



Genetic diversity and symbiotic compatibility among rhizobial strains and *Desmodium incanum* and *Lotus* spp. plants

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Abstract

This work aimed to evaluate the symbiotic compatibility and nodulation efficiency of rhizobia isolated from *Desmodium incanum*, *Lotus corniculatus*, *L. subbiflorus*, *L. uliginosus* and *L. glaber* plants by cross-inoculation. Twelve reference strains and 21 native isolates of rhizobia were genetically analyzed by the BOX-PCR technique, which showed a high genetic diversity among the rhizobia studied. The isolates were also characterized based on their production of indolic compounds and siderophores, as well as on their tolerance to salinity. Fifteen of the 33 rhizobia analyzed were able to produce indolic compounds, whereas 13 produced siderophores. All the tested rhizobia were sensitive to high salinity, although some were able to grow in solutions of up to 2% NaCl. Most of the native rhizobia isolated from *L. uliginosus* were able to induce nodulation in all plant species studied. In a greenhouse experiment using both *D. incanum* and *L. corniculatus* plants, the rhizobia isolate UFRGS Lu2 promoted the greatest plant growth. The results demonstrate that there are native rhizobia in the soils of southern Brazil that have low host specificity and are able to induce nodulation and form active nodules in several plant species.

Keywords: Rhizobia, nodulation, nitrogen, specificity.

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Introduction

In southern Brazil there are large areas dominated by grasslands. These areas are a highly diverse forage resource, and most farms use this forage as a single food source for animals (Overbeck *et al.*, 2007). Native leguminous plants, such as those belonging to the genus *Desmodium*, are frequently found in these areas (Overbeck *et al.*, 2007) and, due to the symbiosis between these plants and rhizobial species, are responsible for an increase in the nitrogen content of the soil-plant system via the mechanism of biological nitrogen fixation (BNF) (Tarré *et al.*, 2001). The introduction of new leguminous species is an alternative approach to maximizing the nitrogen input in these areas (Howieson *et al.*, 2011) and increasing the protein content of pastures.

Although *Lotus* species are non-native plants in the south of Brazil they can be recommended as forage plants in these areas (Dalmarco *et al.*, 2010). Species like *L. corniculatus*, *L. subbiflorus*, *L. uliginosus* and *L. glaber* present favorable adaptation ability in regions with temperate climate (Díaz *et al.*, 2005). *Lotus* species present a high nutritional value as forage plants and, like *Desmodium*, they also use the benefit of BNF through the symbiosis with bacteria belonging to rhizobia genera (Howieson *et al.*, 2011).

Rhizobial species are considered legume endosymbionts that are able to promote plant growth by BNF (Mason-Boivin *et al.*, 2009). In general, the associations between legumes and rhizobia are very specific (Bauer, 1981). There are, however, rhizobial isolates that show low specificity to the host plant and induce nodule formation in several legume species. Hernandez *et al.* (2005) found that rhizobial strains, which presented with intermediary growth rates, were able to induce nodule formation in *L. corniculatus* and *L. glaber*. In the same work, strains with slow growth rates belonging to the genus *Bradyrhizobium*

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were able to induce nodule formation in *L. subbiflorus* and *L. uliginosus*.

BNF by symbiotic rhizobial species is not the only characteristic that aids plant growth and adaptation (Hayat *et al.*, 2010; Granada *et al.*, 2014). Symbiotic rhizobia on non-native plants can induce plant adaptation through both indirect effects, including decreases in plant disease severity and enhancements in mutualistic bacterial interactions, and direct effects, including alterations in ecological and ecosystem processes (Kempel *et al.*, 2012), hormone production and soil nutrient solubilization (Costa *et al.*, 2013; Granada *et al.*, 2013).

The aims of this work were: (1) to analyze the genetic diversity of rhizobial isolates symbiont of *D. incanum*, *L. corniculatus*, *L. subbiflorus*, *L. uliginosus* and *L. glaber* plants; (2) to verify, by cross-inoculation, the symbiotic compatibility between these bacterial isolates and the five plants species; and (3) to identify, in a greenhouse experiment, a rhizobium isolate efficient in promoting the growth of both *L. corniculatus* and *D. incanum* plants.

