60 days after planting.

* The yield response was in the range of 2.8% to 40% (60 to 1000 kg pods/ha) over the uninoculated control.

* Three 70 gram peat packets (containing 10^9-10^{10} Rhizobium cells/gram peat) are required to inoculate one hectare. Manufacturers could sell peat packets at Rs.10-15 each.

* We do not suggest that Rhizobium strain NC 92 is mixed with other strains.

* We recommend that the strain should be applied as a liquid (peat mixed with water) below the seed (3-4 ml/seed). If the seeds are sown by hand, the inoculant could be applied by means of a plastic jerry can, or a similar container, fitted with a small tube (Fig. 1). Less than one man day and 400 litres of water are required to inoculate one hectare by hand. A suitable method of inoculation needs to be devised for mechanised sowing.

Fig.1. A simple procedure to inoculate groundnut. Peat containing rhizobia is mixed with water and added in the furrow before sowing.

Groundnut (Arachis hypogaea L.) is nodulated by a large group of Rhizobium strains classified as the "cowpea miscellany" (Fred, et al., 1932; Buchanan and Gibbons, 1974). Most of the cultivated soils of the tropics appear to have relatively large populations ($>10^3$/g dry soil) of this type of Rhizobium, and groundnut is usually nodulated in these soils. However, the presence of nodules on roots of the groundnut plant does not mean that sufficient N_2 is being fixed for good growth of the host plant (Weaver, 1974). Inoculation with effective Rhizobium strains have increased pod yield in fields where groundnut had not been previously grown (Seeger, 1961; Shimshi et al., 1967; Schiffmann and Alper, 1968; Chesney, 1975; Pettit et al., 1975; Burton, 1976). There are few reports on the effect of Rhizobium inoculation on groundnut where the crop had been previously grown. In the U.S.A. inoculation did not increase pod yields in either Alabama (Hiltbold, A.E. personal communication) or North Carolina (Wynne, J.C. personal communication). At Ludhiana, India, Arora et al. (1970) observed that the seed protein content, but not the pod yield, was increased by inoculation. Subba Rao (1976) observed that Rhizobium inoculation resulted in decreased yields in the All-India coordinated trials conducted over several seasons. However, Sundara Rao (1971) reported pod yield increases after Rhizobium inoculation at Hyderabad (India). Van Der Merwe et al. (1974) conducted eleven seed inoculation trials over three seasons in different locations in South Africa, where groundnuts had been cropped intensively. They obtained increased seed yield in only one trial, conducted at Buffelspoort. Hence these authors suggested that seed inoculation may be superfluous under the existing agricultural practices in South Africa.

We report here the results of Rhizobium inoculation trials conducted in ICRISAT fields at Patancheru, near Hyderabad, India from 1977 to 1981, and at Dharwar, in Karnataka state, during the 1981 rainy season. These trials were conducted in fields where the native Rhizobium population of cowpea miscellany rhizobia ranged from 10^2 to 10^4/g soil, and where uninoculated control plants formed 200 to 600 nodules/plant at 60-70 days after planting. Groundnut had previously been grown recently in most of these fields (Table 1). Several cultivar and Rhizobium strain combinations were tested during these trials.

A. MATERIAL AND METHODS

1. Cultural Practices:

The experiments were conducted during the rainy season (usually mid June to October) and the irrigated postrainy season (mid November to March). The crops were grown on ridges 75 cm apart with an in row spacing of 15 cm. The plants were irrigated during the postrainy season at 7 to 10 days intervals, and were regularly protected by sprays against insect pests. Experiments during the 1977 and 1978 rainy season, and the 1978 postrainy season at ICRISAT were laid out in a randomised block design. The experiment during the 1981 rainy season at Dharwar was laid out in a split plot design with cultivars as main plots and Rhizobium treatments as sub plots. All other experiments were split plot designs with the Rhizobium treatment as the main plots and cultivars as the sub plots. The plot size and harvest areas are shown in Table 1.

