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STUDIES ON SOIL AND RHIZOSPHERE POPULATIONS OF *RHIZOBIUM* sp. NODULATING *CICER ARIETINUM*[1]

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Abstract

Using a plant infection dilution method developed at ICRISAT for counting chickpea rhizobia the population of chickpea *Rhizobium* has been studied in Alfisols, Vertisols and Entisols. Some of the soil samples collected from traditional chickpea growing areas have been found to have low populations of this specific *Rhizobium*. *Rhizobium* numbers decreased with depth in a Vertisol from 10^4 per g soil at 0-15 cm to 10^2 per g soil at 90-120 cm. A 100-fold decrease in population was recorded when wet-land paddy followed chickpea. Of five ICRISAT mandate crops (sorghum, millet, groundnut, chickpea and pigeonpea) in pot culture, chickpea roots are most stimulatory to the multiplication of chickpea rhizobia.

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Introduction

The chickpea Rhizobium is very specific although it occasionally nodulates Sesbania bispinosa and S. sesban (Gaur and Sen, 1979)[1]. There has been a paucity of data on numbers of chickpea rhizobia in soils because of non-availability of a suitable counting method. Population data are likely to serve as an effective tool in predicting inoculation responses in the field. With the development of a method of estimating the most probable number of chickpea rhizobia at ICRISAT by Toomsan et al, 1979[2], we examined the population of chickpea rhizobia in various soils in relation to crop patterns in Central and Northern India.

Materials and Methods

Chickpea Rhizobium in different soils

Vertisol fields at ICRISAT were surveyed in September 1981 (Table 1). Soil samples were taken with a Veihmeyer metal coring tube of 4 cm diameter. The soil samples were collected in fresh polythene bags and stored at 4°C until the time of counting (within 2-3 weeks of sampling). For survey purposes (Table 1) 10 spots were sampled from about a hectare in each field and the samples pooled, mixed and a representative sample used for measurements. Fields in Hissar (Entisol, latitude 19°N) were sampled in October 1979 using a local implement (khurpi) which had about 4" wide cutting front. A 15 cm deep V-shaped notch was made at the sampling spot and a 2-3 cm slice of soil was sampled from the side wall of the notch. Six to ten spots scattered over a field of about 0.1 ha

)le 1: Rhizobium populations in different types of semi-arid tropic soils (top 15 cm)

Field	Vertisol (ICRISAT)	Alfisol (ICRISAT)	Entisol (Hissar)	Alluvial soil (Gwalior)
		$\log_{10}$ MPN[a]		
1	2.68	1.28	3.46	3.58
2	2.34	1.28	3.26	2.94
3	2.68	< 1.0	4.59	3.94
4	3.04	< 1.0	3.60	1.94
5	3.84	< 1.0	3.60	2.94
6	3.34	< 1.0	3.61	2.25
7	2.04	< 1.0	2.60	2.94
8	< 1.0	0.40	6.26[b]	0.94
9	4.38	-	-	2.24
10	2.03	-	-	0.95
11	4.74	-	-	1.25
12	3.57	-	-	2.95

a = most probable number estimated by a soil dilution plant infection technique

b = field under 130 day old chickpea crop.

each comprised a sample for measurements. These samples were brought to ICRISAT at Hyderabad and processed for most probable number (MPN) count within six weeks of sampling after storage in a refrigerator (4-10°C). Soil samples from Gwalior [Alluvial soil (Raychaudhuri et al 1963[3]) latitude 26°N] were collected from farmers fields by co-operators who sent them to us by mail. These samples were also stored at 4-10°C until processing within six weeks of sampling.

For soil sampling over depth in Vertisol fields, a 6 cm diameter Gidding Hydraulic Coring machine mounted on the back of a Land-rover was used. Soil samples from other fields were taken by manual drawing of a 4 cm diameter Veihmeyer tube which had to be extracted by digging. The metal coring tube had a side slit to facilitate removal of soil core. The soil core remained intact when brought out of soil profile and divided into desired lengths (say 0-5, 5-15 etc.). Sampling of paddy field was restricted to the top 15 cm.