Material and Methods

Bacterial isolation

Plants of *D. incanum* were collected at the UFRGS Agronomic Experimental Station (30°05'22" S and 51°39'08" W) in the south of Brazil. Root nodules were detached and their surfaces sterilized by washing in 70% ethanol for 2 min, followed by washing with 2% sodium hypochlorite (v/v) for 2 min and five serial rinses with sterilized distilled water. Rhizobia were isolated according to Somasegaram and Hoben (1985). Pure colonies were grown in yeast mannitol medium (YM) (Vincent, 1970) for 72 h at 28 °C and stored at -20 °C in 50% glycerol.

For the isolation of rhizobia from the root nodules of *L. glaber* Mill (cv Pampa INTA), *L. subbiflorus* Lag (cv El Rincon), *L. corniculatus* (cv São Gabriel), and *L. uliginosus* Schkuhr (cv Maku) plants, the trap plant technique was employed. Seeds of these four *Lotus* species were surface sterilized as described above for *D. incanum* root nodules. Subsequently, these seeds were cropped in 500 mL plastic pots containing the same soil from where the *D. incanum* plants had been collected. The pots were kept in a greenhouse for 60 days. Soil moisture was verified daily, and plants were harvested after 45 days. For plants that demonstrated natural nodulation, rhizobia were isolated and stored according to the same methodology described above.

Rhizobia reference strains recommended for inoculant production in Brazil (belonging to the SEMIA collection) were provided by FEPAGRO (RS, Brazil). Rhizobia reference strains EEL698 and EEL8084 were provided by Dr. Elemar Brose from Estação Experimental de Lages (EEL, SC, Brazil). Strain U510, which is recommended for inoculant production in Uruguay, was provided by Dr.

Carlos Labandera from Ministerio de Ganadería Agricultura e Pesca (MGAP, Uruguay).

Genetic diversity of rhizobial isolates

Each rhizobial strain studied was grown in YM medium before DNA was extracted according to Sambrook and Russel (2001). DNA was amplified by polymerase chain reaction (PCR) using the enterobacterial repetitive sequences BOX A1 primer (CTACGGCAAGGCGACG CTGACG) (Versalovicj *et al.*, 1994). Reactions were performed according to Giongo *et al.* (2010). The resulting DNA fingerprints were transformed into a binary matrix and subjected to a clustering analysis using the UPGMA algorithm and the Jaccard coefficient using PAST software (Hammer *et al.*, 2001).

Cross-inoculation experiment

The ability of the rhizobial isolates and reference strains to form nodules capable of efficiently fixing nitrogen in *D. incanum*, *L. corniculatus*, *L. subbiflorus*, *L. glaber* and *L. uliginosus* plants was evaluated by cross-inoculation using an *in vitro* inoculation technique. This technique consisted of adding 5 mL of Sarruge nutritive solution (Sarruge 1975) (25% diluted, without nitrogen) to a test-tube with a germitest paper tape on the inner side. Seeds of these five plant species were surface sterilized as above described and were then pre-germinated at 28 °C for three days. Individually, seedlings were placed on the germitest paper and inoculated with 2 mL of culture of one bacterial isolate grown in YM medium (with 10^8 cfu mL⁻¹). Each treatment group consisted of three replicates. The seedlings were maintained in a growth chamber with a photoperiod of 12 h of light for 45 days. After this period, the nodulation ability of each bacterial isolate in each plant species was evaluated. If plants presented nodules and leaves with dark green coloration after 45 days, the bacterial isolate was considered able to form efficient (active) nodules in the host plant, and, consequently, was defined as a nitrogen fixer.

Evaluation of siderophores and indolic compound production and saline resistance

Rhizobial strains were grown in YM medium supplemented with tryptophan (2.4×10^{-3} M) for 48 h at 28 °C at 120 rpm. Indolic compound (IC) production was analyzed according to Glickmann and Dessaux (1995). Siderophore production was evaluated by the inoculation of 10 µL of each bacterial culture (as previously described) in agar (1.5%) King B medium (Glickmann and Dessaux, 1995). The medium was five-fold diluted and supplemented with CAS dye (Schwyn and Neilands, 1987). The evaluation of these abilities was only qualitative, and the rhizobial isolates were classified as either producers or non-producers of indolic compounds and/or siderophores.

Saline tolerance was evaluated by the inoculation of 20 μL of each bacterial culture (as previously described) in agar YM plates, to which 1, 2 or 3% NaCl had been added. If a bacterial isolate was able to grow at a particular salt concentration, it was classified as salt-resistant.