2. Cultivars:

Cultivars used in these experiments were Argentine, Ah 8189 (Spanish); MH 2 (Valencia); Kadiri 71-1, Robut 33-1, ICGS 17, ICGS 22, and ICGS 15 (Virginia).

3. Rhizobium Strains:

The following strains were used - NC 92, NC 43.3, NC 7.2, NC 6, NC 70.1 (obtained from Dr. G.H. Elkan, NCSU Raleigh, USA); 3G486, 3G4816 (obtained from USDA, Beltsville), 5a/70 (obtained from Dr. Rina Lobel, The Volcani Center, Israel). Other strains were isolated at ICRISAT from nodules collected from different parts of India.

4. Preparation of Inoculants and Method of Application:

Inoculants, using γ -irradiated peat as a carrier, were used in these experiments (Thompson, 1980). The peat contained 10^9-10^{10} Rhizobium/gram peat and were free of contaminants at the 10^6 dilution level. Granular inoculum was prepared by mixing 70 g of peat with 800 ml of aqueous methyl cellulose (1.5% w/v), and then with 5.5 kg washed river sand, until the sand was evenly coated with the peat. The mixture was then air dried in the shade for 8-12 hours. One to two grams of this sand was placed below the seed at sowing to provide minimum 10^6 Rhizobium/seed. Liquid inoculum was prepared by thoroughly mixing the peat in water (0.7 g/litre) and pouring the mixture below the seed (4-5 ml/seed) into the furrow, to give a population of minimum 10^6 Rhizobium/seed.

B. RESULTS AND DISCUSSION

1. Host Cultivar x Rhizobium strain compatibility

The results of the trials are summarised in Table 1. During the 1977 rainy season the trials were conducted at the ICRISAT Center on alfisol fields. The cultivars, TMV 2 and Robut 33-1 produced higher yields when inoculated with the strain 5a/70 (Table 2). However severe moisture stress during the growing season limited yields. During the rainy seasons of 1979, 1980, and 1981, and the postrainy season of 1978-79, Robut 33-1 inoculated with the strain NC 92 produced significantly

(P>.05) higher pod yields than the uninoculated control (Tables 3-6). The increase in pod yield ranged from 18 to 34%. At Dharwar, Robut 33-1, produced 40% more yield when inoculated with the strain NC 92 (Table 7). There was no significant increase in pod yield among the other cultivar/strain combinations tested, except for Robut 33-1 inoculated with 5a/70 (1980 rainy season) and ICGS 15 inoculated with NC 92 (1981 rainy season). Interestingly, ICGS 15 is a progeny derived from a cross between TMV 7 and Robut 33-1. During 1981 seven Rhizobium strains, excluding the strain NC 92, were tested against 4 cultivars, but there was no significant difference in pod yield as a result of inoculation (Table 8). In all experiments inoculation did not effect shelling percentage.

A pooled analysis of variance done over seven experiments showed a significant (P>.01) increase in yield of Robut 33-1 inoculated with the strain NC 92. The average increase in pod yield over that of the uninoculated control was 16% (Table 9). Since the treatment x experiment interaction was significant (P>.01) an unweighted analysis was preferred for the above test (Cochran and Cox, 1957). A similar compatibility between Robut 33-1 and the strain NC 92 has been reported from Junagadh, India (J.H. Kulkarni, per. comm.). Such a strong cultivar/Rhizobium interaction under field conditions resulting in increased pod yield over seasons has not been reported earlier. However, cultivar x Rhizobium interaction for nodulation has been observed with groundnut grown under sterile conditions in a glasshouse (Burton, 1976; Wynne et al.,1980; Nambiar and Dart, unpublished observations) and in fields at Raleigh, North Carolina (Wynne et al., 1980). It is interesting to note that the strain NC 92 originates from South America, where Arachis also originated. The nodules were collected by Professor W.C. Gregory of NCSU (Raleigh,

U.S.A.) and the strain was isolated by microbiologists at NCSU. The strain was supplied to us as a part of the NCSU/ICRISAT collaborative project on BNF (Biological Nitrogen Fixation).

