Competition among *Rhizobium leguminosarum* bv. *phaseoli* strains for nodulation of common bean

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Six effective *Rhizobium leguminosarum* bv. *phaseoli* strains were examined for nodulation competitiveness on common bean (*Phaseolus vulgaris* L.), using all possible two-strain combinations of inoculum. Nodule occupancy was determined with strain-specific fluorescent antibodies. The strains were divided into three groups according to their overall competitive abilities on pole bean cv. Kentucky Wonder and bush bean cv. Bountiful. Strains TAL 182 and TAL 1472 were highly competitive (greater than 70% nodule occupancy); strains KIM-5, Viking 1, and CIAT 899 were moderately competitive (approximately 50% nodule occupancy); and strain CIAT 632 was poorly competitive (less than 5% nodule occupancy). The competitiveness of the six strains was similar on the two host cultivars. The proportion of competing strains in the inoculum influenced the nodule occupancy of the highly competitive and moderately competitive strains, but not that of the poorly competitive strain. Two outstanding strains (TAL 182 and TAL 1472) were identified as ideal model strains for molecular and genetic studies on nodulation competitiveness.

Key words: *Rhizobium*, *Phaseolus vulgaris*, nodulation, competition.

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Six souches de *Rhizobium leguminosarum* bv. *phaseoli*, qui étaient de bonnes fixatrices d'azote, ont été utilisées pour l'étude de la compétition entre souches pour la formation de nodules sur les racines du haricot (*Phaseolus vulgaris* L.). Les plantes ont été inoculées avec toutes les combinaisons possibles de paires de souches. Les souches occupant les nodules ont été identifiées à l'aide d'anticorps fluorescents. Les souches ont été divisées en trois groupes d'après leur tendance générale à occuper les nodules du haricot à perche cv. Kentucky Wonder et du haricot nain cv. Bountiful. Les souches TAL 182 et TAL 1472 étaient les meilleures compétitrices, occupant plus de 70% des nodules. Les souches KIM-5, Viking 1 et CIAT 899 formaient un groupe de compétitrices modérées, occupant approximativement 50% des nodules. La souche CIAT 632 était une compétitrice médiocre identifiée dans moins de 5% des nodules. Ces résultats s'appliquent aux deux types de plantes. En ce qui concerne les deux premiers groupes, un changement dans les proportions des souches dans l'inoculum a resulté en un changement dans les proportions des souches dans les nodules. Par contre, la présence de la compétitrice médiocre dans les nodules n'a pas été influencée par ces changements dans l'inoculum. Les deux excellentes souches (TAL 182 et TAL 1472) identifiées ici constituent un modèle idéal pour l'étude de la compétition au niveau moléculaire et génétique.

Mots clés : *Rhizobium*, *Phaseolus vulgaris*, nodulation, compétition.

A yield response to inoculation of legumes depends on the use of highly effective rhizobia that are able to compete for nodulation of their hosts. Successful nodulation by an introduced strain in soil containing an indigenous population of rhizobia has been attributed to environmental variables such as soil type, pH, temperature, combined nitrogen, and certain biotic factors (Dowling and Broughton 1986). Numerous competition studies have been done to predict strain performance in the field (Amarger and Lobreau 1982; Beattie *et al.* 1989; Weaver and Frederick 1974) and to enhance nodulation competitiveness (Martinez-Romero and Rosenblueth 1990).

The competitive performance of a strain in the field reflects the effect of various factors on a strain's ability to compete for nodulation. Cultural and environmental factors condition this performance, either amplifying or diminishing a strain's basic competitive ability. Thus, while field studies permit the interplay of all factors that impact on the competition process, the complexity of the natural system does not permit the easy comparison of the competitive abilities of various strains. For detailed molecular and genetic studies on nodulation competitiveness, characterization of the basic competitive ability of model strains in a defined system is necessary. The objective of this study was to characterize the competitive abilities of six *Rhizobium leguminosarum* bv. *phaseoli* strains for nodulation of common bean (*Phaseolus vulgaris* L.), using all possible two-strain combinations of competing strains. The emphasis was on the measurement of the inherent competitive abilities of the strains without the modifying effect of environmental variables. This study led to the identification of two highly competitive and effective strains (TAL 182 and TAL 1472) that are ideal as model strains for genetic manipulation.

