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GENETIC DIVERSITY OF INDIGENOUS COMMON BEAN (*PHASEOLUS VULGARIS* L.) RHIZOBIA FROM THE STATE OF MINAS GERAIS, BRAZIL

Adalgisa Ribeiro Torres[1]; Luciana Cursino[2]; Júpiter Israel Muro-Abad[3]; Eliane Aparecida Gomes[4]; Elza Fernandes de Araújo[1]; Mariangela Hungria[5*]; Sérvio Túlio Alves Cassini[6]

[1]Departamento de Microbiologia, Universidade Federal de Viçosa, Viçosa, MG, Brasil; [2]Department of Plant Pathology and Plant-Microbe Interaction, Cornell University, New York State Agriculture Experimental Station, Geneva, New York, USA; [3]Aracruz Celulose S.A., Centro de Pesquisa e Tecnologia, Aracruz, ES, Brasil; [4]Embrapa Milho e Sorgo, Sete Lagoas, MG, Brasil; [5]Embrapa Soja, Londrina, PR, Brasil; [6]Departamento de Engenharia Ambiental, Centro Tecnológico, Universidade Federal do Espírito Santo, Vitória, ES, Brasil.

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ABSTRACT

We characterized indigenous common bean rhizobia from five districts of the state of Minas Gerais, Brazil. The isolates were trapped by two common bean varieties, the Mineiro Precoce (Andean origin) and Ouro Negro (Mesoamerican origin). Analysis by BOX-PCR of selected isolates detected a high level of genetic diversity.

Key words: BOX-PCR; *Phaseolus vulgaris*; *Rhizobium*.

Commom bean (*Phaseolus vulgaris* L.) grains represent the most important source of protein, especially for low-income people in Latin America, and also in South and West Africa. Mesoamerica and Andean South America are considered the main centers of genetic origin and/or domestication of *P. vulgaris* (6), but the legume has been widely grown in Brazil for thousands of years. Nowadays, Brazil is the largest grower and consumer of common beans worldwide, but national mean yields are low, estimated at 817 kg ha $^{-1}$ in the last crop season (4).

An important aspect of this crop is its capacity of establishing symbiotic associations with rhizobial species capable of nodulating and in most cases fixing atmospheric nitrogen (N_2). However, most of the symbiotic interactions are not effective (13). Studies performed in this last decade have shown that the selection of effective strains adapted to local environmental conditions may represent a successful approach that should be pursued (8). Nevertheless, it is also very important to assess the genetic diversity of indigenous rhizobial communities within the main producing areas, as the composition of the local community can affect the response to inoculation with superior strains. Unfortunately, knowledge of the genetic diversity of indigenous common bean rhizobia in most Brazilian ecosystems is still very poor.

***Corresponding Author. Mailing address:** Embrapa Soja, PO Box 231, 86001-970, Londrina, Paraná, Brazil.; Tel: +55-43 3371-6206, Fax +55-43 3371-6100.; E-mail: hungria@cnpso.embrapa.br

This study aimed at evaluating the genetic diversity of indigenous common bean rhizobia from the state of Minas Gerais, one of the most traditional states in growing this legume in Brazil. We selected BOX-PCR as the molecular typing technique due its reproducibility, easiness, quickness, and high discriminatory power at the strain level (14), yielding results that may give a good correlation with pairwise DNA-DNA analyses (16).