Symbiotic efficiency experiment between rhizobial isolates and *D. incanum* and *L. corniculatus* plants

Six rhizobial isolates were able to nodulate both *L. corniculatus* and *D. incanum* plants (UFRGS Lu2, UFRGS Lu13, UFRGS Ls1, UFRGS Lc336, UFRGS Di9 and EEL 698). These bacterial isolates presented different growth promotion abilities and were selected for the plant inoculation experiment.

This *in vivo* assay was conducted in Leonard jars with sterile substrate composed of vermiculate and sand (3:1) on the top and Sarruge nutritive solution (25% diluted) without nitrogen on the bottom. Each treatment was composed of three replicates of two plants of *D. incanum* (native variety) or *L. corniculatus* (cv São Gabriel) per Leonard jar inoculated with one rhizobial isolate (5 mL of rhizobia culture with 10^8 cfu mL^{-1}). Two control treatments were performed: one without nitrogen (negative control, N-) and another with 25 mg of nitrogen, in the form of NH_4NO_3 , per jar (positive control, N+). The strain recommended for inoculation of *L. corniculatus* plants, SEMIA 816, was also included. The experiment was conducted for a period of 90 days in a completely randomized design. At the end of this period, plants were harvested, and the root nodules were removed and counted. Plants were dried at 60 °C until they reached a constant weight, and root and shoot dry matters were determined. Additionally, in the experiment with *L. corniculatus* plants, the nodule dry matter and the amount of nitrogen accumulated in the shoots were also estimated (Jones *et al.*, 1991). Statistical analyses were performed by ANOVA test, with means compared by the Scott-Knott test ($p < 0.01$) using the Assisat 7.6 beta program (Silva and Azevedo, 2002).

Results

Twenty-one native rhizobia were isolated from the root nodules of *D. incanum* or *Lotus* spp. Twelve rhizobial reference strains were also included in the analysis. The native rhizobial and reference strains, their host plants, indolic compound and siderophore production capabilities and resistance to increasing salt concentrations are displayed in Table 1. With exception of SEMIA 830, all of the rhizobial reference strains (belonging to the SEMIA collection) were able to produce indolic compounds, whereas among the native rhizobia isolated from *D. incanum* and *L. uliginosus* only the isolate UFRGS Lu2 was able to do so. Approximately 39% of the rhizobia studied were able to produce siderophores. All rhizobial isolates were able to grow in up to a 1% salt substrate. Only the native rhizobial

isolates UFRGS Ls1 and UFRGS Lu2 grew in up to 2% salt, and none was able to grow in up to 3% salt.

The diversity of the 33 rhizobia studied was accessed by BOX-PCR analysis (Figure 1). These bacterial isolates were highly diverse, and with the exception of reference strains SEMIA 808 and SEMIA 806, which formed one group with approximately 80% similarity, and the native isolates UFRGS Lu13 and UFRGS Lu8, which presented 100% similarity, there was less than 70% similarity between the bacterial isolates. Bacterial strains isolated from the same plant did not form groups with high genetic similarity.

The ability of the bacterial isolates to form nodules able to fix nitrogen (active) in the five plant species studied was also highly diverse (Table 2). Most of the native rhizobial isolates and reference strains studied were able to form active nodules in their host plant. One rhizobium isolated from *D. incanum*, UFRGS Di9, also nodulated *L. corniculatus*. The reference strains SEMIA 6208 and SEMIA 6209, recommended for the inoculation of *D. ovalifolium*, and SEMIA 656, recommended for the inoculation of *Desmodium* sp., were unable to nodulate any of the five plant species studied. Five of the 11 bacterial isolates from *L. corniculatus* also nodulated *L. uliginosus* and one of them, UFRGS Lc336, was capable of forming active nodules in both plants. The rhizobia isolated from *L. glaber*, in addition to nodulating their host plant, also nodulated *L. uliginosus* plants. The majority of native rhizobial isolates from *L. subbiflorus* and *L. uliginosus* formed active nodules in at least three of the five plant species analyzed. Among these rhizobia, we highlight the isolates UFRGS Ls1, UFRGS Ls69 and UFRGS Lu13, which nodulated all five plant species analyzed, and the isolate UFRGS Lu2, which was also able to form active nodules in all plant species analyzed.

