UNIVERSIDADE FEDERAL DE LAVRAS

ALAN MARIO ZUFFO

APLICAÇÕES DE *Azospirillum brasilense* NA CULTURA DA SOJA

LAVRAS - MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia/Fitotecnia, área de concentração em Produção Vegetal, para a obtenção do título de Doutor.

Orientador
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APROVADA em 28 de abril de 2016.

Dr. Adriano Teodoro Bruzi	UFLA
Dr. Wagner Pereira Reis	UFLA
Dr. Vanoli Fronza	EMBRAPA
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Dr. Pedro Milanez de Rezende
Orientador

LAVRAS – MG
2016

***DEDICO** esta vitória a Deus, em cujas mãos está a nossa origem,*
caminho, direção e destino. Dedico esta, bem como todas as
minhas demais conquistas a todos que um dia sonharam e
hoje compartilham este importante momento comigo.

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da minha longa caminhada, iniciada no momento em
que recebi a vida, a concepção, uma família e os
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e Jordana, ao meu sobrinho Luiz Henrique
e a minha esposa Rosalina Zuffo.

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"Muitas vezes, as pessoas são egocêntricas, ilógicas e insensatas.
Perdoe-as assim mesmo.
Se você é gentil, as pessoas podem acusá-lo de egoísta, interesseiro.
Seja gentil assim mesmo.
Se você é um vencedor, terá alguns falsos amigos e alguns inimigos verdadeiros.
Vença assim mesmo.
Se você é honesto e franco, as pessoas podem enganá-lo.
Seja honesto assim mesmo.
O que você levou anos para construir, alguém pode destruir de uma hora para outra. Construa assim mesmo.
Se você tem Paz e é Feliz, as pessoas podem sentir inveja.
Seja Feliz assim mesmo.
O bem que você faz hoje pode ser esquecido amanhã.
Faça o bem assim mesmo.
Dê ao mundo o melhor de você, mas isso pode nunca ser o bastante.
Dê o melhor de você assim mesmo.
Veja que no final das contas, é entre você e Deus.
Nunca foi entre você e as outras pessoas".
(Madre Teresa de Calcutá)

"Ainda que eu tivesse o dom da profecia, o conhecimento de todos os mistérios e de toda a ciência; ainda que eu tivesse toda a fé, a ponto de transportar montanhas, se não tivesse o amor, eu não seria nada. (...) O amor jamais passará. As profecias desaparecerão, as línguas cessarão, a ciência também desaparecerá. Pois o nosso conhecimento é limitado; limitada é também a nossa profecia. Mas, quando vier a perfeição, desaparecerá o que é limitado."
I Coríntios 13: 2, 8 a 10

