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Rhizobia isolation and selection for serradella (*Ornithopus micranthus*) in Southern Brazil

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Serradella is a plant that belongs to the Fabaceae family, with recognized forage value and has the ability to associate symbiotically with rhizobia. The objective of this study was to isolate, authenticate and select effective rhizobia obtained from native serradella (*Ornithopus micranthus*) to reduce the need for mineral fertilizer. Nodules, roots of serradella and rhizosphere soil samples were collected in seven municipalities from Rio Grande do Sul and Santa Catarina states. Bacterial colonies were isolated and identified based on morphological characteristics. The authentification and initial selection of rhizobia were conducted *in vitro*. The most promising strains from the *in vitro* study were evaluated in a greenhouse experiment for 60 days. Among the 148 bacterial cultures characterized, 113 induced the formation of nodules in serradella while 32 isolate effectively increased fresh mass of plants under *in vitro* conditions. The strains UFRGS Om57, UFRGS Om59 and UFRGS Om148 formed nodules with greater dry mass and produced high dry mass of plants (shoots and roots) which allowed greater accumulation of N in the shoots.

Key words: Plant growth promoting rhizobacteria (PGPR); biological fixation of nitrogen; *Ornithopus micrantus*; pasture.

INTRODUCTION

The serradella [*Ornithopus micranthus* (Benth.) Arechavaleta] is a plant that belongs to the Fabaceae family, with recognized suitability for grazing by cattle. Its leaves are rich in protein, reaching the crude protein content close to 20 % in the vegetative period (Fernandes and Reis, 2001). According to Dartora et al. (2012), the supply of proteins through pastures is important to balance nutritionally pastures which will be offered to ruminants, reducing the need for concentrated feed.

The serradella is adapted to lowland areas which tolerates high moisture content in the soil, with good root

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system and excellent nodulation capacity in flood conditions (Menezes et al., 2001), taking place before the summer crop. Because of its ability to produce green mass in lowland areas, it is used in experimental crops as cover crops in crop rotation systems after the cultivation of rice. It also has great potential for intercropping with ryegrass and great capacity for survival and development in acid soils (Freixial and Barros, 2012), typical in the state of Rio Grande do Sul. It is recommended to make the cuts together with the flowering period, from which its quality has drastically reduced, while wet seasons allow a second cut (Fernandes and Reis, 2001).

In addition to offering green mass and protein, serradella offers nitrogen (N) supply to the soil-plant system. It is also capable of establishing symbiosis with rhizobia which perform biological fixation of atmospheric nitrogen (BFN) and therefore provide essential nutrient to animals and other plants that make up the ecosystem of the pasture.

In a study by Jandrey (2008), the production of dry matter of the aerial part of serradella was 2.2 t ha^{-1}, lower than that obtained with ryegrass but equivalent to birdsfoot trefoil. As for the nitrogen (N) accumulated in the shoots, it was observed by Jandrey (2008) that an average of 63.1 kg N. ha^{-1} accumulate the area where serradella has been grown. This quantity of N is equivalent to that obtained with birdsfoot trefoil and higher than that obtained with ryegrass, showing it efficiency in biological nitrogen fixation.

In the case of grasses cultivation in succession to serradella, the N content found in the shoot of the legume, as well as the N present in the nodes and roots, certainly favor the successor crops during vegetative growth. Other possible benefits of the succession of legume/grass crop system are the production of phytohormones and other plant stimulating substances by rhizobia symbionts with the species that inhabit the rhizosphere of crops that follow them (Yanni and Dazzo, 2010; Yanni et al., 2011).

Despite the great potential of serradella as native pasture in southern Brazil, there is little use of this forage legume in feeding cattle that produce meat or milk in the farms of Rio Grande do Sul. One of the important reasons is that, there are few studies about selection of nitrogen-fixing bacteria for this plant, which hinders the adaptation of it to new environments, and also affects the expression of its maximum productive potential. Currently, in Brazil there are strains of rhizobia such as SEMIA905 and SEMIA929 strains which are recommended for the kind of serradella *Ornithopus sativus*. However, there are no strains recommended for the *Ornithopus micranthus* species, which occurs in lowland areas in succession to rice cultivation. Thus, it is possible to increase the yield of *Ornithopus micranthus* through the selection and use of efficient rhizobia to hold the symbiosis with the legume, and through symbiotic association it provide increased N content attached to the soil-plant system, as well as increasing the green mass contribution of serradella in pasture farming systems.

Rhizobacteria that promote plant growth are inserted into pasture production systems for feeding cattle that produce meat or milk. The present mechanisms that promote plant growth may be recommended for the composition of commercial inoculants for specific crops. In the case of rhizobacteria present in pastures, plants yield increment mechanisms which anticipate the supply of pastures, lengthen the crop production cycle, or increase the supply of pastures to animals at the same time reduce the need for use of mineral fertilizers.

This study aimed to isolate, authenticate and select effective and efficient rhizobia as to the nitrogen fixation in symbiosis with native serradella of the *Ornithopus micranthus* species, which may increase the N content of the leaf tissue and provide a better yield culture.

MATERIALS AND METHODS

Obtainment of rhizobium nodules in serradella plants

Soil samples and serradella plant of the *Ornithopus micranthus* species were collected (*Ornithopus micranthus*) in southern towns Cachoeirinha, Palmeira das Missões, Passo Fundo, Porto Alegre, Santa Vitória do Palmar and São Martinho da Serra, as well as in Correia Pinto town, Santa Catarina. Samples were collected in pastures with the presence of serradella (Table 1). Soil samples were collected with cutting shovel at 0 to 15 cm depth in rhizospheric region of plants. In addition to soil samples, whole serradella plants were also collected, with shoot and root system. In the laboratory, the roots were separated and thoroughly washed with running water for subsequent posting of nodules. Nodules were highlighted, dried on paper towels and packed in glass jars with silica and cotton for preservation. In the greenhouse, to obtain nodules from soil samples, soil samples will be suspended in saline (NaCl 0.85 %). Then, with the aid of a sterile pipette, 5 ml of solution was inoculated in serradella plants (*Ornithopus micranthus*) grown in Leonard jars (Vincent, 1970) of 700 ml, containing a sterilized mixture of vermiculite and sand (2:1). Nutrients were added to plants through the nutrient solution Sarruge 25 % (Sarruge, 1975) without nitrogen, and then sterilized. For disinfection, the serradella seeds were immersed in alcohol (70 %) for 30 seconds, followed by sodium hypochlorite (2.5 %) for 30 seconds, wash for seven consecutive times with sterile distilled water by autoclaving at 120°C for 20 min, and then stored at room temperature (20 to 25°C) for 24 h of germination. Inoculation of soil samples suspensions was performed when plants were seven days with 1 to 3 pairs of leaves. The inoculation was performed under axenic conditions with automatic pipetter (Labmate brand), adding 2 ml of soil solution per pot. At 45 days after inoculation, plants were collected and each shoot was separated from the root system. After washing the roots, nodules obtained in the greenhouse were highlighted, dried on paper towels and placed in glass containers silica and cotton according to the methodology described by Beck et al. (1993).