The six rhizobial isolates that were able to nodulate *D. incanum* and *L. corniculatus* plants were selected for a greenhouse inoculation experiment with these two plants. Data from *D. incanum* plants demonstrated that, with the exception of bacterial isolate UFRGS Lu2, the highest yields of shoot dry matter were produced by rhizobial isolates with lower numbers of nodules per plant (Table 3). Data from root dry matter were similar to those from treatment with nitrogen addition (N+). The most promising rhizobial isolates were EEL698, UFRGS Ls1, UFRGS Lu2 and UFRGS Lu13. Data from *L. corniculatus* plants (Table 4) demonstrated that plants inoculated with the rhizobial reference strain SEMIA 816 showed the highest number of nodules and, consequently, the highest value of nodule dry matter. Plants inoculated with the rhizobial strains SEMIA 816, UFRGS Lu2 and EEL698 showed the highest values of shoot dry matter. The highest values of shoot nitrogen content were observed in those plants inoculated with the rhizobial strains SEMIA 816 and UFRGS Lu2.

Table 1 - Native rhizobial isolates and reference strains. Their host plants and an evaluation of indolic compound and siderophore production and resistance to high salt concentrations are displayed.

Host plant	Rhizobial isolate	Indolic compound production	Siderophore production	Salt concentration (NaCl)		
				1%	2%	3%
<i>Desmodium incanum</i>	UFRGS Di6	-	+	+	-	-
	UFRGS Di7	-	-	+	-	-
	UFRGS Di9	-	-	+	-	-
	UFRGS Di10	-	-	+	-	-
	SEMIA 6208	+	-	+	-	-
	SEMIA 6209	+	+	+	-	-
	SEMIA 656	+	+	+	-	-
<i>Lotus corniculatus</i>	UFRGS Lc10	+	-	+	-	-
	UFRGS Lc14	-	+	+	-	-
	UFRGS Lc22	-	+	+	-	-
	UFRGS Lc36	-	+	+	-	-
	UFRGS Lc322	-	+	+	-	-
	UFRGS Lc336	+	-	+	-	-
	UFRGS Lc340	-	-	+	-	-
	SEMIA806	+	-	+	-	-
	SEMIA808	+	+	+	-	-
	SEMIA816	+	+	+	-	-
<i>Lotus glaber</i>	U 510	+	-	+	-	-
	UFRGS Lg109	-	-	+	-	-
	UFRGS Lg111	+	-	+	-	-
<i>Lotus subbiflorus</i>	SEMIA830	-	-	+	-	-
	UFRGS Ls1	-	+	+	+	-
	UFRGS Ls8	+	-	+	-	-
	UFRGS Ls62	-	-	+	-	-
	UFRGS Ls69	-	+	+	-	-
	SEMIA848	+	+	+	-	-
<i>Lotus uliginosus</i>	EEL 698	-	-	+	-	-
	UFRGS Lu2	+	-	+	+	-
	UFRGS Lu8	-	-	+	-	-
	UFRGS Lu13	-	-	+	-	-
	UFRGS Lu19	-	-	+	-	-
	SEMIA822	+	+	+	-	-
	EEL8084	+	-	+	-	-

+ = Native rhizobial isolate or reference strain able to produce indolic compounds or siderophores and/or resistant to a high salt concentration. - = Native rhizobial isolate or reference strain unable to produce indolic compounds or siderophores and/or not resistant to a high salt concentration.

The native rhizobial isolate UFRGS Lu2, isolated from *L. uliginosus* plants, was able to produce indolic compounds and was the most promising bacterial isolate for use in facilitating the adaptation of *L. corniculatus* plants and the growth promotion of this plant and *D. incanum* plants. Furthermore, it behaved promiscuously because in the *in vitro* nodulation test, it was able to form active nodules in all the five plants studied in this work. The greenhouse experiment with *D. incanum* and *L. corniculatus* plants confirmed the agronomical potential of this isolate, because

plants inoculated with UFRGS Lu2 showed the best results for the evaluated parameters.

Discussion

Bacterial isolates from the same microbial species can be evaluated for intraspecific diversity based on an analysis of repetitive nucleotide sequences, including the BOX motif (Binde *et al.*, 2009; Ishii and Sadowsky, 2009; Li *et al.*, 2009; Díaz *et al.*, 2013). The 33 rhizobial isolates

studied in this work were highly diverse. These data were expected because the rhizobia studied were obtained from different host plant species (Kennedy, 1999; Garbeva *et al.*, 2004) and presented different growth promotion characteristics. This high variability appears to be very common in

studies with native rhizobia. Giongo *et al.* (2008), in their study of *Bradyrhizobium* spp. symbiosis with *Glycine max* plants, and Vargas *et al.* (2009), studying rhizobia symbiosis with clover, demonstrated high genetic diversity using the same technique utilized in this work (BOX-PCR). Gu *et*

