academicJournals

Vol. 10(20), pp. 675-680, 28 May, 2016
DOI: 10.5897/AJMR2016.7911
Article Number: CA2DF1E58675
ISSN 1996-0808

http://www.academicjournals.org/AJMR

**African Journal of Microbiology Research**

*Full Length Research Paper*

# Foliar application of *Azospirillum brasilense* in soybean and seed physiological quality

**Alan Mario Zuffo*, Adriano Teodoro Bruzi, Pedro Milanez de Rezende, Maria Laene Moreira de Carvalho, Everton Vinicius Zambiazzi, Igor Oliveri Soares and Karina Barroso Silva**

Department of Agriculture, Campus Universitário, UFLA, 37200-000, Lavras, MG, Brazil.

Recieved 8 January, 2016,Accepted 18 March, 2016.

**Bacteria of the *Azospirillum* genus have considerable potential for application in agricultural systems, either in co-inoculation and foliar application to increase crop yields, due to its role in the production of phytohormones. The objective of this research was to evaluate the yield and quality of soybean seeds produced under the effect of different doses of *Azospirillum brasilense* bacteria applied to the leaves. Seeds of four soybean cultivars (Anta 82 RR®, BRS Favorita RR®, BRS 780 RR® and BRS 820 RR®) were produced in the 2013/2014 crop year in Lavras, Minas Gerais, Brazil, with application of six doses of inoculants based on *A. brasilense* (0, 300, 400, 500, 600 and 700 mL $ha^{-1}$) of the strains, $AbV_5$ and $AbV_6$. Besides the yield, the mass of a thousand seeds, moisture content, germination, emergence at 7 and 15 days after sowing, emergence speed index, accelerated aging, strength, viability and mechanical damage were evaluated by tetrazolium and the hypochlorite test. Regardless of the soybean cultivar, application of up to 700 $mLha^{-1}$ of *A. brasilense* innoculant at $v_3$ stage (second open trefoil) of the plants, the yield, physiological potential and seeds were not affected.**

**Key words:** *Glycine max* (L.) Merrill, growth promoting bacteria, emergency, viability, vigor.

## INTRODUCTION

In the last decades, the soybean crops [*Glycine max* (L.) Merrill] have expanded to several regions of the world. In this scenario, Brazil stands out in the production of this oilseed, being the second largest producer of soybeans. In 2014/2015 cropping year, the soybean crops occupied an area of 31.33 million hectares, which reached the total production of 93.25 million tons. In the Southeast, the state of Minas Gerais is the largest producer of soybeans, with an area of about 1.31 million hectares (CONAB, 2015).

In commercial crops and seed production fields, inoculation with *Bradyrhizobium japonicum* bacteria is common eliminating the use of nitrogen fertilization due to the efficiency of biological nitrogen fixation (BNF). Recently, it has been speculated that the use of *Azospirillum brasilense* bacteria can increase the soybean crops performance (Hungria et al., 2007). Bacteria of the genus *Azospirillum*, produce phytohormones,

*Corresponding author. E-mail: alan_zuffo@hotmail.com.