The six effective *R. leguminosarum* bv. *phaseoli* strains used in this study were maintained on yeast extract – mannitol (YEM) agar slants (Bohlool and Schmidt 1970) containing 0.05% $CaCO_3$. The strains could be distinguished serologically by epifluorescence microscopy (data not shown) after treatment with strain-specific fluorescent polyclonal antibodies (Schmidt *et al.* 1968) obtained from the NifTAL Project, University of Hawaii. Although TAL

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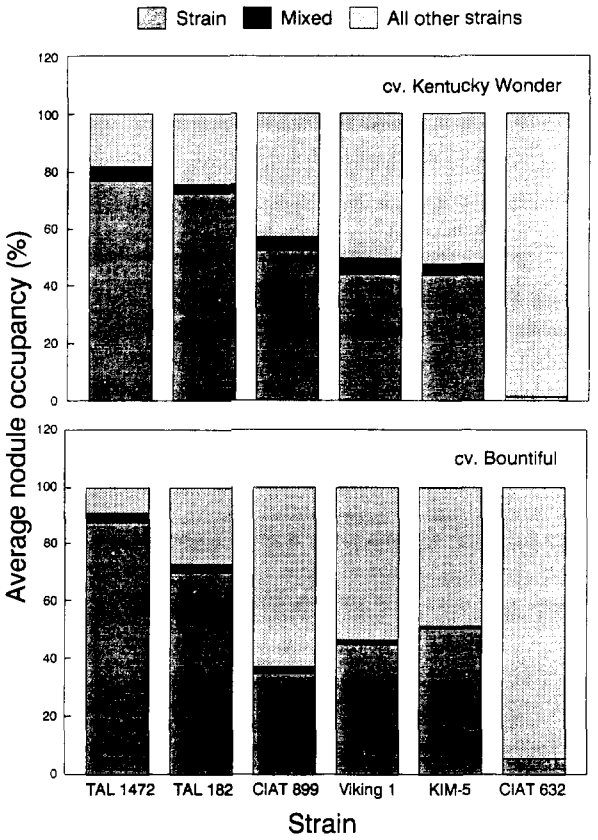

FIG. 1. Overall competitiveness of *R. leguminosarum* bv. *phaseoli* strains on *P. vulgaris* (L.) cv. Kentucky Wonder and cv. Bountiful. The values represent the nodule occupancy of each strain averaged from every combination where the strain competed with the other strains in 1:1 proportion.

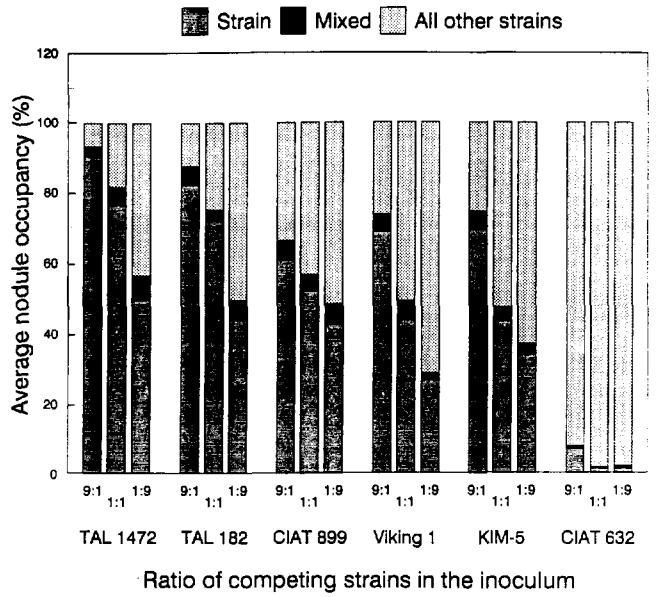

FIG. 2. Effect of inoculum proportions on the competitiveness of *R. leguminosarum* bv. *phaseoli* strains on *P. vulgaris* (L.) cv. Kentucky Wonder. The values represent the nodule occupancy of each strain averaged from every combination where the strain competed with the other strains in specific proportions.

