SCAP
SOCIEDADE DE CIÊNCIAS AGRÁRIAS DE PORTUGAL

Co-inoculation of *Bradyrhizobium japonicum* and *Azospirillum brasilense* in the soybean crop

Coinoculação de *Bradyrhizobium japonicum* e *Azospirillum brasilense* na cultura da soja

Alan M. Zuffo*, Pedro M. Rezende, Adriano T. Bruzi, Natália T. Oliveira, Igor O. Soares, Geraldo F.G. Neto, Bruno E. S. Cardillo and Laís O. Silva

** Departamento de Agricultura, Universidade Federal de Lavras, Campus Universitário, CEP: 37200-000, Lavras, MG, Brazil. E-mail: alan_zuffo@hotmail.com, author for correspondence*

Received/Recebido: 2014.08.21
Accepetd/Aceite: 2014.12.19

ABSTRACT

In soybean, the combined use of *Bradyrhizobium japonicum* and *Azospirillum brasilense* may be a promising strategy, joining biological nitrogen fixation (BNF) and phytohormone production. In this context, the aim of this study was to evaluate the effect of co-inoculation at different application rates of *Bradyrhizobium japonicum* and *Azospirillum brasilense* on the morphophysiological development and nodulation of soybean. The experiment was carried out in a greenhouse at the Federal University of Lavras (UFLA), in the Field Crop sector of the Department of Agriculture, Lavras, MG state, Brazil. A completely randomized experimental design was used with four replications in a 5 x 2 factorial arrangement consisting of five application rates of *Azospirillum brasilense* (0, 0.5, 1.0, 1.5 and 2.0 mL kg^{-1} of seed) and two application rates of *Bradyrhizobium japonicum* (0 and 3.0 mL kg^{-1} of seed), both transmitted via seeds. The soybean cultivar used was 'BRS Favorita RR', grown in 5 dm^3 pots. At the beginning of flowering (R_1), the following features were determined: plant height, number of trifoliate leaves, shoot dry matter, root dry matter, nodules dry matter, root volume, leaf chlorophyll content, and leaf nitrogen content. Application of *Bradyrhizobium japonicum* at the rate of 3 mL kg^{-1} of seed led to the best morphophysiological performance and the greatest nodulation in the potted soybean crop. The use of *Azospirillum brasilense* alone and in co-inoculation with *Bradyrhizobium japonicum* did not have a significant effect on the parameters evaluated.

Keywords: *Glycine max*, phytohormones, inoculation, *Rhizobium*, plant growth bacteria.

RESUMO

Na cultura da soja, a inoculação conjunta de *Bradyrhizobium japonicum e Azospirillum brasilense* pode ser uma estratégia promissora, combinando a FBN e a produção de fito-hormônios. Nesse âmbito o trabalho teve como objetivo avaliar o efeito da coinoculação com diferentes doses de *Bradyrhizobium japonicum* e de *Azospirillum brasilense*, no desenvolvimento morfofisiológico e nodulação da soja. O ensaio foi realizado em estufa na Universidade Federal de Lavras (UFLA), no setor de Grandes Culturas do Departamento de Agricultura Lavras - MG, Brasil. O delineamento experimental utilizado foi inteiramente casualizado, com quatro repetições, em esquema fatorial 5 x 2, sendo utilizadas cinco doses de *Azospirillum brasilense* (0, 0,5, 1,0, 1,5 e 2,0 mL kg^{-1} de semente) e duas doses de *Bradyrhizobium japonicum* (0 e 3,0 mL kg^{-1} de semente), ambas veiculadas via sementes. A cultivar de soja utilizada foi a 'BRS Favorita RR', cultivada em vasos de 5 dm^3. No início da floração (R_1) determinaram-se os seguintes parâmetros: altura de plantas, número de trifólio, peso seco da parte aérea, da parte radicular e dos nódulos, volume radicular, teor de clorofila foliar e teor de nitrogênio foliar. O uso da dose de 3 mL de *Bradyrhizobium japonicum* kg^{-1} de semente, proporciona melhor desempenho morfofisiológico e maior nodulação na cultura da soja cultivada em vaso. A utilização de *Azospirillum brasilense* isoladamente e em coinoculação com *Bradyrhizobium japonicum* não promove efeitos significativos nos parâmetros avaliados.

