Performance of *Rhizobium* strains isolated from *Lathyrus sativus* plants growing in southern Chile

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Introduction

Grass pea (*Lathyrus sativus* L.) is grown in southern Chile by small farmers [(2)], in poorly managed soils characterised by a high level of erosion and low fertility [(3)]. As with any other legume, the symbiotic nitrogen fixation is a process that must be improved. Grain legume crops are rarely inoculated in Chile and empirical evidence suggests that ineffective *Rhizobium* strains may be a significant proportion of the nodulating bacteria. This may be particularly relevant in the case of grass pea, where areas not previously cultivated with this legume are being utilised. Being a low-cost, environmentally friendly, simple technology, the use of inoculants should be promoted. However, there is a lack of an inoculant specific for grass pea in Chile. Consequently, we have undertaken the task of developing a quality inoculant for grass pea, starting with the evaluation of *Rhizobium* strains isolated from well-nodulated grass pea plants sampled in three locations of the La Araucania region of Chile.

Material and Methods

Locations. Sampling of nodulated plants was done in three different locations of La Araucania: Lumaco, Selva Oscura and General Lopez. The soils of Lumaco are inceptisols, severely degraded with low fertility. Besides grass peas, other legumes traditionally found in these soils are peas (*Pisum sativum*), lentils (*Lens culinaris*), broad beans (*Vicia faba*), vetches (*Vicia* spp.) and chickpeas (*Cicer arietinum*). The soils of Selva Oscura are andisols moderately degraded, which quite often present medium-high levels of aluminium saturation. A majority of the soils in Selva Oscura do not have a record of previous grass pea cultivation. Sampling at General Lopez was done in soils of the Carillanca Regional Research Centre, which have a high level of fertility, with a long history of several legume species participating in crop rotations, though rather sparsely over time.

Sampling. Twenty small farms were visited in Lumaco and from the nodules collected, 47 strains were isolated. Three small farms were visited in Selva Oscura, from which eight strains were obtained. Sampling at Carillanca, in four different fields, yielded five additional strains. Therefore, a total of 60 strains were isolated, presumably corresponding to *Rhizobium leguminosarum* biovar *viciae*.

Rhizobia strains isolation. Three random, good-looking, pink nodules were excised from each plant and nodule isolates were obtained by the procedure of Vincent [(5)]. Single colonies were picked and maintained on yeast mannitol agar (YMA) slants at 4ºC for further characterisation. Isolates were designated by two letters (LU = Lumaco, SO = Selva Oscura, CA = Carillanca) and a correlative number.

Evaluation of symbiotic properties of rhizobial isolates. The isolates were characterised according to their colony morphology in YMA [(5)], after 2 and 4 days of growth and then evaluated preliminarily in Leonard jars with two replications, under greenhouse conditions. The best 15 strains were selected for a second evaluation, whose results are reported here. Leonard jars were filled with river sand carefully washed and watered with a nitrogen-free nutrient solution [(4)]. Seedlings of grass pea were transferred aseptically to jars (five per jar) and inoculated with 10 mL of 2 day-old yeast mannitol broth (YMB) cultures of rhizobia to provide approximately 10^8 cells seed^{-1}. An non-inoculated control jar with mineral N (CN), supplied as potassium nitrate solution (0.75 g L^{-1} of N every 15 days), was also evaluated. Seedlings were thinned to uniformity to two per jar and covered with a layer of sterilised coarse sand. Jars were arranged in a completely randomised design with three replicates per treatment. Plants were harvested 45 days after inoculation and symbiotic effectiveness was estimated through the aerial dry weight according to an effectiveness criterion [(1)]. A relative production index was calculated as RP = I/N, where I is the dry matter production by inoculated plants and N is the dry matter production of uninoculated control plants that received fertilizer nitrogen. An RP<1 indicates that nitrogen fixation was insufficient to cover the plants requirement of nitrogen. The size, number and weight of nodules per jar were also recorded (not shown). Data were processed with an analysis of variance and Duncan's multiple range test using SAS 6.12.

