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CHICKPEA *RHIZOBIUM* POPULATIONS: SURVEY OF INFLUENCE OF SEASON, SOIL DEPTH AND CROPPING PATTERN

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Summary—Chickpea *Rhizobium* populations in soil samples from research stations and farmers' fields in different geographic regions of India ranged from <10 to $>10^4$ rhizobia g^{-1} soil. Fields on research stations with a known history of chickpea cropping had more rhizobia (calc. 10^3 to 10^5 rhizobia g^{-1} soil) than the majority of farmers' fields (calc. <10 to 10^3 rhizobia g^{-1} soil). In the absence of chickpea in the cropping pattern, soils generally had $<10^2$ rhizobia g^{-1} and crops in such fields nodulated poorly. However, poor nodulation was also observed when populations of rhizobia were high, indicating that other factors were also important for nodulation. There was no obvious consistent correlation of *Rhizobium* population with pH, electrical conductivity and nitrate–nitrogen status of the soil.

Rhizobium populations declined with soil depth and were highest (about 10^4 rhizobia g^{-1} soil) in the top 30 cm of the profile and lowest, but still present (calc. 10^2–10^3 rhizobia g^{-1} soil), at 90–120 cm—a depth where no nodules are found. Populations fluctuated most in the top 5 cm, being reduced during periods of high soil temperature in summer and recovering after rains. *Rhizobium* populations were at a maximum after chickpea but survived well under pigeonpea, groundnut and maize. When rice followed an inoculated chickpea crop, there was about a 100-fold decrease in the *Rhizobium* population.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most widely grown grain legume in the world, with an annual production of 6.5×10^6 t from 9.8×10^6 ha (FAO, 1985) over a wide range of climatic conditions. In India, chickpea is grown in the post-monsoon, winter season (rabi), from September–October until February–March, on receding residual soil moisture. Chickpeas may have been introduced to India around 600 B.C. (Ramanujam, 1970; cited from van der Maesen, 1972) and we would expect that *Rhizobium* populations in Indian soils, with a history of chickpea cultivation, would have been established long before the systematic production of legume inoculants began in India (Sahni, 1977). Chickpea *Rhizobium* is very specific (Raju, 1936), although it occasionally nodulates *Sesbania bispinosa* and *S. sesban* (Gaur and Sen, 1979).

The little information available on soil populations of rhizobia and how they can be affected by crop, soil type, cultivation and soil chemical properties has been reviewed by Parker *et al.* (1977). Rhizobia proliferate better in the rhizosphere of the host than that of other crops (Toomsan *et al.*, 1983; Reyes and Schmidt, 1979). Acidic soils support low populations of *Rhizobium trifolii*, which increased with liming (Coventry *et al.*, 1985) apparently due to increased soil pH. Intensive cereal cropping and high N-fertilization resulted in low population and less effectiveness of the native *R. trifolii* in the soil (Martensson *et al.*, 1984). Such information on chickpea rhizobia is lacking, due to the non-availability of a suitable method of estimating the population of native soil rhizobia in the presence of other organisms. However, a technique has now been developed (Toomsan *et al.*, 1984) to estimate the number of chickpea rhizobia.

Our objective was to survey fields in various geographical regions in India to assess the chickpea *Rhizobium* populations. The effects of season, soil depth and cropping pattern on the size of the chickpea *Rhizobium* population was also investigated.

