## Scientific Notes

# Viability of liquid medium-inoculation of *Rhizobium etli* in planting furrows with common bean

Dâmiany Pádua Oliveira(1), Bruno Lima Soares(2), Fábio Aurélio Dias Martins(2), Leandro Alex Franceschini(2), Bruno Ewerton da Silveira Cardillo(2), Márcia Rufini(2), Augusto Ramalho de Morais(3), Fatima Maria de Souza Moreira(1) and Messias José Bastos de Andrade(2)

(1)Universidade Federal de Lavras, Departamento de Ciência do Solo, Caixa Postal 3.037, CEP 37200-000 Lavras, MG, Brazil. E-mail: damiany.padua.oliveira@gmail.com, fmoreira@dcs.ufla.br (2)Universidade Federal de Lavras, Departamento de Agricultura, Caixa Postal 3.037, CEP 37200-000 Lavras, MG, Brazil. E-mail: brunolsoares@gmail.com, fabioaureliod@gmail.com, leandrofranceschini@hotmail.com, brunocardillo9@gmail.com, marciarufini@yahoo.com.br, mandrade@dag.ufla.br (3)Universidade Federal de Lavras, Departamento de Estatística, Caixa Postal 3.037, CEP 37200-000 Lavras, MG, Brazil. E-mail: armorais@dex.ufla.br

Abstract – The objective of this work was to evaluate the viability of liquid medium inoculation of *Rhizobium etli* in the planting furrow and to certify the efficiency of its strain UFLA 02-100 as a potential inoculant for common bean (*Phaseolus vulgaris*). The treatments consisted of three application rates of liquid inoculant applied in the planting furrows and one rate applied in common bean seed, besides two controls without inoculation. The inoculant contained $10^9$ cells of *Rhizobium etli* mL$^{-1}$. Regardless of the application method, the yield obtained with the inoculation was equivalent to that of N from urea; however, the application in the furrows, at 0.6 L ha$^{-1}$, is more advantageous due to the operational practicality and reduced costs.

Index terms: *Phaseolus vulgaris*, biological $N_2$ fixation, biotechnology.

# Viabilidade da inoculação líquida com *Rhizobium etli* no sulco de semeadura do feijoeiro-comum

Resumo – O objetivo deste trabalho foi avaliar a viabilidade da inoculação líquida de *Rhizobium etli* no sulco de semeadura e certificar a eficiência da estirpe UFLA 02-100 como inoculante potencial para o feijoeiro-comum (*Phaseolus vulgaris*). Os tratamentos consistiram de três doses do inoculante líquido aplicadas aos sulcos e uma dose aplicada às sementes de feijão-comum, além de dois controles sem inoculação. O inoculante continha $10^9$ células de *Rhizobium etli* mL$^{-1}$. Independentemente do método de aplicação, a produtividade obtida com a inoculação foi equivalente à da aplicação de N da ureia, mas a aplicação ao sulco, com 0,6 L ha$^{-1}$, é mais vantajosa pela praticidade operacional e redução de custos.

Termos para indexação: *Phaseolus vulgaris*, fixação biológica de $N_2$, biotecnologia.

In recent years, numerous studies indicate that common bean (*Phaseolus vulgaris* L.) can truly benefit from biological nitrogen fixation (BNF) under field conditions, as long as good inoculation practices are performed. Nonetheless, questions regarding the conventional use of peat inoculant in seed still persist, and the effects of mineral N fertilization on BNF are still being assessed. Current results with peat inoculant on seed have indicated that the application of 20 kg ha$^{-1}$ N, at planting, favors the symbiosis, which increases yield and reduces costs (Soares et al., 2016). In this sense, other studies have shown that common bean inoculation, complemented by leaf fertilization with Mo, increases the cost-effectiveness of the crop (Valadão et al., 2009; Figueiredo et al., 2016).