Obtaining bacterial isolates

After storage period of 1 to 2 months, nodules were rehydrated in

Table 1. Phenotypic characterization of isolates obtained for serradela (*Ornithopus micrantus*). The parameter growth time (days) refers to the time between the inoculation on solid medium and the final evaluation. All colonies showed convex elevation and entire margin, so these features are not shown in the table.

Isolates groups*	Growth time (Days)	Color	Diameter (mm)	Consistency	Opacity	Form	Locations	Number of Isolates	REI (%) Average
Group 1	10	Milky	1 a 2	Gummy	Opaque	Circular	Cach./P. A./S.V.P.	37	109,19
Group 2	10	Milky	<1	Aqueous	Translucent	Punctiform	Cach./S.V.P.	30	117,17
Group 3	10	Milky	<1	Aqueous	Opaque	Punctiform	Cach./S.V.P.	33	198,64
Group 4	10	Milky	< 1	Gummy	Translucent	Punctiform	Cach./C.P.	8	236,25
Group 5	3 a 4	Pink	3 a 4	Gummy	Opaque	Circular	Cach./P.F.	10	42,00
Group 6	4	Milky	3 a 4	Aqueous	Opaque	Circular	Cach./C.P.	8	215,00
Group 7	10	Transparent	<1	Gummy	Opaque	Punctiform	Cach.	1	520,00
Group 8	10	Transparent	<1	Aqueous	Translucent	Punctiform	Cach.	5	285,00
Group 9	10	Pink	1 a 2	Aqueous	Opaque	Circular	P.M.	3	30,00
Group 10	3 a 4	Pink	3	Aqueous	Opaque	Circular	P.M.	1	15,00
Group 11	8 a 10	Pink	1 a 2	Gummy	Opaque	Circular	P.A./S.M.S.	4	60,00
Group 12	4	Pink	3 a 4	Viscous	Opaque	Circular	P.M.	2	85,00
Group 13	10	Milky	<1	Gummy	Opaque	Punctiform	C.P.	4	83,75
Group 14	10	Milky	<1	Viscous	Translucent	Punctiform	P.M.	1	120,00
Group 15	10	Milky	<1	Butter	Translucent	Circular	C.P.	1	320,00
-	-	-	-	-	-	-	-	148 (Total)	41,88

* Isolates groups with the same morphological characteristics; REI (%) Average: Relative efficiency index (%) for each isolates group, described in Experimental section; Abbreviations: Cach.: Cachoeirinha; C.P.: Correia Pinto; P.F.: Passo Fundo; P.A.: Porto Alegre; P.M.: Palmeira das Missões; S.V.P.: Santa Vitória do Palmar; S.M.S.: São Martinho da Serra.

sterile distilled water for 24 h and kept in the refrigerator for the next day. After rehydration, nodules were separately macerated in sterile test tube with a glass rod sterile in laminar flow hood. With the aid of sterile pipette tips in a laminar flow hood, macerated nodules broths were inoculated into standard petri dishes with 9 cm diameter, containing the yeast extract mannitol agar (YMA) medium which contain 0,0025 % (w/v) congo red (Vincent, 1970), using drop method (Miles & Misra, 1988) and scattering (Buck and Cleverdon, 1960), separately.

Petri dish containing, medium YMA inoculum was stored in an oven at 28°C. Daily evaluation was performed in each of the plates with the aid of a magnifying glass table. As colonies with morphological features of rhizobia emerged, they were immediately transferred to another plate in order to obtain pure colonies with persistent morphology. The isolates were characterized according to time of growth characteristics, color, colony high, colony diameter, colony consistency and opacity of the colony, according to Conn et al. (1957). After phenotypic characterization, each isolate was inoculated into three test tubes containing YMA medium (Vincent, 1970). These inoculated tubes were stored in the Rhizobia Culture Collection of UFRGS.

Authentication and initial selection of rhizobia experiment

The isolates obtained in the previous step were evaluated for the ability to induce the formation of nodules and promote the growth in serradella plants. For this, an *in vitro* experiment was conducted in the laboratory, using 30 ml test tube containing a paper towel strip and 15 ml of nutrient solution Sarruge 25 % (Sarruge, 1975) without nitrogen and sterilized by autoclaving at 120°C for 20 min. For production of culture broths, the rhizobia were inoculated in test tubes with screw containing liquid yeast extract mannitol culture medium (YM) (Vincent, 1970) and placed in an orbital incubator at 28°C with shaking at 120 rpm for seven days. On the day of inoculation, the broth had a cell concentration of 10^8 colony forming units per ml (ufc mL^{-1}), determined in a Neubauer chamber (Moura et al., 1987).

The serradella seeds were sterilized by successive immersions in alcohol (70 %) for 30 seconds, followed by sodium hypochlorite (2.5%) for 30 seconds and wash for seven consecutive times with sterile distilled water.

Then, for pre-germination, the seeds were sterilized and placed on moistened paper towels with sterile distilled water, maintained at 22°C for 24 h. In laminar flow chamber, each 30ml tube received a disinfected pre-germinated serradella seed, with the aid of a clamp fowl. One day after seedling emergence, the isolates were inoculated individually in a laminar flow hood. Each 30 ml tube with a serradella plant received 1 ml of culture broth with an isolate. After inoculation the tubes were stoppered with cotton plugs and kept in lamps for 8 h of light per day in an average ambient temperature of 24°C.

The experiment consist of 150 treatments with three replications arranged in a randomized design. As for the treatments, 148 were composed of isolates from different colonies. Two not-inoculated control treatments were also conducted. One of the Control treatments received an addition of mineral N (Control + N), with an aliquot of 107 µl ml of NH_4NO_3 solution (20 g L^{-1}) equivalent to application of 100 kg of N ha^{-1}. The other control without inoculation did not receive the addition of mineral N (Control - N). After a period of 35 days, the experiment was terminated and the plants were collected. The plant fresh mass, number of leaves, number of nodules and length of shoot and root were quantified. For each inoculated treatment, relative efficiency index (REI) was used to estimate the bacterial efficiency on symbiotic nitrogen fixation (Brockwell, 1966). The REI was measured with the following formula:

$$REI\ (\%) = ((\ FM\ Inoculated) - (FM\ Control\ N\ -)) / ((FM\ Control\ N\ +) - (FM\ Control\ N))x100$$

Where:
FM Inoculated = Plant fresh mass of inoculated treatment
FM Control N - = Plant fresh mass of non-inoculated treatment without the addition of nitrogen
FM Control N + = Plant fresh mass of inoculated treatment with the addition of nitrogen equivalent to 100 kg N ha^{-1}.

Evaluation of symbiotic efficiency of rhizobia in serradella plants

Among the isolates that produced promising results *in vitro* environment, eight were chosen for symbiotic efficiency test in the greenhouse. Besides these, we also tested the two strains currently recommended in Brazil to serradella. In Brazil, there is no isolated released for *Ornithopus micranthus* species, we use SEMIA 905 (*Bradyrhizobium japonicum*) and SEMIA 929 strains (*Bradyrhizobium japonicum*), released by the Ministry of Agriculture, Livestock and Food Supply (MAPA) for the production of inoculants for the species of serradella *Ornithopus sativus*, obtained from the Rhizobia Cultures Collection of the Agricultural Research Foundation of Rio Grande do Sul (FEPAGRO).

