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Response of Groundnut (*Arachis hypogaea* L.) to inoculation with *Rhizobium* strains isolated from wild arboreal legumes

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Introduction

Groundnut (*Arachis hypogaea* L.) is a rich source of protein and is one of the principal oil seed crop of India. Efforts have been continually been concentrated towards increasing its yield. *Rhizobium* inoculation is a cheap and widely accepted technology for achieving maximum yield of crops in nitrogen-deficient soils. The results of inoculation response are not always encouraging and are often inconsistent with groundnuts, because both micro- and macro-symbiont possess promiscuity. The promiscuous types of rhizobia which form nodules on groundnut are not generally efficient and, owing to their large population colonize in the rhizosphere at an early stage. This early infection then encourages the plant to develop immunity to further infection (Dunham & Baldwin 1931) and also the infection sites are covered with the nodules formed by them (Chen 1941). Poor competitive ability of the rhizobia also results in their failure to inoculate plants efficiently (Nicol & Thornton 1941). Hegde (1982), noticed that in India, the necessity to inoculate groundnut has neither been shown conclusively nor investigated thoroughly.

Preliminary studies in our laboratory showed that rhizobial strains isolated from wild arboreal legumes were efficient nitrogen fixers on cowpea (*V. unguiculata*) as compared to the homologous strain. Hence, an attempt was made to study the inoculation response of groundnut to rhizobial strains isolated from wild arboreal legumes along with standard homologous strains.

Materials and methods

Strains used

Details of rhizobial strains used in the present study are given in Table 1.

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Table 1 Details of rhizobial strains used

Host plant	Culture code of isolated rhizobial strain	Location/Source		Nodulation on:		Nitrogenase activity in cowpea (nmol acetylene reduced/g dry wt per h)
				Homologous host	Cowpea	
Acacia nilotica	Acni	Akola	MACS, Pune	+	+	37.6
Albizzia lebbek	Alle	Ratnagiri	MACS, Pune	+	+	32.5
Butea monosperma	Bumo	Lonavala	MACS, Pune	+	+	36.2
Erythrine subrosea	Ersu	Pune	MACS, Pune	+	+	28.3
Glyricidia sepium	Glys	Pune	MACS, Pune	+	+	35.2
Sesbania grandifolia	Segr	Pune	MACS, Pune	+	+	36.9
Arachis hypogaea	NC92	South America	ICRISAT, Hyderabad	+	+	–
Arachis hypogaea	G2	Jalgaon	MACS, Pune	+	+	–

* The cross inoculation group in all cases was cowpea miscellany.

Inoculation

The response of groundnut (cv. 'SB–XI') to inoculation with rhizobial strains from wild arboreal legumes as well as homologous host was studied under pot culture condition using 7-kg capacity plastic pots (30 × 20 cm) and non-sterilized black cotton soil (pH 7.0) in randomised block design during summer season of 1987 in a glass-house. In all, 10 treatments were tested and each treatment was replicated 4 times; 5 plants were kept in each pot. The seeds of groundnut were inoculated with broth having 10^8 cells/ml. The pots were irrigated with tap water on every alternate day. Observations on nodulation, acetylene reduction activity of nodulated roots, shoot weight and competitive ability were recorded 45 days after sowing (DAS). At 90 DAS, observations on shoot weight, pod number and pod dry weight were recorded.

Competition studies

The antisera of the rhizobial strains used in the study were prepared by the standard procedure described by Vincent (1970). For determining the competitive ability of the inoculated test strains with native rhizobia, the nodule occupancy test was carried out. For this purpose, 20 nodules from each plant of individual treatments were randomly selected and from that bulk another 20 randomly-selected nodules were used. Each nodule was crushed in a tube containing some physiological saline. The crushed nodule suspension was then held in a boiling water bath for 30 min and tested against the antiserum of the respective strain by immunodiffusion technique as described by Vincent (1970). On the basis of number of nodules giving positive reaction the percentage, nodule occupancy was calculated.

