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Displacement of native rhizobia nodulating chickpea (*Cicer arietinum* L.) by an inoculant strain through soil solarization*

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Summary. Soil solarization greatly reduced the native chickpea *Rhizobium* population. With inoculation, it was possible to increase the population of the *Rhizobium* in solarized plots. In the 1st year, 47% nodulation was obtained with chickpea inoculant strain IC 59 when introduced with a cereal crop 2 weeks after the soil solarization and having a native *Rhizobium* count of $<10\,g^{-1}$ soil, and only 13% when introduced 16 weeks after solarization at the time the chickpeas were sown, with 2.0×10^2 native rhizobia g^{-1} soil. In the non-solarized plots inoculated with 5.6×10^3 native rhizobia g^{-1} soil, only 6% nodulation was obtained with the inoculant. In the succeeding year, non-inoculated chickpea was grown on the same plots without any solarization or *Rhizobium* inoculation. The treatment that showed good establishment of the inoculant strain in year 1 formed 68% inoculant nodules. Other treatments indicated a further reduction in inoculant success, from 1%–13% to 1%–9%. Soil solarization thus allowed an inoculant strain to successfully displace the high native population in the field and can serve as a research tool to compare strains in the field, irrespective of competitive ability. In year 1, *Rhizobium* inoculation of chickpea gave increased nodulation and increased plant growth 20 and 51 days after sowing, and increased dry matter, grain yield, and grain protein yield at maturity. These beneficial effects of inoculation on plant growth and yield were not measured in the 2nd year.

Key words: Soil solarization – Displacing native rhizobia – Chickpea – *Cicer arietinum* – Survival of inoculant strain – *Rhizobium* spp.

It is generally difficult to displace indigenous rhizobia with inoculant strains, and most nodules on the host legume are formed by native rhizobia (Bohlool and Schmidt 1973; Kvien et al. 1981; Moawad et al. 1984). However, this is only likely to occur where the native *Rhizobium* population is low or absent (Materon and Hagedorn 1982; May and Bohlool 1983). The degree of establishment and the persistence of inoculant rhizobia generally decreases with increasing population density of the native rhizobia (Roughley et al. 1976; ICRISAT 1981). However, some inoculant strains have succeeded in forming the greatest number of nodules even in the presence of active indigenous competing rhizobia, e.g., Viking 1 on French beans (Robert and Schmidt 1983), G 1067 on *Trifolium* (McLoughlin et al. 1984), and NC 92 on groundnuts (Nambiar et al. 1984). The reason(s) for these successes is not well understood. The often poor ability of inoculant strains to compete with the native populations and the importance of identifying competitive strains have recently been reviewed by Schmidt (1988).

High temperatures may adversely affect the survival of rhizobia in soil. When exposed to a continuous incubation temperature of 46 °C all 10 *Rhizobium* strains on different legumes, including one strain (TAL 620, ICRISAT 3889) on chickpeas, died within a week (Somasegaran et al. 1984). In the field, even higher temperatures occur (Somasegaran et al. 1984) but with diurnal changes/fluctuations. Soil solarization, i.e., heating the topsoil by covering it with transparent polythene sheeting during the hot summer period, increased the duration of high soil temperatures and heated the soil to a greater depth than the control soil (Chauhan et al. 1988). Further, solarization of well watered soil markedly reduced chickpea rhizobial populations. Since the inoculant strains became well established when native rhizobial populations were low (Materon and Hagedorn 1982), soil solarization thus made it possible to establish inoculant strains in fields with high native rhizobial populations. The objective of the present study was to test this use of solarization.

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Materials and methods

Soil and solarization

A Vertisol (Typic Pellustert) field, previously depleted of soil N by growing cereal cover crops, was selected at the ICRISAT Center. The soil had a native population of 1.9×10^4 chickpea rhizobia g^{-1}. Before solarization, the soil pH (1:2, soil:water) was 8.1–8.2, electrical conductivity was 0.21–0.33 dS m^{-1}, NO_3^--N was 2.9–4.1 ppm, NH_4^+-N was 6.6–9.0 ppm, Olsen P was 5.3–7.0 ppm, and organic C was 1.0%–1.6% in the top 15 cm of the profile.