Palavras-chave: *Glycine max*, fitohormonas, inoculação, rizóbio, bactérias promotoras de crescimento.

Introduction

Soybean [*Glycine max* (L.) Merrill] is of great importance for the Brazilian economy occupying, Brazil, the second place worldwide in soybean production. This fact is related to a competitive edge associated with scientific advances and the availability of technologies to the productive sector (Hungria *et al.*, 2005). In this context, the introduction of bacteria of the genus *Bradyrhizobium*, which bring about biological nitrogen fixation (BNF), was one of the great driving forces for growing soybean on a large scale in Brazil.

The use of inoculants containing strains of *Bradyrhizobium* spp. has led to approximate annual savings of US$ 3.2 billion in nitrogen fertilizers (Fagan *et al.*, 2007). According to Hungria *et al.* (2006), the amounts of nitrogen fixed by soybean through BNF have been reported up to 300 kg N ha^{-1}, supplying up to 94% of crop needs.

In this context, other alternative technologies have been researched with a view toward better productive results for the soybean crop, e.g., co-inoculation. According to Ferlini (2006) and Bárbaro *et al.* (2008), this consists in the use of different microorganism combinations which produce a synergistic effect, i.e., when used, they go beyond the productive results they obtain in an isolated manner.

Combined use of *Bradyrhizobium japonicum* and *Azospirillum brasilense* has shown good result in soybean (Benintende *et al.*, 2010). Bacteria of the genus *Azospirillum* provide beneficial effects to plants due to their capacity to stimulate the production of plant hormones in expressive quantities, which results in plant growth. Studies have shown the capacity of *Azospirillum brasilense* in producing auxins, gibberellins, and cytokinins under *in vitro* conditions (Crozier *et al.*, 1988; Cacciari *et al.*, 1989; Arshad & Frankenberger, 1997; Masciarelli *et al.*, 2013).

Nevertheless, the results obtained from combined inoculation in leguminous plants may show contradictory responses, i.e., they may both stimulate and inhibit the formation of nodules and root growth in a symbiotic system, varying as a function of the inoculum concentration level and of the inoculation type (Barbaro *et al.*, 2008). Further tests are needed to demonstrate the viability of co-inoculation.

Thus, the use of plant growth promoting bacteria, such as *Azospirillum*, which seek to increase the efficiency of fertilizer use, and also the input of nitrogen through biological fixation, represents an economically viable strategy, besides the environmental benefits associated with the reduction in the use of fertilizers (Hungria, 2011). They thus contribute to satisfying the modern demands of agriculture through economic, social, and environmental sustainability (Chaparro *et al.*, 2012).

In light of the above, the aim of this study was to evaluate the effect of co-inoculation of different application rates of *Bradyrhizobium japonicum* and *Azospirillum brasilense* on the morphophysiological development and nodulation of soybean.

Material and Methods

The experiment was carried out in a greenhouse with controlled temperature of ±27ºC and relative air humidity of ±80% in the Field Crop sector of the Universidade Federal de Lavras (UFLA) in Lavras, MG state, Brazil (21º40′06″ latitude South, 45º00′00″ longitude West, at a mean altitude of 918 m) in the period from November 2013 to January 2014.

The substrate was composed of a Latossolo Vermelho distroférrico típico (Lvdf) (Oxisol) (Embrapa 1999), with a very clayey texture, and washed sand at the proportion of 2:1 v/v, respectively. The soil used in the experiment was collected from the 0-0.20 m layer in an area that does not have a history of agricultural use. The chemical composition of the soil from the experimental area used for collection is shown in Table 1.

