Available online at www.sciencedirect.com
SCIENCE DIRECT®
Applied Soil Ecology 32 (2006) 210–220
Applied Soil Ecology
www.elsevier.com/locate/apsoil

Genetic diversity of rhizobia associated with common bean (*Phaseolus vulgaris* L.) grown under no-tillage and conventional systems in Southern Brazil

G. Kaschuk [a,b,1], M. Hungria [a,*], D.S. Andrade [c], R.J. Campo [a]

[a] *Embrapa Soja, Cx. Postal 231, 86001-970, Londrina, PR, Brazil*
[b] *Department of Microbiology, UEL, Londrina, Brazil*
[c] *IAPAR, Cx. Postal 481, 86001-970 Londrina, Brazil*

Received 23 April 2004; accepted 20 June 2005

Abstract

Brazil is the largest producer and consumer of the common bean (*Phaseolus vulgaris* L.), but yields are often low and may be improved by a higher N supply through symbiosis with rhizobia. One main limitation to the N_2-fixation process is the susceptibility of the symbiosis to environmental stresses frequent in the tropics, such as high soil temperatures and low soil moisture contents. Among other benefits, the no-tillage (NT) system reduces those stresses resulting in higher N_2 fixation rates and yields; however, the effects of NT on rhizobial diversity are poorly understood. This study evaluated the diversity of rhizobia compatible with common bean in cropping areas under the NT or the conventional tillage (CT) systems in Ponta Grossa, State of Paraná, Southern Brazil. Genetic diversity was assessed by DNA analyses using the methodologies of BOX-PCR and RFLP-PCR of the 16S rDNA region. A high level of diversity was observed among the strains and the DNA profiles from the CT system were quite different from those from the NT system. Twenty-three RFLP-PCR profiles were obtained, indicating that many tropical rhizobial species remain to be described. Strain differentiation was achieved in the BOX-PCR analysis; diversity was slightly higher under the NT when compared with the CT system. Surprisingly, the rhizobial grouping based on cluster analysis of the RFLP-PCR of the 16S rDNA region indicated a higher diversity of species under the CT. It could be that the environmental stability offered by the NT system has led to a decrease in the number of species, with the predominance of the most successful ones, although genetic diversity within each species has increased. The results obtained in this study show that we still understand poorly the relation between microbial diversity and soil sustainability and that the complexity of the ecosystems require the evaluation of several parameters to define and monitor soil quality.

Keywords: Bacterial diversity; Common bean; Nitrogen fixation; Rhizobium; Tillage systems

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is a main component in the diet, and often the most important source of protein of over 300 million people in Latin America and South and West Africa. Brazil is the largest producer and consumer of common bean worldwide: 4,286,200 tons were produced in 2003/2004, but with the very low mean yield of only

* Corresponding author. Tel.: +55 43 33716206; fax: +55 43 33716100.
E-mail addresses: hungria@cnpso.embrapa.br, hungria@sercomtel.com.br (M. Hungria).
[1] Present Address: Plant Production Systems Group, WUR, PO Box 430, 6700AK, Wageningen, The Netherlands.

0929-1393/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsoil.2005.06.008

699 kg ha^{-1}. Common bean is not considered an important cash crop, thus poor technology, cropping in soils with low organic matter content and fertility, especially deficient in N, contribute greatly to the low yields obtained in Brazil.

An increase in the N supply through the symbiotic association with efficient rhizobial strains might play a key role in obtaining agricultural and economic sustainability of the common bean crop in the tropics (Hungria et al., 2000, 2003; Mostasso et al., 2002). However, poor nodulation and low N_2-fixation rates have been frequently reported in field experiments performed worldwide (e.g., Graham, 1981; Hardarson, 1993). A contributory factor may be the high sensitivity of the symbiosis to environmental stresses. One main limiting factor in the tropics is the low P availability, that can be considered as the most widespread production constraint in many regions, as the Brazilian Cerrados, and the situation is aggravated by the intense fixation of the nutrient in oxisols (Goedert, 1983). Other major limitations in the tropics are the high soil temperature and lack of moisture (Graham, 1981; Hungria et al., 1993; Hungria and Vargas, 2000). Both high temperatures and shortage of water compromise every step of the development and function of the symbiosis, from rhizobial survival, maintenance of the genome, chemotaxis, the root infection process, nodule formation, to activity of enzymes related both to N_2 fixation and N assimilation (Hungria and Vargas, 2000).