RESUMO

As bactérias promotoras de crescimento do gênero *Azospirillum brasilense* têm grande potencial agronômico, por exercer efeitos fisiológicos benéficos durante o crescimento das plantas. Com o intuito de verificar esses potenciais, quatro estudos foram conduzidos. No primeiro trabalho objetivou-se avaliar o efeito de coinoculação com diferentes doses de *Bradyrhizobium japonicum* e *Azospirillum brasilense* no desenvolvimento morfofisiológico e nodulação da soja. O experimento foi realizado em delineamento inteiramente casualizado, com quatro repetições, em esquema fatorial 5 x 2, sendo cinco doses de *Azospirillum brasilense* (0; 0,5; 1,0; 1,5 e 2,0 mL por kg^{-1} de semente) e duas doses de *Bradyrhizobium japonicum* (0 e 3,0 mL por kg^{-1} de semente), ambas veiculadas via sementes. As doses de *Azospirillum brasilense* isoladas e a coinoculação não promoveram influências nos parâmetros avaliados. No segundo trabalho objetivou-se avaliar a inoculação de *Azospirillum brasilense* associada à *Bradyrhizobium japonicum* nos caracteres agronômicos e produtividade dos grãos em soja. O experimento foi realizado em blocos casualizados, dispostos em esquema fatorial 4 x 2, sendo quatro cultivares (Anta 82 RR®, BRS Favorita RR®, BRSMG 780RR® e BRSMG 820RR®) e dois tratamentos com *Azospirillum brasilense* (sementes inoculadas e não inoculadas), com três repetições em dois anos agrícolas. A inoculação ou não de *Azospirillum brasilense* associado à *Bradyrhizobium japonicum* não afeta os caracteres agronômicos e a produtividade dos grãos em cultivares de soja RR®. No terceiro trabalho objetivou-se avaliar os caracteres agronômicos e produtividade dos grãos na soja em função de doses de *Azospirillum brasilense* aplicadas por pulverização nas folhas. O experimento foi realizado em blocos casualizados, dispostos em esquema fatorial 4 x 6, sendo quatro cultivares (Anta 82 RR®, BRS Favorita RR®, BRSMG 780RR® e BRSMG 820RR®) e seis doses de *Azospirillum brasilense* (0, 300, 400, 500, 600 e 700 mL ha^{-1}), com três repetições em dois anos agrícolas. A pulverização foliar de doses de *Azospirillum brasilense* não afeta os caracteres agronômicos e produtividade dos grãos em cultivares de soja RR®. No quarto trabalho objetivou-se avaliar a produtividade e a qualidade de sementes de soja produzidas sob o efeito de diferentes doses da bactéria *Azospirillum brasilense* aplicadas via foliar. As sementes de quatro cultivares de soja (Anta 82 RR®, BRS Favorita RR®, BRSMG 780RR® e BRSMG 820RR®) foram produzidas no ano agrícola 2013/2014 em Lavras - MG, Brasil, com a aplicação de seis doses de inoculante a base de *Azospirillum brasilense* (0, 300, 400, 500, 600 e 700 mL ha^{-1}) das estirpes (AbV_5 e AbV_6). A aplicação do inoculante com bactérias de *Azospirillum brasilense* não afetou as variáveis estudadas.

Palavras-chave: Bactérias promotoras de crescimento. *Glycine max* (L.) Merrill.

ABSTRACT

The growth promoting bacteria of the genus *Azospirillum brasilense* have great agronomic potential due to their beneficial physiological effects during the plant growth. In order to verify these potential four studies were performed. In the first study the objective was to evaluate the inoculation effect of different doses of *Bradyrhizobium japonicum* and *Azospirillum brasilense* in the morphophysiological development and soybean nodulation. The experiment was performed in design the completely randomized, with four replications, in a 5 x 2 factorial, with five doses of *Azospirillum brasilense* (0, 0.5, 1.0, 1.5 and 2.0 mL per kg^{-1} of seed) and two doses of *Bradyrhizobium japonicum* (0 and 3.0 mL per kg^{-1} of seed), both transmitted via seeds. Doses of isolated *Azospirillum brasilense* and coinoculation did not promote influences on the evaluated parameters. In the second study the objective was to evaluate the inoculation of *Azospirillum brasilense* associated with *Bradyrhizobium japonicum* in agronomic traits and soybean yield. The experiment was performed in randomized blocks in a 4 x 2 factorial, with four cultivars (Anta 82 RR®, BRS Favorita RR®, BRSMG 780RR® and BRSMG 820RR®) and two treatments with *Azospirillum brasilense* (inoculated seed and non inoculated) with three replications in two crop years. The inoculation or not of *Azospirillum brasilense* associated with *Bradyrhizobium japonicum* does not affect the agronomic traits and grain yield in RR® soybean cultivars. In the third study the objective was to evaluate the agronomic traits and grain yield in soybeans due to *Azospirillum brasilense* doses applied by spraying the leaves. The experiment was performed in randomized blocks in a 4 x 6 factorial, with four cultivars (Anta 82 RR®, BRS Favorita RR®, BRSMG 780RR® and BRSMG 820RR®) and six doses of *Azospirillum brasilense* (0, 300 , 400, 500, 600 and 700 mL ha^{-1}) with three replications in two crop years. The spraying leaves in doses of *Azospirillum brasilense* does not affect the agronomic traits and grain yield in RR® soybean cultivars. In the fourth study the objective was to evaluate the yield and quality of soybean seeds produced under the effect of different doses of bacteria *Azospirillum brasilense* applied to the leaves. Seeds of four soybean cultivars (Anta 82 RR®, BRS Favorita RR®, BRSMG 780RR® and BRSMG 820RR®) were produced in 2013/2014 crop year in Lavras, Minas Gerais, Brazil, with the application of six doses of inoculants based of *Azospirillum brasilense* (0, 300, 400, 500, 600 and 700 mL ha^{-1}) of the strains (AbV$_5$ and AbV$_6$). The application of the inoculant with *Azospirillum brasilense* bacteria did not affect the studied variables.