Limestone recommendation was made according to the base saturation method, considering the level of 70% as ideal saturation. The application rate per hectare was transformed in volume of the pot. First, limestone was added to the substrate and allowed to rest for 30 days. Over this time, the soil was mixed and watered. Afterwards, the substrate was placed in pots and then fertilization was made. The nutrients phosphorus (P), potassium (K), and sulfur (S) were supplied at the rates of 200 mg dm^3, 150 mg dm^3, and 50 mg dm^3, respectively, as recommended by Malavolta (1980) and Novais *et al.* (1991). The K was supplied through potassium chloride, and the P and S through single super phosphate.

A completely randomized experimental design was used with four replications in a 5 x 2 factorial arrangement, consisting of five application rates of *Azospirillum brasilense* (0, 0.5, 1.0, 1.5, and 2.0 mL kg^{-1} of seed - strains AbV5 and AbV6), which is respectively, 0, $2x10^4$, $4x10^4$, $6x10^4$ and $8x10^4$ UFC/seed and, two application rates of *Bradyrhizobium japonicum* (0 and 3.0 mL kg^{-1} of seed), with 0 and $1.8x10^6$ UFC/seed. *Azospirillum brasilense* strains were derived from the inoculant Azo® ($1x10^8$ UFC/mL) and *Bradyrhizobium japonicum* inoculant from Nitragin

Table 1 – Chemical composition of the soil Latossolo Vermelho distroférrico típico (Oxisol) (0-0.20 m) before setting up the experiment. Lavras, MG, Brazil.

pH	Ca^{2+}	Mg^{2+}	Al^{3+}	$H^{+}+Al^{3+}$	SB	CEC	P	K	OM	V
$CaCl_2$	------------------ $cmol_c\ dm^{-3}$ ------------------						--mg dm^{-3}--		g kg^{-1}	%
5.4	0.32	0.10	0.10	2.90	0.47	3.37	0.84	20.00	2.36	13.98

H + Al: potential acidity; SB: sum of bases; CEC: cation exchange capacity at pH 7.0; OM: organic matter; V: base saturation.

Cell Tech HC® ($3x10^9$ UFC/mL). The treatments were applied through the seed upon sowing.

The soybean cultivar used was 'BRS Favorita RR', grown in 5 dm^{-3} pots. Seeds were sown on November 28, 2013, distributing six seeds per pot at a depth of 1-2 cm. The plants were later thinned, leaving one plant per pot.

Throughout the crop cycle, weed control was performed manually, keeping the crop free from weed competition. In addition, the following management practices were used: (i) application of the insecticide teflubenzurom at the rate of 50 mL of commercial product ha^{-1}, applied at 35 days after emergence (DAE); ii) application of fungicide, azoxystrobin + cyproconazole at the rate of 300 mL of commercial product ha^{-1} + an additional 0.5% of the adjuvant Nimbus, applied at 50 DAE.

At the beginning of flowering (R_1), the following parameters were determined: plant height – determined from the soil surface to the tip of the apical meristem with the aid of a ruler in millimeters; number of trifoliate leaves; shoot dry matter, root dry matter, and nodules dry matter, with the aid of an air circulation laboratory oven at 60 ºC for 72 hours until obtaining constant weight, with subsequent weighing of the plant residue in a separate manner for dry matter determination (g); root volume (cm^3) – performed by measuring displacement of the water column in a graduated cylinder, i.e., placing the roots, after washing and drying, in a graduated cylinder containing a known volume of water (100 mL). From the difference, a direct reading of root volume was obtained by equivalence of units (1 mL = 1 cm^3), according to the method described by Basso (1999); leaf chlorophyll content – using a SPAD model portable chlorophyll meter, measuring 3 points on each trifoliate leaf, at different parts of the same leaf, always on the leaf blade between the veins on the third trifoliate leaf from above to below; and leaf nitrogen contents were also determined.

