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ORIGINAL PAPER

M. Hungria · R.J. Campo · L.M.O. Chueire
L. Grange · M. Megías

Symbiotic effectiveness of fast-growing rhizobial strains isolated from soybean nodules in Brazil

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Abstract The symbiotic effectiveness of 30 fast-growing rhizobial strains (doubling times of 85–225 min and acid reaction in yeast mannitol medium) isolated from soybean nodules in Brazil and of *Sinorhizobium fredii* reference strains was evaluated under greenhouse and field conditions. Most Brazilian fast-growing strains were genetically related to the *Rhizobium tropici-Rhizobium* genomic species Q-*Agrobacterium* spp. branch and five to the *Bradyrhizobium japonicum* and *B. elkanii* species. Under axenic conditions, some of the fast-growing strains fixed as much N_2 as the *B. japonicum*/*B. elkanii* strains carried in Brazilian commercial inocula. However, in a co-inoculation experiment, very few strains were able to compete against *B. elkanii* strain SEMIA 5019. Although isolated from acid soils (pH 3.0–5.1), the competitiveness of Brazilian fast growers and of *S. fredii* reference strains against *B. japonicum*/*B. elkanii* was low under acid conditions (pH 5.1 and pH 5.4), but increased when the pH was raised to 6.8 and 7.9. Therefore, as the great majority of Brazilian soils are acidic and show a very high population of naturalized *B. japonicum*/*B. elkanii*, the low competitiveness of *S. fredii* and of the Brazilian rhizobial strains investigated in this study, under the given conditions, limits, at this time, their recommendation for use in commercial inocula.

Keywords *Agrobacterium* · *Bradyrhizobium* · Competitiveness · *Glycine max* · Nitrogen fixation

M. Hungria (✉) · R.J. Campo · L.M.O. Chueire
Embrapa-Soja, Cx. Postal 231, 86001-970, Londrina, PR, Brazil
e-mail: hungria@cnpso.embrapa.br
Tel.: +55-43-3716206. Fax: +55-43-3716100

L. Grange
Department of Genetics, Universidade Estadual de Londrina, Londrina, Brazil

M. Megías
Department of Microbiology and Parasitology, Facultad de Farmacia, University of Seville, Apdo. Postal 874, 41080, Seville, Spain

Introduction

The main effective N_2-fixing symbioses with soybean [*Glycine max* (L.) Merrill] are established with rhizobial strains of *Bradyrhizobium japonicum* and *B. elkanii* species, which are characterized by a slow growth rate and alkaline reaction in media containing mannitol as a C source (Jordan 1982; Kuykendall et al. 1992). However, the legume can also associate with fast-growing strains that were first isolated from soybean nodules and soil from the People's Republic of China, within the centre of origin and diversity of this legume (Keyser et al. 1982), and later from other primary and secondary centres of origin of soybean (Xu and Ge 1984; Dowdle and Bohlool 1985; Young et al. 1988; Rodriguez-Navarro et al. 1996). These fast growers were classified as the new species *Rhizobium fredii* (Scholla and Elkan 1984), and later reclassified as *Sinorhizobium fredii* and *S. xinjiangensis* (Chen et al. 1988).

Soybean is an exotic plant in Brazil, but has been intensively cultivated since the 1960s. The first experiments, with disinfected seeds, showed that inoculation was needed, since native rhizobial strains were unable to effectively nodulate soybean (e.g. Lopes et al. 1976; Peres 1979; Vargas and Suhet 1980), and therefore, inoculation with *Bradyrhizobium* has been mandatory. Although originally it was thought that *S. fredii* was specific for Asian soybean lines (Keyser et al. 1982; Stowers and Eaglesham 1984; Devine 1985), more recently it was shown that several North American and Brazilian genotypes are able to form effective nodules with sinorhizobia (Balatti and Pueppke 1992; Chueire and Hungria 1997). The use of these fast growers in commercial inocula would be of great interest, especially because only half the time would be required to produce the 13 million doses annually sold in the country. However, a first attempt has shown that two *S. fredii*/*S. xinjiangensis* strains were not able to outcompete a *B. elkanii* Brazilian commercial strain (Chueire and Hungria 1997).