The no-tillage (NT) management system has become widely adopted in many countries of South America. In Brazil, the area devoted to NT has increased from 2.02 Mha in 1992/1993 to more than 19 million ha today. In comparison with conventional tillage (CT), NT enhances soil moisture content and helps in the regulation of soil temperature, in addition to protecting the soil against erosion by water, improving soil structure and the stability of the aggregates, and, with time, increases the soil organic matter content, often resulting in higher yields (e.g., Derpsch et al., 1991; Bayer et al., 2002; Castro Filho et al., 2002; Hérnandez and Lopéz-Hernández, 2002). Good productivity has also been reported for the common bean under NT and minimum tillage systems (Deibert, 1995).

When compared with CT, NT also benefits the biological N_2 fixation process, most likely due to the lower soil temperatures and higher soil moisture content. There are reports of increases in rhizobial survival, growth and diversity, in induction activity of nodulation genes, in nodulation, nodule distribution in the soil profile, and in N_2 fixation rates (Voss and Sidiras, 1985; Ferreira et al., 2000; Hungria and Vargas, 2000). However, these studies were performed with soybean, an important cash crop in Brazil and the effects of management system on rhizobial genetic diversity were mentioned in just one study (Ferreira et al., 2000).

Common bean is a promiscuous host. It is nodulated by a variety of rhizobia, and in Brazil there are reports of symbioses with *Rhizobium tropici*, *R. etli*, *R. leguminosarum* and *R. giardinii*, with bacteria belonging to the genera *Mesorhizobium* and *Sinorhizobium*, and with other bacteria that may well represent new species (Mostasso et al., 2002; Grange and Hungria, 2004). The sensitiveness and promiscuity of common bean symbiosis may represent an interesting model for the examination of effects of different soil management systems on rhizobial diversity. Therefore, the aim of this research was to characterize rhizobia nodulating common bean grown under NT and CT in Southern Brazil.

2. Material and methods

2.1. Field sites and soil sampling

The sites were located at the Experimental Station of the Instituto Agronômico do Paraná (IAPAR), Ponta Grossa, Paraná, Southern Brazil. The area is located at an altitude of 880 m, 25°13′S and 50°1′W. The average rainfall is 1507 mm year^{-1}, with 123 rainy days year^{-1}; the rainiest month is January (184 mm) and the driest is August (77 mm); according to Koeppen's classification, the climate is type Cfb. The soil, a Dark Red Latosol (Haplorthox) containing (g kg^{-1}), 730 sand, 40 silt and 240 clay.

The area representing the NT system had been planted to maize (*Zea mays* L.) in summer and wild radish (*Rabanus sativus*) or common oat (*Avena sativa*) in winter for 6 years. Two years before collecting the samples common bean was planted on the wild radish residue. The main chemical characteristics were: pH in $CaCl_2$, 4.77; exchangeable Al ($cmol_c$ dm^{-3}) 0.09; cation exchange capacity (CEC) ($cmol_c$ dm^{-3}), 13.66; P ($cmol_c$ dm^{-3}), 2.5. The area representing the CT had been cultivated with common bean in summer for 10 years and left with natural spontaneous vegetation in winter. The main chemical characteristics were: pH in $CaCl_2$, 4.82; exchangeable aluminum ($cmol_c$ dm^{-3}), 0.06; CEC ($cmol_c$ dm^{-3}), 13.66; P ($cmol_c$ dm^{-3}), 2.5. Field sites had 1 ha under NT and 1 ha under CT. The system NT requires the cultivation of cover crops, as wild radish and common oat, and unfortunately we have found no area under NT with more than 2 years of bean