After the collection and tabulation of data, analysis of variance was carried out ($p<0.01$) using the statistical program SISVAR (Ferreira, 2011). For the significant factors, the study of the mean values was carried out through the F test at 1% significance.

Results and Discussion

In Table 2, it may be observed that the parameters of plant height (PH), number of trifoliate leaves (NT), shoot dry matter (SDM), root dry matter (RDM), nodules dry matter (NDM), root volume (RV), chlorophyll content (CC), and leaf nitrogen content (LNC) were significantly ($p<0.01$) affected by the application of *Bradyrhizobium japonicum*. These results corroborate those obtained by Zilli *et al.* (2008) for shoot dry matter and dry matter of nodules.

In relation to the application rates of *Azospirillum brasilense* and to the interaction between the factors, no significant effect was observed, proceeding to study their isolated effects of the factors (Table 2). These results are similar to those obtained by Bárbaro *et al.* (2009), who verified an absence of significant effects of *Azospirillum brasilense* on shoot, root and nodules dry matter of the soybean crop. Gitti *et al.* (2012), when evaluating the effect of seed inoculation with *Azospirillum brasilense* on common bean (*Phaseolus vulgaris* L.), another crop of the *Fabaceae* (also called *Leguminosae*) plant family, concluded that *Azospirillum brasilense* does not significantly affect the development of common bean plants, corroborating the data obtained in this study.

The lack of a significant effect from the use of *Azospirillum brasilense* on the parameters evaluated may be attributed to the efficiency of the bacteria *Bradyrhizobium japonicum* in BNF and in competition with the other bacteria, including the genus *Azospirillum*. Thus, it is found that *Azospirillum brasilense* has no significant effect for soybean under pot growing conditions. However, these results contradict those obtained by Benintende *et al.* (2010). When comparing the effect of this co-inoculation, they observed stimulation of growth, nodulation, and nitrogen accumulation. However, these authors conducted the experiment under field conditions, with soil and climate conditions totally different than those of the present study.

Didonet *et al.* (2000) report that for inoculation with bacteria of the genus *Azospirillum* to be effective, these bacteria must have the ability to compete with the native diazotrophic bacteria and with the soil microflora. In addition to the quality of the inoculant, the inoculation process is of fundamental importance to achieve a high number of viable bacteria. It is thus possible that there was competition between the bacteria of the genus *Bradyrhizobium* or even of native species, impeding the beneficial effect of *Azospirillum brasilense* on the development and nodulation of soybean under the conditions of this study.

In general, it was verified that all the parameters subjected to application of *Bradyrhizobium japonicum* at the rate of 3 mL were higher than the treatment with the absence of bacteria (Figure 1 A, B, C, D, E, F, G, H). Similar reduction in the shoot dry matter and nodules dry matter, as a function of the absence or presence of inoculation with the bacteria *Bradyrhizobium japonicum* was also verified by Zilli *et al.* (2008). This fact was expected because the bacteria of the genus *Bradyrhizobium japonicum* have already been recommended for the inoculation of soybean crop and they are capable of obtaining all the nitrogen (N) the plant needs for its cycle to occur, by means of BNF. For Catroux *et al.* (2001), inoculation of leguminous plants is an agricultural practice recom-

Table 2 – Mean values of plant height (PH), number of trifoliate leaves (NT), shoot dry matter (SDM), root dry matter (RDM), nodules dry matter (NDM), root volume (RV), chlorophyll content (CC), and leaf nitrogen content (LNC) obtained in the trial application rates of *Bradyrhizobium japonicum* and rates of *Azospirillum brasilense* in soybean. Lavras, MG, Brazil, 2013/2014 crop season.

