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Response of Groundnut (*Arachis hypogaea* L.) to *Rhizobium* Inoculation

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Abstract: Biofertilizers play an important role in increasing yield by scavenging atmospheric nitrogen, increasing phosphorus availability or by promoting growth. Groundnut (*Arachis hypogaea L.*) is endowed with nitrogen fixing ability. Though the beneficial effects of *rhizobium* inoculation in legumes are well documented, there is dearth of information regarding response of groundnut to *rhizobium* inoculation. Seed treatment with different cultures of rhizobia viz. IRG 40, IRG-6, NC-92, NRCG-22, NRC-4 and TAL -1000 was studied in bunch type peanut cultivar, SG-99 under field conditions. Inoculation with IRC-6 strain enhanced number of pink colored nodules, nitrate reductase activity and leghaemoglobin content at 50 days after sowing (DAS). Significant variation in nodule numbers per plant,growth parameters, shelling percentage, kernel weight was recorded with *rhizobium* cultures. Marginal increase in pod yield was recorded with Brady*rhizobium* strains IRG-6(6.8%). Yield increase was due to increase in number of mature pods per plant. Number of gynophores and immature pods increased with NC-92 over uninoculated control.*Rhizobium* strains NC-92, NRCG-22, NRC-4 and TAL -1000 were effective nodulators but could not improve yield. This was probably due to competition from ineffective strains and native strains being active and efficient.

Key words: *Arachis hypogaea* % *Rhizobium* % Strains % Nitrate Reductase % Leghaemoglobin

INTRODUCTION

Biological nitrogen fixation is the major source of nitrogen input in agricultural systems.Rhizobia is symbiotic bacteria that elicit on the roots of specific legume hosts the formation of new organs i.e. nodule, within which the bacteria proliferates, differentiate into bacteroids and subsequently the atmospheric nitrogen into ammonia. Peanut (*Arachis hypogaea L.*) a member of family Leguminosae is usually nodulated by rhizobia of genus Bradyrhizobium as demonstrated byVan Rossum *et al.* [1]. The aim of inoculation is to provide sufficient numbers of viable/effective rhizobia to induce rapid colonization of the rhizosphere whereby nodulation takes place as soon as possible after germination and produce optimal yields according to Cat roux *et al.* [2]. Inoculation of legume seed is an efficient and convenient way of introducing viable rhizobia to soil and subsequently to the rhizosphere of legume. However, an indigenous population of rhizobia is generally present having the potential ability to nodulate an introduced peanut crop. Peanut response to inoculation has been inconsistent. Although several studies showed increased yield following inoculation for details references be made to the studies of Sundara Rao [3] Bajpai *et al.* [4], Iswarans and Sen [5], Nagaraja Rao [6]and Bogino *et al.* [7] .

Yield is typically not increased when peanut is planted in fields that have been rotated regularly with peanut. Experiments conducted by ICRISAT at Panancheru and other centers of the All India Co-ordinated Research Projects on oilseeds (AICORPO) showed that yield response to inoculation was observed,in the fields that had not been planted previously with peanut. Indigenous rhizobia in soil present a competition barrier to the establishment of inoculant strains and may cause inoculation failure. Nodulation of peanut by indigenous bacteria is usually assumed to be adequate and inoculation is seldom practiced. However, survival and effective functitioning of rhizobia population are reduced by high soil temperature, salt, osmotic stress, soil acidity and alkalinity, pesticide and fungicide applications and nutrient deficiency stress as reported by Zahran [8]. Therefore, full potential of the symbiotic system may be realized by increasing the number of effective rhizobia through improved inoculant technology. The efficacy of inoculation depends on several factors all of which affect the number of viable rhizobia available for infection of legume roots. Deaker *et al.* [9] have demonstrated the increased number of viable rhizobia per seed by application of inoculants resulting in a continued linear increase in nodulation and yield. In north India,

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groundnut is grown during summer season in rotation with cereals, fodders, potato etc. in multiple cropping systems under input intensive and assured irrigation conditions but on light textured soils which are generally low in organic carbon. The temperature during major part of its growing season is usually very high to impact survival of bacteria. So far, no systematic studies have been conducted in this region and there exist no recommendation of *rhizobium* inoculation in groundnut. The objective of this study was to find out response of groundnut to *rhizobium* inoculation and to compare the effectiveness of different inoculants. Inoculation of groundnut is not a common practice and the aim of this study was to compare the effects of inoculants on nodulation, growth and yield in peanut.