In an attempt to isolate native sinorhizobia strains, a survey was performed in Brazil, and 30 fast-growing

strains (generation times of these isolates varied from 85 to 225 min and, after 4 days of growth in medium containing mannitol as the C source, final pH values ranged from 3.7 to 6.9) were isolated from nodules of Asian and modern soybean genotypes and were preliminarily characterized (Hungria et al. 2001). Later, the 16S rRNA genetic characterization of these strains showed that they did not belong to *S. fredii* species; most were genetically related to the *Rhizobium tropici-Rhizobium* genomic species Q-*Agrobacterium* spp. branch and five to the *B. japonicum* and *B. elkanii* species. In this paper, the symbiotic effectiveness of these 30 fast-growing strains was compared to that of *B. japonicum*/*B. elkanii* strains used in Brazilian commercial inocula, to verify if the indigenous strains could outcompete *Bradyrhizobium*.

Materials and methods

Bacterial strains

Brazilian fast-growing rhizobial strains

Thirty strains, obtained from 12 Brazilian soils located in widely spread states, including undisturbed areas covered with native vegetation and fields traditionally cultivated with soybean and previously inoculated, were used in this study. The sites of isolation and morphological and physiological characteristics of the strains were described before (Hungria et al. 2001), and the doubling times are shown in Table 1. By RAPD, RFLP of the 16S rDNA gene and partial 16S rDNA sequences, 25 strains fit the *R. tropici-Rhizobium* genomic species Q-*Agrobacterium* spp. branch and therefore were named as *Rhizobium* sp.; five strains were genetically related to *B. japonicum* and *B. elkanii*, and were therefore named as *Bradyrhizobium* sp.. Table 1 shows the nomenclature and site of isolation of each strain.

Reference rhizobial strains

The *S. fredii* strain CCBAU 114 (=RT 15) was received from Dr E. T. Wang, Beijing Agricultural University, Beijing, China; strains HH102-2, HH103-2 and SMH12 were provided by Dr J. E. Ruíz-Saínz (University of Sevilla, Spain) and strain USDA 205 by Dr P. van Berkum (USDA, Beltsville, Md.). *B. japonicum* strains SEMIA 5080 (=CPAC 7) and SEMIA 5079 (=CPAC 15) and *B. elkanii* strains SEMIA 587 and SEMIA 5019 (=29w), carried in Brazilian commercial inocula, came from the Embrapa Soja rhizobia germplasm bank

Plant material

Seeds of soybean cultivars BR-16 (genealogy, D 69-B 10-M 58 X Davis) and Davis (genealogy, D 49-2573 X N 45-1497) came from the Embrapa Soja seed germplasm bank.

Symbiotic effectiveness

N_2-fixation capacity

Each strain was grown in yeast mannitol agar medium (Vincent 1970) for 5 days, at 28°C, and cultures were adjusted to a concentration of 10^9 cells ml^{-1}. Soybean seeds of cultivar BR-16 were surface-sterilized (Vincent 1970) and incubated with the inoculum (1 ml $seed^{-1}$) for 30 min. The experiment was performed in modified Leonard jars containing sterile sand and vermiculite; sowing, supply of N-free nutrient solution and plant growth conditions were as described before (Santos et al. 1999). Eight non-inoculated controls, with or without mineral N [80 mg of N (as KNO_3) $plant^{-1}$ $week^{-1}$] were included. Plants were harvested at 4 weeks after emergence and the parameters evaluated were nodule number and dry weight, shoot and root dry weight and total N content of shoots, as described before (Santos et al. 1999). The experiment was performed in a randomized block design, with four replicates, and statistically analysed by the ANOVA procedure and the treatment means compared by Tukey's test ($P \leq 0.05$).

Nodule occupancy

The experiment was carried out as described in the previous section, except that each strain (10^9 cells ml^{-1}) was inoculated in a proportion of 1:1 with *B. elkanii* strain SEMIA 5019 (10^9 cells ml^{-1}) and that the experiment was performed with two cultivars, BR-16 and Davis. In addition to the parameters analysed in the previous experiment, 60 nodules $treatment^{-1}$ were randomly collected for evaluating nodule occupancy. First bacteria were isolated from nodules (Vincent 1970) and growth rate and acid reaction in yeast mannitol agar medium containing bromothymol blue (25 µg ml^{-1}) were verified. Isolates were then analysed for reaction with the antiserum of SEMIA 5019, as described before (Santos et al. 1999).