Inoculations were made in Leonard jars (Vincent, 1970) of 700 ml, containing a mixture of vermiculite and sand in the ratio 2:1, at the top and bottom part of the nutrient solution without nitrogen (Sarruge, 1975).

The inoculations were performed using aliquots of 2 ml broth cultures, with about 10^8 ufc ml^{-1}, of each studied Rhizobium, grown in yeast-mannitol medium for seven days at 28°C, 120 rpm. The experiment consisted of; two control treatments without inoculation, one without nitrogen addition and the other with addition of two aliquots of 5.4 ml of NH_4NO_3 (20 g L^{-1}) solution equivalent to the addition of 250 kg of N ha^{-1}. The ten inoculated treatments were composed by the inoculations of isolates UFRGS OM4, UFRGS Om27, UFRGS Om57, UFRGS Om59, UFRGS Om62, UFRGS Om67, UFRGS Om82, UFRGS Om148, SEMIA 905 and SEMIA 929. Four replications per treatment were performed, arranged in a completely randomized design. The serradella seeds were sterilized and pre-germinated as described in the previous section. At the end of 60 days after emergence of the plants, the shoot was separated from the root system, wrapped in paper bags and subjected to drying in an oven at 65°C to constant weight for 96 h. The shoot was weighed after drying and then grounded for chemical determination of nitrogen accumulation in tissue according to the methodology described by Tedesco et al. (1995). The nodules were detached from the roots, counted and placed in an oven at 65°C for drying and dry weight determination at 48 h. The data obtained were submitted to analysis of variance and average medium test (Scott Knott, 5%), using the statistical program SISVAR (Ferreira, 2000).

The relative efficiency index (REI) of nitrogen fixation of the isolates (Brockwell et al., 1966) was calculated using the formula below:

$$REI = ((Ntotal\ trat. - Ntotal\ T\text{-}N) / (Ntotal\ T+N - Ntotal\ T\text{-}N))x\ 100$$

where:
N total trat = Total nitrogen of the inoculated treatment plant
N total T-N = total nitrogen of the uninoculated and without nitrogen control
N total T+N = total nitrogen of the uninoculated control and that received nitrogen supplementation.

RESULTS

Morphological characterization, authentication and isolated efficiency test

With collection and isolation studies, we obtained 148 isolates with typical bacterial characteristics. The isolates were grouped into 15 different groups, based on colonies morphological characteristics (Table 1). In addition, all serradella isolates obtained in this study showed concave colonies and regular edges.

The composition of the groups is shown in table 2. The groups with the highest number of isolates were; groups 1, 3 and 2 with 37, 33 and 30 isolates respectively. The Relative efficiency index (REI %) average was 41, 88 %. The REI (%) ranges from 15 and 30 % in Group 9 and 10 formed by inefficient isolate up to 320 % in Group 15 (Table 1). The best REI (%) were obtained with an inoculation of isolated UFRGS Om62 and UFRGS Om9, with values above 750%. Figure 1 shows the morphological similarity dendrogram of an isolates group. The lowest similarity observed was 75 % with a comparison of groups 5, 6, 10 and 12 with each other groups. This represents a high similarity between the groups. Of the total isolates, 127 had smaller colonies than 2 mm in eight to ten days of growth, classified in this study as slow-growing colonies. This slow growth accompanied by the formation of small colonies is also presented by strains SEMIA905 and SEMIA929, recommended for inoculant composition for serradella (*Ornithopus sativus*) and belonging to the genus *Bradyrhizobium*.

Moreover, the other 21 isolates were observed with

Table 2. Groups of isolates with identical morphological characteristics.

Group	Isolates
Group 1	UFRGS Om1, UFRGS Om4, UFRGS Om13, UFRGS Om14, UFRGS Om15, UFRGS Om19, UFRGS Om22, UFRGS Om23, UFRGS Om24, UFRGS Om25, UFRGS Om26, UFRGS Om27, UFRGS Om28, UFRGS Om29, UFRGS Om32, UFRGS Om37, UFRGS Om40, UFRGS Om42, UFRGS Om44, UFRGS Om45, UFRGS Om46, UFRGS Om51, UFRGS Om73, UFRGS Om75, UFRGS Om88, UFRGS Om89, UFRGS Om90, UFRGS Om91, UFRGS Om92, UFRGS Om93, UFRGS Om94, UFRGS Om95, UFRGS Om96, UFRGS Om97, UFRGS Om98, UFRGS Om104, UFRGS Om140
Group 2	UFRGS Om2, UFRGS Om3, UFRGS Om16, UFRGS Om20, UFRGS Om21, UFRGS Om43, UFRGS Om47, UFRGS Om48, UFRGS Om49, UFRGS Om52, UFRGS Om76, UFRGS Om77, UFRGS Om85, UFRGS Om86, UFRGS Om114, UFRGS Om115, UFRGS Om116, UFRGS Om117, UFRGS Om118, UFRGS Om119, UFRGS Om120, UFRGS Om121, UFRGS Om122, UFRGS Om123, UFRGS Om124, UFRGS Om126, UFRGS Om127, UFRGS Om128, UFRGS Om138, UFRGS Om142
Group 3	UFRGS Om6, UFRGS Om7, UFRGS Om9, UFRGS Om10, UFRGS Om11, UFRGS Om12, UFRGS Om50, UFRGS Om54, UFRGS Om55, UFRGS Om56, UFRGS Om58, UFRGS Om62, UFRGS Om63, UFRGS Om64, UFRGS Om65, UFRGS Om66, UFRGS Om67, UFRGS Om68, UFRGS Om70, UFRGS Om71, UFRGS Om72, UFRGS Om79, UFRGS Om81, UFRGS Om84, UFRGS Om87, UFRGS Om105, UFRGS Om106, UFRGS Om108, UFRGS Om109, UFRGS Om110, UFRGS Om111, UFRGS Om112, UFRGS Om143
Group 4	UFRGS Om17, UFRGS Om18, UFRGS Om38, UFRGS Om41, UFRGS Om57, UFRGS Om74, UFRGS Om136, UFRGS Om148
Group 5	UFRGS Om35, UFRGS Om53, UFRGS Om101, UFRGS Om102, UFRGS Om103, UFRGS Om107, UFRGS Om113, UFRGS Om125, UFRGS Om137, UFRGS Om145
Group 6	UFRGS Om5, UFRGS Om8, UFRGS Om78, UFRGS Om80, UFRGS Om82, UFRGS Om83, UFRGS Om132, UFRGS Om147
Group 7	UFRGS Om59
Group 8	UFRGS Om33, UFRGS Om60, UFRGS Om61, UFRGS Om69, UFRGS Om146
Group 9	UFRGS Om31, UFRGS Om36, UFRGS Om99
Group 10	UFRGS Om100
Group 11	UFRGS Om34, UFRGS Om39, UFRGS Om129, UFRGS Om130
Group 12	UFRGS Om30, UFRGS Om131
Group 13	UFRGS Om133, UFRGS Om134, UFRGS Om139, UFRGS Om144
Group 14	UFRGS Om135
Group 15	UFRGS Om141

greater than or equal to 3 mm colonies within three to four days of growth, which were classified as intermediate growth isolates. The isolates obtained in laboratory were tested in the authentication trials *in vitro* environment for the ability of nodulation and effect on the growth and development of plants. Of the 148 inoculated treatments, 113 were able to nodular the plants of serradella. Isolates that showed higher total fresh weight of plant at the end of the 35 days of experiment were the ones that formed 3 to 5 nodules plant. These are the cases of isolated UFRGS Om9, UFRGS Om62, and UFRGS Om67, from Cachoeirinha (Table 3). These three isolates are highly recommended for studies in the field but when high efficiency is maintained, it will be recommended to the composition of rhizobial inoculants for *Ornithopus micrantus*.