With the help of scientists of the All-India Coordinated Project on Oilseeds (AICORPO) we are currently examining whether this compatibility between Robut 33-1 and NC 92 exists at other locations in India. The cv Robut 33-1 is a released variety in Andhra Pradesh State, and if this cultivar/NC 92 compatibility persists over locations, inoculation of Robut 33-1 with the strain NC 92 should help to increase groundnut production in India. To achieve this will require good quality inoculant strains.

2. Methods of field inoculation of rhizobia:

Direct application of cultures of Rhizobium to seed is the most common form of legume inoculation. However shelled groundnuts are too fragile to be inoculated directly. Moreover, seed treatment with fungicides toxic to rhizobia poses another difficulty in direct seed inoculation of groundnut (Curley and Burton, 1975). Alternately Rhizobium can be added to the soil as a granulated formulation (Bonnier, 1960; Fraser 1966, 1975) or in a liquid added below the seed hole (Schiffmann and Alper, 1968; Weaver, 1969; Brockwell et al., 1980). During the 1977 and 1978 rainy seasons, and the 1977-78 postrainy season, an NC 92 granular inoculum was effective (Tables 1, 2 & 3), whereas during the 1979-80, 1980-81 postrainy seasons, and the 1980 rainy season, granular inoculum was found to be ineffective (Tables 5 and 8). It is possible that sand in the granular formation inhibits capillary movement of water to the seed, and under a water stress situation this leads to poor

germination. During the 1980 rainy season, as a result of an initial water stress, seeds inoculated with a granular formation had poor germination (73%) as compared to 98% in liquid inoculated seeds (Table 10). However, when adequate water was available germination was not affected by granular inoculum. Peat granules, as used by commercial companies in the USA, may be better carriers than sand. Direct seed inoculation was also found to decrease seed germination during the 1980 rainy season (Table 10).

3. Effect of mixing inoculant strains

Commercial inoculants sometimes contain two or more strains of Rhizobium in order to safeguard the failure of a single strain. However, during the 1980 and 1981 rainy seasons, we found that inoculation with a single strain of NC 92 was superior to a mixture of the strains NC 92, 5a/70, and IC 6006. In Alabama, USA, commercial Rhizobium mixture on groundnut produced fewer nodules and a lower plant weight than a single strain (A.E. Hiltbold, personnel communication). There are several dangers in using mixed strains in an inoculum e.g., (i) control of production quality is more difficult to ensure as each strain must be grown in a broth separately, and then added to the carrier, (ii) Differential multiplication rates of the strains may result in large differences in the final prepared inoculant, (iii) Competition between the strains in forming nodules may result in the least effective combination dominating others following inoculation (Date & Brockwell, 1976).

We are currently investigating the competitive ability of different inoculum strains in forming nodules with several promising groundnut lines.

C. CONCLUSION

Our results indicate that substantial increases in groundnut pod yields can be obtained by using a combination of the cultivar Robut 33-1 (Kadiri-3), and a *Rhizobium* inoculant using the strain NC 92. The responses were obtained in fields where groundnuts had been grown earlier and the uninoculated plants were well nodulated by the native *Rhizobium* population. To our knowledge, this is the first report of a strong host-cultivar *Rhizobium* compatibility observed over several seasons in a field grown groundnut crop resulting in increased pod yield.