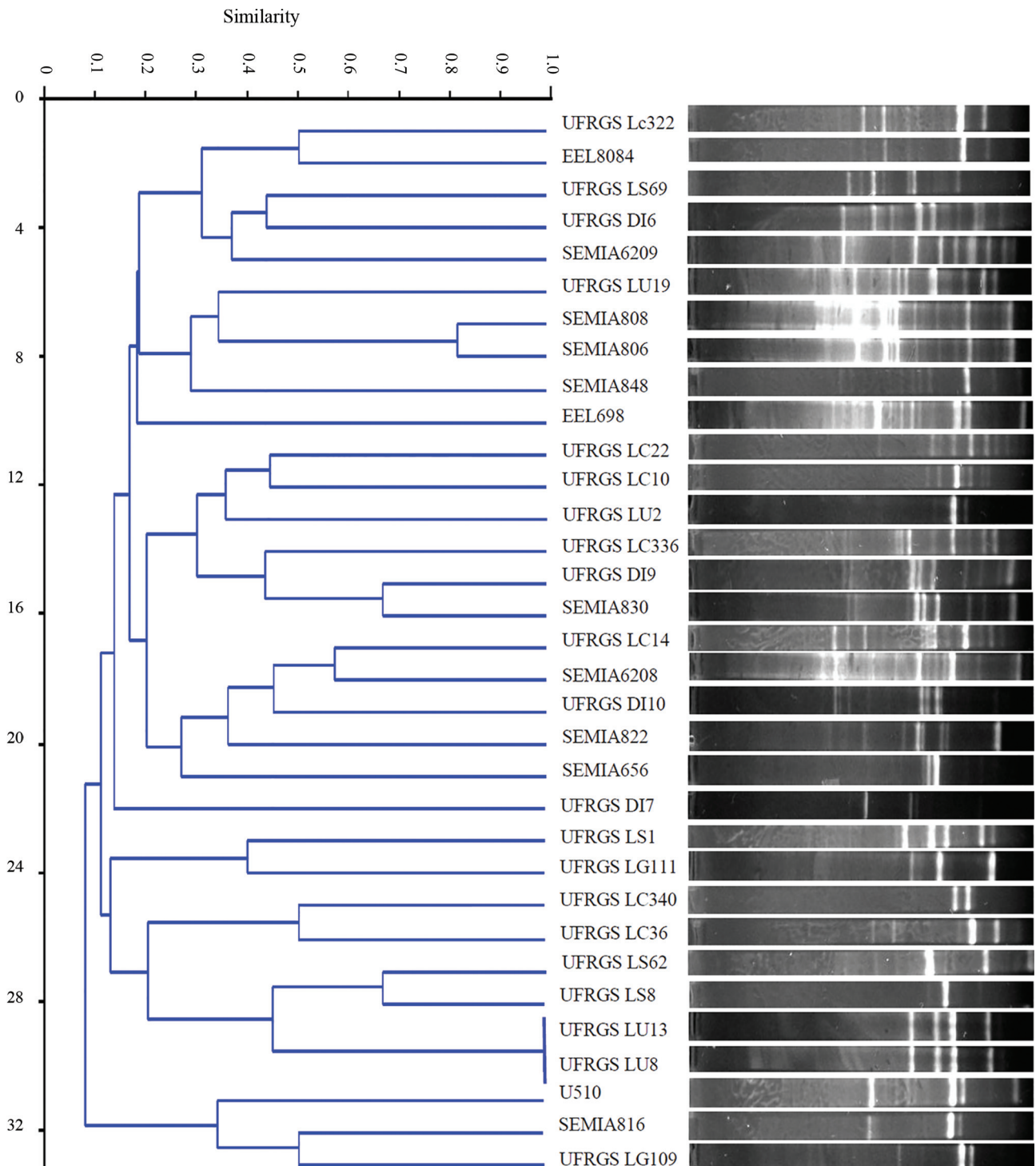


Figure 1 - Dendrogram of genetic similarity based on UPGMA cluster analysis using the Jaccard coefficient (PAST software). Data were obtained through BOX-PCR analysis of 21 rhizobia isolated from the root nodules of *D. incanum*, *L. corniculatus*, *L. subbiflorus*, *L. uliginosus*, *L. glaber* and 12 rhizobia reference strains.

Table 2 - Cross-inoculation results among the native rhizobial and reference strains and the five plant species studied in this work.