The isolates UFRGS Om25, UFRGS Om26 and UFRGS Om94 formed 12, 3; 12, 3 and 9, 5 nodules per plant, respectively (Table 3). Isolates with the highest number of nodules were less efficient for total fresh mass growth parameter. These results corroborate Souza et al. (2008), which indicate the dry weight of nodules as the best parameter to evaluate nodulation, instead of the number of nodules. Thus, the number of nodules parameter is interesting for the rhizobia authentication, especially in the study of species that form low weight small nodules, but it is not

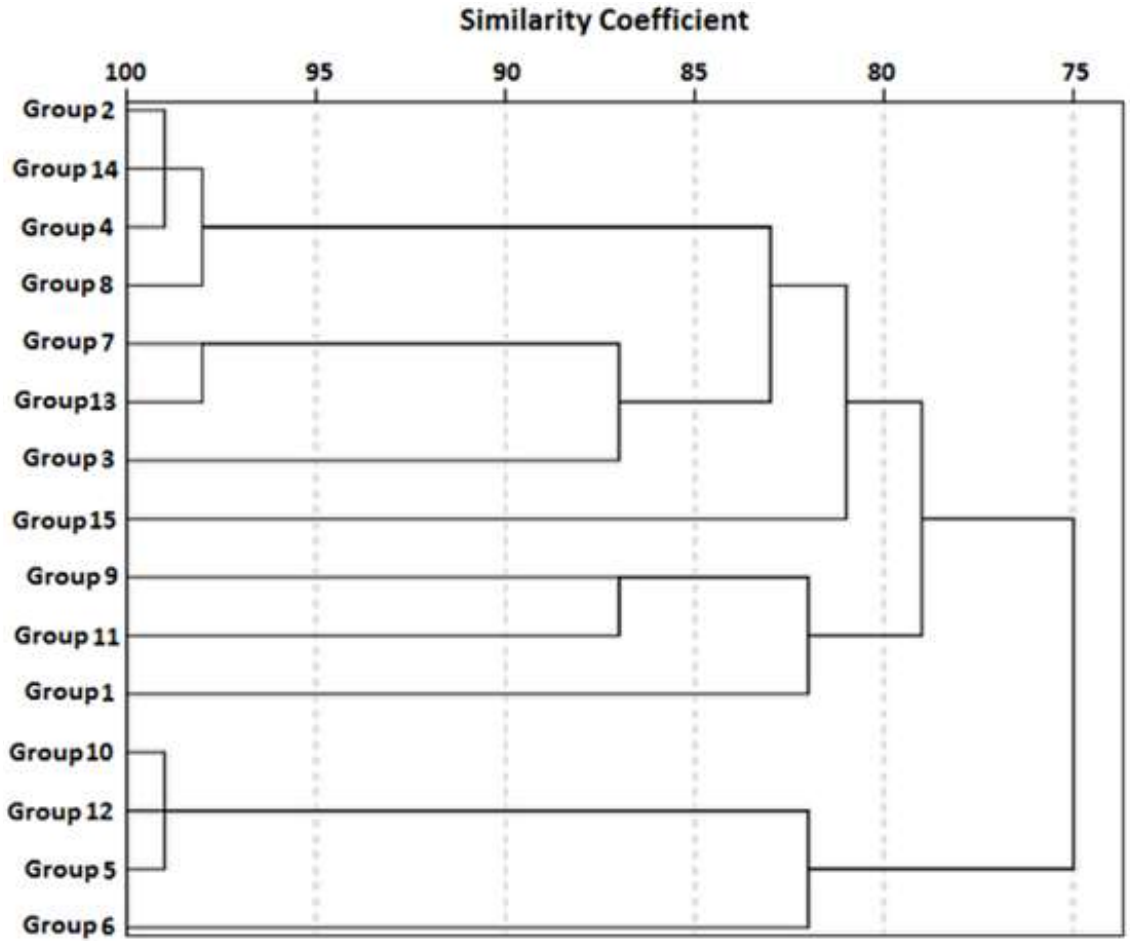

Figure 1. Dendrogram of similarity for the isolates groups, obtained through the Nearest-neighbor method based on morphological characteristics.

suitable to quantify the efficiency of nodulation. As for the total fresh weight parameter, 32 treatments that were superior to the Control treatments wereobtained. 29 of those are from Cachoeirinha, the other three were obtained from collections in the cities Correia Pinto, Passo Fundo and Santa Vitória do Palmar. The best yields were observed in treatments UFRGS Om9 and UFRGS Om62, which showed high total fresh weight of 0,167 and 0,190g, respectively. The best yields treatments (UFRGS Om9 and UFRGS Om62) belonging to group 3 are characterized by slow growth, milky color, diameter smaller than 1 mm, aqueous consistency, opaque and punctiform (Table 1).

The UFRGS Om67 treatment was slightly lower as to the total fresh weight, but overcame those mentioned above, as to the number of leaves, shoot length and root length. Other 16 isolates were able to nodulate plants of serradella, but induced results of total fresh weight, number of leaves, shoot length and root length equal or inferior than the treatment Control - N. It is undesirable that the plants studied are infected by these bacteria, since this interaction does not set up a symbiosis, to the extent that the plant is not benefited by the infection and although it spends photo assimilating metabolites for maintenance of the inefficient nodules. These rhizobia are able to colonize and nodulate serradella plants, but do not contribute to their income, it account for a physiological cost which is not compensated in terms of income. In these cases, it is recommended to exclude these nodulating bacteria from the studies and promote the inoculation of legumes grown with infective rhizobia which are efficient to fix atmospheric N so that, these efficient rhizobia prevail in the sites of infection and promote better yields to plants (Figure 2).The study found positive effect of serradella inoculation with different rhizobia as the isolates UFRGS Om9, UFRGS Om62, and UFRGS Om67 induced significant increases in the yield of serradella plants, especially regarding the

Table 3. Plant fresh mass, relative efficiency index, number of leaves, number of nodules, shoot and root length of serradella (*Ornithopus micrantus*), inoculated with isolates and evaluated after cultivation in vitro by a period of 35 days.