MATERIALS AND METHODS

Soil sampling for Rhizobium

Soil samples were collected from a wide range of soil types and locations (Table 1). Samples were obtained with a 2.5 cm dia. tube at Morena and a 4 cm dia. tube elsewhere. The top 15 cm of the soil profile was sampled in all places except in the sandy soils of Haryana and Rajasthan, where samples were taken from the top 30 cm, because chickpea is generally sown deeper than 10 cm in these soils. Random samples were collected from several points in each field (Table 1). Field size was usually about 0.1 ha, except at ICRISAT Center where it was 1 ha. Soil samples from each point in a field were bulked, broken into pieces smaller than 1 cm and subsampled for the different analyses. Although chickpea had been traditionally grown in the regions visited, some of the sampled fields had not had any recent history

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Table 1. Data for the locations sampled for MPN estimates of chickpea *Rhizobium*

Location and latitude in India	Soil type	Date of collection	Range of soil pH	Range of electrical conductivity	Number of fields sampled	No. of points per bulked sample
Haryana Agricultural University (HAU), Haryana, 29°N Hisar,	Sandy loam (Entisol)	March 1980	7.7–8.3	0.15–0.28	10	6–10
Farmers' fields in Haryana* and Rajasthan,* 28°N	Desert sand	February 1982	7.7–8.8	<0.15–0.20	26	5
Agricultural Research Station (ARS), Morena,* Madhya Pradesh (MP), 26°N	Loam	June 1982	6.4–8.1	<0.15–0.58	18	5
Farmers' fields around Gwalior*, Madhya Pradesh (MP), 26°N	Sandy loam,	January 1982	6.4–8.8	<0.15–0.31	23	5
ICRISAT Center,* Patancheru, Andhra Pradesh (AP), 17°N	Black clay (Vertisol)	September 1981	7.7–8.6	<0.17–0.40	28	10
	Red soil (Alfisol)†	September 1981	6.3–7.8	<0.15	7	10
	Transition soil†	September 1981	7.9–8.5	0.19–0.29	6	10

*Key words used in the text to indicate different locations.
†Since both types of soils traditionally do not grow chickpea the data in Fig. 1 have been presented together as Alfisol.

of chickpea. In a standing crop of chickpea, samples were taken in the middle of two plant rows that were at least 30 cm apart, and roots and nodules were removed from the sampled soil, if any, before they were analyzed for MPN. Unless otherwise indicated, none of the sampled fields had been deliberately inoculated with *Rhizobium*.

Survey

During the two survey trips to farmers' fields in Madhya Pradesh (Gwalior region), Haryana and Rajasthan, soil and chickpea plants were sampled from fields at a predetermined regular distance. At each stop, fields with an apparently normal chickpea crop, depending on age, etc., was selected for sampling. In Gwalior, only the fields where the farmer was available to provide the field history were sampled. This was generally possible within 2 km of a predetermined stop. The distances between stops was 10–20 km in Gwalior and 10–80 km in Haryana and Rajasthan. All the fields sampled in Haryana and Rajasthan had sandy soils and discussion with farmers revealed that chickpea cultivation was highly dependent on good and timely rainfall, which has a low probability in this region (Sarkar *et al.*, 1978).

Assessment of nodulation

About 10 plants in the Gwalior region and at least 20 plants in the Haryana and Rajasthan regions were carefully uprooted from each field with a crowbar to recover maximum roots and nodules. The uprooted plants represented at least four randomly selected spots in a field. Observations on nodule colour and numbers were made after washing the root system whenever required.

Soil sampling over depth at ICRISAT Center

Three fields, one each on a Vertisol, Alfisol and a transition soil (probably a fine mixed hyperthermic deep Aquic Ustorthent) (Soil Survey Staff, 1975), each cropped for at least 8 yr, were sampled at various depths on several occasions. A 6 cm dia. tube, with a side slit to assist in the removal of the soil core, was driven to the desired depth with a hammer or a hydraulic coring machine (Giddings Machine Co., Fort Collins, Colorado, U.S.A.). With the machine it was possible to take out a complete soil core in one attempt. The complete core was then cut into pieces of the desired length and the pieces placed in separate polythene bags. All samples were analysed separately without bulking.

Preparation of soil samples for Rhizobium *estimation and chemical analysis*

Soil samples were broken into small pieces by hand when wet, or they were ground in a mortar and pestle when dry, taking the necessary precautions to prevent cross-contamination of soil samples. A portion of each soil sample was air-dried in the shade, ground further and sieved (< 2 mm) before chemical analysis.