The field was prepared in 60-cm ridges and furrows, and was irrigated. The plots to be treated by solarization were covered with clear polythene sheeting of 400 gauge thickness 4 days after the irrigation and left for 50 days, from 22 April to 11 June 1986 (Chauhan et al. 1988). In the following year the land was left fallow, without solarization during the summer.

Soil temperature

Soil thermometers with metal jackets were used to measure the temperatures at depths of 5, 10, and 20 cm in the solarized and non-solarized plots. The thermometers pierced the polythene sheeting to the desired depth, both on top of the ridges and in the furrows. The joints were sealed with silicone rubber sealant. Daily readings were taken at 0830 and 1500 h. On 4 clear days, temperatures were recorded at hourly intervals to measure the diurnal fluctuations in the soil and the atmospheric air temperature.

Rhizobium strain

Rhizobia that nodulate chickpea are very specific and do not show a cross-inoculation affinity with any of the members of known cross-inoculation groups (references in Rupela and Saxena 1987). According to the 9th edition of Bergey's Manual of Systematic Bacteriology these have been called *Bradyrhizobium* sp. (Cicer), but in the present paper the species is termed chickpea *Rhizobium*. Strain IC 59 was obtained from a *Rhizobium* culture collection maintained at the ICRISAT Center. Earlier unpublished studies showed that this strain was efficient in pot culture at the ICRISAT Center and in multilocational field studies in India conducted by the All India Coordinated Pulse Improvement Project (AICPIP) under the auspices of the Indian Council of Agricultural Research (ICAR).

Chickpea Rhizobium count

Soil samples (pool of four cores $plot^{-1}$ from the top 15 cm of the profile) were collected after solarization and before the chickpeas were sown about 4 months after the solarization, using a 40-mm diameter soil corer. The population of chickpea rhizobia (most probable number) in these samples was determined by using a plant infection technique, as described by Toomsan et al. (1984).

Experimental design

The six treatments in the study comprised applications of *Rhizobium* at the time of sowing both sorghum and chickpeas, application of *Rhizobium* at the sowing of chickpeas only, and a non-inoculated control, each with and without solarization. These treatments were applied in field plots of 6×6 m in a random block design with six replications. The plots were separated by 2-m buffer zones.

Cover crop and application of Rhizobium strain to the soil

Solarization is known to increase soil nitrate levels by increased mineralization (Chauhan et al. 1988). To deplete this nitrate in the solarized plots and minimize its effects on chickpea growth and nodulation, a cover crop of sorghum was grown after the solarization and before the chickpeas were sown. As noted above, the inoculant strain was introduced along with sorghum in selected treatments, 2 weeks after soil solarization (on 28 June 1986), along the furrows that were opened on top of the ridges; a liquid suspension of peat-based inoculant strain IC 59 in water was used (1 g carrier with 1.4×10^9 rhizobia $liter^{-1}$ of water). The suspension was applied evenly at the rate of 165 ml m^{-1} row. Seeds of the sorghum hybrid CSH 6 were then placed in the opened furrows and covered with soil. This cover crop was rainfed, except for one sprinkler irrigation provided soon after sowing. About 430 mm rain fell during the crop growth period. Throughout the season, no rainwater was allowed to flow across the plots. During routine hand-weeding and interculture operations care was taken to minimize any mixing of soil and of rhizobia between plots. The sorghum was harvested 110 days after sowing.

Chickpea 1986–1987 (year 1)

After the sorghum was harvested on 6 October 1986, the land was plowed and remade into 60-cm ridges. It was hoped that the buffer zones between the plots would minimize the transfer of soil from one treatment plot to the next by farm machinery. The chickpea genotype Annigeri was sown in two rows per ridge at 30×10 cm spacing on 25 October 1986. The inoculant strain was applied at the time the chickpeas were sown, as described for sorghum. Thus half the total of 24 inoculated plots were treated with inoculant *Rhizobium* sp. for the first time. A post-sowing irrigation was provided, through a sprinkler system equivalent to a total of 42 mm rain, between 7 and 15 days after sowing. No further irrigation was provided, though moisture stress, as evidenced by dark green foliage, was observed about 60 days after sowing. Routine plant protection and weeding operations were performed. The chickpeas were harvested on 16 February 1987.