Treatment	Plant fresh mass (mg)	Relative efficiency index (%)	Number of leaves	Number of nodules	Shoot lenght (cm)	Root lenght (cm)
Control N +	33^{d}	100	20.3^{b}	0.0^{e}	7.6^{b}	9.3^{b}
UFRGS Om62	190^{a}	885	22.0^{b}	5.0^{d}	6.6^{c}	7.4^{c}
UFRGS Om9	167^{a}	770	18.0^{c}	3.0^{d}	4.1^{c}	4.9^{d}
UFRGS Om67	130^{b}	585	29.3^{a}	4.3^{d}	11.0^{a}	14.3^{a}
UFRGS Om59	117^{c}	520	26.0^{a}	6.0^{c}	8.4^{a}	9.6^{b}
UFRGS Om4	113^{c}	500	32.0^{a}	6.0^{c}	10.4^{a}	12.8^{a}
UFRGS Om57	110^{c}	485	26.3^{a}	6.0^{c}	9.5^{a}	11.5^{a}
UFRGS Om82	100^{c}	435	25.0^{a}	4.3^{d}	8.5^{a}	12.1^{a}
UFRGS Om148	100^{c}	435	29.3^{a}	6.0^{c}	9.3^{a}	11.9^{a}
UFRGS Om125	93^{c}	400	23.3^{b}	7.3^{c}	9.1^{a}	12.2^{a}
UFRGS Om142	93^{c}	400	22.7^{b}	3.0^{d}	10.0^{a}	11.9^{a}
UFRGS Om74	93^{c}	400	23.3^{b}	3.3^{d}	8.6^{a}	10.3^{b}
UFRGS Om60	90^{c}	385	25.0^{a}	4.3^{d}	8.5^{a}	11.4^{a}
UFRGS Om61	87^{c}	370	23.0^{b}	3.3^{d}	8.3^{b}	9.5^{b}
UFRGS Om58	83^{c}	350	22.3^{b}	5.3^{d}	7.4^{b}	11.2^{b}
UFRGS Om143	80^{c}	335	26.5^{a}	4.5^{d}	9.8^{a}	12.3^{a}
UFRGS Om68	80^{c}	335	24.0^{b}	4.7^{d}	7.6^{b}	6.3^{c}
UFRGS Om80	80^{c}	335	22.3^{b}	5.3^{d}	9.6^{a}	13.4^{a}
UFRGS Om141	77^{c}	320	21.3^{b}	3.3^{d}	6.8^{b}	13.5^{a}
UFRGS Om126	73^{c}	300	21.5^{b}	5.5^{c}	11.5^{a}	10.0^{b}
UFRGS Om146	73^{c}	300	26.3^{a}	4.3^{d}	9.6^{a}	11.6^{a}
UFRGS Om83	73^{c}	300	26.7^{a}	4.7^{d}	9.3^{a}	10.9^{b}
UFRGS Om12	70^{c}	285	33.0^{a}	4.0^{d}	8.2^{b}	10.8^{b}
UFRGS Om13	70^{c}	285	27.7^{a}	5.0^{d}	7.9^{b}	9.0^{b}
UFRGS Om81	70^{c}	285	19.0^{b}	4.0^{d}	8.3^{b}	11.2^{b}
UFRGS Om2	67^{c}	270	25.5^{a}	4.0^{d}	7.8^{b}	9.5^{b}
UFRGS Om25	67^{c}	270	25.7^{a}	12.3^{a}	7.2^{b}	11.6^{a}
UFRGS Om73	67^{c}	270	23.7^{b}	4.3^{d}	7.6^{b}	8.4^{b}
UFRGS Om69	67^{c}	270	22.7^{b}	3.7^{d}	7.2^{b}	7.3^{c}
UFRGS Om65	63^{c}	250	22.3^{b}	3.3^{d}	5.9^{c}	9.3^{b}
UFRGS Om124	63^{c}	250	23.3^{b}	3.3^{d}	8.9^{a}	11.0^{b}
UFRGS Om72	60^{c}	235	21.0^{b}	6.0^{c}	8.7^{a}	10.1^{b}
UFRGS Om5	60^{c}	235	25.3^{a}	3.3^{d}	7.6^{b}	8.9^{b}

Table 3. Contd.

UFRGS Om27	57[d]	220	28.0[a]	8.5[c]	9.0[a]	16.7[a]
UFRGS Om64	57[d]	220	17.0[c]	4.0[d]	5.3[c]	8.0[c]
UFRGS Om76	53[d]	200	21.0[b]	2.6[d]	9.6[a]	7.4[c]
UFRGS Om18	53[d]	200	26.0[a]	4.0[d]	7.4[b]	8.0[c]
UFRGS Om11	53[d]	200	28.7[a]	6.3[c]	7.3[b]	8.0[c]
UFRGS Om134	53[d]	200	16.3[c]	4.3[d]	7.9[b]	6.5[c]
UFRGS Om42	50[d]	185	23.7[b]	3.3[d]	6.8[b]	9.3[b]
UFRGS Om24	50[d]	185	19.7[b]	8.0[c]	4.4[c]	7.1[c]
UFRGS Om123	50[d]	185	17.5[c]	5.0[c]	8.0[b]	10.0[b]
UFRGS Om45	50[d]	185	24.3[b]	5.7[c]	7.2[b]	9.9[b]
UFRGS Om75	50[d]	185	16.5[c]	2.0[e]	11.3[a]	16.5[a]
UFRGS Om20	50[d]	185	23.0[b]	5.3[d]	9.2[a]	9.8[b]
UFRGS Om118	50[d]	185	22.0[b]	2.3[e]	7.1[b]	12.5[a]
UFRGS Om6	50[d]	185	24.7[a]	3.7[d]	7.5[b]	8.1[c]
UFRGS Om131	47[d]	170	16.7[c]	0.0[e]	7.0[b]	7.3[c]
UFRGS Om111	47[d]	170	15.0[c]	0.0[e]	6.1[b]	10.6[b]
UFRGS Om38	47[d]	170	29.0[a]	2.7[d]	6.7[b]	10.2[b]
UFRGS Om138	47[d]	170	21.0[b]	3.0[d]	6.9[b]	6.5[c]
UFRGS Om132	43[d]	150	17.3[c]	0.0[e]	7.4[b]	8.8[b]
UFRGS Om17	43[d]	150	27.3[a]	4.7[d]	7.9[b]	8.8[b]
UFRGS Om117	43[d]	150	19.0[b]	2.3[e]	4.9[c]	6.1[c]
UFRGS Om147	43[d]	150	25.0[a]	3.0[d]	6.1[b]	13.8[a]
UFRGS Om122	43[d]	150	15.5[c]	3.0[d]	6.7[b]	8.8[b]
UFRGS Om116	40[d]	135	17.3[c]	1.0[e]	7.2[b]	10.5[b]
UFRGS Om63	40[d]	135	18.0[c]	3.0[d]	6.3[b]	7.4[c]
UFRGS Om19	40[d]	135	24.0[b]	5.0[d]	5.5[c]	11.6[a]
UFRGS Om140	40[d]	135	14.5[c]	0.0[e]	6.4[b]	10.4[b]
UFRGS Om94	40[d]	135	22.7[b]	12.3[a]	10.1[a]	14.0[a]
UFRGS Om37	40[d]	135	25.0[a]	6.3[c]	5.3[c]	12.1[a]
UFRGS Om56	40[d]	135	19.0[b]	3.3[d]	6.7[b]	7.6[c]
UFRGS Om70	40[d]	135	17.0[c]	2.0[e]	5.6[c]	4.7[d]
UFRGS Om133	37[d]	120	18.3[c]	3.7[d]	7.1[b]	10.8[b]
UFRGS Om119	37[d]	120	19.0[b]	3.0[d]	7.4[b]	9.2[b]
UFRGS Om84	37[d]	120	20.3[b]	4.0[d]	7.2[b]	6.7[c]
UFRGS Om10	37[d]	120	22.3[b]	7.7[c]	5.9[c]	8.8[b]
UFRGS Om14	37[d]	120	21.0[b]	3.5[d]	6.6[b]	7.4[c]

