

## Full Length Research Paper

## Symbiotic efficiency of pea (*Pisum sativum*) rhizobia association under field conditions

Aleksander Westphal Muniz<sup>1\*</sup>, Murilo Dalla Costa<sup>2</sup>, Enilson Luiz Saccol de Sá<sup>3</sup>, Ana Beatriz Fiuza<sup>1</sup> and Edemar Brose<sup>4</sup>

<sup>1</sup>Embrapa, Rodovia AM 10, Manaus 69010-970, Brazil.

<sup>2</sup>Epagri, Rua João José Godino, Lages 88502-970, Brazil.

<sup>3</sup>UFRGS, Av. Bento Gonçalves 7712, Porto Alegre 91540-000, Brazil.

<sup>4</sup>Rua Papagaio 185, Bombas 88215-000, Brazil.

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The objective of this study was to evaluate the symbiotic efficiency of rhizobia under field conditions for peas (*Pisum sativum* L.). Ten treatments were evaluated and divided into eight strains of rhizobia and two uninoculated strains as controls (with and without the addition of mineral N). The variables analyzed were: Nodulation, dry mass of the aerial part, total N of the aerial section, pea production and symbiotic efficiency. The inoculated rhizobia strains had effects on the number and mass of nodules, accumulated N in the aerial part and pea grains production. The strain EEL7802 presented the highest symbiotic efficiency for peas. The inoculation may allow cost reduction due to the equivalence with nitrogen fertilization. Studies in other soil types are needed to confirm the efficiency of this strain.

**Key words:** *Pisum sativum*, *Rhizobium leguminosarum*, nodulation, nitrogen biological fixation.

### INTRODUCTION

Pea (*Pisum sativum* L., Fabaceae) is a grain legume, characterized by its high nutritional value and its potential use for human and animal feeding. The pea grains can be used for immediate consumption or canned for long-term storage, and can also be used for the preparation of instant soups. The huge industrial demand for peas has increased the cultivated area in Brazil by 36% between 2001 (1893 ha) and 2010 (2569 ha). In addition, pea production was 4442 and 5909 tonnes in 2001 and 2010, respectively. Despite this increase in production, Brazil still imports more than 80% of the consumed peas (FAO,

2012).

Pea crops, like other legume crops, are able to establish symbiosis with rhizobia (Van Rhijn and Vanderleyden, 1995). The pea plant absorbs nitrogen (N) obtained by the biological fixation by *Rhizobium leguminosarum* bv. *viciae* through symbiosis. Under conditions of low N availability in the soil, symbiosis can provide up to 80% of the N amount required for the growth of the pea plant (Voisin et al., 2002).

Before the 1990s, the number of rhizobial strains recommended for association with pea plant in Brazil was

\*Corresponding author. E-mail: [aleksander.muniz@embrapa.br](mailto:aleksander.muniz@embrapa.br).

**Table 1.** Pea rhizobia strains by Epagri collection.

Strain	Source	Collection year
SEMIA 3007	*Embrapa, Londrina, Paraná state	-
USA 212-7	*Embrapa, Londrina, Paraná state	-
EEL 5501	São Jorge, Rio Grande do Sul state	2001
EEL 3001	São Jorge, Rio Grande do Sul state	2001
EEL 7802	Raul Basso farm, Vacaria, Rio Grande do Sul state	2002
EEL 1002	Rodrigo Barison Farm, Vacaria, Rio Grande do Sul state	2002
EEL 6802	Raul Basso farm, Vacaria, Rio Grande do Sul state	2002
EEL 13402	Bonanza farm, Lagoa Vermelha, Rio Grande do Sul state	2002

\*Embrapa = Brazilian Agricultural Research Corporation, National Rhizobia Collection.

**Table 2.** Soil analysis of the experimental area at Triunfo Farm, Lages-SC.

Clay %	Water:pH 1:1	SMP Index	P mg.dm <sup>3</sup>	K 108	OM %	Al 3.2	Ca 0.5	Mg 0.8	H+Al 19.4	CEC 21.0
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\*OM = Organic matter; CEC = Cation exchange capacity; cmol = centimole.

few, and this was based on the studies conducted in Europe and Brazil (Conceição et al., 1981; Jensen, 1987). The isolation and selection of efficient N fixing rhizobial strains was initiated in Santa Catarina in 2001 (Brose and Muniz, 2008). However, most of the studies were performed under controlled conditions that use sterile substrates (sand and vermiculite) in pots and nutritious solution for the growth of the pea plants. However, this procedure is not recommended for the inoculation and culture of the rhizobial strains by the Ministry of Agriculture, Government of Brazil. The Agriculture Ministry requires field trials for the official recommendation of the rhizobial strains (SEMIA 3007 and USA 212-7). Therefore, the objective of this study was to evaluate the symbiotic efficiency of several rhizobial strains on pea plant under field conditions.