Symbiotic performance in soils of different pH

For the evaluation of effects of pH on nodulation and N_2 fixation, samples of an oxisol with pH 5.1 received sufficient lime for 4 months, according to the soil chemical analyses, to reach pH values of 6.8 and 7.9. Pots of 5 kg capacity were filled with 4 kg soil (with an established population of 10^4 cells *Bradyrhizobium* g^{-1}, after counting in soybean plants), with four replicates per treatment. Plants were harvested 6 weeks after emergence and evaluated for all parameters described.

Symbiotic performance in the field

A field experiment was performed in an oxisol at Londrina, in the State of Paraná, Brazil, with four Brazilian rhizobial strains, three *S. fredii* and the four Brazilian commercial strains. Three months before planting, lime was applied according to the soil chemical analysis, and at the time of sowing the pH was 5.4. The experimental plots measured 3.0×2.0 m, with 0.5 m between rows, and plots were separated by 2.0 m with terracing. Five days before sowing, the plots received 84 kg P ha^{-1}, 60 kg K ha^{-1} and 40 kg ha^{-1} of micronutrients (containing: Zn, 9.0%; B, 1.8%; Cu, 0.8%; Fe, 3.0%; Mn, 2.0%; Mo, 0.10%); fertilizers were incorporated into the soil. The soil rhizobial population was estimated at 10^4 cells g^{-1} soil by the most probable number technique with counting in soybean plants (Vincent 1970). Inocula of *B. elkanii* SEMIA 587, *B. japonicum* SEMIA 5080, *S. fredii* SMH12 and of the strains 5, 16 and 24 from this study were prepared to a density of 10^9 cells ml^{-1} in yeast mannitol medium, adding 100 ml inoculum kg^{-1} seeds. Two non-inoculated controls were included, with or without 200 kg N ha^{-1}, applied as urea, and split into two doses of 100 kg N ha^{-1}, at sowing and at growth stage R2 (open flower at one of the two uppermost nodes on the main stem with a fully developed leaf). The experiments were performed in a randomized block design, with six replicates. At R2, 15 plants of each treatment were harvested and nodule number and dry weight were determined; also, 60 nodules were randomly chosen per treatment for agglutination analysis, using the antisera of the corresponding strains for that treatment, as described. At the final harvest, yield and N content of grains were evaluated, taking into account a moisture content of 13%. The results were statistically analysed by the ANOVA procedure and the treatment means were compared by Tukey's test ($P \leq 0.05$).

Table 1 N_2-fixation capacity of soybean cultivar BR-16 inoculated with 30 Brazilian rhizobial strains and with reference strains of *Bradyrhizobium japonicum*, *B. elkanii* and *Sinorhizobium fredii*. Plants grown in modified Leonard jars and harvested 4 weeks after emergence. *PR* Paraná, *RS* Rio Grande do Sul, *DF* Distrito Federal, *AM* Amazonas, *CT* conventional tillage, *NT* non-tillage, *U* uncropped soils covered with native vegetation, *LSD* Least significant difference, *CV* coefficient of variation