Table 3. Contd.

UFRGS Om71	37[d]	120	17.0[c]	3.0[d]	6.6[b]	5.0[d]
UFRGS Om135	37[d]	120	12.0[c]	2.0[e]	5.7[c]	5.3[d]
UFRGS Om77	37[d]	120	16.0[c]	1.3[e]	6.2[b]	7.2[c]
UFRGS Om79	37[d]	120	22.3[b]	5.0[d]	7.6[b]	7.6[c]
UFRGS Om33	33[d]	100	23.0[b]	3.3[d]	6.3[b]	7.1[c]
UFRGS Om3	33[d]	100	20.3[b]	6.7[c]	4.4[c]	8.0[c]
UFRGS Om1	33[d]	100	19.7[b]	4.7[d]	5.9[c]	7.4[c]
UFRGS Om21	33[d]	100	20.5[b]	3.0[d]	7.2[b]	5.7[d]
UFRGS Om16	33[d]	100	25.0[a]	3.5[d]	7.4[b]	7.6[c]
UFRGS Om40	33[d]	100	18.5[c]	0.0[e]	5.9[c]	6.0[c]
UFRGS Om7	33[d]	100	24.0[b]	4.3[d]	5.5[c]	7.9[c]
UFRGS Om129	33[d]	100	12.3[c]	0.0[e]	7.2[b]	4.6[d]
UFRGS Om78	33[d]	100	13.0[c]	0.7[e]	6.2[b]	4.3[d]
UFRGS Om87	30[d]	85	26.0[a]	3.7[d]	9.3[a]	9.9[b]
UFRGS Om54	30[d]	85	22.3[b]	4.7[d]	6.1[b]	5.2[d]
UFRGS Om110	30[d]	85	18.0[c]	3.7[d]	4.5[c]	9.2[b]
UFRGS Om89	30[d]	85	21.7[b]	4.0[d]	9.4[a]	10.2[b]
UFRGS Om97	30[d]	85	18.0[c]	5.7[c]	7.7[b]	10.5[b]
UFRGS Om26	30[d]	85	19.5[b]	9.5[b]	4.8[c]	10.9[b]
UFRGS Om127	27[d]	70	15.7[c]	1.7[e]	6.4[b]	8.3[b]
UFRGS Om52	27[d]	70	23.0[b]	7.3[c]	7.8[b]	9.3[b]
UFRGS Om22	27[d]	70	14.7[c]	4.0[d]	5.1[c]	5.0[d]
UFRGS Om106	27[d]	70	15.3[c]	5.7[c]	6.6[b]	10.3[b]
UFRGS Om36	27[d]	70	16.3[c]	0.0[e]	3.9[c]	7.6[c]
UFRGS Om137	27[d]	70	8.0[d]	0.0[e]	3.0[d]	3.6[d]
UFRGS Om86	27[d]	70	24.3[b]	3.0[d]	9.6[a]	11.5[a]
UFRGS Om105	27[d]	70	15.3[c]	5.0[d]	5.8[c]	8.8[b]
UFRGS Om23	27[d]	70	19.5[b]	6.7[c]	4.8[c]	5.5[d]
UFRGS Om115	27[d]	70	15.0[c]	0.0[e]	4.8[c]	7.3[c]
UFRGS Om34	27[d]	70	15.7[c]	0.0[e]	4.9[c]	7.8[c]
UFRGS Om41	23[d]	50	17.0[c]	1.3[e]	3.7[c]	3.8[d]
UFRGS Om47	23[d]	50	15.7[c]	2.0[e]	3.4[d]	3.1[d]
UFRGS Om55	23[d]	50	15.3[c]	2.7[d]	5.2[c]	4.4[d]
UFRGS Om90	23[d]	50	19.7[b]	6.7[c]	8.2[b]	10.4[b]
UFRGS Om44	23[d]	50	14.7[c]	0.0[e]	4.1[c]	6.9[c]
UFRGS Om46	23[d]	50	16.7[c]	4.3[d]	4.0[c]	4.8[d]

Table 3. Contd.

