

## NEW SEROGROUP AMONG *CICER*-RHIZOBIA FROM GREY AND BROWN SOIL IN SEMI-ARID REGION OF RAJASTHAN

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Chickpea (*Cicer arietinum* L.) is mostly cultivated in semi-arid regions of tropics and forms symbiosis with highly host specific root nodule bacteria, which henceforth will be called as *Cicer*-rhizobia (Gaur and Sen, 1979). In the cultural and physiological characteristics, *Cicer* rhizobia form a bridge between the fast growing rhizobia of cl ver, pea and beans and the slow growing rhizobia of soybean, lupin and cowpea miscellany (Gaur and Sen, 1981). *Cicer*-rhizobia are not indigenous to a few Indian soils particularly the desert rhegosolic and the red sandy soils (Gaur and Sen, 1979), while the rhizobia of cowpea miscellany occur in almost all the soils (Sundara Rao et al. 1972). Although the strains of *Cicer*-rhizobia isolated from alluvial soil of Durgapura (Rajasthan) cross reacted with the antibodies produced against strains from other locations having similar soil type, yet represented separate serotype (Gaur and Sen, 1989). Serological reaction of *Cicer*-rhizobia from grey and brown soil in the semi-arid region of Rajasthan was not known and hence appeared to be of interest because this soil is of different genesis than the alluvial soil. Besides, for legume inoculation trial, prior knowledge of the antigenic relationship between the indigenous population of strains and the inoculant strain is essential, if the immunological probe is to be employed successfully for monitoring the population of inoculant strain. With these objectives in view, representative strains of *Cicer*-rhizobia from grey and brown soils were examined for their serological relation ship with each other and 17 other serotypes, identified among strains from alluvial soil in an earlier study (Gaur and Sen, 1989).

Eight strains of *Cicer*-rhizobia, isolated from chickpea cv NP 53 and G 130 growing in the grey and brown soil of Sukhadia University (now the Rajasthan Agricultural University) Regional Research Station Farm, Sumerpur, Rajasthan (Table 2) and 17 strains representing 17 different serotypes among the population of *Cicer*-rhizobia from alluvial soils (Table 1) were used. The authenticity of all these strains has been described earlier (Gaur and Sen, 1981).

The antigenic relationship of the 8 strains from grey and brown soil with the 17 representatve strains from alluvial soil was examined through Ouchterlony's gel

Table 1. Serogroups, serotypes and the representative strains of *Cicer* rhizobia from alluvial soils, which were used as reference strains\*

Serogroup	Serotype	Representative strain	Numbers of strains represented
I	1	Ca 122	1
	2	Ca 8	1
II	3	Ca 78	4
	4	Ca 170	2
	5	Ca 165	1
	6	Ca 9	17
III	7	Ca 85	1
	8	Ca 12	7
	9	Ca 182	3
IV	10	Ca 26	3
	11	Ca 98	5
	12	Ca 1	20
V	13	Ca 90	3
VI	14	Ca 121	1
	15	Ca 120	1
	16	Ca 151	3
VII	17	Ca 153	3

\*After Gaur and Sen, 1989

immunodiffusion technique (Vincent, 1970). Since none of the 8 strains cross reacted with the antibodies of the representative strains of 17 serotypes for the strain specific slow diffusing thermostable surface antigens, the antigenic constitution of all the 8 strains was studied using antibodies prepared against each of them. Broken (B) and heat treated cells (H) were employed against the whole or the absorbed antisera. The procedures for the preparation of antigens, antisera and antigen-antibody reactions were according to Gaur and Sen (1984).