Strains representative of described rhizobial genera/species capable of nodulating common bean were used for comparison. The following strains were provided by Dr. P. van Berkum (USDA, Beltsville, MD, USA): *Sinorhizobium meliloti* USDA 1002^T (=ATCC 9930, =LMG 6133, =3DOa2); *Rhizobium leguminosarum* bv. trifolii USDA 2145 (=ATCC 14480, =LMG 8820, =3DIK22a); *R. leguminosarum* bv. phaseoli USDA 2671 (=RCR 3644); *Bradyrhizobium japonicum* USDA 94; *Mesorhizobium loti* NZP 2213^T (=ATCC 33669, =LMG 6125, =USDA 3471); *M. ciceri* USDA 3383; *S. fredii* USDA 205^T (=ATCC 35423, =LMG 6217, =PRC 205). Dr. E. Martínez, Cuernavaca, Mexico sent the strains *R. tropici* type A CFN 299 (=USDA 9039, =LMG 9517), type B CIAT 899^T (=UMR 1899, =USDA 9030, =TAL 1797, =HAMBI 1163, =SEMIA 4077, =ATCC 49672) and *R. etli* bv. phaseoli CFN 42^T (=USDA 9032). Dr. Noelle Amarger (INRA, Dijon, France) sent the strain H152^T of *R. giardinii* bv. giardinii.

Soil samples were collected from five different areas in the state of Minas Gerais, Brazil (Table 1) from the 0–20 cm layer, with a soil corer that was cleaned with alcohol (95%) and flamed between samplings. Two bean varieties from contrasting centers of genetic origin were used as trap host: Mineiro Precoce (Andean region, beige seeds) and Ouro Negro (Mesoamerican region, black seeds), both previously reported as good hosts for N_2–fixing bacteria (5). Seeds were surface-disinfested according to Vincent (1970) and pre-germinated at 28°C in Petri dishes containing sterile 0.5% (w/v) agar in water. Seedlings were then transplanted to vases containing 500 g of each soil sample. Plants were grown under greenhouse conditions at 28/22°C (day/night) and received N-free nutrient solution (3). After four weeks, the rhizobia were isolated on yeast extract-mannitol agar (YMA) medium (21), from fresh root nodules. The experiment was set up as a randomized complete block design with 3 replicates for each treatment.

The rhizobia were isolated from fresh root nodules on YMA medium. The purity of the rhizobial cultures was confirmed by repeatedly streaking the bacteria on YMA medium and by determining colony morphology, Congo red absorption (25 mg/ml), and acid/alkali production on YMA containing 0.01% bromothymol blue (1). Each colony was then transferred to YM liquid broth (YMB), and after further growth, the bacteria were dried on porcelain beads and stored at 4°C, as described by Hunt *et al*. (10). Working cultures were maintained on YMA slants at 4°C.

Table 1. Localization and relevant soil management of the sites in the state of Minas Gerais, Brazil, used in this study

No	Site	Geographic coordinates	Soil management
1	Capinópolis	18°41'S 49°38'W	Cropped with common beans
2	Janaúba	15°47'S 43°18'W	Cropped with common beans
3	Maria da Fé	22°17'S 45°23'W	Cropped with common beans
4	Patos de Minas	18°36'S 46°31'W	Cropped with common beans
5A	Viçosa	20°45'S 42°51'W	Cropped with common beans
5B	Viçosa	20°45'S 42°51'W	Undisturbed area covered with native vegetation

Twenty isolates trapped by both varieties, Ouro Negro and Mineiro Precoce, were randomly selected for BOX PCR analysis: 4 from Viçosa, 3 from Janaúba, 4 from Maria da Fé, 5 from Patos de Minas, and 4 from Capinópolis. Rhizobial DNA was extracted as described by Ausubel *et al*. (2). PCR amplification was carried out with BOX A1R (5´-CTACGGCAAGGCGACGCTGACG-3´) primer (Invitrogen), following the procedure described by Versalovic *et al.* (20). The fingerprints obtained in PCR procedures were analyzed using BioNumerics software (Applied Mathematics, Kortrijk, Belgium, version 4.6), with the UPGMA algorithm [unweighted pair-group method with arithmetic mean, (18) and Jaccard coefficient (J) (11)]. The genetic diversity of the rhizobial communities was estimated using the Shannon diversity index:

$$H = -\sum_{i=1}^{n}\left(\left(\frac{x_i}{x_0}\right)\ln\left(\frac{x_i}{x_0}\right)\right)$$ (17), where H is the genetic diversity, x_i the number of isolates in each group, and x_0 the total number of isolates.