Chickpea 1987–1988 (year 2)

The land remained fallow without solarization after the 1st year's harvest of the chickpea crop on 16 February 1987. A normal weed flora was present when the land was prepared for sowing the non-inculated chickpeas on 25 November 1987. The seeds were sown on ridges 75 cm apart at 37.5×10 cm spacing. The crop was harvested 117 days after sowing. During the crop growth period, about 245 mm rain fell.

Nodulation, N_2 fixation, and plant growth of chickpeas

Roots, nodules, and tops were sampled from all the replicate plots for all treatments 20 and 51 days after sowing in year 1 and 47 days after sowing in year 2. Twelve plants per plot were carefully excavated up to 20 cm in depth for this purpose. Following an acetylene reduction test and recording of the fresh mass, all the nodules were preserved at −20 °C in 20% glycerol solution for serotyping. The plants sampled during year 2 were not subjected to the acetylene reduction assay. All plant samples were dried at about 70 °C, weighed, and ground in a Wiley mill mounted with a 0.2-mm sieve for determination of the N content.

All freshly excavated roots and nodules from each plot were subjected to an acetylene reduction assay in 6-liter airtight plastic containers, as described by Dart et al. (1972). Gas samples were taken after 30 min and the ethylene content was determined using a Pye Unicam gas chromatograph with a flame ionization detector.

Serotyping of nodules

Preparation of whole-cell antigen and antiserum were carried out as described by Vincent (1970), and the agglutination titer of the serum prepared was ≤1/100. All the stored nodules in both years were washed with sterile water and subsampled. For the serotyping, the alkaline phosphatase-based double-antibody sandwich form of direct enzyme-linked immunosorbent assay (ELISA) was used (Kishinevsky and Bar-Joseph 1978).

Results and discussion

Soil temperature

A temperature of 50 °C or more was recorded on 45 of the 50 days of solarization and the temperature at the top of the ridges was generally 0.5°–1 °C higher than that in the furrows. The soil temperature ranged from 42° to 55 °C in the top 20 cm of the profile between 1100 and 1800 h on 6 June 1986 (Fig. 1), with the highest temperature at a depth of 5 cm and the lowest at 20 cm. Over the rest of the day the highest temperature occurred at a depth of 20 cm and the lowest at 5 cm, with a range of 33°–46 °C. The temperature at the 10-cm depth was generally between those of the 5-cm and the 20-cm depths; in the top 10 cm of the profile the temperature reached ≥ 50 °C for about 5 h a day in the solarized plots. In the non-solarized plots the soil temperature in the top 10 cm of the profile ranged from 42° to 48 °C even in the hottest period of the day (1100–1800 h). Soil temperatures above 45 °C may kill rhizobia (Somasegaran et al. 1984), and the soil temperatures in solarized plots may decrease the survival of rhizobia well below that in the non-solarized plots.