## MATERIALS AND METHODS

The experiment was conducted through September to November 2005 in an Argisol. The Argisol represented the soil type specific for the growth of the pea plant in Brazil. Six strains of *R. leguminosarum* bv. *viciae*, which were previously selected through greenhouse experiments were obtained and evaluated from the diazotrophic bacterial collection of Epagri (Table 1) (Brose and Muniz, 2008).

Rhizobial strains, SEMIA 3007 and USA 212-7, which were previously recommended for the production of pea inoculants, were used as controls (Brasil, 2009). In addition, two non-inoculated controls were used: One without N fertilization and the other with a dose of 200 kg N ha<sup>-1</sup> applied as urea by broadcasting method. Soil fertilization and correction were performed by using phosphorous pentoxide (220 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) and potassium oxide (90 kg K<sub>2</sub>O ha<sup>-1</sup>) according to the recommendations of the Brazilian Society of Soil Science (2004). This recommendation was based on the chemical

analysis presented in Table 2. The chemical analysis was performed according to Embrapa (1997).

Pea seeds (cultivar: Spencer) were inoculated with the rhizobial bacterial broth in plastic packaging containing sterile peat. The inoculant was prepared at a ratio of 3:1 of peat and log phase cultures in 79 liquid media (Vincent, 1975). Two hundred grams of the inoculant was used per 20 kg of pea seeds. Planting was carried out on September 23, 2005 at the Triunfo Farm in the municipality of Lages, Santa Catarina. After 60 days of germination, the pea plants were collected to evaluate nodulation, dry mass of the aerial part, and the total N accumulated in the aerial part. After 60 days of flowering, the pea crop was harvested to determine the yield and the total N accumulated in the grain. The relative efficiency of the biological N fixation was calculated according to the following formula (Brockwell et al., 1966):

$$Ef(\%) = \frac{N_{inoc} - TestSN}{TestCN - TestSN} \times 100$$

Ef (%) = Relative efficiency in biological nitrogen fixation; N<sub>inoc</sub> = Total nitrogen in the inoculated treatment grains; TestCN = Total nitrogen in the control grains with addition of nitrogen; TestSN = Total nitrogen in the grains of the control treatment without addition of nitrogen. The Ef classes considered for strains selection were: 0 to 20% = inefficient; 21 to 40% = very low; 41 to 60% = low; 61 to 80% = average; 81 to 100% = high; and > 100% = very high.

The experimental design consisted of six randomized blocks. The area of each plot was 24 m<sup>2</sup> with a space of 40 cm between the rows. The results obtained were analyzed for variance analysis and Tukey's mean separation test. Statistical analysis was performed using the Matlab software. The values were considered statistically significant at p < 0.10, which was adopted as per the recommendations of the Brazilian Ministry of Agriculture (Brazil, 2009).

## RESULTS AND DISCUSSION

The number of nodules was higher in the pea plants

**Table 3.** Number of nodules (NNOD), dry mass of nodules (MNOD), total nitrogen of aerial part (NTPA), dry mass of aerial part(MSPA), total nitrogen of grain (NTG), grain mass (MG) and relative efficiency (EF) in pea cv. 'Spencer'.

Strain	NNOD	MNOD	MSPA	NTPA	NTG	MG	EF
	N° planta <sup>-1</sup>	mg planta <sup>-1</sup>		mg N planta <sup>-1</sup>	Kg N ha <sup>-1</sup>	Kg ha <sup>-1</sup>	%
SEMIA 3007	36.5 <sup>bc</sup>	83.1 <sup>cb</sup>	3400 <sup>a</sup>	105.9 <sup>ab</sup>	188.4 <sup>a</sup>	1040 <sup>ab</sup>	66.4
USA 212-7	30.9 <sup>bcd</sup>	165.3 <sup>a</sup>	2838 <sup>a</sup>	80.2 <sup>b</sup>	188.5 <sup>a</sup>	1016 <sup>ab</sup>	31.4
EEL 5501	50.8 <sup>ab</sup>	139.2 <sup>ab</sup>	3352 <sup>a</sup>	108.3 <sup>ab</sup>	186.6 <sup>a</sup>	1059 <sup>ab</sup>	64.6
EEL 3001	21.9 <sup>cd</sup>	21.6 <sup>d</sup>	4217 <sup>a</sup>	100.0 <sup>ab</sup>	163.9 <sup>a</sup>	1006 <sup>ab</sup>	41.9
EEL 7802	25.3 <sup>cd</sup>	36.9 <sup>cd</sup>	3298 <sup>a</sup>	91.7 <sup>ab</sup>	206.0 <sup>a</sup>	1170 <sup>a</sup>	84.1
EEL 1002	53.5 <sup>ab</sup>	42.7 <sup>cd</sup>	4033 <sup>a</sup>	108.0 <sup>ab</sup>	175.7 <sup>a</sup>	1066 <sup>ab</sup>	53.7
EEL 6802	67.4 <sup>a</sup>	46.6 <sup>cd</sup>	4603 <sup>a</sup>	129.1 <sup>a</sup>	181.7 <sup>a</sup>	1049 <sup>ab</sup>	59.7
EEL 13402	45.5 <sup>abc</sup>	49.4 <sup>cd</sup>	4037 <sup>a</sup>	113.1 <sup>ab</sup>	174.2 <sup>a</sup>	1063 <sup>ab</sup>	52.2
TEST. SN	10.6 <sup>d</sup>	26.4 <sup>cd</sup>	3125 <sup>a</sup>	86.1 <sup>b</sup>	122.1 <sup>a</sup>	762 <sup>b</sup>	0.0
TEST. CN	10.3 <sup>d</sup>	41.2 <sup>cd</sup>	3812 <sup>a</sup>	113.3 <sup>ab</sup>	221.9 <sup>a</sup>	1177 <sup>a</sup>	100.0
CV (%)	41.00	53.75	27.73	22.19	19.73	20.73	