In tropical soils, where acidity conditions are more accentuated, the use of adapted strains is necessary to maximize the contribution of BNF to crops. Researches on peat inoculation have shown a high performance of the *R. tropici* strains CIAT 899 and H 12, and also of the *R. freirei* strain PRF 81. These strains are already recommended for use; nonetheless other strains, such as UFLA 02-127 (*R. leguminosarum* bv. *phaseoli*), UFLA 02-68 (*R. etli* bv. *mimosa*), UFLA 04-173 (*R. miluonense*), and UFLA 02-100 (*R. etli*), have shown a high competitive ability, and presented an

Pesq. agropec. bras., Brasília, v.53, n.3, p.394-398, Mar. 2018
DOI: 10.1590/S0100-204X2018000300015

equivalent (Ferreira et al., 2009) and even greater effectiveness than that of the native rhizobia population (Soares et al., 2006; Nogueira et al., 2017). Actually, field experiments with this last strain (UFLA 02-100) show similar results to that of CIAT 899 (frequently used in commercial inoculants), and to those of control treatments fertilized with mineral N (Soares et al., 2006; Nogueira et al., 2017). All these studies show the possibility of economic savings in relation to N fertilizers.

Despite the efficiency of peat inoculation in seed, there are many producers that question the practicality of this operation in large scale, especially with the current trend of increasing the amounts and types of products applied to seed, such as fungicides, micronutrients, and microorganisms. For this reason, soybean producers and large producers of common bean have shown interest in the use of liquid inoculant in the planting furrow.

The possible incompatibility between products used in seed treatment (mainly of fungicides) and rhizobium survival from the inoculant is a matter of great concern for these producers. The pertinent literature, however, shows some disagreement on this issue (Araújo & Araújo, 2006; Oliveira et al., 2016). In cases of incompatibility between products, the producer should treat seed by himself, since it would be risky to acquire it already treated. Seed inoculation with peat or liquid inoculant subjected to drying should be undertaken in the shade, with an interval for proper adherence, since rhizobium is greatly exposed to the incompatible product, strengthening the possibility of deleterious effect of the practice (Araújo & Araújo, 2006; Araújo et al., 2007). In fact, it has been shown that under liquid inoculant and Mo applications to seed, the number of *Bradyrhizobium* cells can be significantly reduced (Albino & Campo, 2001), with negative effects on BNF. Besides, planting with moist or wrinkled seed can cause difficulties to the sowing operation, which requires an adequate time for drying the inoculated seed.

One of the limitations of liquid application to the planting furrows is related to the small number of research studies involving this practice, and to the difficulties in determining the best application rates for good performance of BNF (Vieira Neto et al., 2008a, 2008b; Zilli et al., 2010). Publications involving this alternative method of inoculation for common bean could not be found; therefore, the present work can help the producer in deciding on which method to adopt for this crop.

The objective of this work was to evaluate the viability of liquid medium inoculation in planting furrows, and to certify the efficiency of the UFLA 02-100 strain of *Rhizobium etli* as a potential inoculant for common bean.

The field experiment was carried out in the dry season of 2014, in the municipality of Lavras, in the state of Minas Gerais, Brazil, in a Latossolo Vermelho eutroférrico (Santos et al., 2013), i.e., Eutric Acrudox, according to the soil taxonomy. Results of the chemical and physical analyses of the soil, before planting, are shown in Table 1. A randomized complete block design with six treatments was used, with four replicates. The treatments involved three application rates of liquid inoculant in planting furrows, and one rate applied in seed, and two controls without inoculation. Crop management was performed under no-till, over corn residues, with periodic sprinkler irrigation. The population of native rhizobium capable of nodulating the crop was of approximately $10^3$ colony forming units (CFU) per gram of soil, estimated according to Rufini et al. (2011).

Each experimental unit consisted of six rows of 4.0 m length each, spaced at 0.6 m, with an useful area corresponding to the four center rows. All plots received 20 kg ha$^{-1}$ N (urea), 110 kg ha$^{-1}$ $P_2O_5$, and 40 kg ha$^{-1}$ $K_2O$. In addition, N-fertilized control plots received another 20 kg ha$^{-1}$ N, at planting, and 40 kg ha$^{-1}$ as topdressing, between the V3 and V4 stages. Planting was performed with a “handjab planter” at density of 15 seed per linear meter, using the common bean BRSMG Madrepérola cultivar.