Strain	Doubling time (min)	State[a]	Cropping sys-tem	Nodulation		Shoot	
				Number (no. plant^{-1})	Dry weight (mg plant^{-1})	Dry weight (g plant^{-1})	Total N (mg N plant^{-1})
Rhizobium spp.							
1	95	PR	CT	105	443	2.21	63.9
2	105	PR	CT	92	435	2.49	65.4
3	110	PR	CT	112	520	2.80	69.9
4	115	PR	CT	89	536	3.18	76.8
5	90	PR	CT	113	526	2.82	75.0
6	100	PR	CT	7	7	0.56	3.2
7	105	PR	CT	10	14	0.51	3.9
9	90	PR	U	3	5	0.47	2.4
10	95	PR	U	54	148	0.68	17.0
12	110	PR	NT	108	389	3.08	79.8
13	85	PR	NT	109	429	3.49	70.7
14	85	PR	CT	66	353	2.04	55.3
15	85	PR	NT	35	85	0.78	21.1
17	85	RS	U	16	48	0.62	9.2
18	95	RS	U	13	36	0.62	7.6
19	105	RS	U	64	167	1.08	28.7
20	95	RS	U	62	255	1.73	52.9
21	100	DF	U	16	39	0.66	7.7
22	100	DF	U	86	507	3.10	91.8
23	90	AM	U	72	350	1.81	56.8
24	85	AM	U	111	522	2.96	86.7
25	105	AM	U	72	414	2.53	62.5
26	165	PR	NT	74	508	2.21	65.7
27	155	PR	NT	90	490	2.32	73.2
29	160	PR	NT	82	370	1.99	55.1
Bradyrhizobium spp.							
8	205	PR	NT	103	353	2.29	66.6
11	240	PR	NT	86	359	2.82	84.4
16	225	PR	NT	100	371	2.77	70.1
28	170	PR	NT	88	450	2.18	60.6
30	130	PR	NT	90	410	1.96	42.8
S. fredii							
USDA 205				82	193	1.24	17.6
CCBAU 114				30	150	0.91	18.9
B. japonicum/B. elkanii							
SEMIA 587				76	535	3.08	92.7
SEMIA 5019				72	416	2.32	72.0
SEMIA 5079				65	349	2.82	87.6
SEMIA 5080				90	409	3.04	99.4
Control							
C–N[b]				0	0	0.23	1.80
C+N[b]				0	0	2.80	90.3
LSD (5%)[c]				23	103	0.40	9.6
CV (%)				24	21	11	8

[a] Sites of isolation (States of Brazil); more details are in Hungria et al. (2001)
[b] Control plants with (*C+N*) or without (*C–N*) mineral N (80 mg N plant^{-1} week^{-1}); data not included in the statistical analysis
[c] LSD between treatments, with four replicates per treatment

Host range

This experiment was performed under sterile conditions, in glass jars containing N-free nutrient solution, as described before (Andrade and Hamakawa 1994). Plants of alfalfa (*Medicago sativa*), common bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*), crotalaria (*Crotalaria juncea*), siratro (*Macroptilium atropurpureum*) and velvet bean *(Mucuna cochinensis)* were inoculated with each strain, with three replicates per species per strain.

Results

N_2-fixation capacity

In the experiment in which soybean cv. BR-16 was inoculated with each strain individually and grown in sterile substrate receiving N-free nutrient solution, several Bra-

Table 2 Nodule number and dry weight (*NDW*), and nodule occupancy (*NO*) by Brazilian rhizobial isolates and by *S. fredii* and *B. elkanii* reference strains and total N accumulated in shoots (*TNS*) of soybean cultivars BR-16 and Davis inoculated with a mixture of each strain with *B. elkanii* strain SEMIA 5019. Plants grown in modified Leonard jars and harvested 6 weeks after emergence. For other abbreviations, see Table 1