MATERIALS AND METHODS

Field experiments were conducted at Punjab Agricultural University, Ludhiana in 2006 and 2007 to study the effect of seed treatments with different cultures of Rhizobia viz. IRG 40, IRG-6, NC-92, NRCG-22, NRC-4 and TAL-1000. The cultures were procured from Directorate Groundnut Research, Junagarh, Gujarat. For each treatment, 7 rows each of 5m length were sown at spacing of 30 cm x15 cm on 19 June in 2006 and 5 June in 2007. Treatments were replicated thrice and randomized complete block design was used. The soils of the experimental area were light textured with organic carbon and nitrogen content of 0.25%. For fertilizer application and irrigation schedule Package and Practices were followed.

Preparation of Solution of Culture and Seed Treatment: Inoculations with bradyrhizobium / *rhizobium* were done about 1 hour before commencement of sowing. Solution of 10% jaggery or sugar was prepared in water. For treating 100 kg seed of groundnut, approximately one liter of this solution was sufficient. Two hundred grammes of carrier based culture of bradyrhizobium/*rhizobium* containing 10^9-10^{10} cells /g carriers was added and mixed with 10% cold solution of jaggery or sugar to make slurry .Seeds were spread on the hard floor or polythene sheet and thoroughly mixed with slurry of inoculant strains. For uniform application of inoculation, small batches of seed may be treated.

Nodulation, nitrate reductase and leghaemoglobin randomly selected plants from each replication were carefully uprooted and washed from each treatment at 50 days after sowing (DAS) to record growth parameters, nodulation status and number of gynophores with developing pegs and without pegs. From each treatment nodules were excised and the number with pink and white pigmentation was counted. The activity of nitrate reductase was assayed in leaves and nodules as per method given by Jaworski [10]. The leghaemoglobin concentration of freshly excised nodules was determined by the method of Wilson and Reisenauer [11].

At The Time of Harvest Following Parameters Were Studied:

Growth Parameters: Five plants were selected randomly from each plot. Plant height, number of primary and secondary branches per plant were counted.

Yield Attributes: At 120 DAS /harvest time from randomly selected five plants from each treatment the number of mature or fully developed pods, immature pods and gynophores were counted.

Seeds: After harvesting, the kernels were removed from the pods. Hundred kernels were taken from each replicate randomly and their weight was determined in grams (g).

Seed Yield: Pod yield at harvest was recorded from the net area (3.5 x 5 m) and expressed as kg/ha.

Shelling Percentage: A sample of 250g of pods from the produce of whole plot in each treatment was taken and shelled to determine the shelling percentage calculated as given under:

Weight of kernels/ weight of pods x 100

Oil Content: From the seeds oil content was estimated by the NMR method given by Folch *et al.* [12]. Data on different parameters were subjected to statistical analysis.

RESULTS AND DISCUSSION

Symbiotic Effectiveness of the Bradyrhizobial Strains: Non significant differences have been recorded for the plant height, root length and number of primary and secondary branches with inoculations at 50 days after sowing .Number of leaves per plant were highest with inoculation treatment IRG-6 followed by NC-92. Nodulation has improved with the application of cultures in SG-99 (Table1). Number of nodules per plant were 70.6 with NC-92, 63.2 with IRG-6 and almost comparable in uninoculated control and TAL-1000.Variation in number of gynophores with pegs and without pegs were recorded in the present investigation. Number of gynophores with the pegs were highest with IRG-6 treatment and equal in IRG-40 and NC-92. In rest of the inoculation the number of gynophores without pegs were lower than the control.

Table 1: Effect of culture inoculations on growth parameters and nodulation in groundnut cultivar SG-99 at 50 DAS.

				Number of branches/plant				Number of gynophores	
Tr.No.	Treatments	Plant height (cm)	Root length (cm)	Primary branches	Secondary branches	Number of leaves/plant	Nodules/ plant	With pegs	Without pegs
T1	IRG-40	20.4	9.3	4.5	2.3	53.1	56.2	6.2	7.6
T2	IRG-6	19.9	8.0	4.7	3.1	62.9	63.2	10.4	7.8
T3	NC-92	19.4	9.5	4.5	2.6	59.7	70.6	6.2	9.5
T4	TAL-1000	18.1	9.3	4.2	2.1	43.7	45.3	3.7	6.9
T5	NRC-4	15.2	8.4	4.5	2.1	50.7	31.1	3.96	5.3
T6	NRCG-22	16.5	8.1	4.2	1.6	44.9	48.0	3.97	6.5
T7	Control	21.2	8.4	4.4	2.5	51.1	46.0	4.73	8.3
C.D@ 5%		NS	NS	NS	NS	6.46	4.6	1.37	2.21

Table 2: Effect of culture inoculations on number of pigmented nodules, nitrate reductase and leghaemoglobin content in groundnut cultivar SG-99 at 50 DAS.