Keywords: Growth promoting bacteria. *Glycine max* (L.) Merrill.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

A soja [*Glycine max* (L.) Merrill] é a principal oleagina cultivada no mundo, o que torna a espécie com grande interesse a pesquisa, relacionada à redução do uso de fertilizantes sem perdas na produção dos grãos. Nesse aspecto, as bactérias promotoras de crescimento do gênero *Azospirillum brasilense* têm grande potencial agronômico, por exercer efeitos fisiológicos benéficos durante o crescimento das plantas.

Dessa forma, a substituição de insumos minerais por organismos biológicos a fim de reduzir a contaminação do ambiente está relacionada à busca pela sustentabilidade do sistema agrícola, além da crescente demanda de alimentos saudáveis. Assim, um dos grandes desafios da pesquisa é o desenvolvimento de técnicas de manejo que visam ao uso dos fatores biológicos no incremento da produção.

As bactérias do gênero *Azospirillum* apresentam considerável potencial de aplicação em sistemas agrícolas. Quando inoculadas em diversas espécies (especialmente gramíneas), apresentam mecanismos que influenciam o desenvolvimento da planta e a fixação biológica do nitrogênio, devido à estimulação da produção de fitohormônios pelas plantas, melhora dos parâmetros fotossintéticos, condutância estomática e elasticidade da parede celular, alterando as variáveis de produção dessas culturas. Contudo, com exceção de trabalhos de coinoculação entre a bactéria *Azospirillum brasilense* com *Bradyrhizobium japonicum*, pesquisas com *Azospirillum brasilense* de forma isolada em. leguminosas ainda são muito incipientes.

Nas últimas décadas, o consumo de fertilizantes no Brasil cresceu 6% ao ano (maior crescimento mundial), sendo que no período de 1990 até 2010, o crescimento acumulado foi de 216%. O Nitrogênio (N) tem previsão para o ano de 2016, de um consumo de 3,1 mil toneladas, sendo que 38% deste total serão adquiridos através de importação (AGÊNCIA NACIONAL PARA DIFUSÃO DE ADUBOS - ANDA, 2013). Desse modo, o uso de bactérias, promotoras do crescimento de plantas, que visam a aumentar a eficiência de utilização dos fertilizantes, e que eventualmente aportem nitrogênio via fixação biológica, representa uma estratégia economicamente viável, além dos consequentes benefícios ambientais associados à redução no uso de fertilizantes (HUNGRIA, 2011; HUNGRIA et al., 2010).

O *Azospirillum brasilense*, por ser um promotor de crescimento das plantas, confirma a importância de novos trabalhos que abordem o seu uso na cultura da soja. Com o intuito de verificar esses potenciais, quatro estudos foram conduzidos. No primeiro trabalho objetivou-se avaliar o efeito de coinoculação com diferentes doses de *Bradyrhizobium japonicum* e *Azospirillum brasilense* no desenvolvimento morfofisiológico e nodulação da soja. No segundo trabalho objetivou-se avaliar a inoculação de *Azospirillum brasilense* associada à *Bradyrhizobium japonicum* nos caracteres agronômicos e produtividade dos grãos em soja. No terceiro trabalho objetivou-se avaliar os caracteres agronômicos e produtividade dos grãos na soja em função de doses de *Azospirillum brasilense* aplicadas por pulverização nas folhas. No quarto trabalho objetivou-se avaliar a produtividade e a qualidade de sementes de soja produzidas sob o efeito de diferentes doses da bactéria *Azospirillum brasilense* aplicadas via foliar.

Portanto, a presente proposta de pesquisa se justifica devido ao papel estratégico dos microrganismos como ferramenta para o aumento da produtividade agrícola, especificamente o gênero *Azospirillum* como aliado na redução de custos e consequente aumento na sustentabilidade ambiental.