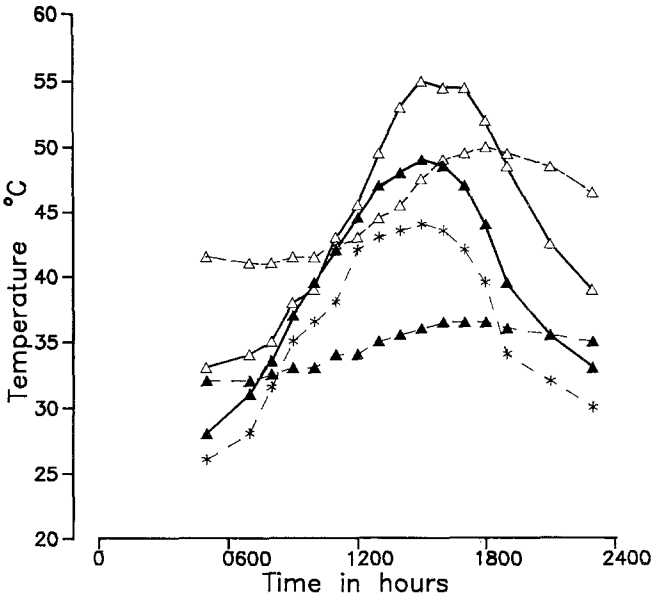

Fig. 1. Diurnal changes in soil temperatures in solarized (△) and non-solarized (▲) plots at soil depths of 5 cm (———) and 20 cm (– – –), and atmospheric air temperature (∗–∗) on 6 June 1989, 5 days before the end of the solarization period

Solarization and Rhizobium population

Solarization substantially decreased the native *Rhizobium* population in the top 15 cm of the profile when measured soon after solarization, from 2.2×10^3 to < 10 rhizobia g^{-1} soil in the solarized plots. The extent of the decrease was similar to that reported by Chauhan et al. (1988) for a different Vertisol field at the ICRISAT Center. At the time the chickpeas were sown, the population of native rhizobia increased in all plots. From < 10 rhizobia g^{-1} of soil in the solarized plots, the number swelled to 200 cells (2.31 $\log_{10}$) in the non-inoculated plots and 5.6×10^3 (3.75 $\log_{10}$) rhizobia in the inoculated plots. The population in the plots inoculated with sorghum when chickpeas were sown was, respectively, twofold and fivefold higher in the solarized and non-solarized inoculated plots compared with the population measured before the solarization.

Cover crop of sorghum

The significant soil chemical change brought about by solarization was a substantial increase in soil nitrate. The level was at least double in the solarized compared with the non-solarized treatments (Chauhan et al. 1988). A sorghum cover crop was therefore grown soon after solarization to deplete the excess NO_3-N. The planting of sorghum also filled the large gap between solarization during the summer (April to June), and the normal time for sowing chickpeas (October/November).

In the solarized plots the sorghum produced about twice the above-ground biomass and threefold more seed yield than in the non-solarized plots, from which 2800 kg biomass ha^{-1} and 360 kg grain ha^{-1} were obtained. Data on the growth of sorghum as influenced by solarization are being reported separately (O. P. Rupela, N. Seetharama, and M. R. Sudarashana).

Nodulation, N_2 fixation, and plant growth of chickpeas

The solarized non-inoculated plots had the fewest nodules and the smallest nodule mass both 20 and 51 days after sowing (Tables 1 and 2). Twenty days after sowing the solarized plots with dual inoculation, both sorghum and chickpea, had statistically more nodules per plant than the solarized non-inoculated control. The nodule count 51 days after sowing was similar with and without chickpea inoculation. The treatment with a dual application of *Rhizobium* sp., however, showed the maximum numbers of nodules (Table 1). With dual inoculation the nodule mass was also at a maximum. These differences did not appear among the non-solarized treatments (Table 2).

Acetylene reduction activity plant^{-1} h^{-1} was generally similar both in the plots with and without *Rhizobium* inoculation (Table 2). However, in the solarized inoculated plots the N concentration in shoots and the N uptake per plant were, respectively, 10% and 40% greater than in the solarized non-inoculated plots. The N uptake in the non-solarized inoculated plots was 8% greater than that in the non-solarized non-inoculated plots (Table 2). The application of the *Rhizobium* inoculant increased plant growth by 4%–9% by 20 days after sowing and 9%–20% by 51 days after sowing over the non-inoculated treatments (Table 2).

The chickpea growth in year 2 was superior to that in year 1 due to the apparently better soil moisture conditions. About 240 mm rain fell 2–5 weeks after sowing. In year 1, only 42 mm rain fell during the crop growth period. Unlike year 1, the *Rhizobium*-inoculated plots were only marginally superior to the non-inoculated plots for nodulation and plant growth 47 days after sowing, and for grain protein and protein yield at the final harvest (Table 3).