UFRGS Om88	23^{d}	50	20.0^{b}	4.3^{d}	8.9^{a}	9.9^{b}
UFRGS Om112	23^{d}	50	13.3^{c}	0.7^{e}	4.1^{c}	5.5^{d}
UFRGS Om113	20^{d}	35	2.5^{d}	0.0^{e}	2.7^{d}	3.3^{d}
UFRGS Om43	20^{d}	35	16.0^{c}	0.0^{e}	3.9^{c}	8.4^{b}
UFRGS Om39	20^{d}	35	15.7^{c}	0.0^{e}	3.7^{c}	5.0^{d}
UFRGS Om103	20^{d}	35	12.0^{c}	0.0^{e}	4.5^{c}	7.8^{c}
UFRGS Om98	20^{d}	35	7.5^{d}	3.0^{d}	4.1^{c}	6.7^{c}
UFRGS Om85	20^{d}	35	15.7^{c}	3.0^{d}	6.5^{c}	6.0^{c}
UFRGS Om50	20^{d}	35	24.3^{a}	3.7^{d}	7.0^{b}	6.7^{c}
UFRGS Om15	20^{d}	35	16.7^{c}	3.3^{d}	4.8^{c}	5.0^{d}
UFRGS Om120	20^{d}	35	16.0^{c}	0.7^{e}	4.9^{c}	7.0^{c}
UFRGS Om99	20^{d}	35	12.0^{c}	0.0^{e}	7.1^{b}	9.3^{b}
UFRGS Om91	20^{d}	35	19.7^{b}	3.7^{d}	9.9^{a}	8.9^{b}
UFRGS Om130	20^{d}	35	6.0^{d}	0.0^{e}	2.0^{d}	4.0^{d}
UFRGS Om29	20^{d}	35	21.7^{b}	5.3^{d}	6.2^{c}	8.1^{c}
UFRGS Om128	17^{d}	20	16.3^{c}	2.0^{e}	7.5^{b}	4.9^{d}
UFRGS Om92	16^{d}	15	21.0^{b}	4.0^{d}	7.1^{b}	10.5^{b}
UFRGS Om93	16^{d}	15	16.3^{c}	3.7^{d}	6.7^{b}	10.0^{b}
UFRGS Om144	16^{d}	15	9.3^{d}	0.0^{e}	2.3^{d}	3.7^{d}
UFRGS Om114	16^{d}	15	14.3^{c}	1.0^{e}	4.2^{c}	2.8^{d}
UFRGS Om95	16^{d}	15	15.7^{c}	6.0^{c}	5.9^{c}	7.3^{c}
UFRGS Om51	16^{d}	15	17.0^{c}	0.0^{e}	5.3^{c}	7.8^{c}
UFRGS Om100	16^{d}	15	13.0^{c}	1.3^{e}	5.9^{c}	10.3^{b}
UFRGS Om28	16^{d}	15	17.3^{c}	7.0^{c}	5.1^{c}	10.3^{b}
UFRGS Om109	16^{d}	15	14.7^{c}	0.7^{e}	7.4^{b}	8.8^{b}
UFRGS Om8	16^{d}	15	16.0^{c}	4.0^{d}	4.8^{c}	5.9^{c}
UFRGS Om108	13^{d}	0	15.3^{c}	1.7^{d}	3.9^{c}	7.1^{c}
UFRGS Om139	13^{d}	0	7.3^{d}	0.0^{e}	3.7^{c}	5.7^{d}
UFRGS Om30	13^{d}	0	22.3^{b}	0.0^{e}	4.6^{c}	8.5.^{b}
UFRGS Om96	13^{d}	0	14.3^{c}	1.7^{e}	5.1^{c}	7.1^{c}
UFRGS Om136	13^{d}	0	12.5^{c}	0.0^{e}	3.0^{d}	2.4^{d}
UFRGS Om101	10^{d}	-15	8.0^{d}	0.0^{e}	2.0^{d}	3.1^{d}
UFRGS Om35	10^{d}	-15	8.0^{d}	0.0^{e}	1.9^{d}	3.4^{d}
UFRGS Om53	10^{d}	-15	8.0^{d}	0.0^{e}	1.9^{d}	3.8^{d}
UFRGS Om48	10^{d}	-15	14.8^{c}	3.6^{d}	4.3^{c}	5.5^{d}
UFRGS Om104	10^{d}	-15	12.7^{c}	0.0^{e}	5.8^{c}	7.5^{c}

Table 3. Contd.

UFRGS Om31	10[d]	-15	8.0[d]	0.0e	2.0[d]	4.2[d]
UFRGS Om107	10[d]	-15	6.3[d]	0.0e	1.7[d]	3.0[d]
UFRGS Om32	10[d]	-15	7.3[d]	0.0e	2.1[d]	4.0[d]
UFRGS Om145	7[d]	-30	8.0[d]	0.0e	1.3[d]	2.7[d]
UFRGS Om121	7[d]	-30	8.0[d]	1.0e	2.8[d]	3.1[d]
UFRGS Om102	7[d]	-30	6.7[d]	0.0e	1.2[d]	2.7[d]
UFRGS Om49	7[d]	-30	18.0[c]	3.3[d]	2.9[d]	4.1[d]
UFRGS Om66	0[d]	-30	11.5[d]	3.0[d]	3.9[c]	7.5[c]
Control N -	13[d]	0	11.0[d]	0.0e	3.7[c]	7.2[c]
CV (%)	61.53	-	17.84	51.59	27.95	28.87

Averages followed by the same letter in the column do not differ among themselves by Scott-Knott test at 5% probability. Control N +: non-inoculated control treatment with the addition of the equivalent to 100 kg of N ha^{-1}; Control N -: non-inoculated control treatment without addition of nitrogen.

increase of root and shoot dry weight.

Evaluation of symbiotic efficiency of rhizobia in serradella plants

The results produced by serradella plants grown in the greenhouse are presented in Table 4. It observed a higher dry weight of shoot (DWS) and root dry weight (RDW) from uninoculated treatment + Nitrogen (Control + N) which is equivalent to the application of 250 kg N ha^{-1}. The inferior yield to the Control + N are, the UFRGS Om57, Om59 and Om148 treatments, which exceeded the Control treatment - N and all other inoculated treatments as DWS and RDW, surpassing the strains SEMIA 905 and SEMIA 929, currently licensed by MAPA (Ministry of Agriculture, Livestock and Food Supply) for the production of inoculants for serradella. Despite the lower total fresh weight of UFRGS Om57, Om59 and Om148 treatments compared to the Control + N, it was observed that these isolates were equivalent to Control + N on the leaf N accumulation, which received N dose equivalent to 250 $kg.ha^{-1}$. This shows the great efficiency of the isolated UFRGS Om57, Om59 and Om148 in biologically fixing atmospheric nitrogen in serradella plants, enabling the elimination of mineral nitrogen fertilization, without prejudice to the total nutrient content of the leaf. In the assessment made at the end of 60 days after plant emergence, the average number of nodes per pot ranged from 0.0 (SEMIA905 and SEMIA929 strains) and 143.0 (UFRGS Om59). The plants of higher dry weight of nodules were inoculated with rhizobia UFRGS Om57, Om59 and Om148, precisely those that also had higher root dry weight (RDW), dry weight of shoot (DWS) and higher total nitrogen content of shoot (N Total). It is inferred that, the most massive nodules rhizobia were the same as an induced greater mass of plants and nitrogen content in the leaves.The treatments UFRGS OM4, Om27 and Om82, was found to have a large number of nodes (NN) and early nodular (EN) in the treatments which the shoot dry weight, root dry weight and N Total were equivalent to Control - N, therefore unsatisfactory. This indicates that many nodules and early nodular recorded did not set atmospheric N and so the physiological costs of these nodules were not converted to increase yield of plants. Thus, in this study we did not observe the connection between the number of nodes and the mass increase of plants or N Total of leaves.

The relative efficiency index (REI %) presented in percentage shows how efficient the symbiont organism is to fix atmospheric N for the legume in association. In percentage terms, the N content obtained in the leaves of each inoculated treatment is compared to the respective values obtained in the leaves of Control + N and Control - N treatments, to estimate the potential of each isolate to fix atmospheric N under axenic conditions. Isolates UFRGS Om57; Om59 and

Figure 2. Serradella inoculated with effective rhizobia, 35 days after emergence (left) and serradella not inoculated, 35 days after emergence (right).

Table 4. Dry weight of shoot (DWS), root dry weight (RDW), nodules dry mass (NDM), total nitrogen of shoots (N Total), Relative Efficiency Index (REI), number of nodules (NN) and number of early nodules (EL) of serradela (*Ornithopus micranthus*), grown in the greenhouse. Average of four replications, with two plants per pot.

