

Competition for Nodulation of *Rhizobium* spp. (*Cicer Arietinum* L.) Inoculated on Different Cultivars of Chickpea*

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Abstract

In this work we have evaluated the competition ability for nodulation of the strains of *Rhizobium* spp. (*Cicer arietinum* L.) IC2091, 27A2, 27A15 inoculated on different Chickpea genotypes and the effect of inoculation in a fertile neutral soil (pH = 7.2). The inoculation was carried out under field conditions and the nodule occupancy evaluated by the indirect immunofluorescent technique. All the strains increased the number of nodules in inoculated plants. The strain 27A15 showed a serological diversity with the native strains and a nodule occupancy from 83.4 to 100 per cent in inoculated plants. A percentage of cross reaction with the native strains affect the results for IC2091 and 27A2. The inoculation generally increased the grain yield production. The different strain/cultivar combinations exhibited a significative variation for the nodulation and productivity.

Introduction

The ability to survive and nodulate under diverse soil conditions, in the presence of competing indigenous populations have been investigated in several *Rhizobium* strains suitable for inoculant production. (H. Jansen, van Rensburg and B.W. Strijdom, 1982, T.J. McLoughlin and L.K. Dunican, 1985.).

Recent studies have demonstrated the plant host genotype influence on nodulation and Nitrogen-fixation efficiency in *Medicago sativa*, *Phaseolus vulgaris*, and other legumes (R.W. Miller and J.C. Sirois, 1982; M.J. Sadowsky, P.B. Cregan, H.H. Keiser 1988; H.H. Keyser et al., 1988) and the selection of strain/cultivar combinations with good symbiotic effectiveness in field conditions should be prerequisite in inoculant production.

In the mediterranean area *Cicer arietinum* is an important crop but very few

*Reviewed

information about the ecology of his related symbiont and the effect of the inoculation are available.

Recent studies by immunofluorescence, immunodiffusion, and intrinsic antibiotic resistance have demonstrated the antigenic difference of chickpea *Rhizobium* with any other species and grouped isolates of *Rhizobium* spp. (*Cicer arietinum* L.) obtained from different culture collections into serogroups. (M.T. Kingsley and B.B. Bohlool 1983). J.F. Arzac and J.C. Cleyet-Marel (1986), have used the immunofluorescence to quantify the effect of soil conditions and host plant genotype on competition ability of some strains.

Competition studies and evaluation of plant genotype interaction on chickpea-*Rhizobium* nodulation have been carried out in field conditions in Italian soils by R. Di Bonito, J.C. Cleyet-Marel and G. Porreca. (1988).

In this work we have evaluated the competitive ability for nodulation of three *Rhizobium* strains inoculated on *Cicer arietinum* in a soil where native populations are present. We have evaluated also the effect of inoculation in different plant host genotypes in order to select the most efficient strain-cultivar associations.

Materials and Methods

The strains used as inoculants were: N27A2, N27A15, and IC2091, furnished respectively by the Nitragin Co Milwaukee, WI, USA and J.C. Cleyet-Marel, INRA Montpellier, France. The strains did not exhibit differences for their N₂-fixing ability, after evaluation of the acetylene reduction activity (ARA) and nitrogen content of INRA 199 cultivar in Leonard jars (R. Di Bonito, G. Porreca, M.L. Belli, in press). We inoculated with a single strain treatment the cultivars Calia, Califfo, Sultano, ILC 482, obtained from the Plant Breeding Laboratory, ENEA, Roma, Italy and INRA 199, furnished by the INRA-ENSA, Montpellier, France. The inoculum was peat carried and added to the seeds with a 10⁶ bacteria/seed concentration. A spring sowing (March 15, 1989) was carried out under field conditions in a randomized block design with three replicates including controls without inoculation in a fertile neutral soil (pH=7.2). Ten plants for each replicate were collected before flowering for the evaluation of number and weight of nodules. At this stage it is possible to collect an intact root apparatus for the evaluation of nodulation.