The strain UFLA 02-100 was isolated from acid soils from the Amazon region, and selected for its high efficiency in common bean BNF, with a performance comparable to that of strains frequently used in commercial inoculants for this crop (Soares et al., 2006; Ferreira et al., 2009; Nogueira et al., 2017). The inoculant was prepared in liquid medium “79”, and its quality was monitored by counting the number of CFUs, that should be according to the legal minimum number of viable *Rhizobium* cells, which is approximately $10^9$ mL$^{-1}$ inoculant (Brasil, 2011). The inoculant rate applied in seed was equivalent to 7.5 mL kg$^{-1}$, and inoculation occurred a short time before planting. The application rates in the furrows were determined to obtain populations equivalent to ½, 1, and 2 times the reference application rate of 1.2 L ha$^{-1}$ commonly recommended for commercial inoculants. The inoculant distribution in the furrows was performed with a manual backpack sprayer, with a flat fan spray nozzle XR 110.02, and spray volume equivalent to 20 L ha$^{-1}$.

At full blossom (R6 stage), a sample of 10 plants was removed at random from rows 2 and 3 of each plot, for the determination of the number of nodules (NN), dry matter of nodules (DMN), shoot dry matter (SDM), shoot-N concentration (SNC), and shoot-N accumulation (SNA). At maturation (R9 stage), final stand (FS), grain yield and its primary components (number of pods per plant, grains per pod, and mean 100 grain weight, W100), and grain-N concentration (GNC) and accumulation (GNA) were determined in 10 plant samples from the rows 4 and 5. The final stand was obtained by counting the number of plants in the useful area of the plot. Grain yield was obtained from total weight of grains produced in the useful area of the plot (lines 4 and 5), with moisture corrected to 130 g kg$^{-1}$. The SNC, GNC, SNA, and GNA were calculated according to Soares et al. (2006).

Data were subjected to analysis of variance, using the software Sisvar 4.0, after meeting the presupositions of normality (Shapiro-Wilks test) and homoscedasticity (Bartlett test). NN and DMN were transformed into $(x+1)^{0.5}$ prior to the analyses. The means were grouped by the Scott-Knott test, at 5% probability.

NN and DMN did not differ between treatments with or without inoculation (Table 2), indicating the presence of native bacteria capable of nodulating with an effectiveness similarly to that of the introduced strain UFLA 02-100. This has been a frequent result in the literature. Using this same strain inoculated in common bean, Soares et al. (2006) obtained NN equivalent to that of native rhizobia. Ferreira et al. (2009) and Figueiredo et al. (2016) found a similar performance not only in relation to nodulation, but also to yield and grain-N accumulation. However, BNF of the native population, in the present study, appears to have been less effective, leading to a lower grain yield, which is certainly a result of reduced GNA and W100.

**Table 1.** Results of the chemical and physical analyses of soil samples from the experimental area (0–0.20 m soil depths), collected before planting.

| pH in $H_2O$ | P-Melich 1 | K | Ca | Mg | Al | H + Al | SB | T | m | BS | OM | Clay | Silt | Sand |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (mg dm$^{-3}$) | (cmol$_c$ dm$^{-3}$) | | | | | | | (%) | | | (g kg$^{-1}$) | | |
| 5.9 | 37.15 | 0.41 | 4.2 | 1.3 | 0.0 | 3.24 | 5.91 | 9.15 | 0.0 | 65.0 | 3.6 | 560 | 40 | 400 |

SB, sum of exchangeable bases; T, cation exchange capacity at pH 7; m, exchangeable aluminum saturation; BS, base saturation; and OM, organic matter.

**Table 2.** Liquid inoculant (LI) treatment effects on the number of nodules (NN), dry matter of nodules (DMN), shoot dry matter (SDM), shoot-nitrogen concentration (SNC), shoot-nitrogen accumulation (SNA), final stand (FS), grain yield (GY) and its primary components[(1)] – grain-nitrogen concentration (GNC) and grain-nitrogen accumulation (GNA) – of common bean (*Phaseolus vulgaris*) BRSMG Madrepérola cultivar[(2)].