For MPN estimates of rhizobia, samples were stored at 4°C without drying and processed within 2 weeks, except those received from Hisar that were stored for 4 weeks and, in these cases the populations may not have been affected by this storage period (B. Toomsan, unpublished Ph.D. thesis, University of Manitoba, Canada, 1981). A 20 g soil sample was suspended in 180 ml of sterile tapwater and shaken on a wrist-action shaker for 10 min or on a reciprocal shaker for 5 min. A serial 10-fold soil dilution series was then made and 1 ml aliquots used to inoculate cotyledon-free chickpea seedlings growing in 200 × 25 mm test tubes with three tubes per dilution (Toomsan *et al.*, 1984). Observations on the presence or absence of nodules in each test tube were made after 40–45 days and were used to calculate the MPN on the basis of the weight of oven-dried soil.

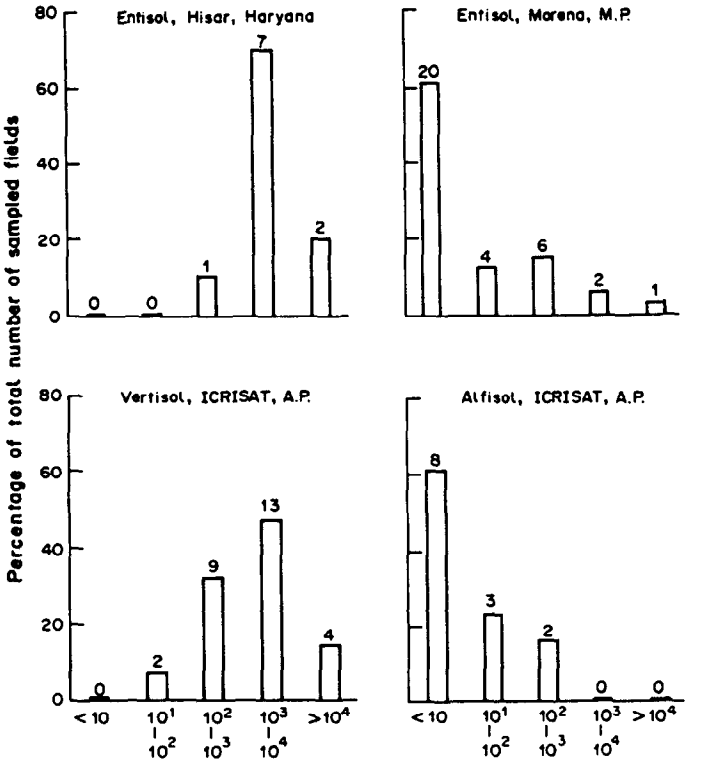

Fig. 1. Distribution of chickpea *Rhizobium* populations in fields at 3 research stations in India. Figures on the bar are the numbers of fields observed.

Soil pH and electrical conductivity (EC) were measured in a 1:2 soil:water suspension. The phenol disulphonic acid colorimetric method was used for determination of NO_3-N (Jackson, 1973).

RESULTS

Rhizobium *populations at research stations*

At Hisar, where chickpea has been extensively grown for many years, rhizobia were plentiful in all fields, with 9 of the 10 fields carrying at least 10^3 rhizobia g^{-1} (Fig. 1). The five fields growing chickpea at the time of sampling generally had higher populations than the other fields, perhaps due to a beneficial rhizosphere effect on rhizobia (Reyes and Schmidt, 1979).

At Morena, where chickpea cultivation commenced only in October 1981, most of the fields carried < 10 rhizobia g^{-1}. Eight of the 9 fields that had $> 10^2$ rhizobia g^{-1} soil, had been cropped in the previous season (Fig. 1).