Soil samples were broken into small pieces by hand or mortar, pestle, before grinding in grinders sterilized with 75% ethanol between samples.

Rhizosphere effect on chickpea Rhizobium population

The experiment was conducted on both Alfisol and Vertisol soils in a glasshouse during mid-July to end of August, 1980 with ambient temperatures 25-30°C. Soil from the top 15 cm was collected, ground and sieved through 4 mm sieve to remove grit and gravel. Four kg. soil was placed in 18 cm diameter pots

and water holding capacity of soils was determined. For each soil, 3 replicate pots sown to 10 seeds/pot of groundnut (cv. TMV-2), pearl millet (cv. MHB-3), sorghum (CSH-6), pigeonpea (cv. ICP-1), and chickpea (cv. K-850). An unplanted set with germinating weeds removed served as fallow treatment. The Alfisol field had no previous history of chickpea cultivation and a plant dilution infection count found less than 10 chickpea Rhizobium per gram dry soil. Each Alfisol pot was inoculated with Rhizobium strain 9036 at the rate of 1.2×10^7 cells/pot at sowing by suspending 1 g of peat inoculum (2.4×10^9 cells/g peat) in 1 litre of tap water, shaking vigorously and watering 5 ml of this suspension on to each pot. The plants were thinned to 3 per pot within a week of emergence. Three weeks after planting the pots were watered to 80% of their estimated water holding capacity and then watered once a week to the same level. The plants were harvested 6 weeks after planting and separated into shoots and roots. The soil was emptied from the pot into an alcohol-sterilized tray and the roots carefully removed. The soil attached to the root was considered to be the rhizosphere soil and the remainder to be bulk soil. Nodules were carefully removed from the roots of chickpea, groundnut and pigeonpea using a pair of scissors to cut the nodule and part of the attached root. For the most probable number estimate (MPN) of chickpea rhizobia, all the roots from a pot were put in a plastic bag, 180 ml sterilized tap water added, and shaken in 'Colworth' 400 Stomacher for 5 minutes. The soil suspension was then diluted

in a tenfold series and one ml from each dilution used to inoculate plants in each of 3 test tubes. Roots were then separated from the suspension, washed and dried at 70°C. The dry weight of the rhizosphere soil was determined by drying the suspension at 105°C in an oven before weighing.

For estimating the MPN of chickpea rhizobia per g of dry soil, 20 g of soil was added to 180 ml of sterilized tap water and shaken for 2-5 minutes on a horizontal shaker or a Stomacher before serial dilution as above.

The plants were grown in a light chamber with 16 hr day (9000-12000 lux) at <30°C and observed for presence or absence of nodules after 6-7 weeks. The MPN (Fisher and Yates, 1963)[4], was calculated as described by Toomsan et al, 1979[2]. The MPN count was expressed on dry soil or dry root basis.

Results and Discussion

The populations of chickpea rhizobia in the top 15 cm soil have varied widely. Chickpea is not generally grown in Alfisols in India and low numbers (<10 rhizobia per g dry soil) were found (Table 1). However once introduced by growing inoculated chickpea they can survive in reasonable numbers for over two years even in the absence of its specific host (unpublished data). Chickpea has been grown for centuries in Vertisols in South and Central India and in Entisols in North India including Hissar

and Gwalior. The Rhizobium populations in these soils are expected to have been established very well before the systematic production of legume inoculants started in India in the early sixties (Sahni, 1976)[13]. Most fields (Table 1) sampled in these areas have population ranging 10^3 to 10^5. Limited work done at ICRISAT on the effect of storage temperature on chickpea Rhizobium population in Vertisols strongly indicates that transit conditions and six week storage at ambient temperature would not have affected the Rhizobium survival. It is surprising to note (Table 1) that about 33% fields in Gwalior have population level of 100 or less although they are traditional chickpea growing areas. These fields were found to be normal for pH (8-8.3), electrical conductivity (.15 to .22 m mhos/cm^2) of soil when measured. We are now looking at the numbers in different soils in relation to soil pH, soil moisture and soil salinity which are known to affect survival of rhizobia (Richmond 1926[5], Wilson 1930[6], Foulds 1971[7], Chatel and Parker 1973[8], Pillai and Sen 1966[9]).