Some previous studies have examined the diversity of rhizobia associated with common bean in different producing areas of Brazil (8, 12, 15). Although a great diversity in morphological, physiological, genetic and symbiotic properties has been found among common bean rhizobial strains from Brazil, there are few reports (19) on genetic variability among indigenous rhizobia from the state of Minas Gerais.

We obtained 77 indigenous rhizobial isolates in this study: 33 from Capinópolis, 8 from Janaúba, 4 from Maria da Fé, 22 from Patos de Minas and 10 from Viçosa. The Mineiro Precoce variety trapped 58% of the isolates, while Ouro Negro trapped 42%. In the soils of Janaúba, Patos de Minas and Viçosa, the ability to trap indigenous common bean rhizobia was similar for both varieties. However, variety Mineiro Precoce was more effective in trapping rhizobia from Capinópolis, while Ouro Negro showed a better performance with Maria da Fé (data not shown).

It is well known that common bean seeds contain a variety of flavonoids related to the seed color, responsible for the rhizobial *nod*-gene inducing activity and that may affect nodulation (9). However, in our study there is no recognizable effect of either the variety or the sampling site on rhizobial diversity. In another study using ERIC-PCR, Grange and Hungria (7) also did not detect differences between black- or beige-seeded common beans in their capacity to trap rhizobia from the soils of two other Brazilian states, Paraná and Pernambuco.

All isolates produced a single morphologically homogeneous group of colonies, which were white, displayed fast growth (three days), and had a high exopolysaccharide production and acidic changes in growth culture medium.

A high level of polymorphism was observed in the BOX-PCR analysis, as in the clustering analysis using the UPGMA algorithm and the Jaccard coefficient, the profiles were joined at a very low final level of genetic similarity, of only 10% (Fig. 1). Four rhizobial groups were clearly observed. Group 1 clustered 8 isolates and *M. loti* reference strain USDA 3471 with 27% similarity and could be split into 2 subgroups, in addition to a single isolate [21] trapped by Mineiro Precoce grown on a Capinópolis soil. This group included isolates from Capinópolis, trapped by both varieties and isolates from Viçosa, trapped by Ouro Negro. Group 2 included 12 isolates and the *R. tropici* type A reference strain CFN 299, at a 32% level of similarity. However, the analysis showed that the isolates were different from the *R. tropici* type B reference strain CIAT 899. Within this group, some isolates displayed complete similarity, *e.g.*, isolates 10, 11, 12 and 13, and 7 and 14. Three of these isolates [11, 12 and 13] were trapped by Ouro Negro grown in Maria da Fé and Patos de Minas soils. Only one of these isolates [10] which exhibited complete profile similarity was isolated from Mineiro Precoce plants grown in soil samples of Maria da Fé. It is noteworthy that two isolates within group 2 [7, 14] showed complete similarity of profiles although isolated from different sample locations. Groups 3 and 4 clustered only type and reference strains at 29% and 38% similarity, respectively. It should also

be mentioned that within group 2, some isolates differed only by one or two bands, *e. g.*, isolates 6, 7, 9 and 14. Finally, isolate 8 from Janaúba, trapped by Mineiro Precoce, was quite distinct from all groups. None of the isolates in this study clustered with *Azorhizobium caulinodans*, *Bradyrhizobium japonicum*, *B. liaoningense*, *Mesorhizobium ciceri*, *Rhizobium etli* bv. phaseoli and *R. giardinii* bv. giardinii, *R. leguminosarum* bv. phaseoli, *R. leguminosarum* bv. trifolii, *R. tropici* type B, *Sinorhizobium* (=*Ensifer*) *fredii* or *S. meliloti*.