All the 8 strains from grey and brown soil of Sumerpur showed identity with the 17 representative serotypes from alluvial soil (Table 1) in their fast diffusing thermolabile intracellular antigens. However for the strain specific slow diffusing thermostable antigens, none of the strains showed any cross reaction with these 17 serotypes. This demonstrated that the antigenic constitution of these 8 strains from grey and brown soil was different from the antigenic constitution of the strains from alluvial soils. Thus the former strains can be placed in separate serogroup, having limited relationship with the 7 serogroups, represented by the 17 serotypes from alluvial soils (Table 1). Nevertheless, the identity in the intracellular antigens among all the strains further confirms them to be of taxonomic importance (Gaur and Sen, 1984, 1989). Taxonomic significance of the fast diffusing intracellular antigens have also been reported among rhizobia of other host legumes (Vincent and Humphrey, 1970; Vincent et al., 1973; Humphrey and Vincent, 1975).

Table 2. Antigenic constitution and the inter-relationship between the 8 strains of *Cicer* rhizobia, isolated from grey and brown soil of Sumerpur, Rajasthan (antiserum and antigen No. correspond with the strain No.)

Chickpea cultivar	Antiserum tested*	Antigen absorbed*	Precipitin lines formed with test antigen*					
			Ca 172,173,176,177		Ca 174		Ca 179,180,181	
			B	H	B	H	B	H
NP 53	Ca 172, 173, 176, 177	Nil	a	a	s	s	s	s
			b	b	b	b	—	—
			c	—	c	—	c	—
			d	—	d	—	d	—
		Ca 172, 173, 176,177	—	—	—	—	—	—
		Ca 174	a	a	—	—	s	s
		—	—	—	—	—	—	
		—	—	—	—	—	—	
		Ca 179, 180, 181	a	a	s	s	—	—
		b	b	b	b	—	—	
	Ca 174	Nil	s	s	a	a	s	s
			b	b	b	b	—	—
			c	—	c	—	c	—
			d	—	d	—	d	—
		Ca 172, 173, 176,177	—	—	—	—	—	—
		Ca 174	—	—	—	—	—	—
		—	—	—	—	—	—	
		—	—	—	—	—	—	
		Ca 179, 180, 181	s	s	a	a	—	—
		b	b	b	b	—	—	
C 130	Ca 179, 180, 181	Nil	s	s	s	s	a	a
			—	—	—	—	b	b
			c	—	c	—	c	—
			d	—	d	—	d	—
	Ca 172, 173, 176,177	—	—	s	s	a	a	
	—	—	—	—	b	b		
	—	—	—	—	—	—		
	Ca 174	s	s	—	—	a	a	
	—	—	—	—	b	b		
	—	—	—	—	—	—		
Ca 179, 180, 181	—	—	—	—	—	—		

\*Each tested or absorbed individually. Common alphabet in a row denotes the identical antigens, denotes partially identical antigen, other had no cross reaction; Common alphabet in the column denotes the antigen forming precipitin band at the identical position. Order of antigen bands from the outer antigen well to inner antiserum well in the immunodiffusion reaction : a, b, c, d

Immunodiffusion reactions between these 8 strains showed variations among the strains and revealed the existence of 3 new serotypes (Table 2). All the 3 strains, Ca 179, 180 and 181 from chickpea cv G130 were completely identical to each other and represented a single serotype (Ca 179), while of the remaining 5 strains from the other cultivar NP 33 of chickpea, Ca 172, 173, 176 and 177 showed complete identity with each other and represented another serotype, Ca 172. The fifth strain Ca 174 represented a separate serotype. For the most slow diffusing antigen 'a', all the 3 serological types among these 8 strains showed cross reaction of partially identical nature; however for antigen 'b', there was complete identity between the strains of 2 serotypes, identified among strains from cv NP 53 but not with strains from cv G 130. These results further lend support to the earlier findings about the propensity of chickpea cultivars for certain serological types of *Cicer-rhizobia* (Gaur and Sen, 1986) and also the serological diversity even at the same location in the population of this specific group of rhizobia (Gaur and Sen, 1989). It also suggested that the serological diversity in *Cicer-rhizobia* is further accelerated by the soil type and because of such diversity, immunological probe can successfully be used to monitor the population of inoculant strain in for ecological studies these soils.

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