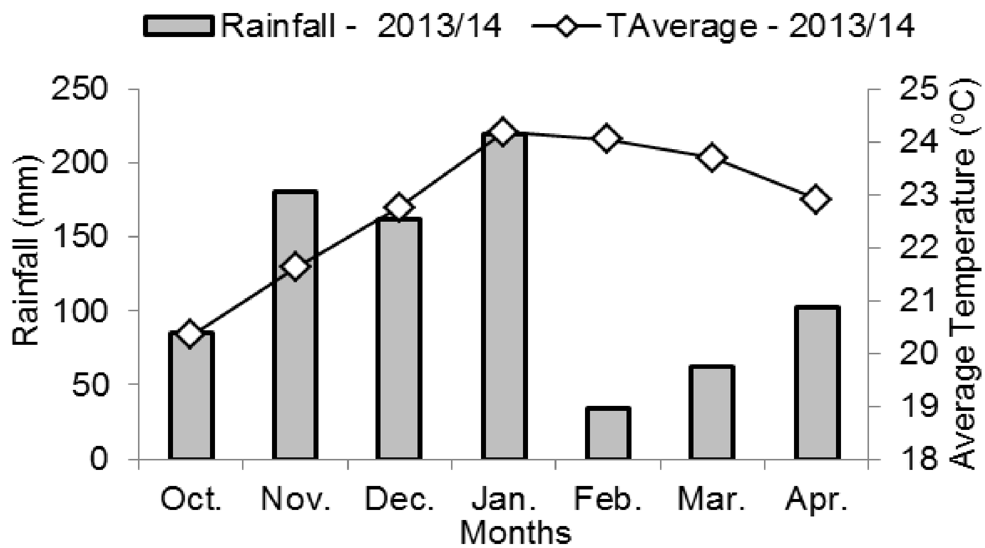


**Figure 1.** Monthly means for rainfall and air temperature in Lavras, MG, Brazil, in 2013/2014 crop year during soybean production. Source: National Meteorology Institute (INMET).

including auxins, gibberellins, cytokinins, that under "*in vitro*" conditions (Araujo, 2008; Masciarelli et al., 2013), can reduce the need of chemical inputs application, provide the fixing of atmospheric nitrogen, reduce biotic and abiotic stresses, and increase crop yield (Hungria, 2011). These benefits can improve the physiological potential of seeds. Cassán et al. (2009) found that in corn (*Zea mays* L.) and soybean crops, the treatment of seeds with *A. brasilense* strains led to significant increase in germination and vigor. However, in wheat crop(*Triticum aestivum* L.), Brzezinski et al. (2014) found that the sanitary quality of wheat seed is not influenced by *A. brasilense* inoculation. The inoculation of wheat seeds with *A. brasilense* favors the vigor (accelerated aging) and seedlings shoot mass.

According to Azevedo et al. (2007), little has been studied on the effects of cultural practices on the physiological quality of soybean seed. It is known that high quality seeds are desirable for the success of agriculture. Thus, the objective of the research was to evaluate the yield and quality of soybean seed produced under the effect of different doses of *A. brasilense* bacteria inoculation applied to the leaves.

## MATERIALS AND METHODS

The seeds were produced in 2013/2014 crop year, in Lavras - MG, Brazil, at the Scientific and Technological Development Center of Agriculture - at UFLA, located at 21°12'S latitude, 44°58'W longitude and altitude of 918 m in soil classified as distroferric Red Latosol - LVdf.

The climate is Cwa, according to the Köppen classification, with an average annual temperature of 19.3°C and normal annual rainfall of 1,530 mm (Dantas et al., 2007). During the seed production process, the climatic data was collected at the meteorological station of the National Institute of Meteorology (INMET) located at the Federal University of Lavras-UFLA and are presented in Figure 1.

The experiment was performed in a randomized block design with three replications. The treatments were arranged in a 4 x 6 factorial, four soybean cultivars seeds were produced (Anta 82 RR®, BRS Favorita RR®, BRS 780 RR®, BRS 820 RR®) with the application of six doses of the inoculants based on *A. brasilense* (0, 300, 400, 500, 600 and 700 mL ha$^{-1}$) applied to the leaves in the $V_3$ stage (second open trefoil). Each plot consisted of four sowing rows of 5 m in length, spaced at 0.50 m, and the area of each plot was of 10 m$^2$ (5 m x 2 m). The two central rows were considered as useful area.

The sowing was performed in November 2013. The fertilization consisted of 350 kg ha$^{-1}$ of $N$-$P_2O_5$-$K_2O$ (02-30-20), applied via groove. The *Bradyrhizobium japonicum* bacteria were inoculated via groove after soybean seeding. The dose of *B. japonicum* was 18 mL p. c. kg$^{-1}$ of seed – strains SEMIA 5079, containing 10.8 x 10$^6$ CFU/seed of Nitragin Cell Tech HC® (3x10$^9$ CFU/mL). the inoculant Azo® (1 x 10$^8$ CFU/mL) strains estirpes $AbV_5$ and $AbV_6$ were used. The application of microorganisms was performed using a motor-driven backpack sprayer, coupled to a bar with four spray nozzles XR 110.02, applying spray volume equivalent to 150 L ha$^{-1}$. The legumes were harvested manually and threshed by stationary threshing in order to simulate the mechanical harvesting held by the harvester. The grain yield (kg ha$^{-1}$), being standardized for grainmoisture of 13% was determined. Subsequently, the seeds were separated from impurities with the assistance of sieves, and packaged in 'Kraft' model paper bags. Seeds with moisture content above 13% were placed in the shade to slow drying. After determination of the adequate moisture content, mixing of samples and separation in sieves were done/performed. For the laboratory tests, seeds classified in the 6.00 mm diameter sieve were used. Laboratory evaluations were performed using a completely randomized design, all the tests were performed with two subsamples of 50 seeds per replication, totaling 300 seeds per treatment. The physiological and physical quality of the seeds were determined using the following determinations:

**Thousand seeds mass and moisture content**

Recommendation of Brasil (2009) was followed.