Strain	BR-16				Davis			
	Nodulation			TNS	Nodulation			TNS
	Number (no. plant^{-1})	NDW (mg plant^{-1})	NO (%)	(mg N plant^{-1})	Number (no. plant^{-1})	NDW (mg plant^{-1})	NO (%)	(mg N plant^{-1})
Rhizobium spp.								
1	138	676	0	146.1	99	656	38	131.2
2	176	668	0	127.3	105	425	24	135.5
3	128	593	0	123.0	120	654	30	132.1
4	130	554	0	130.2	109	700	24	147.5
5	132	625	0	149.7	118	685	22	129.3
6	140	540	0	119.0	117	659	12	134.8
7	160	540	0	117.8	152	681	2	131.7
9	123	630	0	158.2	113	712	2	146.5
10	121	620	0	145.4	104	698	10	118.3
12	137	652	0	165.8	98	681	7	139.3
13	132	660	15	175.0	117	685	14	142.2
14	117	650	0	149.4	116	666	15	119.1
15	154	654	0	147.2	58	300	0	66.5
17	120	568	75	152.5	97	607	43	152.3
18	119	580	0	128.6	116	689	0	140.9
19	125	692	0	152.2	100	694	12	144.4
20	120	627	0	144.3	110	742	0	138.2
21	90	286	0	58.6	112	642	0	115.1
22	114	685	0	150.0	100	637	0	132.2
23	151	702	0	145.2	112	660	5	144.3
24	115	677	0	138.1	108	533	5	129.4
25	144	610	0	136.1	121	720	13	144.7
26	123	590	0	135.2	107	640	5	138.2
27	125	608	0	138.8	110	600	5	122.2
29	118	572	0	130.2	129	640	2	138.1
Bradyrhizobium spp.								
8	121	595	38	146.1	100	467	35	144.5
11	101	548	43	144.5	112	621	38	138.2
16	118	682	0	148.6	109	740	0	144.2
28	119	600	0	140.0	118	610	2	125.6
30	115	580	0	129.1	115	622	0	135.5
S. fredii								
205	101	510	0	120.9	90	510	5	112.7
114	95	493	0	118.1	81	440	0	109.4
B. elkanii								
5019	130	610	–	127.2	108	693	–	134.2
LSD (5%)[a]	44	181	6	24.8	36	165	7	19.7
CV (%)	24	20	9	9	26	17	12	9

[a] Least significant difference between treatments, with four replicates per treatment

zilian rhizobial isolates, including strains with a higher genetic relatedness with *Rhizobium* and with *Bradyrhizobium*, showed good nodulation and fixed as much N as the four *B. japonicum*/*B. elkanii* strains officially recommended for use in commercial inocula (Table 1). Higher N_2-fixation capacity, with plants accumulating >70 mg N plant^{-1}, was observed when cultivar BR-16 was inoculated with strains 4, 5, 11, 12, 13, 16, 22, 24 and 27; of these, all but strains 11 and 16 showed higher genetic relatedness with *Rhizobium*. Twenty-two of the 30 strains allowed an accumulation of N which was statistically higher than in plants inoculated with *S. fredii* strains USDA 205 and CCBAU 114 and six strains (6, 7, 9, 17, 18 and 21) showed poor symbiotic performance (Table 1).

Nodule occupancy

Also under sterile conditions and in the absence of mineral N, the majority of the rhizobia were poor competitors when co-inoculated with *B. elkanii* strain SEMIA 5019 (Table 2). In cultivar BR-16, just four strains showed some competitiveness against *B. elkanii*,

Table 3 Effects of soil pH on nodule number and occupancy and total N accumulated in shoots of soybean cultivar BR-16 inoculated with seven Brazilian rhizobial isolates and with *S. fredii* and *B. elkanii* reference strains. Experiment performed in pots with non-sterile soil (10^4 cells of *Bradyrhizobium* g^{-1}). Plants harvested 7 weeks after emergence

Strain	pH	Nodule number (no. plant^{-1})	Nodule occupancy (%)	Total N in shoots (mg N plant^{-1})
Rhizobium spp.				
4	5.1	79	4	88.7
	6.8	120	25	115.9
	7.9	82	35	94.4
5	5.1	121	15	94.2
	6.8	130	40	102.3
	7.9	115	45	115.0
12	5.1	88	5	121.3
	6.8	102	39	182.1
	7.9	80	42	144.2
13	5.1	78	9	95.6
	6.8	116	39	133.2
	7.9	107	52	119.2
17	5.1	52	39	72.8
	6.8	84	41	101.2
	7.9	88	54	88.9
22	5.1	104	4	136.3
	6.8	95	12	186.4
	7.9	109	24	179.8
24	5.1	88	5	126.2
	6.8	107	8	194.3
	7.9	115	15	198.1
27	5.1	132	05	104.2
	6.8	109	12	152.3
	7.9	117	18	141.9
Bradyrhizobium spp.				
8	5.1	108	12	42.5
	6.8	126	50	65.8
	7.9	117	55	48.2
11	5.1	97	8	128.2
	6.8	118	30	175.6
	7.9	85	40	154.2
16	5.1	99	23	119.2
	6.8	115	50	141.8
	7.9	95	55	135.5
S. fredii				
USDA 205	5.1	64	5	26.7
	6.8	70	10	38.9
	7.9	60	15	42.1
CCBAU 114	5.1	48	8	22.6
	6.8	53	8	35.3
	7.9	50	15	41.0
HH102-2	5.1	95	5	75.5
	6.8	98	20	94.4
	7.9	99	40	144.7
HH103-2	5.1	101	8	74.2
	6.8	135	30	104.6
	7.9	106	42	175.8
SMH12	5.1	88	4	60.2
	6.8	93	20	154.6
	7.9	107	35	68.4
B. elkanii				
SEMIA 5019	5.1	139	48	188.1
	6.8	155	50	206.2
	7.9	150	45	180.6
LSD (5%)[a]		28	9	22
CV (%)		22	19	9