Nitrogenase activity

Nitrogenase activity of nodulated roots given in terms of acetylene reduction was determined by using a gas chromatograph (Model Chemito 3800) with Propak T column and FID.

Results

The nodule number, dry weight of nodules, nitrogenase activity and the dry weight of shoot were signicantly influenced by rhizobial inoculation on 45 DAS (Table 2). A significant increase in the nodule number was registered by strain Glys, followed by strains Bumo and Acni; strain NC92 was equal with Glys, Bumo and Acni. The nodule dry weights due to strains Alle, Ersu, Segr, Bumo and Acni were significantly greater than the absolute control. Both homologous strains NC92 and G2 recorded less nodule weight as compared to the majority of the wild rhizobial cultures. The nitrogenase activity was significantly higher in plants inoculated with strain Bumo. As compared to Acni, Bumo and Glys, both NC92 and G2 strains were inferior in terms of nitrogenase activity. Except for Alle, all other strains increased the shoot dry weight significantly over the absolute control. Both Bumo and Acni strains were significantly superior and registered more shoot weight as compared to homologous strains NC92 and G2. The increase in shoot dry weights due to Glys, NC92, G2 and Ersu strains were comparable with one another.

At 90 DAS the shoot dry weight was significantly affected by the application of

Table 2 Response of groundnut (cv. 'SB-XI') to rhizobial inoculation. (Basal dose of 80 kg P_2O_5/ha was also applied in treatments having *Rhizobium* strains)

Treatments	45 days after sowing				90 days after sowing			Competition
	Number of nodules/ pot	Nodule dry wt. (mg/pot)	Nitrogenase activity (μmol acetylene/g dry wt. nodules/h)	Shoot dry wt. (g/pot)	Shoot dry wt. (g/pot)	Number of pods/pot	Dry pod wt. (g/pot)	(%)
Acni	967.5	246.2	316.6	14.7	16.0	7.5	0.77	50
Alle	713.7	357.6	146.3	7.9	14.2	6.0	0.68	18
Bumo	1007.5	270.1	334.2	15.4	16.3	5.5	0.89	68
Ersu	708.7	305.2	227.2	10.9	15.2	4.2	0.59	37
Glys	1023.7	183.6	316.8	12.7	15.4	11.5	0.86	58
Segr	690.0	283.0	217.3	8.8	13.4	11.0	0.62	27
NC92	852.5	227.3	298.5	12.3	13.9	10.0	0.53	56
G2	638.7	101.8	248.0	11.3	13.6	11.0	0.78	48
*Cc	452.5	211.6	205.5	8.5	12.7	10.0	0.70	48
**Abc	407.5	86.7	117.8	5.7	9.4	4.0	0.32	–
S.E. (±)	64.17	52.21	41.59	0.78	0.78	2.51	0.11	–
C.D. at 5%	186.21	151.51	120.67	2.27	2.25	–	–	–

* Cc = Chemical control (40 kg N/ha + 80 kg P_2O_5/ha).
** Abc = Absolute control.

rhizobial strains (Table 2). All the rhizobial strains and chemical fertilizers increased the shoot dry weight significantly over absolute control. Strain Bumo was significantly superior and produced more shoot weight than NC92 and G2, whereas both Acni and Bumo strains gave more shoot weight over G2 strain of groundnut. The pod number and their dry weights were not significantly affected by different treatments (Table 2). Strain Glys resulted in the formation of a maximum number of pods. Good pod numbers were also seen due to application of strain Segr and strain G2. All the treatments increased the dry weight of pods over the control. Strains Bumo, Glys and Acni showed more pod weight over NC92, whereas only Bumo and Glys strains showed more pod weight than G2.

The competitive ability of the introduced strains over the native population in forming nodules is given in Table 2. The competitive ability ranged from 18 to 68%. The highest competition was found with Bumo (68%), followed by Glys (58%) and NC92 (56%).

The correlation analysis revealed that a significant correlation existed between number of nodules and shoot weight, number of nodules and pod weight, and shoot weight and pod weight (Table 3).