Rhizobial isolate	<i>Desmodium incanum</i>		<i>Lotus corniculatus</i>		<i>Lotus glaber</i>		<i>Lotus subbiflorus</i>		<i>Lotus uliginosus</i>	
	Nod	Fix	Nod	Fix	Nod	Fix	Nod	Fix	Nod	Fix
UFRGS Di6	+	-	-	-	-	-	-	-	-	-
UFRGS Di7	+	-	-	-	-	-	-	-	-	-
UFRGS Di9	+	+	+	-	-	-	-	-	-	-
UFRGS Di10	+	+	-	-	-	-	-	-	-	-
SEMIA6208	-	-	-	-	-	-	-	-	-	-
SEMIA6209	-	-	-	-	-	-	-	-	-	-
SEMIA656	-	-	-	-	-	-	-	-	-	-
UFRGS Lc10	-	-	+	+	-	-	-	-	+	-
UFRGS Lc14	-	-	+	+	-	-	-	-	-	-
UFRGS Lc22	-	-	+	+	-	-	-	-	+	-
UFRGS Lc36	-	-	+	+	-	-	-	-	+	-
UFRGS Lc322	-	-	+	+	-	-	-	-	-	-
UFRGS Lc336	+	-	+	+	-	-	-	-	+	+
UFRGS Lc340	-	-	+	+	-	-	-	-	+	-
U510	-	-	+	+	-	-	-	-	-	-
SEMIA816	-	-	+	+	-	-	-	-	-	-
SEMIA806	-	-	+	+	-	-	-	-	-	-
SEMIA808	-	-	+	+	-	-	-	-	-	-
UFRGS Lg109	-	-	-	-	+	+	-	-	+	+
UFRGS Lg111	-	-	-	-	+	+	-	-	+	+
SEMIA830	-	-	-	-	+	+	-	-	-	-
UFRGS Ls1	+	+	+	+	+	-	+	+	+	+
UFRGS Ls8	+	+	-	-	-	-	+	+	-	-
UFRGS Ls62	+	+	+	-	-	-	+	+	-	-
UFRGS Ls69	+	+	+	-	+	-	+	-	+	-
EEL698	+	+	+	+	-	-	+	+	-	-
SEMIA848	-	-	-	-	-	-	+	+	-	-
UFRGS Lu2	+	+	+	+	+	+	+	+	+	+
UFRGS Lu8	+	+	+	-	-	-	+	+	+	+
UFRGS Lu13	+	+	+	+	+	-	+	+	+	+
UFRGS Lu19	+	+	-	-	+	-	+	-	+	+
EEL8084	-	-	+	+	-	-	-	-	+	+
SEMIA822	-	-	-	-	-	-	-	-	+	+

+ = Native rhizobial isolate or reference strain that was able to nodulate and/or fix atmospheric nitrogen in the leguminous plant studied. - = Native rhizobial isolate or reference strain that was unable to nodulate and/or fix atmospheric nitrogen in the leguminous plant studied. Nod = Nodulation. Fix = active nodules.

al. (2007), in their study of rhizobial symbionts of *Desmodium* spp. in China, also found a high level of variability in the tested isolates using the PCR-RFLP technique.

The native rhizobial isolates UFRGS Lu13 and UFRGS Lu8 exhibited 100% similarity, but they presented different symbiotic characteristics. These data indicated that bacterial isolates may differ in other genomic regions than those amplified by the BOX A1 primer. Similar results were obtained by Grange and Hungria (2004) and Granada

et al. (2014) leading to infer that bacterial strains with the same DNA profile can display different growth promotion and symbiotic characteristics.

The cross-inoculation of rhizobial isolates and four *Lotus* species and *D. incanum* plants showed that some native rhizobial isolates were able to nodulate more than one plant species studied. Some studies have already found related rhizobial species that were able to nodulate and/or form active nodules in different plant species. Gossmann *et al.* (2012), for example, found rhizobial isolates that nodu-

Table 3 - Greenhouse experiment with the inoculation of *D. incanum* plants and rhizobial isolates or reference strains that are able to nodulate both *D. incanum* and *L. corniculatus* plants.