At ICRISAT Center, in common with other areas in southern India, the traditional soils for chickpea cultivation are Vertisols, and most of these soils were well populated. Only two of the 28 Vertisol fields had $< 10^2$ rhizobia (Fig. 1). However, the Alfisol, which is not suitable for the crop because of its low water storage capacity (Russell, 1980), had few chickpea rhizobia.

Rhizobium *populations and nodulation in farmers' fields*

In Rajasthan, a majority of the fields yielded < 10 rhizobia g^{-1} and the few with high populations were those cropped with chickpea. It is notable, however, that many cropped fields also had low populations (Table 2).

Table 2. Numbers of farmers' fields with different populations of chickpea rhizobia (and range of nodules plant^{-1})

Region	Chickpea cultivation at sampling	*Rhizobium* population g^{-1} soil					Total fields
		< 10	10–10^2	10^2–10^3	10^3–10^4	$> 10^4$	
Rajasthan	+	9 (0–6)	2 (1–9)	2 (3–11)	0	1 (7)	14
	–	3	1	0	0	0	4
Haryana	+	0	0	4 (5–18)	3 (7–19)	1 (10)	8
	–	0	0	0	0	0	0
Gwalior	+	1 (<1)	1 (7)	2 (3–5)	4 (5–29)	1 (11)	9
	–	3	2	1	3	0	9

Table 3. Changes in chickpea *Rhizobium* population with time and soil depth in a Vertisol field at ICRISAT Center

Depth (cm)	Log_{10} MPN g^{-1} dry soil				
	November 1978 (after maize)	March 1979 (after chickpea)	June 1979 (fallow)	August 1979 (fallow)	December 1979 (standing chickpea)
0–5	3.49	4.62	3.78	3.91	3.89
5–15	3.49	5.34	3.55	4.34	3.99
15–30	3.32	3.85	3.65	4.30	3.75
30–60	ND	3.81	3.28	3.86	3.52
60–90	ND	2.53	2.73	3.31	2.74
90–120	ND	2.13	2.10	3.02	2.23
SE	±0.14	±0.21	±0.23	±0.16	±0.24

ND = Not determined.

All farmers' fields sampled in Haryana grew chickpea and all had populations $>10^2$ g^{-1} (Table 2). The trend was similar to that at Hisar Research Station (Fig. 1), which is situated in the chickpea growing area of Haryana state.

At Gwalior, the situation was intermediate; 7 of the 18 farmers' fields had $<10^2$ rhizobia g^{-1} soil and two of these were reclaimed from ravines and did not have a history of chickpea cropping (Table 2). The results suggest that the numbers could be low even in the presence of a crop obviously because of lack of rhizobia in the soil before sowing chickpea.

Poor nodulation (<10 plant^{-1}) was generally seen in fields with $<10^2$ rhizobia g^{-1} soil, and moderate to good nodulation in fields with about 10^3 rhizobia g^{-1} soil (Table 2). However, fields with high population (10^3 rhizobia g^{-1} soil) having poorly nodulated plants were also seen. But none of the fields having $<10^2$ rhizobia g^{-1} soil had good nodulation.

Soil chemical properties

Chickpea was either known to grow on all the 84 research station fields (Fig. 1) and 44 farmers' fields, or the fields were apparently suitable for growing chickpea. The pH in these fields ranged from 6.4 to 8.8, EC from <0.15 to 0.58 (mmhos cm^{-1}) and NO_3–N from <0.15 to 26 μg g^{-1} (Table 1). Fields with such levels of pH and EC have been seen growing a normal chickpea crop.

Rhizobium populations at various depths and times

Populations of *Rhizobium* at various depths were measured for three fields at ICRISAT Center on five different occasions. In each field, the numbers of rhizobia declined with depth (Tables 3, 4 and 5).