Table 3 Correlation coefficients between different parameters

	Number of nodules	Nodule weight	Nitrogenase activity	Shoot weight	Number of pods	Pod weight
Number of nodules	–	−0.0227	0.5225	0.8770**	−0.3000	0.8092**
Nodule weight	–	–	−0.7569*	0.1472	0.0103	0.0571
Nitrogenase activity	–	–	–	0.3125	0.0909	0.3338
Shoot weight	–	–	–	–	−0.3404	0.9387**
Number of pods	–	–	–	–	–	−0.4000

* Significant at 1% level.
** Significant at 5% level.

Discussion

According to Allen & Allen (1981), the groundnut is nodulated by rhizobia that also nodulate many species of tropical leguminous plants and these rhizobia are classified as the cowpea miscellany. In the light of this statement the use of wild rhizobia in the present study has been justified, because all the strains isolated from tropical arboreal legumes belonged to cowpea miscellany group and were efficient nitrogen fixers as seen in our laboratory studies under aseptic conditions.

Improvement in almost all the characters of groundnut by the wild arboreal rhizobium over the homologous strains and chemical as well as absolute controls is a new observation. The superiority of wild rhizobia over the homologous strains may be attributed to three possible reasons: i.e. the possibility of secretion of some beneficial compounds which may be helping in improving plant nutrition; secondly, the presence of some superior genetic characters in wild rhizobia which may be responsible for improved host–bacterium interaction; and thirdly their competitive ability.

The increase in nitrogenase activity and yield due to rhizobial inoculation in the present study is also supported by the findings of Subba Rao (1976). Increase in shoot dry weight and pod weight over chemical and absolute controls due to rhizobial inoculation in the present study are in agreement with Schiffmann (1961). However, the results showed that pod weight was increased due to rhizobial inoculation which is contradictory to the findings of Arora *et al.* (1970).

The results of correlation coefficient studies clearly revealed that nodule number can, however, be used as a criterion to predict the inoculation response. Similar opinions were put forward by (Nambiar 1985; Wange 1985). The strains which are efficient also showed high competition with the native rhizobia in this study. Consideration of competitive ability especially in case of highly promiscuous legumes such as groundnut and other cowpea group of legumes while screening for the most efficient rhizobial strains was also advocated by Gaur *et al.* (1974).

The three rhizobial strains isolated from wild arboreal legumes viz., Bumo, Glys and Acni possess the two important characteristics of efficient strains such as high nitrogenase activity and competitive ability, essential for obtaining optimum symbiotic association leading to high yield of crop. The results obtained needs further testing and confirmation at field level.

The results of this study thus opens a new area in which superior strains from wild legumes possessing good qualities can be explored and used for improving the yield of cultivated legumes.

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Summary

The rhizobial strain isolated from wild arboreal legumes viz. *Butea monosperma, Glyricidia sepium* and *Acacia nilotica* effectively increased nodulation, shoot weight, pod weight, nitrogenase activity and also showed high competitive ability as compared to the homologous strains NC92 and G2 as well as chemical and absolute controls in groundnut. Thus, the findings of present study open a new field for exploring superior rhizobia from wild legumes, which can be used for improving the yield of cultivated legumes.

Résumé

Réponse de l'arachide (Arachis hypogaea L.) *à l'inoculation par des souches de* Rhizobium *isolées de légumes arboricoles sauvages*

Les souches rhizobiennes isolées de légumes sauvages arboricoles comme *Butea monosperma, Glyricidia sepium* et *Acacia nilotica* ont effectivement augmenté la nodulation, le poids de la racine, le poids des cosses, l'activité de nitrogénase. Elles ont aussi montré une aptitude à la forte compétition par comparaison avec les souches homologues NC92 et G2 de même qu'un contrôle chimique et absolu de l'arachide. Aussi, les résultats de la présente étude ouvrent un nouveau champ pour l'exploration des rhizobia supérieurs de légumes sauvages, qui peuvent être utilisés pour l'amélioration du rendement de légumes cultivés.