| Treatment | NN | DMN | SNC | SDM | SNA | FS | PP | GP | W100 | GNC | GNA | GY |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (unit per plant) | (mg per plant) | (%) | -(g per plant)- | | (plant per ha × 1,000) | --(unit)-- | | -(g)- | (%) | ---(kg ha$^{-1}$)--- | |
| LI at 0.6 L ha$^{-1}$ in furrow | 27a | 52a | 2.6a | 9.2a | 90.5a | 226a | 16a | 3.8a | 28a | 4.0a | 68.8a | 1,826a |
| LI at 1.2 L ha$^{-1}$ in furrow | 28a | 50a | 2.6a | 9.4a | 83.3a | 208a | 19a | 3.6a | 28a | 3.6a | 75.4a | 1,861a |
| LI at 2.4 L ha$^{-1}$ in furrow | 28a | 45a | 2.9a | 11.8a | 82.8a | 207a | 17a | 3.8a | 29a | 4.0a | 67.5a | 1,733a |
| LI in seed at 7.5 mL kg$^{-1}$ | 19a | 35a | 2.9a | 9.2a | 85.5a | 214a | 17a | 3.9a | 28a | 3.6a | 71.5a | 1,751a |
| Control without N | 22a | 40a | 3.0a | 9.7a | 81.5a | 204a | 16a | 3.7a | 24b | 3.5a | 28.9b | 801b |
| Control with N fertilization | 6b | 5b | 2.8a | 10.3a | 74.8a | 187a | 17a | 3.7a | 29a | 3.9a | 54.5a | 1,662a |
| Mean | 22 | 38 | 2.8 | 9.9 | 83.0 | 208 | 17 | 3.8 | 28 | 3.8 | 61.1 | 1,606 |
| Coefficient of variation (%) | 21.4 | 25.1 | 11.8 | 25.2 | 26.5 | 10.4 | 18.9 | 7.9 | 18.5 | 13.3 | 28.6 | 25.3 |

[(1)] Number of pods per plant (PP), grains per pod (GP), and mean 100 grain weight (W100). [(2)] Means followed by equal letters belong to the same group, according to the Scott-Knott's test, at 5% probability.

A lower nodulation in the treatment that received 80 kg $ha^{-1}$ N confirms the considerations of Moreira & Siqueira (2006), who attributed the reduction of nodule formation to excess mineral N in the soil due to the lack of stimuli related to nutrient deficiency in the plant. This negative effect, however, was not observed on FS and SDM. It is possible that the high-water availability during the crop establishment and growth, provided by the sprinkler irrigation, was decisive for the absence of saline effect from the nitrogen fertilization.

Neither nodulation nor BNF were affected by the inoculation method, which can be inferred by the SNC and SNA variables (Table 2). In fact, SNC, which is one of the traits closely related to BNF efficiency, was greater than 2.8%, within the sufficiency range indicated by Oliveira et al. (2016). Moreover, the effectiveness of the UFLA 02-100 strain on GNA, W100, and grain yield, whose values were similar to those of mineral fertilization with 80 kg $ha^{-1}$ N, is noteworthy. The higher values of these variables compared to those of the control without additional N indicate a greater use of N for nutrition and grain filling, which resulted in yields of 1,600 kg $ha^{-1}$, which represents twice the mean value of the absolute control.

These results clearly show the important contribution of the UFLA 02-100 strain to common bean and support the considerations of high efficiency of bacteria in relation to the nitrogen fertilizer control reported by other authors (Soares et al., 2006; Nogueira et al., 2017). They also justify the continuity of studies with this strain, which has a considerable potential for future approval as an inoculant strain.

Research studies with liquid medium inoculation in the planting furrows for common bean were not found, which made it impossible to compare our results. This fact, however, reinforces the relevance of the present study. In soybean, liquid medium inoculation in the furrow has been reported not only as practical, but also as compatible with fungicide treatment in seed (Zilli et al., 2010). Potential advantages of application of liquid inoculant in the planting furrows had also been reported for soybean grown in native field (Voss, 2002).