**Germination test**

This was done in the paper roll form with a water volume in the amount of 2.5 times the dry mass of the substrate at 2°C. The evaluations were performed on the 8th day after sowing, according to the criteria established by the Rules for Seed (BRASIL, 2009).

**Emergence test under controlled conditions**

The substrate used was composed of soil and sand (2:1). After sowing, the trains were kept in a greenhouse at 25°and relative air humidity of ±70%. After the emergence of the first seedling (visible cotyledon), daily evaluations were made for the number of emerged plants, with the final counting 15 days after sowing. For the speed emergence index (SEI) calculation, the Maguire (1962) formula was adopted.

**Accelerating aging test**

This was realized according to Marcos Filho (1999) recommendations, with 42 g seeds per treatment on an adapted stainless steel grille in *gerbox* boxes, with 40 mL of water in the bottom, maintained at 41°C for 72 h in a BOD and were evaluated at germination.

**Tetrazolium test**

The seeds were placed in a *germitest* paper moistened for 16 h at 25°C. After this period, they were submerged in 0.075% tetrazolium salt solution for three hours at 40°C in an incubation chamber. The viability, the damage percentage and the vigor were determined according to Neto et al. (1998).

**Sodium hypochlorite test**

For the sodium hypochlorite test, the seeds were kept for 10 min in a sodium hypochlorite solution (0.2%) and evaluated in accordance with Krzyzanowski et al. (2004).

**Statistical analysis**

The variance analysis was realized adopting the statistical model and the analysis procedure similar to Ramalho et al. (2012). The means were grouped by the Scott-Knott test (1974). The statistical analysis was realized with SISVAR® statistical package (Ferreira, 2011).

## RESULTS AND DISCUSSION

The inoculation doses with isolated *A. brasilense* bacteria and the interaction of cultivars (C) and bacteria (A) did not lead to a significant difference on the studied tests (Table 1). According to this fact, it can be inferred that the physiological quality of the soybean seeds, on average, does not depend on the *A. brasilense* inoculation dose and on the studied cultivar. Similar results were also obtained by Zuffo et al. (2015a) and Zuffo et al. (2015b). The authors did not observe the *A. brasilense* bacteria on the soybean agronomical characters.

A possible explanation for this fact is that the used doses did not promote any effect on the physiological quality because of the absence of effect on agronomical characters, as verified by Gitti et al. (2012), Zuffo et al. (2015a, b). With no influence on agronomical characteristics on the field, the plant produced seeds with a similar physiological potential. On the other hand, when different seeds from different cultivars were evaluated, differences on the physiological quality were observed (Table 1). The cultivar effect on the soybean seed physiological quality was verified by Zambuzzi et al. (2014). The cultivars have different characteristics related to the genetic background, growth habit, maturation group and other attributes, promoting the existence of some variations.

For cultivars, a high amplitude between the means for thousand seed mass was observed, from 187.22 g to 127.77 g for BRS Favorita RR® and Anta 82 RR®, respectively (Table 1), however, all the cultivars presented satisfactory productive performance, with results above the average mean for the crop in Minas Gerais state – 2658 kg ha$^{-1}$ – achieved during the 2014/2015 crop year (CONAB, 2015).

The moisture contents of seeds from different cultivars were similar (Table 1). According to Leoffler et al. (1998), the uniformity on the moisture content is very important to standardize the evaluations and provide consistent results.

In a general manner, the studied cultivars presented a lower germination percentage as compared to the required standard to commercialize seeds in Brazil, which is 80%, established by the normative instruction no. 45 (BRASIL, 2013). Seeds with low or medium germination can generate less competitive seedlings in the field (Neto et al., 2010). The main reason for the low germination can be related to the higher percentage of dead and infected seeds verified during the germination test (Figure 2). According to Binotti et al. (2008), the seed pathogens can increase the deterioration and reduce the vigor and germination of the seeds. During the emergence tests, 7 and 15 days after emergence, higher means were observed as compared to germination. The cultivars Anta 82 RR® and BRS 780 RR® demonstrated more emergence percentage. The higher means from emergence test, when compared with germination test have already been mentioned in literature by Henning and Neto (1980), Bizzetto and Homechin (1997) and Zambiazzi et al. (2014). The authors related that the seedlings, on emergence, releases the infected tegument on the soil, but during the germination test, on the paper, the tegument remains associated with the cotyledons and the fungus promote the seed deterioration.