Treatment	Number of nodules per plant	Root dry matter (mg)	Shoot dry matter (mg)
N+*	-	12.56 ± 1.8 a	26.33 ± 4.9 a
EEL698	6 ± 1.7 c	16.87 ± 4.0 a	33.00 ± 8.6 a
UFRGS Ls1	6.25 ± 1.5 c	14.83 ± 7.1 a	30.5 ± 0.5 a
UFRGS Lu2	18.25 ± 3.0 b	12.12 ± 2.0 a	28.00 ± 6.3 a
UFRGS Lu13	5.25 ± 0.9 c	20.50 ± 1.3 a	27.00 ± 6.2 a
UFRGS Di9	26 ± 4.3 a	13.62 ± 4.2 a	22.75 ± 2.4 b
UFRGS Lc336	6 ± 2.0 c	11.00 ± 3.0 a	17.75 ± 6.0 b
N- *	-	8.56 ± 1.9 b	15.22 ± 2.2 b

Control treatments were performed without nitrogen (N-) or with 25 mg of nitrogen, as NH₄NO₃, per jar (N+). Each treatment was composed of three replicates of two plants of *D. incanum* (native cultivar) in Leonard jars inoculated with one rhizobia culture (5 mL of one rhizobia culture with 10⁸ cfu mL⁻¹). Means followed by different letters in the same column differ by the Scott-Knott test (p < 0.01).

late and were efficient nitrogen fixers in five *Lotus* species (*L. japonicum*, *L. pedunculatus*, *L. filicaulis*, *L. burttii* and *L. glaber*). Ruiz-Díez *et al.* (2012) studied cross-inoculation with 12 traditional cultivated legumes, including pea (*Pisum sativum* L.), lentil (*Lens culinaris* Medik.), chickpea (*Cicer arietinum* L.), vicia (*Vicia* spp.), clover (*Trifolium* spp.), alfalfa (*Medicago sativa* L.) and lupin (*Lupinus* spp.). The rhizobial isolates studied in this work also showed a high variability in their nodulation patterns.

The reference strains SEMIA 808 and SEMIA 806 presented a highly similar pattern in the cross inoculation and BOX analysis. The major characteristics that differentiate these SEMIA strains were the abilities to produce indolic compounds and siderophores. The same was ob-

served for isolates UFRGS Lu 8 and UFRGS Lu13, as they presented similar plant grow promotion characteristics and patterns in the BOX analysis, but they differ in the pattern of cross inoculation. Binde *et al.* (2009) reported that BOX analysis is a powerful fingerprinting tool, revealing strong genetic diversity among rhizobial strains. It is, however, necessary to use other techniques (such as a 16S rDNA sequencing) to achieve the level of bacterial genus and species identification (Binde *et al.*, 2009; Ishii and Sadowsky, 2009, Granada *et al.*, 2014).

As shown with our data, Parker *et al.* (2006) also suggested that the success of plant invasions relies on symbiotic nitrogen-fixing bacteria. However, these bacteria promote plant growth and environmental adaptation not only via their nitrogen fixation ability but also via their production of hormones and solubilization of nutrients (Joseph *et al.*, 2007; Granada *et al.*, 2014). The production of indolic acetic acid by rhizobia can aid non-native plant species in resisting both biotic and abiotic stresses (Bianco and Defez, 2007; Sziderics *et al.*, 2007), because it is well documented as an indispensable phytohormone with the ability to regulate many aspects of plant development (Teale *et al.*, 2006; Spaepen *et al.*, 2007). In our work, among the studied plant growth promotion characteristics, the production of indolic compounds was the characteristic most common among the rhizobial isolates, which may be reflected in the growth of the studied plants. Furthermore, previous studies have reported that phytohormones of the indolic group stimulate seed germination, increase the rate of root formation, control processes of vegetative growth, tropism, florescence, and fructification of plants, affect the biosynthesis of various metabolites, and provide plant resistance to stress factors (Tsavkelova *et al.*, 2005; Zahir *et al.*, 2010; Bhattacharjee *et al.*, 2012).

Table 4 - Greenhouse experiment with the inoculation of *L. corniculatus* plants with rhizobial isolates or reference strains that are able to nodulate both *L. corniculatus* and *D. incanum* plants.