Sources of variation	PH	NT	SDM	RDM	NDM	RV	CC	LNC
	cm	unit		g		cm^3	-	g kg
Bradyrhizobium japonicum (B)								
P (Value) (B)	<0.01**	<0.01**	<0.01**	<0.01**	<0.01**	<0.01**	<0.01**	<0.01**
Azospirillum brasilense (A)								
($mL\ kg^{-1}$)								
0	55.37	16.12	7.85	3.79	0.97	28.75	35.30	23.97
0.5	54.75	16.62	7.83	3.86	0.90	30.25	35.70	23.46
1.0	53.62	16.25	7.99	3.50	0.83	26.62	35.56	23.27
1.5	53.37	15.75	6.82	3.06	0.78	25.12	35.42	22.55
2.0	53.75	15.25	6.71	3.40	0.75	24.87	34.20	22.41
Average	54.17	16.00	7.44	3.52	0.85	27.12	35.24	23.13
P (Value) (A)	0.8461^{ns}	0.8215^{ns}	0.1766^{ns}	0.3074^{ns}	0.1221^{ns}	0.2220^{ns}	0.9457^{ns}	0.8827^{ns}
B x A	0.9904^{ns}	0.6959^{ns}	0.6375^{ns}	0.9269^{ns}	0.1519^{ns}	0.1122^{ns}	0.5712^{ns}	0.9981^{ns}
CV (%)	7.58	15.01	18.03	23.06	21.34	19.65	11.27	14.82

** significant at 1% by the F test. ns – not significant.

mended when there are no specific bacteria in the soil capable of nodulating the leguminous plant that is grown, and when the N levels in the soil are low.

When analyzing the dry matter of nodules (Figure 1E), higher mean values are observed with the use of the bacteria in seed inoculation. With the increase in nodulation, greater nitrogen content was determined in the leaf tissue (Figure 1H) and, consequently, an increase in chlorophyll content (Figure 1G).

It is known that N is the component responsible for various reactions in plants, in addition to being part of the structure of chlorophyll, enzymes, and proteins. Chlorophylls are active in conversion of light radiation into chemical energy in the form of ATP (adenosine triphosphate) and NADPH (reduced nicotinamide adenine dinucleotide phosphate) (Blankenship, 2009). The chlorophylls are thus related to the photosynthetic efficiency of plants, from their growth to their adaptability to different environments. Therefore, greater contents of nitrogen in the leaf tissues led to a greater quantity of chlorophyll, resulting in an increase in the photosynthetic rate in the plant, in which they bring about gains in the production of photoassimilates and, consequen-