For the speed emergence index, the same trend related to the emergence test was observed. This fact

**Table 1.** Mean values for yield, thousand seeds mass (TSM), moisture content (MC), germination (GERM), emergence 7 days after emergence – DAE (E7DAE), emergence 15 DAE (E15DAE), speed emergence index (SEI), accelerated aging (AAG), vigor percentage by tetrazolium ($Tz_{(1-3)}$), viability percentage by tetrazolium ($Tz_{(1-5)}$) and damage percentage by hypochlorite (HYPO) obtained in tests with different doses of inoculant with *Azospirillum brasilense* bacterias applied at $V_3$ stage in soybean cultivars during the 2013/2014 crop year. Lavras, MG, Brazil.

| Source of variation | Yield (kg ha$^{-1}$) | TSM (g) | MC (%) | GERM (%) | E7DAE (%) | E15DAE (%) | SEI (%) | AAG (%) | $Tz_{(1-3)}$ (%) | $Tz_{(1-5)}$ (%) | HYPO (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cultivar (C) | | | | | | | | | | | |
| Anta 82 RR® | 3262$^{c}$ | 127.77$^{c}$ | 8.27$^{a}$ | 62$^{a}$ | 90$^{a}$ | 93$^{a}$ | 72.61$^{a}$ | 79.38$^{a}$ | 93.03$^{a}$ | 96.93$^{a}$ | 7.00$^{b}$ |
| BRS Favorita RR® | 4202$^{a}$ | 187.22$^{a}$ | 8.43$^{a}$ | 65$^{a}$ | 86$^{b}$ | 88$^{b}$ | 69.32$^{b}$ | 61.38$^{c}$ | 88.47$^{a}$ | 95.56$^{a}$ | 11.22$^{a}$ |
| BRS 780 RR® | 3814$^{b}$ | 168.88$^{b}$ | 8.39$^{a}$ | 59$^{b}$ | 92$^{a}$ | 94$^{a}$ | 75.69$^{a}$ | 72.00$^{b}$ | 92.55$^{a}$ | 96.81$^{a}$ | 6.98$^{b}$ |
| BRS 820 RR® | 4385$^{a}$ | 164.88$^{b}$ | 8.58$^{a}$ | 59$^{b}$ | 88$^{b}$ | 89$^{b}$ | 70.54$^{b}$ | 44.16$^{d}$ | 89.51$^{a}$ | 95.26$^{a}$ | 10.94$^{a}$ |
| P (Value) | <0.01** | <0.01** | 0.10$^{ns}$ | <0.01** | <0.01** | <0.01** | <0.01** | <0.01** | 0.12$^{ns}$ | 0.53$^{ns}$ | <0.01** |
| *Azospirillum brasilense* (A) (mL ha$^{-1}$) | | | | | | | | | | | |
| 0 | 4037 | 160.00 | 8.46 | 63 | 88 | 90 | 71.91 | 63.25 | 90.53 | 97.52 | 8.87 |
| 300 | 3784 | 161.66 | 8.54 | 60 | 89 | 91 | 71.88 | 66.66 | 88.09 | 94.91 | 8.91 |
| 400 | 4003 | 165.00 | 8.39 | 58 | 87 | 91 | 69.16 | 64.33 | 93.22 | 97.82 | 8.87 |
| 500 | 3903 | 157.50 | 8.37 | 61 | 91 | 92 | 73.25 | 59.83 | 91.98 | 95.80 | 9.00 |
| 600 | 3969 | 170.00 | 8.37 | 62 | 90 | 91 | 71.98 | 65.41 | 90.64 | 94.90 | 9.41 |
| 700 | 3800 | 165.00 | 8.38 | 60 | 90 | 92 | 74.06 | 65.91 | 90.86 | 95.89 | 9.14 |
| P (Value) | 0.30$^{ns}$ | 0.66$^{ns}$ | 0.87$^{ns}$ | 0.12$^{ns}$ | 0.32$^{ns}$ | 0.86$^{ns}$ | 0.18$^{ns}$ | 0.24$^{ns}$ | 0.56$^{ns}$ | 0.38$^{ns}$ | 0.83$^{ns}$ |
| C x A | 0.11$^{ns}$ | 0.30$^{ns}$ | 0.44$^{ns}$ | 0.07$^{ns}$ | 0.61$^{ns}$ | 0.52$^{ns}$ | 0.64$^{ns}$ | 0.06$^{ns}$ | 0.88$^{ns}$ | 0.77$^{ns}$ | 0.79$^{ns}$ |
| CV (%) | 8.39 | 11.65 | 4.49 | 7.05 | 5.27 | 4.98 | 6.38 | 11.31 | 7.38 | 4.36 | 12.61 |