Treatment	DWS (mg)	RDW (mg)	NDM (mg)	N Total(mg)	REI (%)	NN*	EL*
Control + N	1997^{a}	767^{a}	0^{c}	23.9^{a}	100.0	0^{b}	0^{b}
UFRGS Om148	1237^{b}	234^{b}	45^{a}	27.7^{a}	117.3	127^{a}	29^{a}
UFRGS Om59	1089^{b}	303^{b}	34^{a}	26.7^{a}	112.9	143^{a}	43^{a}
UFRGS Om57	967^{b}	188^{b}	32^{a}	24.9^{a}	104.5	132^{a}	39^{a}
SEMIA 929	654^{c}	125^{c}	0^{c}	6.0^{c}	19.3	0^{b}	0^{b}
UFRGS Om67	609^{c}	158^{c}	23^{b}	14.7^{b}	58.6	86^{a}	32^{a}
UFRGS Om62	570^{c}	190^{b}	22^{b}	13.7^{b}	53.7	115^{a}	44^{a}
UFRGS Om82	338^{c}	133^{c}	14^{c}	7.8^{c}	27.1	79^{a}	28^{a}
UFRGS Om27	240^{c}	95^{c}	14^{c}	3.5^{c}	7.7	116^{a}	25^{a}
UFRGS Om4	235^{c}	81^{c}	6^{c}	2.7^{c}	4.2	71^{a}	43^{a}
SEMIA 905	196^{c}	196^{b}	0^{c}	1.6^{c}	-0.6	0^{b}	0^{b}
Control - N	205^{c}	97^{c}	0^{c}	1.8^{c}	0.00	0^{b}	0^{b}
CV (%)	61.01	36.07	62.13	56.96	-	44.73	28.6

Control + N: treatment not inoculated, fertilized with nitrogen dose (N) equivalent to 250kg N.ha^{-1}; Control - N: treatment not inoculated, without N fertilization

Om148, (RER%) indexes exceeding 100%: 104.5%; 112.9 % and 117.3 %, respectively, were observed. With these results, it is shown that these symbiotic associations were efficient in the increase of total N content in the leaves, being superior to the strains of SEMIA collection and thus promising for future studies in order to be recommended for the production of commercial inoculants to serradella.

SEMIA905 and SEMIA929 strains did not form nodules in serradella (*Ornithopus micranthus*) nor induced growth in serradella plants studied. Thus, based on the present results, it is inferred that in serradella plants (*Ornithopus micranthus*) better yields are obtained with the placement of the isolated UFRGS Om57, Om59 and Om148.

DISCUSSION

Rhizobacteria that promote plant growth are inserted into pasture production systems for, feeding cattle that produce meat or milk. As they present mechanisms that promote plant growth, they may be recommended for the composition of commercial inoculants for specific crops. In the case of the use of rhizobacteria in pastures, these plants yield increment mechanisms should somehow be able to anticipate the supply of pastures, lengthen the crop production cycle, or increase the supply of pastures to animals the same time they keep or reduce the need for use of mineral fertilizers.

With this study we found positive effect of inoculation of different rhizobia on the yield of serradella (*Ornithopus micranthus*). Isolated UFRGS Om57; Om59 and Om148 induced significant increases in the yield of serradella plants, especially regarding the increase of the dry weight of shoot and root dry weight. For the isolated UFRGS Om57, UFRGS Om59 and UFRGS Om148, the relative efficiency index (RER %) for accumulation of total nitrogen (N) in shoots was greater than 100%. These results demonstrate the high efficiency of these isolated (Table 3) which were higher than those found in the literature, as the study of symbiosis between rhizobia grown forage legumes (Scheffer-Basso et al., 2001; Frizzo, 2007) and (Scheffer-Basso et al., 2001). Fontoura et al. (2011) obtained similar results with the inoculation of rhizobia in *Lotus glaber*.

The use of these high performance diazotrophic bacteria significantly increase in N supply to the soil, eliminating the mineral nitrogen fertilizer without damage to the crop yield and to the N supply to the soil. Additionally, some rhizobia are known to produce phyto stimulating substances, such as hormones from the auxin group (Anjum et al., 2011; Spaepen and Vanderleyden, 2011), cytokinins (Senthilkumar et al., 2009) and gibberellins (Erum and Bano, 2008), which are associated with an increase in the yield of legumes and non-legumes. Thus, investment in the rhizobia inoculants that optimize the quality and quantity of pastures offered to cattle that produce milk or meat, may increase the productive performance of the animals.

Moreover, due to the specificity between the host plant and the rhizobium, for both rhizobia fasteners of atmospheric N, and for rhizobia producers of plant stimulating substances, the study of the interaction and the effect of inoculation of these organisms for the yield of plants are required. In this study it was observed that the specificity within the *Ornithopus* gender, as strains recommended for the composition of commercial inoculants for use in pastures composed of *Ornithopus sativus* showed no nodulation and had poor performance on the biological nitrogen fixation and growth promotion of the *Ornithopus micranthus* species (Table 3). Based on these results, we highlight the importance of studies on specificity between bacteria and plant; there is no affinity between SEMIA905 and SEMIA929 strains and serradella *Ornithopus micranthus*.

As for savings promoted with the use of effective rhizobia, we can assume a scenario based on economic data seen in the first quarter of the year 2016 in the state of Rio Grande do Sul, Brazil: considering the price of the US dollar (US$) in R$ 3.487 in March 2016 which state that, the price of urea in the Brazilian domestic market was approximately US$ 200.00 ton^{-1}. Based on this study after 60 days, the inoculation of serradella with fixing bacteria of N (UFRGS Om57; Om59 and Om148) would supply the equivalent of 250 kg $N.ha^{-1}$ (Table 3), representing a saving of U$ 111.11 ha^{-1} in this scenario. Knowing that the average productivity of dairy herd of Rio Grande do Sul state lies at 7.9 liters of milk / cow / day (IBGE, 2013); that in March 2016, the average price of a liter of milk in the state of Rio Grande do Sul was $ 0.31 (CEPEA/ESALQ, 2016); and assuming a stocking of 7 Units Animals/ha, at the end of 60 days, milk production in 1 ha would have a gross value of US$ 1,028.58. The value of N fixed biologically would be equivalent to 10.8% of the gross value of the production obtained by the exploration of the dairy cattle in 1 ha, for 60 days. This economy would surely bring greater economic sustainability for dairy cattle farm in the state of Rio Grande do Sul, favoring the permanence of small milk producers in the dairy business.

Conclusions

We obtained and described 148 isolate, of which 113 were capable of forming nodules in association with *Ornithopus micranthus*. The isolates UFRGS Om57, UFRGS Om59 and UFRGs Om148 were equivalent to Control + N on the leaf N accumulation, which received N dose equivalent to 250 $kg.ha^{-1}$ in greenhouse. This shows the great efficiency of the isolated UFRGS Om57, UFRGS Om59 and UFRGS Om148 in biologically fixing atmospheric nitrogen in serradella plants, enabling the elimination of mineral nitrogen fertilization, without prejudice to the total nutrient content of the leaf.

The isolates that had better performance were characterized with slow growth in culture medium and punctiform colonies with diameter smaller than one millimeter. These characteristics are typical example of genus *Bradyrhizobium*.

Conflict of Interests

The authors have not declared any conflict of interests.

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