182 and TAL 1472 originally cross-reacted, strain-specific fluorescent antibodies were obtained after reciprocal adsorption with the heterologous cells, as in Robert and Schmidt (1983).

The strains were evaluated for nodulation competitiveness on common bean (*P. vulgaris* L.). The competitive abilities of the six strains were compared in pairwise inoculations of pole bean cv. Kentucky Wonder (Burpee Seed Co., Clinton, IA) and bush bean cv. Bountiful (NifTAL Project, University of Hawaii) in all possible combinations. The seeds were surface sterilized in 30% H_2O_2 for 20 min and rinsed five times with sterile water. After 2 days of germination on moist sterile filter paper, one seedling was transferred aseptically to each jar. Each strain was grown in 10 mL of YEM broth for 2 days at 28°C. The cultures were diluted to 10^6 cells mL^{-1} and mixed in different proportions (9:1; 1:1; 1:9), and 1 mL was inoculated onto each plant. All plant tests were done in five replicate jars (Leonard 1943) filled with a 3:1 (v/v) mixture of vermiculite–perlite. The nitrogen-free nutrient solution (Hoagland and Arnon 1938) was modified to provide the plants with quarter-strength salts and full-strength trace elements. The jars were completely randomized in a growth chamber (25:18°C light:dark temperature, 16-h day length, 500 $\mu E\ m^{-2}\ s^{-1}$ light intensity at plant level). The controls consisted of uninoculated plants as well as plants inoculated singly with each of the competing strains.

Nodules were collected 4 weeks after inoculation and frozen at −20°C until use. Nodule occupancy by each strain was determined using strain-specific fluorescent antibodies. Ten nodules per plant were selected at random for a total of 50 nodules per strain combination. The nodules were crushed singly in H_2O, and smears were made on duplicate slides. The two slides were stained separately with fluorescent antibody (FA) specific for each of the competing strains.

The intrinsic competitive ability of a strain was evaluated on the basis of its nodule occupancy when it competed (1:1 proportion) with another in a wide spectrum of strain combinations. Competitiveness groups were derived by averaging the percent nodule occupancy of each strain against that of all the other competing strains in the various treatment combinations. The six strains could be grouped according to their competitive abilities on two cultivars (Fig. 1). Two strains (TAL 182 and TAL 1472) were highly competitive, occupying >70% of the nodules. Three strains (CIAT 899, Viking 1, and KIM-5) were moderately competitive, occupying approximately 50% of the nodules. One strain (CIAT 632) was poorly competitive, occupying <5% of the nodules. The overall competitive ability of a strain was similar on pole bean cv. Kentucky Wonder and bush bean cv. Bountiful, indicating that these two host plants did not selectively influence nodule occupancy by specific strains. In other studies, the differential competitive abilities of various *R. leguminosarum* bv. *phaseoli* strains were also observed in the field. Introduced strains showed nodule occupancies of 10–15% (Beynon and Josey 1980), 3–68% (Beattie *et al.* 1989), and 100% (Robert and Schmidt 1983) in the presence of indigenous populations. One of the highly competitive strains identified in this study, TAL 182, was also found to dominate in the nodules when inoculated in the field (Abaidoo and van Kessel 1989; Abaidoo *et al.*