**Significant at 1% according to F test, ns – not significant. Means followed by the same lower case in the column are from the same group, according to Skott Knott test (1974) at 5% of probability.

was expected because the seedlings that emerge first can grow more and have more biomass because of photosynthesis in the first growth stages. Besides this, in field conditions, the seedlings from seeds with a higher speed emergence index promote a faster closure between the field lines, leading to better weed control (França et al., 2010). For the accelerating aging test, the cultivar BRS 820 RR® followed by BRS Favorita RR® was more sensitive to high temperature and relative humidity conditions. For the vigor percentage and seed viability, measured by tetrazolium, no significant difference between cultivars was observed. For the mechanical damages, moisture deterioration and stink bug damage were found to be the main problems for the soybean; different damages were observed according to cultivar (Figure 3). Thus, cultivars have distinct genetic characteristics that can lead to higher or lower susceptibility to damages from bugs, harvest or adverse conditions. For the damage percentage by hypochlorite, Anta 82 RR® and BRS 780 RR® presented lower means. This data support the results obtained for mechanical damages by tetrazolium, demonstrating consistency between the tests, but without observing effect of different doses of inoculant with *A. brasilense* on the physical and chemical seed quality.

## Conclusion

Despite the soybean cultivar, the inoculant application with *A. brasilense* bacteria up to 700 mL/ha applied on plants at $V_3$ stage did not affect the yield, physiological potential and seed

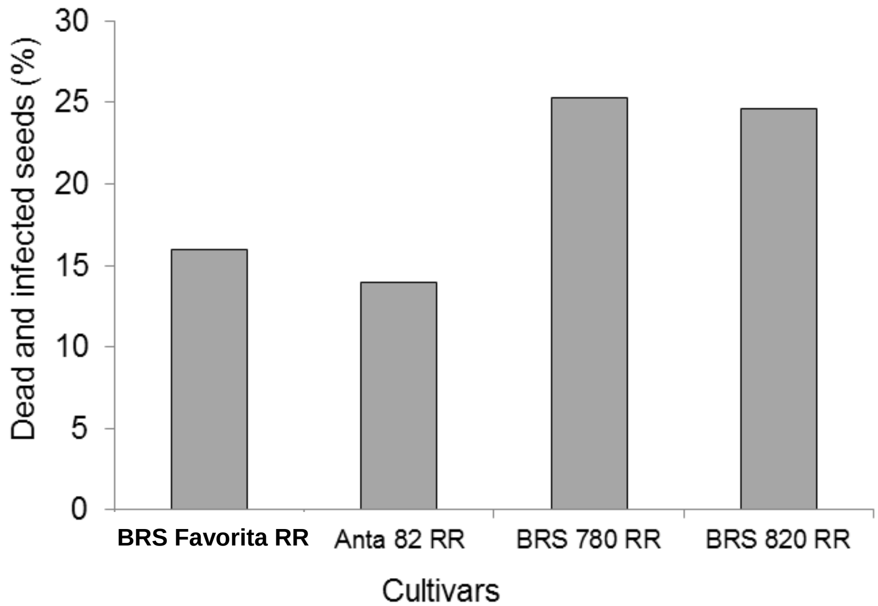


**Figure 2.** Total percentage of dead and infected seeds during germination test for each cultivar during the 2013/2014 crop year. Lavras. MG. Brazil.