In the Vertisol, there was no significant change in the population at 0 to 30 cm in the profile when sampled in November 1978 (before sowing chickpea); but, in the presence of chickpea during November to March, the numbers at 0 to 15 cm increased about 100-fold (Table 3). The numbers at 15 to 30 cm depth were not greatly affected by chickpea cropping. *Rhizobium* populations generally decreased beyond 60 cm and were lowest below 90 cm at all samplings. Maximum numbers were present in March, just after the chickpea harvest, but decreased during the dry summer (June sampling). The slight increase during the rainy season in June–July did not persist until the December sampling.

Inoculated chickpea was sown in the Alfisol in July 1978 (Table 4) and, although populations were not measured, the absence of nodules from uninoculated

Table 4. Changes in chickpea *Rhizobium* population with time and soil depth in an Alfisol field at ICRISAT Center

Depth (cm)	Log_{10} MPN g^{-1} dry soil				
	January 1979 (first inoculated chickpea in July 1978)	March 1979 (standing groundnut)	June 1979 (after groundnut)	August 1979 (standing pigeonpea)	December 1979 (standing pigeonpea)
0–5	4.87	4.81	4.48	4.73	4.52
5–15	4.83	4.61	4.00	4.55	3.89
15–30	4.36	3.89	3.87	4.02	3.96
30–60	ND	3.61	3.01	3.11	3.16
SE	±0.22	±0.23	±0.18	±0.17	±0.18

ND = Not determined.

Table 5. Changes of chickpea *Rhizobium* population with time and soil depths in a transition soil field at ICRISAT Center

Depth (cm)	Log_{10} MPN g^{-1} dry soil				
	January 1979 (after rice)	March 1979 (after inoculated chickpea)	June 1979 (fallow)	August 1979 (standing rice)	December 1979 (after rice)
0–5	0.43	3.94	2.54	1.75	2.87
5–15	0.32	4.06	2.19	1.75	2.98
15–30	1.25	3.57	1.42	ND	0.92
30-60	ND	3.08	0.45	ND	0.82
SE	±0.45	±0.26	±0.28	0	±0.17

ND = not determined.

control plots suggested an absence of native rhizobia. The number of rhizobia in January, following the inoculated chickpea crop, was $>10^4$ g^{-1} dry soil in the top 30 cm and showed a 2.5–7-fold decrease in the top 15 cm by June. At each subsequent sampling from March to December, numbers of rhizobia declined with depth and were substantially fewer at 30–60 cm.

In the transition soil, where rice was grown, the initial population in the 0 to 15 cm profile was <10 rhizobia g^{-1} soil (Table 5). However, in March 1979, following the harvest of inoculated chickpea, the numbers markedly increased. In June the numbers decreased, but a further marked decline was observed in the rainy season when the field was flooded for rice cultivation and the soil was waterlogged. A 15-fold increase was seen after rice, but the population remained at $<10^3$ g^{-1} dry soil.

DISCUSSION

This is the first study of chickpea *Rhizobium* populations in soils in regions where chickpeas have been grown for centuries. The largest populations of chickpea *Rhizobium* were observed in fields at Hisar with a long history of chickpea cultivation. However, at Morena, in the absence of the chickpea host, the native population was generally 10^2 g^{-1} soil. Vegetables were grown at the Morena farm until 1980 and the large number of fields with low numbers is probably related to the absence of chickpea in the cropping pattern. A similar trend was noticed with Alfisols and Vertisols at ICRISAT Center, which are located adjacent to each other. The frequent absence of rhizobia in the Alfisol did not seem to be due to an unfavourable environment because the Alfisol field which grew a well-nodulated crop of chickpea in 1977 and 1978 (Table 4) had about 10^3 rhizobia g^{-1} dry soil even 6 yr later in 1985, without an intervening chickpea crop. Such Alfisol fields contain about 10^4 g^{-1} soil of cowpea group rhizobia nodulating siratro (*Macroptilium atropurpureum*) (Kumar Rao *et al.*, 1982).