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Table 1: Summary of inoculation trials conducted during 1977-81

Year	Season	Soil type	Early cropping history of groundnut[b]	Plot size (Sq.mt.)	Harvest area (Sq.mt.)	Cultivar	Strain	Method of inoculation
1	2	3	4	5	6	7	8	9
1977	Rainy	Alfisol	-	32	9	TMV 2 Kadiri 71-1	Sa/70	Granular
1977	Rainy	Alfisol	-	42	16	Kadiri 71-1 Robut 33-1 TMV 2	Sa/70	Granular
1977	Rainy	Vertisol	-	32	9	Kadiri 71-1 TMV 2	Sa/70 IC 6006	Granular
1978	Rainy	Alfisol	-	47	26	Robut 33-1 Argentine Ah 8189	Sa/70 ICG 60 IC 6006	Granular
1978	Rainy	Alfisol	Yes	41	19	MH 2 Argentine Robut 33-1	Sa/70 ICG 60 IC 6006 Mixture	Granular
1978-1979	Post Rainy	Alfisol	No	54	36	MH 2 Robut 33-1 Ah 8189	NC 92 IC 6009 Mixture	Granular/ [c]Liquid (10 ml)
1979	Rainy	Alfisol	Yes	24	11	Kadiri 71-1 Robut 33-1 Ah 8189	Sa/70 IC 6006 NC 43.3 NC 7.2 NC 92	Granular

1	2	3	4	5	6	7	8	9
1979-1980	Post Rainy	Alfisol	Yes	21	14	Robut 33-1	NC 92	Granular/ Liquid (10 ml)[c]
1980	Rainy	Alfisol	Yes	30	14	Robut 33-1	Sa/70 NC 92 IC 6006 Mixture	Granular Liquid (10+10 ml)/ seed slurry
1980-1981	Post Rainy	Alfisol	Yes	34	16	Robut 33-1	NC 92	Granular/ Liquid (2-4 ml)[c]
1981	Rainy	Alfisol	Yes	34	18	Robut 33-1 J 11 ICGS 15	Sa/70 NC 92 IC 6006 Mixture	Liquid (3-4 ml)[c]
1981	Rainy Site 1	Alfisol	Yes	34	16	Robut 33-1 J 11 ICGS 22 ICGS 17	3G486 NC 6 47A1 8A23 NC 70.1 3G4816 IC 6001	Liquid (3-4 ml)[c]
1981	Rainy Site 2	Alfisol	Yes	8	4	Robut 33-1 J 11	NC 92 Sa/70	Liquid (3-4 ml)[c]
1981	Rainy (Dharwad Center)[a]	Alfisol	-	18	10	Robut 33-1 J 11 TMV 2	NC 92 Sa/70	Liquid (3-4 ml)[c]

a - All other trials were conducted at ICRISAT Center, Patancheru.
b - Groundnut grown at least once during 1977-81; - Not known
c - ml of peat in water added per seed at sowing is given in brackets. During the 1980 rainy season an additional inoculum of 10 ml was added 10 days after sowing.

Table 2: Groundnut yield response (Kg pod/ha) to *Rhizobium* (strain 5a/70) inoculation in the 1977 rainy season, ICRISAT Center

Treatment	Cultivar		
	Kadiri 71-1	Robut 33-1	TMV 2
Uninoculated	95	233	156
Inoculated	114	291	206
SE for treatment mean	± 12.1		
CV (%)	23		

Table 3: Groundnut yield response (Kg pod/ha) to *Rhizobium* inoculation in the 1978-79 postrainy season, ICRISAT Center

Treatment	Cultivar		
	MH 2	Robut 33-1	Ah 8189
Uninoculated	2220	3500	2830
IC 6009	1880	3330	2860
NC 92	1940	4500	2690
SE for treatment mean	± 291.2		
CV (%)	15		

Table 4: Groundnut yield (Kg pod/ha) to *Rhizobium* inoculation in the 1979 rainy , ICRISAT Center

Trea	Cultivar		
	Kadiri 71-1	Robut 33-1	Ah 8189
5a/70	360	800	420
IC 6006	480	800	290
NC 43.3	460	960	470
NC 7.2	450	950	420
NC 92	570	1160	480
Uninoculated	500	870	470
SE for treatment mean	± 24.3		
CV (%)	Main plots 32.5, Sub plots 20		