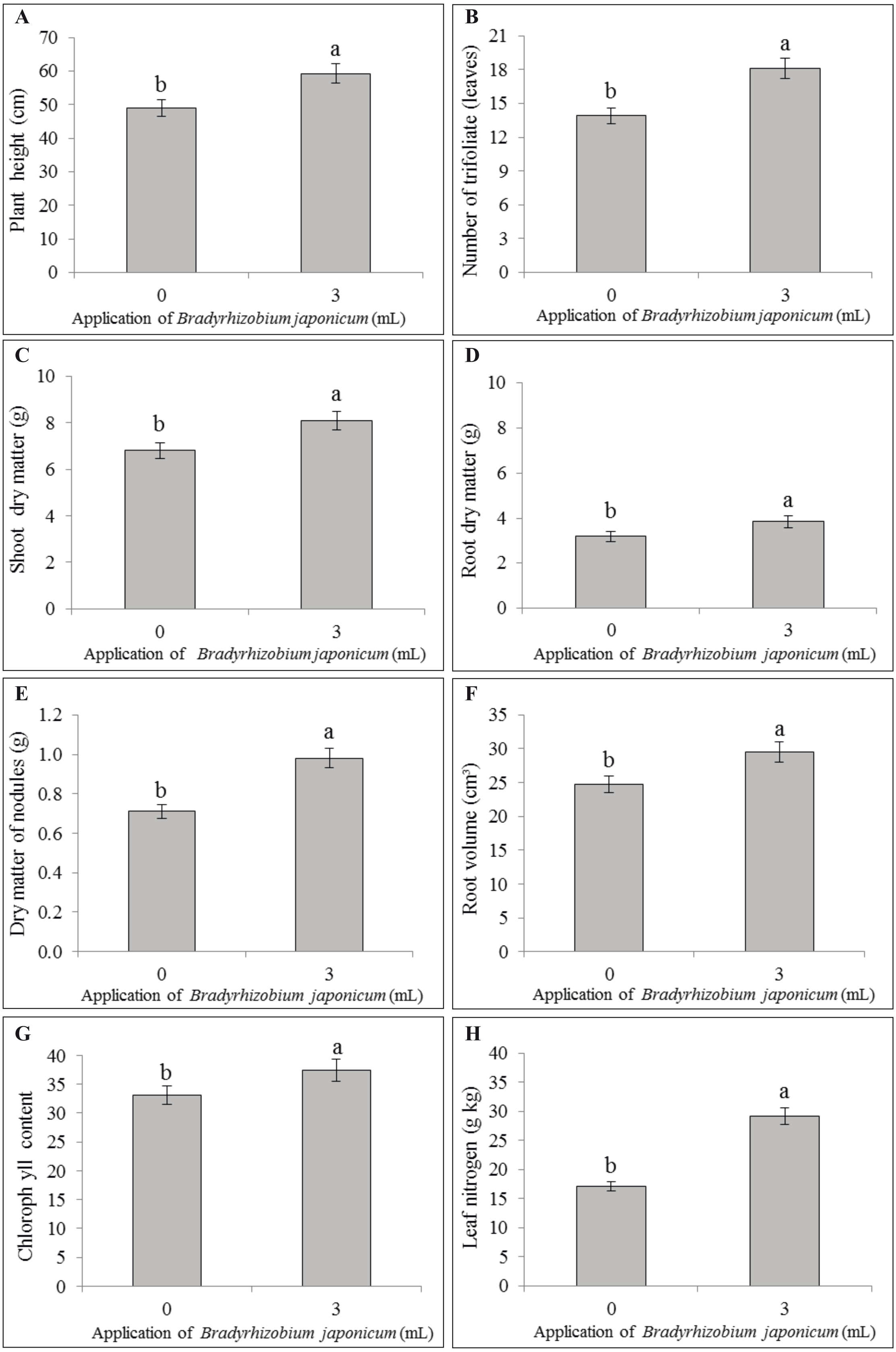

Figura 1– Plant height, shoot dry matter, root dry matter, dry matter of nodules, root volume, chlorophyll content, leaf nitrogen, subjected to application of *Bradyrhizobium japonicum* in soybean. Lavras, MG, Brazil, 2013/2014 crop season. Mean values followed by the same lowercase letter do not differ from each other in a significant manner by the F test at 1%. Each column represents the mean of four replicates (± standard deviation).

tly, better development of plant height (Figure 1A), number of trifoliate (1B), shoot dry matter (Figure 1C), root dry matter (Figure 1D), and root volume (Figure 1F).

In contrast, it was seen that plants not inoculated with *Bradyrhizobium japonicum* nor with nitrogen fertilization, were well developed. This fact is probably related to the native bacteria in the soil, as well as the organic matter present in the soil since upon removing the soil and turning it over so as to mix the limestone, there is an increase in the sites exposed to microbial attack and subsequent mineralization, as documented by Miranda & Macedo (2001).

Nevertheless, in all the parameters evaluated the treatment with absence of *Bradyrhizobium japonicum* inoculation obtained values lower than that with the presence of the bacteria. These results the low content of organic matter (OM) in the soil did not provide enough nitrogen for plant development in this treatment (Table 1). Furthermore, in spite of verifying nodules (dry matter of the nodules, Figure 1E), even with the absence of inoculation, these bacteria were probably in the soil used. This also suggests low efficiency of this nodulation since there were no significant results in the parameters evaluated.