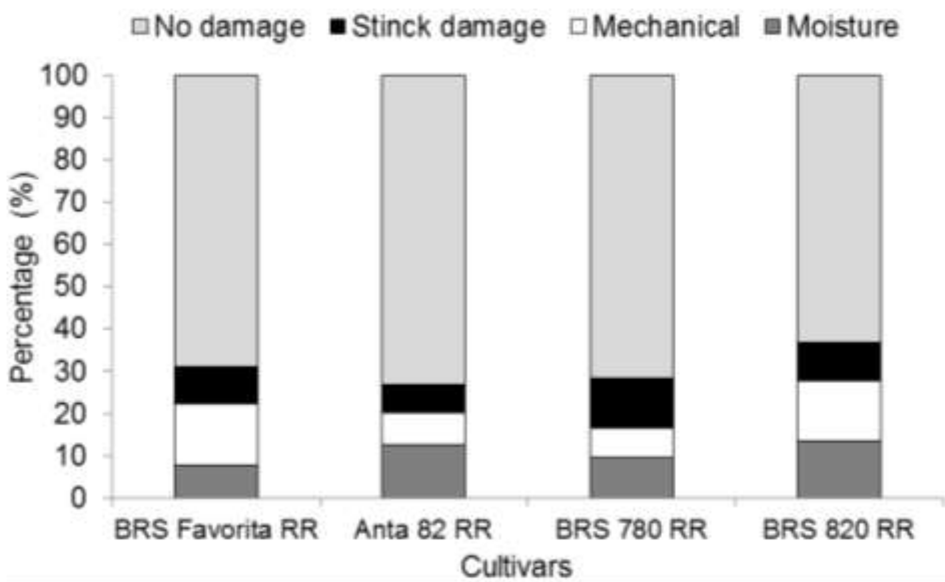


**Figure 3.** Damages and their respective percentages (%) observed during tetrazolium test for each cultivar during the 2013/2014 crop year. Lavras. MG. Brazil.

damage incidence.

## Conflict of Interests

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

The authors thank National Council of Scientific and Technological Development (CNPq), Coordination for the Improvement of Higher Personel Education (CAPES) and Foundation for Supporting Research in Minas Gerais

(FAPEMIG) for the scholarships and financial support.

**REFERENCES**


Araujo SC (2008). Realidade e perspectivas para o uso de *Azospirillum* na cultura do milho. Rev. Inf. Agron. 122:4-6.

Azevedo de DMP, Leite LFC, Teixeira Neto ML, Dantas JS (2007). Atributos físicos e químicos de um Latossolo Amarelo e distribuição do sistema radicular da soja sob diferentes sistemas de preparo no cerrado maranhense. Rev. Cienc. Agron. 38(1):32-40.

Binotti FFS, Haga, KIH, Cardoso ED, Alves CZ, Sá ME de, Arf O (2008). Efeito do período de envelhecimento acelerado no teste de condutividade elétrica e na qualidade fisiológica de sementes de feijão. Acta Sci. Agron. 30(2):247-254.

Bizzetto A, Homechin M (1997). Efeito do período e da temperatura de armazenamento na qualidade fisiológica e sanitária de sementes de soja com altos índices de *Phomopsis sojae* (Leh.). Rev. Bras. Sem.19(2):296-303.

Brasil, Ministério da Agricultura e Reforma Agrária (2009). Regras para análise de sementes. Brasília, Secretaria Nacional de Defesa Agropecuária. Brasil. Ministério da Agricultura, Pecuária e do Abastecimento. Instrução Normativa n. 45, de 17 de setembro de 2013. Padrões de identidade e qualidade para produção e comercialização de sementes. Available in: <http://www.abrasem.com.br/wpcontent/uploads/2012/10/Instru%C3%A7%C3%A3o-Normativa-n%C2%BA-45-de-17-de-Setembro-de-2013-Padr%C3%B5es-de-Identidade-e-Qualiidade-Prod-e-Comerc-deSementes-Grandes-Culturas-Republica%C3%A7%C3%A3o-DOU-20.09.13.pdf>. Access: Retrieved 10 october, 2015.

Brzezinski CR, Zucareli C, Henning FA, Prando JAAM, Henning AA (2014). Nitrogênio e inoculação com *Azospirillum* na qualidade fisiológica e sanitária de sementes de trigo. Rev. Cienc. Agr. 57(3):257-265.

Cassán F, Perrig D, Sgroy V, Masciarelli O, Penna C, Luna V (2009). *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (*Glycine max* L.). Eur. J. Soil Biol. 45(1):28-35.