Table 5: Effect of different method of inoculation on groundnut (cv Robut 33-1) yield (Kg pod/ha) in the 1980 rainy season, ICRISAT Center

Treatment	Method of inoculation	
	Granular	Liquid
5a/70	1290	1770
NC 92	1020	1640
IC 6006	1000	1630
Mixture (5a/70 + NC 92 + IC 6006)	1050	1520
Uninoculated	1350	
SE for method of inoculation	± 133.8	
SE for uninoculated and inoculated	± 77.4	
CV (%)	15	

Table 6: Groundnut yield response (Kg pod/ha) to Rhizobium inoculation in the 1981 rainy season, ICRISAT Center

Treatment	Cultivar		
	Robut 33-1	J 11	ICGS 15
5a/70	2440	1710	1800
NC 92	2760	1870	2390
IC 6006	2070	1680	1920
Mixture (5a/70 + NC 92 + IC 6006)	2710	1600	1940
Uninoculated	2350	1950	1970
SE for treatment mean	± 187.8		
CV (%)	Main plots 13; Sub plots 12		

Table 7: Groundnut yield response (Kg pod/ha) to Rhizobium inoculation in the 1981 rainy season, Dharwar

Treatment	Cultivar		
	Robut 33-1	J 11	TMV 2
NC 92	2150	1850	1640
5a/70	1630	1920	1460
Uninoculated	1530	1710	1270
SE for treatment mean	± 176.5		
CV (%)	23		

Table 8: Response of groundnut yield (Kg pod/ha) to *Rhizobium* inoculation in the 1981 rainy season, ICRISAT Center

Treatment	Cultivar			
	Robut 33-1	J 11	ICGS 22	ICGS 17
3G486	2990	1840	2120	2150
NC 6	2870	2140	2350	2630
47A1	3160	2140	2250	2420
8A23	2910	2060	2160	2640
NC 70.1	2850	2180	2510	2770
3G4816	3090	2030	2340	2800
IC 6001	3060	1920	2160	2250
Uninoculated	2990	1990	2370	2470
SE for treatment mean		±180.8		
CV (%)		Main plots 13; Sub plots 11		

Table 9: Summary of response of cv Robut 33-1 inoculated with *Rhizobium* strain NC 92

Season	POD YIELD (kg/ha)		
	Uninoculated	Inoculated	SE
Postrainy[a] (1978/79)	3500	4500	±291.2
Rainy[a] (1979)	870	1160	± 24.3
Postrainy[a] (1979/80)	4280	4400	±104.7
Rainy[a] (1980)	1350	1640	± 77.4
Postrainy[a] (1980/81)	3210	3300	± 78.8
Rainy season[a] (Site.1) (1981)	2350	2760	± 187.8
Rainy season (Site.2) (1981)	1100	1160	± 34.5
Rainy season[b] (1981)	1530	2150	± 176.5
Mean	2274	2634	± 56.3

a Trial conducted at ICRISAT Center

b Trial conducted at Dharwar

Table 10: Effect of methods of inoculation on % germination of groundnut - 1980 rainy season

Method of inoculation	% Germination	
	Location	
	ICRISAT	Solipur
1. Control	83	77
2. Seed	46	71
3. Liquid in furrow	98	79
4. Granular	73	56
SE for treatment mean	± 3.1	± 3.0
CV (%)	8	13

1. Control - Uninoculated seeds treated with Thiram fungicide.

2. Seed slurry - seeds mixed with peat in aqueous methyl cellulose (1.5%) and air dried. They were later treated with Thiram.

3. Liquid - Peat mixed in water and 7-10 ml added below the seed hole just before sowing the fungicide treated seed.

4. Granular - sand coated with peat in aqueous methyl cellulose (1.5%) and air dried. 1-2 g of this sand was placed below the seed at sowing.