[a] Least significant difference between treatments, with four replicates per treatment

and two of them were genetically closer to *Bradyrhizobium*. Cultivar Davis was more compatible with the fast growers, and nodule occupancy by eight strains, six of them classified as *Rhizobium* sp., was 20% or higher, and they occupied a maximum of 43% of the nodules. It was also interesting to observe that the competitive strain 17 showed, in the previous experiment (Table 1), poor nodulation and symbiotic performance, but was able to stimulate nodulation when co-inoculated with *B. elkanii* (Table 2).

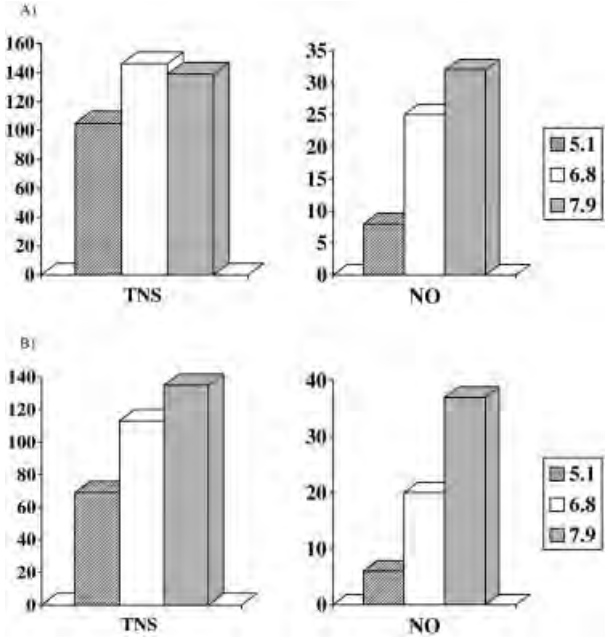

Fig. 1 Effects of soil pH on total N accumulated in shoots (*TNS*, mg N plant^{-1}) and nodule occupancy by inoculated strain (*NO*, %) of plants inoculated with: **A** 30 Brazilian rhizobial strains; **B** *Sinorhizobium fredii* strains HH102-2, HH103-2 and SMH12. Experiment performed in pots with non-sterile soil, with four replicates per treatment and plants were harvested 7 weeks after emergence

Symbiotic performance in soils with different pHs

Although isolated from acid soils (pH 3.0–5.1), nodule occupancy by Brazilian strains in non-sterile soil of pH 5.1 was low, but increased considerably with massive liming that raised the pH to 6.8 and 7.9. Table 3 shows the increases verified in nodule occupancy due to a rise in the pH for the Brazilian strains that showed the best symbiotic performance under axenic conditions (strains 4, 5, 11, 12, 13, 16, 22, 24 and 27, Table 1), and for two other strains (8 and 17) with high competitiveness (Table 2). However, although nodule occupancy was increased at pH 7.9, in general the highest total N accumulated in tissues of plants inoculated with Brazilian strains was maximized at pH 6.8 (Table 3). In contrast, with plants inoculated with *S. fredii* reference strains the highest levels of N accumulation were obtained at pH 7.9 (except for strain SMH12), and this was statistically superior for strains HH102-2 and HH103-2 (Table 3). Nodule occupancy by *B. elkanii* SEMIA 5019 was not altered by the pH, but total N accumulated in shoots was also higher at pH 6.8 (Table 3). The mean values of total N accumulated in shoots and nodule occupancy of plants inoculated with the 30 Brazilian strains and *S. fredii* strains tested before in alkaline Spanish soils [HH102-2, HH103-2 and SMH12, Buendía-Clavería et al. (1994)] are shown in Fig. 1. Competitiveness for both groups of strains increased at pH 7.9, but maximum rates of N_2 fixation with the Brazilian strains occurred at pH 6.8.