CONAB - Companhia Nacional de Abastecimento (2015). Acompanhamento de safra brasileira: grãos, nono levantamento, Junho 2015. Companhia Nacional de Abastecimento, Brasília: CONAB.

Dantas AA, Carvalho LG, Ferreira E (2007). Classificação e tendências climáticas em Lavras, MG. Cienc. Agrotec. 31(6):1862-1866.

Gitti D de C, Arf O, Kaneko FH, Rodrigues RAF, Buzetti S, Portugual JR, Corsin DCDC (2012). Inoculation of *Azospirillum brasilense* cultivars of beans types in winter crop. Rev. Agrar. 5(15):36-46.

Felisberto G, Bruzi AT, Zuffo AM, Zambiazzi EV, Soares IO, Rezende PM, Botelho FBS (2015). Agronomic performance of RR soybean cultivars using to different pre-sowing desiccation periods and distinct post-emergence herbicides. Afr. J. Agric. Res. 10(34):3445-3452.

Ferreira DF (2011). Sisvar: A computer statistical analysis system. Cienc. Agrotec. 35(6):1039-1042.

França-Neto JB, Krzyzanowski FC, Costa NP (1998). O teste de tetrazólio em sementes de soja. Londrina, Brasil, Embrapa Soja (Embrapa Documentos 116).

França-Neto JB, Krzyzanowski FC, Henning AA (2010). A importância do uso de semente de soja de alta qualidade. Inf ABRATES 20:37-38.

Henning AA, França Neto, JB (1980). Problemas na avaliação da germinação de semente de soja com alta incidência de *Phomopsis* sp. Rev. Bras. Sementes 2:9-22.

Hungria M, Campo RJ, Mendes IC (2007). A importância do processo de fixação biológica do nitrogênio para a cultura da soja: Componente essencial para a competitividade do produto brasileiro. Londrina, Brasil, Embrapa Soja, (Embrapa Documentos 283) 80 p.

Hungria M (2011). Inoculação com *Azospirillum brasilense*: inovação em rendimentoa baixo custo. Londrina, Brasil, Embrapa Soja (Embrapa Documentos 325)

Maguire JD (1962). Speed of germination-aid in selection and evaluation for seedling emergence and vigor. Crop Sci. 2:176-177.

Marcos Filho J (1999). Teste de envelhecimento acelerado. In: Krzyzanowski FC, Vieira RD, França Neto, JB (Ed.). Vigor de sementes: conceitos e testes. Londrina: ABRATES, Paraná.

Masciarelli O, Urbani L, Reinoso H, 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. J. Microbiol. 51(5):590-597.

Krzyzanowski FC, França-Neto JB, Costa NP (2004). Teste do hipoclorito de sódio para semente de soja. Londrina: Embrapa Soja (Circular técnica, 37).

Ramalho MAP, Ferreira DF, Oliveira AC (2012). Experimentação em genética e melhoramento de plantas. 2.ed. Lavras: Universidade Federal de Lavras.

Scott AJ, Knott MA (1974). A Cluster Analysis Method for Grouping Means in the Analysis of Variance. Biometrics 30(3):507-512.

Soares IO, Rezende PM, Bruzi AT, Zambiazzi EV, Felisberto G, Zuffo AM, Silva KB, Gwinner R (2015). Adaptability of soybean cultivars in different crop years. Genet. Mol. Res. 14(3):8995-9003.

Zambiazzi EV, Bruzi AT, Carvalho MLM de, Soares IO, Zuffo AM, Rezende PM de, Miranda, DH (2014). Potassium Fertilization and Physiological Soybean Seed Quality. Agric. Sci. 5(11):984-991.

Zuffo AM, Rezende PM, Bruzi AT, Oliveira NT, Soares IO, Neto GFG, Cardillo BES, Silva LO (2015a). Co-inoculation of *Bradyrhizobium japonicum* and *Azospirillum brasilense* in the soybean crop. Rev. Cienc. Agrar. 38(1):87-93.

Zuffo AM, Bruzi AT, Rezende PM, Bianchi MC, Zambiazzi EV, Soares IO, Ribeiro ABM, Vilela GLD, Gesteira GS (2015b). Morphoagronomic and productive traits of RR soybean due to inoculation via Azospirillum brasilense groove. Afr. J. Microbiol. Res. 10(13):438-444.