Based on the effect of inoculation in the furrows on the studied variables, mainly on grain yield (Table 2), our results for common bean are even more encouraging due to the fact that low-application rates of the inoculant were already sufficient to bring about yields much higher than the mean productivity of the crop (932 kg $ha^{-1}$) in the state of Minas Gerais, Brazil. Application rates greater than 0.6 L $ha^{-1}$ in the furrows provided a similar yield to that obtained with the inoculation in the seed, but with higher costs. Using a commercial inoculant for soybean, at 150, 300, and 450 mL $ha^{-1}$, Vieira Neto et al. (2008a, 2008b) obtained similar yields to that with inoculation in the seeds. The authors recognized the viability of furrow applications, even at the lowest rate.

Further studies should evaluate commercial inoculant strains already available in the market, and application rates appropriate for the best productive performance. Applications of these strains should also be compared with that of UFLA 02-100 in the planting furrows. This information will surely assist the producer in choosing the most profitable method and rhizobium strain for growing common bean.

## Acknowledgments

To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), and to Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Fapemig), for financial support and scholarships; to Universidade Federal de Lavras, for providing the experimental field.

## References

ALBINO, U.B.; CAMPO, R.J. Efeito de fontes e doses de molibdênio na sobrevivência do *Bradyrhizobium* e na fixação biológica de nitrogênio em soja. **Pesquisa Agropecuária Brasileira**, v.36, p.527-534, 2001. DOI: 10.1590/S0100-204X2001000300018.

ARAÚJO, A.S.F. de; ARAÚJO, R.S. Sobrevivência e nodulação do *Rhizobium tropici* em sementes de feijão tratadas com fungicidas. **Ciência Rural**, v.36, p.973-976, 2006. DOI: 10.1590/S0103-84782006000300039.

ARAÚJO, F.F. de; CARMONA, F.G.; TIRITAN, C.S.; CRESTE, J.E. Fixação biológica de $N_2$ no feijoeiro submetido a dosagens de inoculante e tratamento químico na semente comparado à adubação nitrogenada. **Acta Scientiarum**. **Agronomy**, v.29, p.535-540, 2007. DOI: 10.4025/actasciagron.v29i4.416.

BRASIL. Instrução Normativa SDA nº 13, de 24 de março de 2011. Aprova as normas sobre especificações, garantias, registro, embalagem e rotulagem dos inoculantes destinados à agricultura, bem como as relações dos micro-organismos autorizados e recomendados para produção de inoculantes no Brasil, na forma dos Anexos I, II e III, desta Instrução Normativa. **Diário Oficial da União**, 25 mar. 2011. Seção 1, p. 3.

FERREIRA, P.A.A.; SILVA, M.A.P.; CASSETARI, A.S.; RUFFINI, M.; MOREIRA, F.M. de S.; ANDRADE, M.J.B. Inoculação com cepas de rizóbio na cultura do feijoeiro. **Ciência Rural**, v.39, p.2210-2212, 2009. DOI: 10.1590/S0103-84782009000700041.

FIGUEIREDO, M.A. de; OLIVEIRA, D.P.; SOARES, B.L.; MORAIS, A.R. de; MOREIRA, F.M. de S.; ANDRADE, M.J.B. de. Nitrogen and molybdenum fertilization and inoculation of common bean with *Rhizobium* spp. in two Oxisols. **Acta Scientiarum**. **Agronomy**, v.38, p.85-92, 2016. DOI: 10.4025/actasciagron.v38i1.26661.

MOREIRA, F.M.S.; SIQUEIRA, J.O. **Microbiologia e bioquímica do solo**. 2.ed. atual. ampl. Lavras: UFLA, 2006. 729p.

NOGUEIRA, C. de O.G.; OLIVEIRA, D.P.; FERREIRA, P.A.A.; PEREIRA, J.P. de A.R.; VALE, H.M.M. do; ANDRADE, M.J.B. de; MOREIRA, F.M. de S. Agronomic efficiency of *Rhizobium* strains from the Amazon region in common bean. **Acta Amazonica**, v.47, p.273-276, 2017. DOI: 10.1590/1809-4392201603422.

OLIVEIRA, D.P.; FIGUEIREDO, M.A. de; SOARES, B.L.; TEIXEIRA, O.H.S.; MARTINS, F.A.D.; RUFINI, M.; MORAIS, A.R. de; MOREIRA, F.M. de S.; ANDRADE, M.J.B. de. Seed treatment with fungicides does not affect symbiosis between common bean (*Phaseolus vulgaris* L.) and rhizobia. **Agronomy Journal**, v.108, p.1-8, 2016. DOI: 10.2134/agronj2016.02.0105.