Some Alfisol fields at ICRISAT Center contained no recoverable chickpea *Rhizobium*, even though they were within 200 m of Vertisol fields containing 10^3–10^5 rhizobia g^{-1} soil. This suggests that there is relatively little aerial movement of *Rhizobium*, even though the prevailing winds are in a direction favouring dust movement to the Alfisol fields. This did occur, particularly during land operations with heavy machinery. Perhaps the rhizobia, carried with the dust, die as a result of desiccation and high temperatures (Boonkerd and Weaver, 1982; Pena-Cabriales and Alexander, 1983).

The large difference in the *Rhizobium* populations in the sandy soils of Haryana (generally 10^3 g^{-1} soil) and neighbouring Rajasthan (generally <10 g^{-1} soil) is surprising. Both soils have high temperatures in summer. In regions where cropping depends solely on rainfall, it is generally possible to grow only one crop in a year. Discussions with farmers revealed that, even if there is adequate rainfall every year, chickpea in both the areas are normally grown on a given field once in 3 yr because of the rotation followed. Infrequent cultivation of chickpea in the surveyed region of Rajasthan with unreliable rainfall may be the major reason for low chickpea *Rhizobium* numbers.

Variations in soil pH, EC, moisture and NO_3–N generally could not account for variation in *Rhizobium* numbers. However, the range of soil factors measured in this study were generally within the limits of host-plant tolerance, and thus unlikely to affect the persistence of chickpea rhizobia. We have insufficient data to adequately relate soil properties to *Rhizobium* numbers between locations.

There was no clear relationship between *Rhizobium* numbers and the extent of nodulation in the farmers' fields, except that nodulation was invariably poor if the *Rhizobium* population was $<10^2$ g^{-1} soil. Fields in which a response to artificial inoculation with *Rhizobium* was seen in our experiments possessed such low levels of native populations (Rupela and Saxena, 1987). Increased yield with inoculation of lucerne was recorded by Vojinovic (1976) when the numbers of *R. meliloti* in the soil were <25 g^{-1} dry soil.

Chickpea rhizobia proliferate in the rhizosphere of the host (Toomsan *et al.*, 1983). Although the soil samples from farmers' fields in which chickpea was growing were taken from the middle of two plant rows, a favourable effect of the rhizosphere on the population data (Table 2) cannot be ruled out. Even so, chickpea fields with <10 rhizobia g^{-1} soil were recorded (Table 2), suggesting the virtual absence of native rhizobia at the time of sowing. Low populations of native *R. trifolii* were observed by Coventry *et al.* (1985) even after one crop of subterranean clover.

The amount of chickpea roots decreases with depth (Sheldrake and Saxena, 1979), which in turn might reduce *Rhizobium* proliferation in the rhizosphere. However, it is significant that even though rhizobia survive down to 1 m in Vertisols, careful excavation of root systems did not reveal any nodules below 45 cm. Movement of top soil to the lower depths through the deep cracks formed in Vertisols, when the chickpea is being harvested or during cultivation, may be important in maintaining rhizobia in the deeper zones. These cracks may penetrate to at least 50 cm.

During the summer, chickpea *Rhizobium* populations declined 7-fold in the Alfisol and 62-fold in the Vertisol. However, the residual populations remained large. Flooding resulted in a 100-fold reduction, to *Rhizobium* levels of $<10^2$ g^{-1}, suggesting that artificial inoculation may be necessary when chickpea is grown after flooding. Boonkerd and Weaver (1982) have made similar observations for cowpea rhizobia.

In conclusion, fields with a low native population are found in regions where chickpea has been grown for centuries, which seemed due to the absence of the chickpea host in the cropping pattern. The *Rhizobium* population in fields at research stations was generally greater than in the farmers' fields. At least 10^3 rhizobia g^{-1} soil could survive up to a depth of 60 cm in the Alfisol and the Vertisol. There was no relationship between *Rhizobium* numbers in soil and the extent of nodulation; hence the nodulation observations cannot be taken as a measure of a need for inoculation. Chickpea following rice may require inoculation for adequate nodulation.

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