Numbers of chickpea rhizobia declined with depth in both Alfisols and Vertisols (Table 2). The numbers were highest at 5-15 cm and lowest but still present at 90-120 depth. Although in Vertisols at ICRISAT most of the chickpea nodules are confined to 0-15 cm soil profile (unpublished data) rhizobia specific to chickpea live in adequate numbers at more than a meter depth (Table 2). Nodules have been seen even at 60 cm depth in Entisol at Hissar and about a meter deep in pigeonpea in Vertisols at ICRISAT (J.V.D.K. Kumar Rao, personal communication). It should be interesting to study the reasons for failure of nodulation of chickpea at 30 cm and below.

Table 2: Changes in chickpea *Rhizobium* population with soil depth at ICRISAT

Soil depth (cm)	Vertisol	Alfisol
	$\log_{10}$ MPN/g dry soil	
0 - 5	4.62	4.60
5 - 15	5.34	4.71
15 - 30	3.85	3.96
30 - 60	3.81	3.69
60 - 90	2.53	2.65
90 - 120	2.13	2.43

The population of Rhizobium through the season was followed in fields of each of three different soil types, Alfisol, Vertisol and in paddy field. The changes in numbers with time and crop history are presented in Table 3. Irrespective of the crop, chickpea Rhizobium population remained reasonably constant throughout the year in a Vertisol and in an Alfisol after introduction of Rhizobium. Population levels were found to be greatly different with sampling time in a transition soil under paddy (Table 3). The numbers were lowest before chickpea planting and highest after harvest of chickpea which had been inoculated with Rhizobium. The numbers declined during summer fallow and further declined when paddy was being grown. Waterlogged conditions might have played a role in this reduction as some recovery was observed in November (Table 3). The population of Rhizobium leguminosarum was found to be greatly reduced due to flooding of pots for two weeks by Vandecaveye, 1927[10]. Kumar Rao et al 1981[11] have also reported low population of cowpea group of rhizobia in soils under paddy.

The effect of rhizosphere on chickpea Rhizobium population was studied in pot culture using an Alfisol and a Vertisol soil. Nodulation of three legumes, chickpea, pigeonpea and groundnut was normal in both soils. The root weights of three legumes were

Table 3: Changes in chickpea Rhizobium population in the top 15 cm over time, ICRISAT centre.

Field	$\log_{10}$ MPN per g dry soil				
	Jan.	March	June	Aug.	Nov.
Vertisol	3.49 (after maize harvest)	4.98 (after chickpea harvest)	3.67 (during fallow)	4.13 (during fallow)	3.94 (standing chickpea)
Alfisol	4.85 (standing inoculated chickpea)	4.71 (standing groundnut)	4.24 (after groundnut harvest)	4.64 (standing pigeonpea)	4.21 (standing pigeonpea)
Paddy field	0.40 (after paddy harvest)	4.00 (after chickpea harvest)	2.36 (during fallow)	1.75 (standing paddy)	2.98 (after paddy harvest)

also similar in both soils. Root weights of sorghum and millet were slightly more in the Vertisol as compared to Alfisol. The numbers of chickpea rhizobia were highest in chickpea rhizosphere and significantly different from the other five crops in Alfisol soil (Table 4). The Rhizobium numbers colonising on roots of other crops were not significantly different. The numbers per g rhizosphere soil was also significantly higher in chickpea. No stimulation was seen in millet rhizosphere, while groundnut and pigeonpea roots stimulated the population 10-fold. In the non-rhizosphere soil of the pots no significant differences were seen, whether cropped or left fallow.