The diversity of local and introduced strains and the nodule occupancy on inoculated plants were evaluated using serological techniques. The indirect fluorescent antibody technique was performed using antisera against the strains at the dilution 1:300, as described by J.F. Arzac and J.C. Cleyet-Marel, 1986. At harvest the grain yield was evaluated and statistical analysis of the results performed by Duncan's Multiple Range Test.

Results

The strains 27A2, 27A15 and IC2091 generally increased the number and weight of

Table 1. Nodulation of Chickpea genotypes by strains of *Rhizobium* spp. (*Cicer arietinum*). The numbers followed by the same letter do not differ significantly ($p=0.05$) within a given column.

Strains	Nodules present on plants inoculated by the strains:											
	Calia		Califfo		Sultano		ILC 482		INRA 199			
	No/plant	Fresh W. (g)	No/plant	Fresh W. (g)	No/plant	Fresh W. (g)	No/plant	Fresh W. (g)	No/plant	Fresh W. (g)		
IC2091	14.4b	0.44c	7.8ab	0.12a	2.5c	0.03ab	18.8c	0.87a	9.3c	0.11c		
N27A2	16.5a	1.20a	15.0a	1.02a	4.8b	0.03ab	14.5b	0.62b	20.9a	1.71a		
N27A15	9.2c	0.70b	8.0ab	0.28b	12.8a	0.62a	22.5a	0.12c	12.2b	0.43b		
Uninoculated	1.2d	0.06d	1.1b	0.02d	0.6d	0.01b	0.6d	0.01c	0.0d	0.00d		

nodules as showed in Table 1 but significative differences ($p=0.05$) were observed only for some cultivar/strain combinations.

The results of serological test are reported in Table 2. The nodules of not inoculated plants showed a percentage of positive reaction between 0 and 25 per cent with IC 2091 and 27A2 antisera and no reaction with 27A15 antisera. On the inoculated plants the nodules exhibited a percentage of positive reaction between 71.5 and 100 per cent with the corresponding antisera and no statistical significative differences were detected.

The grain yield analysis is showed in Table 3. The inoculation generally increased the productivity but each cultivar exhibited a different response to the strains used as inoculants. ILC 482 showed a significative increase ($p=0.05$) with all the strains while Calia, Califfo and Inra 199 exhibited a positive response only with some combination. Noo significative increase was observed in Sultano, where a low number of nodules was formed by all the strains used as inoculants.

Table 2. Nodule occupancy in inoculated and not inoculated plants

Cultivars	% Of nodules showing a positive reaction with the antisera*					
	Strains					
	IC 2091**		27A2**		27A15**	
	I	U	I	U	I	U
CALIA	94.2	0	96.5	25	100	0
CALIFFO	84.8	0	100	0	83.4	0
SULTANO	100	20	71.5	0	91.7	0
ILC 482	94.7	20	95.0	20	98.0	0
INRA 199	—	—	—	—	—	—

I:Inoculated

U:Uninoculated

* The nodules were analysed with indirect immunofluorescent technique.

** Cultures used as inoculants, in a single strain treatment.

Table 3. Productivity of Chickpea cultivars inoculated with different *Rhizobium* spp. (*Cicer arietinum*). The numbers followed by the same letter do not differ significantly ($p=0.05$) with a given column.

Strains	Grain Yield (kg/ha)				
	Cultivars				
	Calia	Califfo	Sultano	ILC 482	INRA 199
IC2091	3361b	2695b	2639a	4805a	5878a
N27A2	2667c	3367a	3195a	4806a	3972bc
N27A15	3972a	2889b	2667a	4333a	4778ab
Uninoculated	3334b	2250b	2389a	3667b	3500c

Discussion

The response to the inoculation of certain inoculum/cultivar combinations suggest a strong strain/plant genotype interaction on nodulation process and productivity of plants under field condition. The significant differences on the number of nodules formed by different strains in the same cultivar (Calia, ILC 482 and INRA 199) are not correlated with the variation in the grain yield and a higher number of nodules correspond in some cases to a lower productivity.

The immunofluorescence seems to be a good method to detect the competition ability for 27A15 strain and a percentage of cross-reaction with native strains affect the results of nodule occupancy for IC2091 and 27A2.

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