Conclusions

Inoculation of soybean with *Bradyrhizobium japonicum* significantly affects all the variables studied, and a better morphophysiological performance and a greater nodulation of soybean was observed. The use of *Azospirillum brasilense* singly or in co-inoculation with *Bradyrhizobium japonicum* does not have a significant effect on the variables evaluated.

Acknowledgments

The authors express their thanks to the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), to the CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), to the FAPEMIG (Fundação de Amparo à Pesquisa do estado de Minas Gerais) for financial support and for granting Doctoral and Master's Degree scholarships and to the Federal University of Lavras for logistic support.

References

Arshad, M. and Frankenberger, J.R. (1997) - Plant Growth-Regulating Substances in the Rhizosphere: Microbial Production and Functions. *In*: Donald, L.S. (Ed.) - *Advances in Agronomy*. Academic Press, p. 45-151.

Bárbaro, I.M.; Brancalião, S.R.; Ticelli, M.; Miguel, F.B. and Silva, J.A.A.D. (2008) - Técnica alternativa: co-inoculação de soja com *Azospirillum* e *Bradyrhizobium* visando incremento de produtividade. Acessed in: <http://www.infobibos.com/Artigos/20084/coinoculacao/index.htm>.

Bárbaro, I.M.; Machado, P.C.; Junior, B.; Souza, L.; Ticelli, M.; Miguel, F.B. and Da Silva, J.A.A. (2009) - Produtividade da soja em resposta á inoculação padrão e co-inoculação. *Colloquium Agrariae*, vol. 5, n. 1, p. 1-7.

Basso, S.M.S. (1999) - *Caracterização morfológica e fixação biológica de nitrogênio de espécies de Adesmia DC. e Lotus* L.. PhD dissertation. Porto Alegre, Universidade Federal do Rio Grande do Sul, 268 p.

Benintende, S.; Uhrich, W.; Herrera, M.; Gangge, F.; Sterren, M. and Benintende M. (2010) - Comparación entre coinoculación com *Bradyrhizobium japonicum* y *Azospirillum brasilense* e inoculación simple con *Bradyrhizobium japonicum* en la nodulación, crecimiento y acumulación de N en el cultivo de soja. *Agriscientia*, vol. 23, n. 2, p. 71-77.

Blankenship, R.E. (2009) - Fotossíntese: As Reações Luminosas. In: Taiz, L. and Zeiger E. (Ed.) - *Fisiologia Vegetal*. 4ª ed. Porto Alegre, Artmed, p.147-181.

Cacciari, I.; Lippi, D.; Pietrosanti, T. and Pietrosanti, W. (1989) - Phytohormone-like substances produced by single and mixed diazotrophic cultures of *Azospirillum* and *Arthrobacter. Plant and Soil*, vol. 115, p. 151-153.

Catroux, G.; Hartmann, A. and Revellin, C. (2001) - Trends in rhizobial inoculant production and use. *Plant and Soil*, vol. 230, n. 1, p. 21-30.

Chaparro, J.M.; Sheflin, A.M.; Manter, D.K. and Vivanco, J.M. (2012) - Manipulating the soil microbiome to increase soil health and plant fertility. *Biology and Fertility of Soils*, vol. 48, n. 5, p. 489-499.

Crozier, A.; Arruda, P.; Jasmim, J.M. ; Monteiro, A.M. and Sandberg, G. (1988) - Analysis of Indole-3-Acetic Acid and Related Indoles in Culture Medium from *Azospirillum lipoferum* and *Azospirillum brasilense. Applied and Environmental Microbiology*, vol. 54, n. 5, p. 2833-2837.

Didonet, A.D.; Lima, O.S.; Candaten A.A. and Rodrigues. O. (2000) - Realocação de nitrogênio e de biomassa para os grãos em trigo submetidos à inoculação de *Azospirillum. Pesquisa Agropecuária Brasileira*, vol. 35, n. 2, p. 401- 411.