Table 4 Yield, total N accumulated in grains (*TNG*) and nodule occupancy by reference strains of *B. elkanii* (SEMIA 587), *B. japonicum* (SEMIA 5080), *S. fredii* (SMH12) and three Brazilian rhizobial strains when inoculated onto soybean cultivar BR-16. Experiment performed in an oxisol of Londrina, with an established population of *Bradyrhizobium* (10^4 cells g^{-1} soil) and pH 5.4. For other abbreviations, see Table 1

Treatment	Yield (kg ha^{-1})	TNG (kg N ha^{-1})	Nodule occupancy (%)
Rhizobium spp.			
5	3,278	191	9
22	3,144	193	7
Bradyrhizobium spp.			
8	3,104	191	11
16	3,182	184	5
S. fredii			
USDA 205	2,998	133	8
HH103-2	3,005	142	7
SMH12	3,111	175	5
Brazilian *B. japonicum/B. elkanii* commercial strains			
SEMIA 587	3,270	172	48
SEMIA 5019	3,292	183	38
SEMIA 5079	3,499	195	56
SEMIA 5080	3,533	193	35
Control treatments			
Control[a]	3,105	168	–
C+N[a]	3,525	203	–
LSD (5%)[b]	296	15	9
CV (%)	9	7	28

[a] Non-inoculated controls, –N and +N (200 kg N ha^{-1}, split twice)
[b] LSD between treatments, with six replicates per treatment

Symbiotic performance under field conditions

For the field experiment, two rhizobial and two bradyrhizobial Brazilian strains were selected. The experiment was performed in an oxisol of pH 5.4 with an established population of *Bradyrhizobium*, and the highest grain yield and total N accumulated in grains was achieved by plants inoculated with the commercial strains SEMIA 5080 and SEMIA 5079 (Table 4). No statistical difference was detected between the non-inoculated control without mineral N and the plants inoculated with four Brazilian rhizobial strains used in the field experiment (Table 4), probably due to poor competitiveness of the strains, with occupancy values of <11%. Inoculation with *B. elkanii* strains SEMIA 587 and SEMIA 5019 allowed higher yields than with the fast growers, although the values were statistically similar (Table 4).

Host range

None of the strains nodulated mucuna, alfalfa or common bean, whereas 22 (with the exclusion of 1, 2, 5, 14, 15, 16 19, 20) nodulated cowpea, 21 (with the exclusion of 7, 9, 14, 15, 17, 18 19, 20, 21) nodulated siratro and three (3, 20 and 22) nodulated crotalaria (data not shown).

Discussion

Soybean nodulation with *S. fredii*/*S. xinjiangensis* strains could be potentially useful in Brazil, since an evaluation of 80 cultivars of the Brazilian germplasm bank showed that 66% of them formed effective nodules with both species (Chueire and Hungria 1997). According to Devine (1984), effective nodulation of cv. Peking by *S. fredii* USDA 205 is controlled by a recessive allele. Thus the generalized response of Brazilian genotypes to *Sinorhizobium* might result from a restricted genetic basis, since Brazilian soybean cultivars originate from an extremely narrow range of ancestors (Hiromoto and Vello 1986). However, in the study performed in Brazil, it was shown that *S. fredii* strain USDA 205 and *S. xinjiangensis* strain CCBAU 114 were not able to outcompete a *B. elkanii* commercial strain. There are reports of *S. fredii* in Brazilian soils, isolated from *Leucaena* spp. and from common beans (Moreira et al. 1993; de Lajudie et al. 1994; Straliotto et al. 1999); however, these strains are not able to nodulate soybean (data not shown). The idea was then to isolate indigenous *S. fredii* strains from soybean nodules. Thirty strains characterized by a fast growth rate and acid production in yeast mannitol medium were isolated (Hungria et al. 2001), but none were genetically related to *S. fredii*; 25 strains fit the *R. tropici-Rhizobium* genomic species Q-*Agrobacterium* spp. branch and five strains were genetically related to *B. japonicum* and *B. elkanii*. However, those five strains showing relatedness with *Bradyrhizobium* were characterized by a fast growth rate (130–240 min) and absence of alkaline reaction in yeast mannitol medium (Hungria et al. 2001). Physiological modifications resulting from adaptation to local conditions, as has been extensively reported in Brazil (e.g. Hungria et al. 1996, 1998; Nishi et al. 1996; Boddey and Hungria 1997; Santos et al. 1999; Hungria and Vargas 2000), might be the case for our strains.