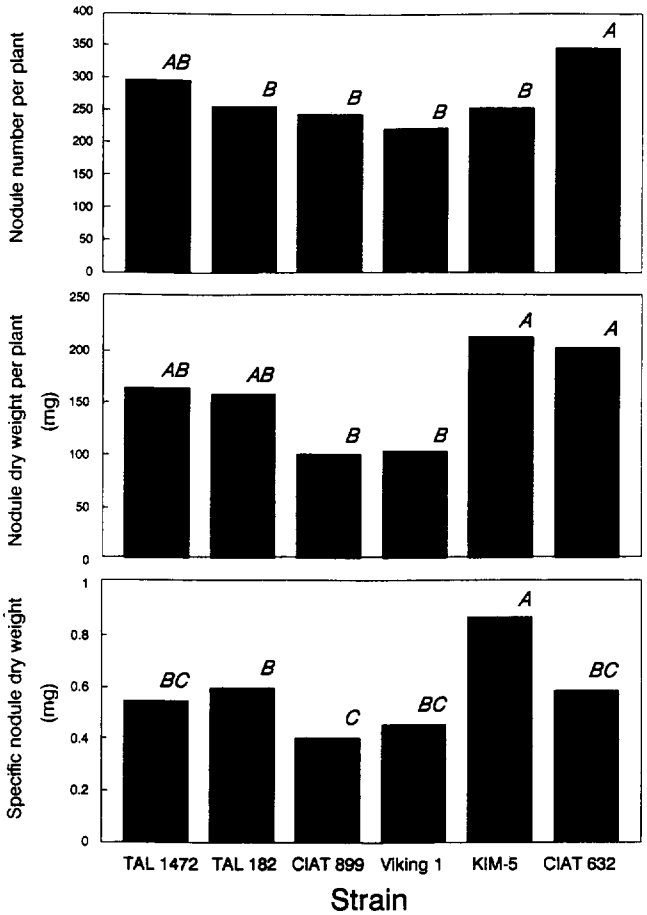

FIG. 3. Nodulation characteristics of *R. leguminosarum* bv. *phaseoli* strains on *P. vulgaris* (L.) cv. Kentucky Wonder. Bars with the same letters are not significantly different by Duncan's multiple-range test ($P < 0.05$).

1990). However, KIM-5 (Josephson and Pepper 1984; Beattie *et al.* 1989) and Viking 1 (Robert and Schmidt 1983), which are considered highly competitive in the field, were only moderately competitive when challenged by the strains used in this study.

To determine if the competition pattern of the strains was altered by the inoculum input ratio, cv. Kentucky Wonder was inoculated with pairs of strains in three proportions. The overall relationship between the inoculum ratio and nodule occupancy was derived by averaging the nodule occupancies of each strain from all the strain combinations within one inoculum ratio (Fig. 2). There was a general tendency for nodule occupancy to change with the relative proportion of the competing strains in the inoculum. In all cases, inoculation in a 9:1 ratio in favor of a strain increased the average nodule occupancy by this strain, whereas inoculation in a 1:9 ratio in favor of the other strain decreased the average nodule occupancy by the first strain. However, the result of this shift was notable only in the highly competitive and moderately competitive strains. Nodule occupancy of the moderately competitive strains when inoculated in 9:1 ratio in their favor became comparable with that of the highly competitive strains when inoculated in equal proportions. In contrast, strain CIAT 632 was consistently a poor competitor, occupying <10% of the nodules even when it outnumbered its competitor in the inoculum. Thus, competitiveness appears to be a flexible character, but only those strains that possess a basic ability to compete can attain significant nodule occupancy. Moreover, this plasticity may only be observed under controlled conditions. In the field, there is no evidence that strain competitiveness is a function of cell numbers in the rhizosphere (Abaidoo *et al.* 1990; Moawad *et al.* 1984; Robert and Schmidt 1983).