Empresa Brasileira de Pesquisa Agropecuária (Embrapa). Centro Nacional de Pesquisa de Solos. Sistema brasileiro de classificação de solos. Rio de Janeiro: Embrapa, 1999.

Fagan, E.B.; Medeiros, S.L.P.; Manfron, P.A.; Casaroli, D.; Simon, J.; Dourado, Neto D. and Müller L. (2007) - Fisiologia da fixação biológica do nitrogênio em soja - Revisão. *Revista da FZVA*, vol. 14, n. 1, p. 89-106.

Ferlini, H.A. (2006) - Co-Inoculación en Soja (*Glicyne max*) con *Bradyrhizobium japonicum* y *Azospirillum brasilense. Articulos Técnicos – Agricultura*. Acessed in: http://www.engormix.com/co_inoculacion_soja_glicyne_s_articulos_800_AGR.htm.

Ferreira, D.F. (2011) - Sisvar: A computer statistical analysis system. *Ciência e agrotecnologia*, vol. 35, n. 6, p. 1039-1042.

Gitti, D.C.; Arf, O.; Kaneko, F.H.; Rodrigues, R.A.F.; Buzetti, S.; Portugual, J.R. and Corsin D.C.D.C. (2012) - Inoculation of *Azospirillum brasilense* cultivars of beans types in winter crop. *Revista Agrarian*, vol. 5, 5: 36-46.

Hungria, M.; Franchini, J.C., Campo, R.J. and Graham, P.H. (2005) - The importance of nitrogen fixation to soybean cropping in South America. In: Werner, D. and Newton, W.E. (Ed.). *Nitrogen fixation in agriculture: forestry ecology and environment*. Dordrecht, Kluwer Academic Publishers, p. 25-42.

Hungria, M.; Francini, J.C.; Crispino, C.C.; Moraes, J.Z.; Sibaldelli, R.N.R. and Arihara, J. (2006) - Nitrogen nutrition of soybean in Brazil: Contributions of biological N2 fixation and N fertilizer to grain yield. *Canadian Journal of Plant Science*, vol. 86, n. 4, p .927-939.

Hungria, M. (2011) - Inoculação com *Azospirillum brasilense*: inovação em rendimento a baixo custo. Embrapa Soja – Documentos 325.

Malavolta, E. (1980) - Elementos de Nutrição Mineral de Plantas. São Paulo: Ceres, 251 p.

Masciarelli, O.; Urbani L.; Reinoso, H. and Luna, V. (2013) - Alternative Mechanism for the Evaluation of Indole-3-Acetic Acid (IAA) Production by *Azospirillum brasilense* Strains and Its Effects on the Germination and Growth of Maize Seedlings. *Journal of Microbiology*, vol. 51, n. 5, p. 590-597.

Miranda, C.H.B. and Macedo, M.C.M. (2001) - *Fixação de nitrogênio pela soja em sistemas de cultivo contínuo e rotacionado com pecuária nos cerrados*. Campo Grande: 29p. (Boletim de Pesquisa e Desenvolvimento n. 14).

Novais, R.F.; Neves, J.C.L. and Barros, N.F. (1991) - Ensaio em ambiente controlado. In: Oliveira, A.J.E.A. (Ed.) - *Métodos de Pesquisa em Fertilidade do Solo*. Brasília: EMBRAPA-SEA, p. 189-253.

Vianello, R.L. and Alves, A.R. (1991) - Meteorologia básica e aplicações. Viçosa: UFV, 449 p.

Zilli, J.E.; Marson, L.C.; Campo, R.J.C. ; Marson, B.F.; Hungria, M. and Gianluppi, V. (2008) - Inoculação de *Bradyrhizobium* em soja por pulverização em cobertura. *Pesquisa agropecuária brasileira*, vol. 43, n. 4, p. 541-544.