\*Averages with the same letter in the column do not statistically differ from each other by the Tukey's test ( $p < 0.10$ ). CV = Coefficient of variation.

inoculated with the rhizobial strain EEL 7802 than in those inoculated with other strains (Table 3). The mass of the pea nodules ranged from 21.6 to 165.3 mg plant<sup>-1</sup> and was higher with the USA 212-7 and EEL 5501 rhizobial strains than with other strains. Pea nodulation was also observed in the non-inoculated samples, indicating the presence of autochthonous noduliferous bacterial population in the soil, since there was no previous record of pea cultivation in the study area. The dry mass of the aerial plant part did not vary much between inoculated treatments and controls. The total N of the aerial plant part varied between 80.2 and 129.1 mg plant<sup>-1</sup>. Inoculation of the EEL 7802 strain resulted in higher N fixation in the aerial part than in the non-inoculated plants and plants without N fertilization. It was found that the number and mass of nodules of the inoculated pea plants had no correlation with pea grain production, which is in accordance with the observation that the efficiency of N fixation and the competitive capacity of a strain are not necessarily correlated (Romdhane, 2007).

Variations were observed in the N content of the aerial plant part and in the grain yield of the pea plants inoculated with different rhizobial strains. However, no differences were observed in the dry mass production of the aerial plant parts. These results could be attributed to the interaction between the soil and the symbiotic performance of the strains inoculated with the studied pea variety (He et al., 2011). Consequently, soil N levels have an effect on the nodulation and biological N fixation, which can be promoted at relatively low levels; however, soil N levels are suppressed at high nutrient concentrations (Eaglesham, 1989). Therefore, efficient strain selection should also consider the adaptation of rhizobia to the N availability conditions and the soil environment (Chen et al., 2004). Therefore, relatively high levels of organic matter (Table 2) in the experimental area might have been a source of N in the soil, and

hence influenced N accumulation in the aerial part of the pea plant.

Grain production in the pea plants that received N fertilization did not differ much from that obtained in the plants inoculated with any rhizobial strain. These results suggest that similar grain yield can be obtained without the need of nitrogen fertilization. Hence, inoculation of pea seeds with rhizobia can be regarded as a more economical form of pea production. Grain productivity in the pea plants inoculated with the EEL 7802 rhizobial strain was 53% higher than in the control without N application, although no differences were observed in the N content of the grains. Experiments with N application in different varieties of pea plants have shown contrasting results (Mckenzie et al., 2001), where the production of grains in different cultivars ranged from 390 kg ha<sup>-1</sup> in the 'Kodama' cultivar (Moreira et al., 2006) to 1.266 kg ha<sup>-1</sup> (De Souza Romero et al., 2008) in the 'Maria' cultivar. Furthermore, experiments with inoculation of pea seeds with *Rhizobium* did not consistently reveal increase in the grain yield (Mckenzie et al., 2001; Ahmed et al., 2007). However, previous study had shown that inoculation with *Rhizobium* increased pea productivity (Rani et al., 2016; Huang et al., 2017).

The relative efficiency of the symbiotic N fixation of the rhizobial strains was high with respect to inoculation of the EEL7802 strain, which demonstrated highest relative efficiency of N fixation during symbiosis with the Spencer pea variety (Table 3).

## Conclusions

The inoculation of pea seeds with rhizobial strains produced grain amounts similar to the amount produced upon the addition of 200 kg N. ha<sup>-1</sup>, and hence, was considered a more economical practice. The EEL7802

strain showed the highest symbiotic efficiency with the pea plant under field conditions in the tested environment, and field-efficiency studies in other ecosystems are necessary to confirm the efficiency of the EEL7802 strain.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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