RUFINI, M.; FERREIRA, P.A.A.; SOARES, B.L.; OLIVEIRA, D.P.; ANDRADE, M.J.B. de; MOREIRA, F.M. de S. Simbiose de bactérias fixadoras de nitrogênio com feijoeiro-comum em diferentes valores de pH. **Pesquisa Agropecuária Brasileira**, v.46, p.81-88, 2011. DOI: 10.1590/S0100-204X2011000100011.

SANTOS, H.G. dos; JACOMINE, P.K.T.; ANJOS, L.H.C. dos; OLIVEIRA, V.A. de; LUMBRERAS, J.F.; COELHO, M.R.; ALMEIDA, J.A. de; CUNHA, T.J.F.; OLIVEIRA, J.B. de. **Sistema Brasileiro de Classificação de Solos**. 3.ed. rev. e ampl. Brasília: Embrapa, 2013. 353p.

SOARES, A.L. de L.; FERREIRA, P.A.A.; PEREIRA, J.P.A.R.; VALE, H.M.M. do; LIMA, A.S.; ANDRADE, M.J.B. de; MOREIRA, F.M. de S. Eficiência agronômica de rizóbios selecionados e diversidade de populações nativas nodulíferas em Perdões (MG). II – Feijoeiro. **Revista Brasileira de Ciência do Solo**, v.30, p.803-811, 2006. DOI: 10.1590/S0100-06832006000500006.

SOARES, B.L.; FERREIRA, P.A.A.; RUFINI, M.; MARTINS, F.A.D.; OLIVEIRA, D.P.; REIS, R.P.; ANDRADE, M.J.B. de; MOREIRA, F.M. de S. Agronomic and economic efficiency of common-bean inoculation with rhizobia and mineral nitrogen fertilization. **Revista Brasileira de Ciência do Solo**, v.40, p.1-13, 2016. DOI: 10.1590/18069657rbcs20150235.

VALADÃO, F.C. de A.; JAKELAITIS, A.; CONUS, L.A.; BORCHARTT, L.; OLIVEIRA, A.A. de; VALADÃO JUNIOR, D.D. Inoculação das sementes e adubações nitrogenada e molíbdica do feijoeiro, em Rolim de Moura. **Acta Amazonica**, v.39, p.741-747, 2009. DOI: 10.1590/S0044-59672009000400002.

VIEIRA NETO, S.A.; PIRES, F.R.; MENEZES, C.C.E. de; MENEZES, J.F.S.; SILVA, A.G. da; SILVA, G.P.; ASSIS, R.L. de. Formas de aplicação de inoculante e seus efeitos sobre a nodulação da soja. **Revista Brasileira de Ciência do Solo**, v.32, p.861-870, 2008a. DOI: 10.1590/S0100-06832008000200040.

VIEIRA NETO, S.A.; PIRES, F.R.; MENEZES, C.C.E.; SILVA, A.G.; ASSIS, R.L. de; SILVA, G.P.; MENEZES, J.F.S. Formas de aplicação de inoculante e seus efeitos na cultura da soja. **Bioscience Journal**, v.24, p.56-68, 2008b.

VOSS, M. **Inoculação de rizóbio no sulco de semeadura para soja, em um campo nativo, no norte do Rio Grande do Sul**. Passo Fundo: Embrapa Trigo, 2002. 5p. (Embrapa Trigo. Comunicado técnico online, 108). Available at: <http://www.cnpt.embrapa.br/biblio/p_co108.htm>. Accessed on: June 22 2017.

ZILLI, J.É.; GIANLUPPI, V.; CAMPO, R.J.; ROUWS, J.R.C.; HUNGRIA, M. Inoculação da soja com *Bradyrhizobium* no sulco de semeadura alternativamente à inoculação de sementes. **Revista Brasileira de Ciência do Solo**, v.34, p.1875-1881, 2010. DOI: 10.1590/S0100-06832010000600011.

Received on March 20, 2017 and accepted on July 4, 2017