Results and Discussion

Table 1. Dry matter production per jar and relative production index of two grass pea plants inoculated with fifteen strains of *Rhizobium leguminosarum* biovar *viciae*, isolated from three locations in southern Chile.

Origin	Strain	Dry matter (g jar^{-1})	Relative production index
Lumaco	LU-29	3.22 a*	1.43
Carillanca	CA-60	2.68 ab	1.19
Selva Oscura	SO-51	2.56 ab	1.14
Control	CN	2.26 bc	1.00
Lumaco	LU-23	2.07 bcd	0.92
Selva Oscura	SO-53	1.96 bcde	0.87
Lumaco	LU-24	1.57 bcdef	0.70
Selva Oscura	SO-47	1.54 cdef	0.68
Selva Oscura	SO-48	1.52 cdef	0.67
Lumaco	LU-19	1.41 def	0.62
Lumaco	LU-45	1.41 def	0.62
Lumaco	LU-02	1.40 def	0.62
Selva Oscura	SO-50	1.20 ef	0.53
Lumaco	LU-01	1.11 f	0.49
Lumaco	LU-28	1.06 f	0.47
Lumaco	LU-27	0.85 f	0.38

* Means sharing same letter are not significantly different according to Duncan's NMRT, $P \leq 0.05$.

Strains varied considerably in dry matter production, as shown in Table 1. Only one strain (LU-29) yielded significantly more dry matter than the control receiving nitrogen. Seven strains (CA-60, SO-51, LU-23, SO-53, LU-24, SO-47, SO-48) performed similarly to the control, and the remaining seven were significantly inferior. Twelve strains gave a relative production index lower than 1, suggesting that grass pea roots are being infected by poorly effective bacteria in terms of nitrogen fixation. In fact, several strains gave rise to small, pale nodules (data not shown). At the same time, this result suggests that much can be done regarding grass pea inoculation in southern Chile. Even when *Rhizobium leguminosarum* biovar *viciae* promiscuously nodulates the Vicieae, which comprises genera *Pisum*, *Lens*, *Vicia* and *Lathyrus* [6], it is widely recognised that maximum benefit from symbiotic nitrogen fixation can be achieved only when rhizobia and host plant present a high affinity. Evaluation of strains LU-29, CA-60 and SO-51 will continue, as they are good candidates to conform a future commercial inoculant, specific for the grass pea cultivated in the region of La Araucania. Interestingly, these strains come from locations with quite different soil and environmental conditions.

Acknowledgements
FNDR Project BIP 20155696-0 granted by Gobierno Regional de La Araucania.

References

1. Bordeleau L, Anton H & Lachance R. 1977. Effets des souches de *Rhizobium meliloti* et des coupes successives de la luzerne (*Medicago sativa*) sur la fixation symbiotique d'azote. Can J Plant Sci 57, 433-439.
2. Mera M, Montenegro A, Espinoza N & Gaete N. 2000. Research backs grass pea exports by small Chilean farmers. Lathyrus Lathyrism Newsletter 1, 31.
3. Montenegro A. 1991. Diagnóstico preliminar de los tenores de nitrógeno, fósforo, potasio, materia orgánica y pH de los suelos de la IX Región. Investigación y Progreso Agropecuario Carillanca 10(3), 3-11.
4. Norris DO and Date RA. 1976. Legume bacteriology. *In*: Shaw NH & Bryan W (eds.). Tropical Pastures Research; Principles and Methods. p 134-174. Commonwealth Bureau of Pastures and Field Crops, Hurley, UK.
5. Vincent JM. 1975. Manual Práctico de Rhizobiología. Hemisferio Sur, Buenos Aires, Argentina. 200 p.
6. Young JPW. 1996. Phylogeny and taxonomy of rhizobia. Plant and Soil 186, 45-52.