Table 1. *Rhizobium* population (no. g^{-1} soil), nodule numbers (plant^{-1}), and the percentage nodulation obtained from the inoculant strain with and without inoculation and soil solarization

Treatment[a]	*Rhizobium* population at chickpea sowing time, year 1 (log_{10})	Nodules (no. plant^{-1})		Nodulation by inoculant strain[b] (%)	
		Year 1, 51 DAS	Year 2, 47 DAS	Year 1, 51 DAS	Year 2, 47 DAS
Solarized					
SCP	3.75	27	39	47.0 (43.0)	68.3 (56.2)
CP	2.10	24	36	13.2 (20.7)	9.3 (16.0)
C	2.31	20	35	1.0 (3.2)	1.0 (3.2)
Non-solarized					
SCP	4.04	32	41	6.2 (14.2)	2.0 (6.6)
CP	4.09	31	36	6.6 (11.9)	1.9 (7.7)
C	3.75	31	35	0 (0)	0.6 (3.2)
±SE	0.212	2.0	1.4	(3.70)	(4.77)
CV (%)	16	18	9	(59)	(75)

[a] SCP, inoculant applied when both sorghum and chickpeas were sown; CP, inoculant applied when chickpeas were sown; C, non-inoculated control; CV, coefficient of variation
DAS, days after sowing
[b] Data analysed after angular transformation; transformed values in parentheses

Table 2. Nodulation, N_2 fixation, shoot mass, and shoot N content of chickpeas with and without *Rhizobium* inoculation and soil solarization in year 1

Treatment	Nodules, 20 DAS (no. plant^{-1})	Nodule fresh mass (mg plant^{-1})		Acetylene reduction activity (μM C_2H_4 plant^{-1} h^{-1})		Dry shoot mass (mg plant^{-1})		Shoot N content, 51 DAS	
		20 DAS	51 DAS	20 DAS	51 DAS	20 DAS	51 DAS	%	mg plant^{-1}
Solarized									
SCP	27	224	653	1.8	1.8	189	2490	3.9	97
CP	15	197	616	1.6	2.0	181	2140	3.8	82
C	14	193	514	1.7	1.8	183	1790	3.5	64
Non-solarized									
SCP	32	228	649	1.7	2.0	161	2220	3.8	85
CP	31	231	667	1.7	2.1	175	2520	4.0	101
C	28	204	626	1.6	2.6	154	2170	3.9	86
±SE	1.6	16	61.9	0.18	0.37	7.4	143	0.10	6.4
CV (%)	16	19	24	27	44	10	16	6	18

See footnote a to Table 1

Dry matter, grain yield, per cent grain protein, and protein yield

In year 1, the nodulation and the early plant growth differences between treatments in the solarized plots were reflected in the final yield. An increased yield due to the *Rhizobium* inoculation was even observed in some non-solarized plots, but the difference was statistically significant only for grain yield; in the solarized plots significantly improved yields over the respective non-inoculated plots were recorded both for dry matter and grain yield. The mean increase ranged from 15% to 19% in the solarized inoculated treatments and from 13% to 14% in the non-solarized inoculated treatments (Table 3). The percentage of grain protein also improved with inoculation, by 4% – 5%, and resulted in significantly more grain protein yield over the non-inoculated plots, both with and without solarization. Although inoculation treatment improved the yield components, the plants in the solarized plots yielded less as a whole. This may have been due to excessive depletion of nutrients in the solarized plots where sorghum was grown in the previous season and the solarized plots produced twofold more biomass. However, the amounts of soil N and soil P were similar in both the solarized and non-solarized plots at the time the chickpeas were sown. In year 2, the treatment differences were not apparent for any yield parameters studied (Table 3).