Some of the rhizobial strains from this study showed a high capacity for N_2 fixation with soybean, while others were poor symbionts. However, only four isolates, two of them showing 16S rRNA identity with *Bradyrhizobium*, were competitive against *B. elkanii* strain SEMIA 587 when cultivar BR-16 received a mixed inoculum. Previous results showed that, in controlled environments, *B. japonicum* outcompetes *S. fredii* (Dowdle and Bohlool 1985; McLoughlin et al. 1985; Cregan and Keyser 1988; Chueire and Hungria 1997). According to Buendía-Clavería et al. (1994), competitiveness is related to pH, since at pH 4.9 *B. japonicum* was more competitive, whereas at pH 8.1 *S. fredii* was more competitive, with both species occupying a similar percentage of nodules at pH 6.6. In this study, although the strains had been isolated from acid soils (pH 3.0–5.1), low competitiveness was observed under acid conditions (pH 5.1 under greenhouse conditions, and pH 5.4 in the field), and nodule occupancy increased when the pH was raised to 6.8 and 7.9, for both the Brazilian rhizobia and *S. fredii* reference strains. Competitiveness of *B. elkanii* SEMIA 5019 was not affected by the pH. The low competitiveness under acid conditions can also explain why, when isolated from pots receiving a nutrient solution with pH 6.8, the strains occupied 17–24% of the nodules (Hungria et al. 2001), while, in the field, this percentage decreased to 3–5% (Chueire and Hungria 1997). The mechanisms of rhizobial competitiveness under acid pH are still not clear, and at least for the symbiosis with common bean (*Phaseolus vulgaris*) there is a strong evidence that *R. tropici* is more competitive than the other bean rhizobial species under acidic conditions (Graham et al. 1994; Anyango et al. 1995; Hungria and Vargas 2000). Therefore, for the soybean, it could be that *B. japonicum*/*B. elkanii* species are more competitive, under acid conditions, than *S. fredii*.

Some of the claimed advantages of using fast-growing strains for soybean inoculation include the facility of commercial production, easier establishment in soils, displacement of indigenous *B. japonicum* strains and easier manipulation of genes (Cregan and Keyser 1988; Chatterjee et al. 1990; Buendía-Clavería et al. 1994). At this time, there is still no proof that the growth rate in yeast mannitol medium is related to ability to establish in soils. The main advantage in using *S. fredii* in Brazil, where 13 million doses of inocula are commercially produced annually with a shelf-life of just a few months, would be a reduced production time with decreased possibility of contamination. Therefore, as the great majority of Brazilian soils are acidic and show a very high population of naturalized *B. japonicum*/*B. elkanii*, the low competitiveness of *S. fredii* and of the Brazilian rhizobial strains investigated in this study, under the conditions described, limits, at this time, their recommendation for use in commercial inocula. Most tropical soils have developed from old geological formations and this, combined with climatic conditions, has resulted in highly weathered acidic soils. Half of the world's soybean grains are produced today in acidic soils of South America, including Brazil, Argentina, Paraguay, Uruguay and Bolivia. Furthermore, soybean has also been produced in acid soils of several African countries. Therefore the low competitiveness of fast-growing soybean rhizobial strains shown in this paper could limit their use in all those countries. However, as genetic manipulation is easier with the fast growers, the search for more competitive strains is a goal of our laboratory.

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