		Pigmentation		Nitrate reductase (µmole/h/g F.W)		Leghemoglobin content (mg/g nodule wt.)
Tr.No.	Treatments	Pink	White	Leaves	Nodules	Nodules
T1	IRG-40	23	33.2	0.418	1.30	1.2
T2	IRG-6	28	35.2	0.549	1.78	1.8
T3	NC-92	21	49.6	0.450	1.04	1.4
T4	TAL-1000	17.2	28.1	0.460	1.2	1.3
T5	NRC-4	14	17.1	0.439	1.36	1.5
T6	NRCG-22	18	30	0.408	1.03	1.3
T7	Control	14	32	0.487	1.29	1.2
C.D @5%		0.019	0.493	0.127	0.131	0.02

Table 3: Influence of culture inoculations on their potential to enhance yield and yield attributes in groundnut cultivar SG-99

			No. of branches/plant		No. of pods/plant						
Tr. No.	Treatment	Plant height (cm)	Primary	Secondry	Mature	Immature	Gynophores	Pods yield (kg/ha)	Shelling (%)	100-seed wt.(g)	Oil content (%)
T1	IRG-40	31.1	4.5	1.5	18.2	6.56	12.0	1807.3	65.2	52	48.7
T2	IRG-6	27.3	4.8	1.0	22.8	5.60	9.5	2103.7	66.2	53	46.7
T3	NC-92	31.6	4.8	1.5	24.6	6.57	10.6	1974.0	65.8	54	49.3
T4	TAL-1000	28.6	4.5	1.2	20.7	9.20	14.4	1882.3	65.0	51	46.6
T5	NRC-4	30.0	4.0	1.06	21.1	6.06	8.46	1907.7	64.0	51	46.2
T6	NRCG-22	26.0	3.2	1.1	18.0	5.60	9.16	1832.6	64.5	46	46.3
T7	Control	33.5	4.4	1.46	14.4	4.8	8.70	1970.3	67.2	46	46.4
C.D@5%		1.85	0.586	0.227	2.49	0.789	1.46	38.4	1.54	1.77	1.56

Only pink and white pigmented nodules were observed at 50DAS. IRG-6 showed effective pink pigmentation followed by IRG-40. Number of white pigmented nodules were higher than the pink pigmented nodules (Table 2). Numbers of white pigmented nodules were maximum in NC-92 and least in NRC-4. IRC-6 inoculation recorded highest nitrate reductase activity both in the leaves and nodules over the uninoculated control. Significant variation for nitrate reductase activity and leghaemoglobin content was observed with the culture inoculations in SG-99, a bunch type variety of groundnut. Leghaemoglobin content was highest with IRG-6 followed by NRC-4 over the control.

Yield and Yield Attributes: Plant height did not improve with the inoculation treatments but the number of branches on per plant basis varied significantly at physiological matutity. Number of primary branches were 4.8 with NC-92 and 4.5 with IRG-40 and TAL-1000.Number of secondary branches were 1.5 with IGR-40 and NC-92 inoculations as compared to the control. However, the numbers of primary and secondary branches were higher than the uinoculated control in NC-92. Number of mature pods were higher in all the treatments over the control. Mature pods were > 20 with IRG-6, TAL-1000 and NRC-4. Immature pods were highest in TAL-1000 and equal in number with IRG-40 and NC-92. Gynophore number was

highest in TAL-1000 followed by IRG-40.However the number of gynophores were less than the control withNRC-4.

Pod yield (2104 kg/ha) with IRG-6 inoculation was significantly higher (6.8%) than the control. 100-seed weight was 54 g with NC-92, 53 with IRG -6 and comparable in NRCG-22 with the uninoculated control. This is in accordance with the findings of Chetti *et al.* [13] that had significant beneficial effect on 100 seed weight and pod yield. The variation in pods per plant, seed weight, shelling percent and pod yield due to different treatments were statistically significant. (Table 3). The maximum oil content was recorded with NC-92 followed by IRG-40 than without inoculation (46.4%). Not much variation was found with other cultures as depicted in Table 3.

Pod yield improvement with IRG-6 and NC-92 was due to the increase in number of primary branches, mature pods, 100-seed weight, more nodules with pink pigmentation, higher activity of nitrate reductase and leghaemoglobin content. *rhizobium* strains IRG-40, NRCG-22, TAL1000 were effective nodulators but could not improve yield over uninoculated control in SG-99. Lack of response to inoculations may be because of several factors, adequate nodulation by indigenous rhizobia,unfavourable conditions for survival of introduced rhizobia strains and inability of inoculant strains to compete with endogenous strains for nodule sites. Natural nodulation was adequate as evidenced by the number of root nodules on the plants in uninoculated plots. In this case it appears that the inoculant strains were not sufficiently competitive or more precisely, that the indigenous rhizobia were already maximally effective .Further research will determine the effectiveness of alternative inoculant strains and conditions for improving groundnut production.

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