Tracing the inoculant strain

Although the antiserum had a low agglutination titer (≤1/100), it was successfully used in the ELISA. The serum showed no positive reaction with any of the nodules formed by native rhizobia in the same field, on plants away from the experimental area, nor did it show any cross-reaction with several other *Rhizobium* strains in our collection. In year 1, most nodules were formed by the inoculant strain (47%) when it was introduced, soon after solarization, with the sowing of the sorghum, and subsequently with the sowing of chickpeas. In year 2, this percentage increased to 68%. In other treatments, nodula-

Table 3. Dry matter (kg ha^{-1}), grain yield (kg ha^{-1}), grain protein (%), and protein yield (kg ha^{-1}) of chickpeas with and without soil solarization and *Rhizobium* inoculation

Treatment	Year 1				Year 2			
	Dry matter	Grain yield	Grain protein	Protein yield	Dry matter	Grain yield	Grain protein	Protein yield
Solarized								
SCP	1830	1190	13.5	159	3970	2110	15.8	405
CP	1780	1160	14.1	164	3940	2020	16.0	392
C	1550	1000	13.6	137	3980	2110	15.6	401
Non-solarized								
SCP	2040	1340	14.7	197	3660	2020	15.7	388
CP	2050	1360	14.9	202	3810	1980	15.8	381
C	1800	1190	14.3	170	3660	1960	15.6	373
±SE	71	49	0.36	8.9	116	65	0.23	14.9
CV (%)	9	10	6	13	7	8	4	9

See footnote a to Table 1

tion obtained with the inoculant strain decreased from 6.2% – 13.2% in year 1 to 1.9% – 9.3% in year 2 (Table 1).

Previous unpublished studies (O. P. Rupela, M. R. Sudarshana, and R. Gururaja) indicated that after a considerable reduction in the native chickpea *Rhizobium* population in solarized plots, the numbers continued to increase even in the absence of the chickpea host. It was for this reason that we decided to introduce the inoculant strain into the soil as early as possible. Sowing a sorghum cover crop provided a good vehicle for the application and spread of the inoculant strain in soil. Chickpea rhizobia are known to survive well and even multiply in the rhizosphere of sorghum (Toomsan et al. 1983). Thus the inoculant strain may have become established in large numbers before the chickpeas were sown about 4 months later. The MPN of native chickpea *Rhizobium* in the non-inoculated plots increased from $<10\,g^{-1}$ soil soon after the solarization to $1.3-2.0\times10^2\,g^{-1}$ soil at the time the chickpeas were sown (within about 4 months). This increase obviously occurred in the sorghum rhizosphere. With inoculation following solarization, the increase over the same period was about 28-fold over the non-inoculated treatment, and was reflected in the increased number of nodules formed by the inoculant strain in this treatment. The inoculant strain, when first introduced with the chickpeas 4 months after the solarization, thus faced a large native population and succeeded in forming only 13% of the total possible nodules. This effect was observed in spite of the application of a high population of the inoculant strain using the liquid inoculation method, which is considered a superior method of *Rhizobium* application (Brockwell et al. 1980).

In year 2, the percentage nodulation obtained with the inoculant strain was also high (68.3%) when it was introduced soon after solarization. Where the inoculant was introduced about 4 months after solarization, the success of the inoculation remained very poor (<10% nodules). Clearly, the inoculant strain became well established and had even survived well for at least one year when it was measured. These measurements were continued beyond the present study.

In the solarized plots most of the 47% nodulation obtained with dual inoculation was apparently formed by the rhizobia applied with the sorghum, because the inoculant rhizobia applied when the chickpeas were sown formed only 13% nodules. This indicates that resident rhizobia, whether indigenous or introduced (established by special methods such as solarization), are likely to form the most nodules on the host. This may be attributed to the special advantage of resident rhizobia in making contact with the rhizosphere in large numbers. A further increase in nodulation by the inoculant strain, from 47% in year 1 to 68% in year 2, probably reflects rhizosphere multiplication of the inoculant strain in year 1 chickpeas and also those rhizobia that originated from year 1 degenerating nodules.

The reduction in native rhizobia by soil solarization and the successful establishment of inoculant *Rhizobium* as demonstrated in these studies indicates that solarization can be used as a new research tool to evaluate inoculant strains for N_2 fixation without confounding their ability to compete. Several inoculant strains can now be introduced to field soils (with a high native population of homologous rhizobia) after solarization and compared for their survival, persistence, and effectiveness under field conditions.

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