Treatment	Number of nodules per plant	Nodules dry matter (mg plant ⁻¹)	Root dry matter (mg plant ⁻¹)	Shoot dry matter (mg plant ⁻¹)	Nitrogen content (mg plant ⁻¹)
N+ *	-	-	1065.5 ± 193.5 a	1008.5 ± 85.8 a	5.63 ± 0.4 c
SEMIA 816	538.5 ± 79.9 a	109.5 ± 14.1 a	627.25 ± 97.1 b	1120.25 ± 134.0 a	14.22 ± 2.3 a
EEL 698	290.25 ± 93.1 b	71.5 ± 29.8 b	365 ± 70.9 c	875 ± 118.3 a	10.1 ± 2.5 b
UFRGS Ls1	231 ± 97.2 b	51.5 ± 6.2 c	189.25 ± 31.8 c	332.5 ± 93.0 b	2.63 ± 1.1 d
UFRGS Lu2	280.5 ± 79.8 b	83.5 ± 33.0 b	335.25 ± 72.8 c	992.75 ± 115.3 a	12.53 ± 0.6 a
UFRGS Lu13	334 ± 32.6 b	53.25 ± 13.3 c	221 ± 54.8 c	454.75 ± 81.5 b	2.48 ± 0.6 d
UFRGS Di9	143.75 ± 25.6 c	40.75 ± 6.1 c	292.25 ± 92.7 c	265 ± 83.8 b	1.31 ± 0.6 d
UFRGS Lc336	155 ± 63.7 c	36 ± 16.2 c	182.5 ± 67.9 c	337.75 ± 68.2 b	2.41 ± 0.7 d
N- *	-	-	304.75 ± 64.1 c	207.25 ± 65.3 b	0.57 ± 0.1 d

* Control treatments were performed without nitrogen (N-) or with 25 mg of nitrogen, as NH₄NO₃, per jar (N+). Each treatment was composed of three replicates of two plants of *L. corniculatus* cv São Gabriel in each Leonard jar inoculated with one rhizobia culture (5 mL of one rhizobia culture with 10⁸ cfu mL⁻¹). Means followed by different letters in the same column differ by the Scott-Knott test (p < 0.01).

The salinization of soils constitutes one of the most serious forms of edaphic degradation (Pisinaras *et al.*, 2010). Excess levels of salt have a negative effect on soil rhizobial populations by both direct toxicity and osmotic and ionic stress (Dhanapackiam and Muhammad-Ilyas, 2010). This soil condition affects the rhizobial infection process by inhibiting root hair growth (Manchanda and Garg, 2008). Rhizobial salt resistance may therefore help plant development by allowing the establishment of an efficient nitrogen fixation process (Bui, 2013). Trabelsi *et al.* (2010) studied the diversity and salt tolerance of *Sinorhizobium* populations isolated from Tunisian soils and showed that some isolates were able to grow in concentrations of up to 1.2% salt. The resistance to salt concentrations up to 1.2% was also noted in studies by Singh *et al.* (2008). The evaluation of rhizobial resistance to salt stress in the present study demonstrated that only two rhizobial isolates were able to grow in concentrations of up to 2% NaCl (UFRGS Lu2 and UFRGS Ls1). It is worthy of note that UFRGS Lu2 was the rhizobial isolate that presented the best results regarding plant growth promotion parameters as evaluated in the greenhouse experiment using both *D. incanum* and *L. corniculatus* plants.

The greenhouse inoculation of *D. incanum* and *L. corniculatus* with native rhizobia demonstrated that this inoculation can improve the growth of both plant species. Rodriguez-Echeverría *et al.* (2012) also reported that inoculation of native and non-native plant species with native rhizobia isolates is beneficial for both plants. Furthermore, they highlight that inoculation with an exotic rhizobia strain may disrupt the native rhizobia population, which indeed would be prejudicial to plant development.

Typically, rhizobial inoculation experiments in leguminous plants demonstrate that a larger number of nodules in the roots results in a higher plant growth promotion effect (Shamseldin and Werner, 2005; Li *et al.*, 2008). These data agree with our results with *L. corniculatus* plants, because the plants inoculated with rhizobium reference strain SEMIA 816 showed the highest number of nodules, the highest yield of shoot dry matter and the highest nitrogen content. However, our data with *D. incanum* plants was different: plants inoculated with the rhizobial isolate EEL 698 showed only few root nodules (six) but resulted in the highest shoot dry matter yield.

The ability to select rhizobia capable of nodulating and efficiently fixing nitrogen in several leguminous plants aids in the adaptation of non-native plant species (Barrett *et al.*, 2012; Knoth *et al.*, 2012). When nitrogen fixation by indigenous rhizobia is limited, field inoculation with efficient and competitive strains is an economically feasible way to increase production. When the inoculant is composed by native strains of rhizobia the success of inoculation should be increased, since local strains are better adapted than those present in commercial inoculants (Mnasri *et al.*, 2007). This adaptation should result in sustainable food and

energy crop production, which, in turn, yields high productivity while minimizing inputs that are both economically and ecologically costly.

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