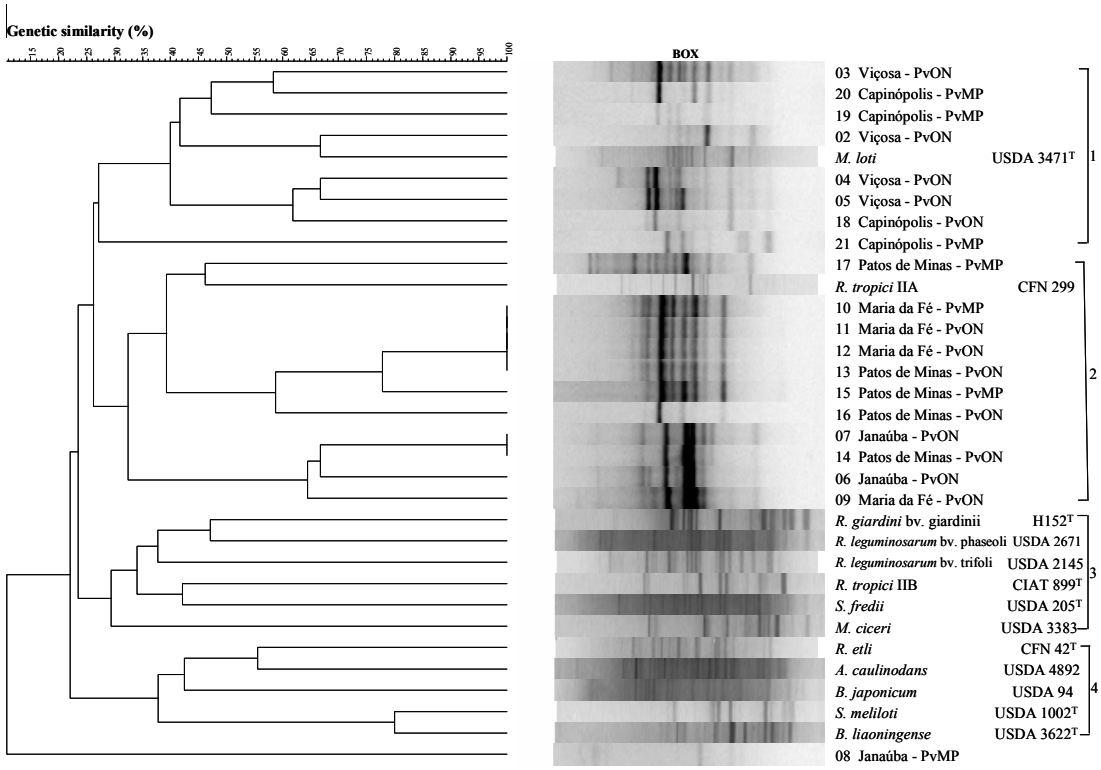

Figure 1. Dendrogram showing the bean rhizobial isolates from the state of Minas Gerais and some reference and type strains used in this study after cluster analysis of BOX-PCR products using the UPGMA method and the Jaccard coefficient. PvON- *Phaseolus vulgaris* cv. Ouro Negro; PvMP- *Phaseolus vulgaris* cv. Mineiro Precoce.

Despite the high polymorphism observed with BOX primer, diversity index (*H*) was slightly higher in Janaúba, Maria da Fé and Patos de Minas (0.37) than in Capinópolis and Viçosa (0.35). Indeed, in our study, the genetic characterization using BOX-PCR was very effective for differentiating the isolates.

Indigenous bean rhizobia can be recovered from both uncropped and cropped soils in Brazil (7). In this study, none of the areas had a previous history of inoculation with rhizobia, thus the strains obtained represent the indigenous diversity.

It would now be important to perform long-term field trials with these isolates to establish strategies towards increasing the contribution of the biological N_2 fixation to the common bean N nutrition and to find inoculants to specific locations of Minas Gerais.

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