In Vertisol soil, the numbers of chickpea and groundnut rhizobia were significantly higher than numbers on roots of other three crops (Table 4). The numbers followed the same trend in an Alfisol with chickpea highest (4.0×10^5) followed by groundnut and the other three crops were not significantly different from each other. The numbers in non-rhizosphere soil of different crops did not differ from each other or from the fallow pots. All the crops had a stimulatory rhizosphere effects on chickpea Rhizobium. (90, 59, 6, 12 and 22 fold for chickpeas, groundnuts, pigeonpea, sorghum and pearl millet respectively). This may explain the survival of chickpea rhizobia in soils where chickpea has not been grown for a long time. It also gives a hope of introducing chickpea rhizobia with a preceding cereal.

Table 4: The number of chickpea rhizobia per g dry root, rhizosphere non-rhizosphere and fallow soil of five ICRISAT's mandate crops.

Crops	Alfisol			Vertisol		
	Root	Rhizo-sphere soil	Non-rhizo-sphere soil	Root	Rhizo-sphere soil	Non-rhizo-sphere soil
			$\log_{10}$ MPN			
Chickpea	7.37[a]	5.56[a]	3.95[a]	6.86[a]	5.60[a]	3.65[a]
Groundnut	5.39[b]	3.86[b]	2.86[a]	6.43[a]	5.10[a]	3.33[a]
Pigeonpea	5.53[b]	3.96[b]	2.95[a]	5.13[b]	4.24[b]	3.45[a]
Sorghum	5.68[b]	3.83[b]	3.11[a]	5.44[b]	4.09[b]	3.01[a]
Pearl Millet	4.62[b]	2.91[b]	2.89[a]	5.47[b]	4.23[b]	2.89[a]
Fallow	-	-	2.43[a]	-	-	3.13[a]

a-b Means within column followed by same letter are not significantly different by Duncan's new multiple range test at P = 0.05

Diatloff 1969[12] has tried to establish soybean rhizobia with a preceding wheat or non-host legume crops.

From the work reported here, it appears that fields with low numbers of chickpea rhizobia can exist even in traditionally chickpea growing states though they are not new lands under plough. It is a common practice to grow a legume after the main crop of paddy, if water is limiting, in large areas in Bihar, Orissa, UP and MP in India (B.M. Sharma, personal communication). Responses to inoculation in soils (including paddy soils) with poor native Rhizobium population have been obtained at ICRISAT (ICRISAT 1977[14], ICRISAT 1978[15]).

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DISCUSSION

K. Kavimandan : Why did you remove cotyledons and do chick pea cotyledons produce nodule inhibiting substances?

P. Rupela : To shorten plant size so that it could be accomodated in a tube of size 200 x 25 mm. We don't think that cotyledons produce nodule inhibiting substances. We do not have any evidence.

. Bhagwat : With the recent reports on autoregulation of nodulation by some legumes, how is MPN of Rhizobia relevant for less nodulation below 12-15 cm in the soil?

.P. Rupela : Autoregulation model proposed from Kettering laboratory, I think, is of relevance in growth pouch culture studies and I don't think it can be extended to natural conditions in the soil.

.A. Bhagwat : Why is such an effect not observed in Haryana soils?

.P. Rupela : There can be a requirement of specific oxygen tension for nodulation which is provided by light (sandy loam) soils of Hisser and not by heavy soils (vertisols) at ICRISAT.

.K. Kavimandan : Just by number of rhizobia how can you predict whether inoculation will respond or not? Even one cell of rhizobia can be stimulated by the plant.

Rupela : We say that the method has a potential in predicting responses in a given field. This ofcourse needs to be tested. A strong possibili is indicated from the Rhizobium inoculation trials (12 in numbers) at ICRISAT. We could obtain responses only whem soil rhizobia were less than 100 per gram of soil.

K. Kavimandan : How many nodules were produced by inoculated strains?

P. Rupela : Nodule numbers per plant with and without Rhizobium inoculation would obviously be varying with the trial. To give you an idea, in one of the trials in a field with less than 100 rhizobia per g of soil we had 4-5 nodules per plant without Rhizobium inoculation and 16-18 nodules per plant with Rhizobium inoculation.