TABLE 1. Effectiveness index* of *R. leguminosarum* bv. *phaseoli* strains on four *P. vulgaris* (L.) cultivars 5 weeks after inoculation

	Bush beans†		Pole beans‡		
Strain	BL	BR	KW	MW	Mean
TAL 1472	3.8	2.8	4.0	6.8	4.4*a*§
TAL 182	3.2	3.8	3.1	6.8	4.2*a*
KIM-5	3.0	3.4	2.6	6.4	3.8*ab*
Viking 1	2.3	3.9	2.0	6.1	3.6*ab*
CIAT 899	2.6	3.7	4.2	5.2	3.9*ab*
CIAT 632	2.6	2.9	2.4	4.8	3.2*b*
Mean	2.9*B*§	3.4*B*	3.0*B*	6.0*A*	

*The effectiveness index was calculated by dividing the shoot dry weight of the inoculated plant by the uninoculated nitrogen-free control.
†BL, cv. Blue Lake; BR, cv. Brazil.
‡KW, cv. Kentucky Wonder; MW, cv. Manoa Wonder.
§Strain by cultivar interaction was not significant. Values followed by the same lower case letter in a column or the same upper case letter in a row are not significantly different by Duncan's multiple-range test ($P < 0.05$).

The nodulation characteristics of the strains were also evaluated on cv. Kentucky Wonder by single-strain inoculation. The plants were harvested 5 weeks after inoculation, and total number of nodules and nodule dry weight were measured. Nodule dry weights were determined after drying at 75°C to constant weight, and specific nodule dry weight was determined by dividing the total dry weight of nodules by the total number of nodules. Figure 3 shows significant differences among strains in their nodule number ($P < 0.05$), nodule dry weight ($P < 0.01$), and specific nodule dry weight ($P < 0.01$). CIAT 632 induced a significantly higher nodule number than four other strains (TAL 182, CIAT 899, Viking 1, and KIM-5). Inoculation with CIAT 632 and KIM-5 resulted in significantly higher nodule dry weights than inoculation with two other strains (CIAT 899 and Viking 1). The highest specific nodule dry weight was obtained with KIM-5. However, although the strains differed in their nodulation characteristics, no correlation between competitiveness and nodulation characteristics was found. The highly competitive strains were generally intermediate in their nodulation characteristics. This lack of correlation was also observed in studies with other legume species (George *et al.* 1987; Pinto *et al.* 1974).

The strains were evaluated for effectiveness on two pole beans, cv. Manoa Wonder (Department of Horticulture, University of Hawaii) and cv. Kentucky Wonder, and on two bush beans, cv. Brazil (NifTAL Project, University of Hawaii) and cv. Blue Lake (Burpee Seed Co., Clinton, IA), by single-strain inoculation of plants grown in Leonard jars. Five replicates of uninoculated plants per cultivar served as controls. The plants were harvested 5 weeks after inoculation, and the shoot dry weight was determined after drying

at 75°C to constant weight. The effectiveness index was expressed as the proportionate increase in shoot dry weight of the inoculated plant over the uninoculated control. Strain effectiveness on four cultivars of *P. vulgaris* L. is given in Table 1. There were no significant cultivar by strain interactions, but effectiveness was significantly influenced by strain ($P < 0.05$) and cultivar ($P < 0.001$). The six strains showed varying levels of effectiveness: high (TAL 1472 and TAL 182), intermediate (CIAT 899, Viking 1, and KIM 5), and low (CIAT 632), while the pole bean cv. Manoa Wonder showed the highest effectiveness. With the cultivars used in this study, there was a correspondence between the strain groupings based on effectiveness and on competitiveness.

Of the six *R. leguminosarum* bv. *phaseoli* strains, TAL 182 and TAL 1472 are ideal model strains for the study of the molecular basis of competitiveness. The superior competitive ability of TAL 182 was demonstrated under different soil types and environmental variables (Abaidoo and van Kessel 1989; Abaidoo *et al.* 1990; Somasegaran and Bohlool 1990), and its high effectiveness was also shown on several cultivars of common bean (Table 1; Pacovsky *et al.* 1981; Somasegaran and Bohlool 1990). The contrast provided by the highly competitive - poorly competitive strain combination of TAL 182 or TAL 1472 versus CIAT 632 also represents a good model for autecological studies.

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