2 REFERENCIAL TEÓRICO

2.1 Cultura da soja: origem, evolução e importância econômica

A soja é a principal *commodity* agrícola e oleaginosa cultivada no mundo. Por ser uma planta milenar, há controvérsia quanto ao seu centro de origem. Provavelmente, o seu centro de origem primário é o continente asiático, na região do Norte da China, no vale do Rio Amarelo; já o Nordeste da China, é considerado como centro secundário de diversidade (CHUNG; SINGH, 2008; LI et al., 2008). Originou-se do cruzamento de duas espécies selvagens naquele país há milhares de anos.

A disseminação da soja ocorreu com os comércios mercantis, que eram realizados através das navegações, sendo que em 1765 foi introduzida nos Estados Unidos da América (EUA). Inicialmente, teve a finalidade de cultura forrageira, posteriormente, em 1941, objetivou-se a produção de grãos, e esta superou a área cultivada com forragem (CHUNG; SINGH, 2008).

A soja chegou ao Brasil vinda dos Estados Unidos da América (EUA) em 1882 (EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA - EMBRAPA, 2004) e, desde então, vem sendo cultivada e melhorada para as diversas regiões do país. Até meados dos anos 1960, essa leguminosa não tinha valor comercial entre as demais culturas. No entanto, com o avanço nas pesquisas, no final da década de 1960, a soja obteve um desempenho agronômico satisfatório. A princípio, a produção concentrou-se na região Centro-Sul até o início dos anos 1980. Após esse período, o cultivo da soja se expandiu na região Centro-Oeste, tornando-se a cultura de maior importância econômica.

Atualmente, o Brasil é o segundo maior produtor mundial e o maior da América Latina. Com uma área de 33,13 milhões de hectares cultivados, com produção de soja que supera 98,98 milhões de toneladas de grãos. A região Centro-Oeste é responsável por 46,4% da produção nacional e o Estado de Mato Grosso é o maior produtor da oleaginosa no cenário nacional, contribuindo com 28,2% (COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB, 2016).

A produção nacional da soja abrange todas as regiões do país, concentrando-se nas regiões Centro-Oeste e Sul, com 45 e 35%, respectivamente, da área correspondente à cultura. Juntas essas regiões respondem por 80% da produção nacional de grãos de soja. Os Estados de Mato Grosso, Paraná, Rio Grande do Sul e Goiás são responsáveis por 71,7% da produção nacional (CONAB, 2016).

A soja é uma planta herbácea, ereta, de ciclo anual, pertence à classe *Dicotyledoneae*, ordem Rosales, família Leguminosae, subfamília Papilionaceae, tribo Phaseoleae, gênero *Glycine* (Moench) e, algumas características podem ser alteradas pelas condições ambientais (CHUNG; SINGH, 2008; DONG et al., 2004; SANTOS, 2008). Dessa forma, a cultura possui ampla plasticidade. Para Komatsu, Guadagnin e Borgo (2010), a plasticidade é a capacidade de se adaptar às condições ambientais e de manejo, por meio de modificações na morfologia da planta e nos componentes da produtividade agrícola.

A oleaginosa tem destaque economicamente, devido a sua importância na alimentação, por ter alto valor nutricional e um baixo custo. O maior interesse comercial é como fonte primária de óleo e proteína vegetal, mas a composição química das sementes de soja varia de acordo com a localização, clima, fertilidade, ataques de insetos, cultivares e com as práticas de manejo (ORF, 2013).

As sementes apresentam alto valor de proteínas (40%), lipídios (20%) (MORAES et al., 2006), além de celuloses, açúcares, cinzas e fibras. A combinação das proteínas e dos lipídios constituem cerca de 60% do peso seco dos grãos da soja. Conforme o relatório do U.S. Department of Agriculture -

USDA (2013), os óleos vegetais comestíveis mais produzidos no mundo, na safra 2012/2013 foram o da palma e da soja, com a produção mundial de 55,3 e 42,9 milhões de toneladas, respectivamente; sendo que o óleo de soja apresenta 15,6% de ácido graxo saturado (AGS), 22,8% de ácido graxo monoinsaturado (AGM) e 57,7% de ácido graxo poli-insaturado (AGP).

O óleo de soja apresenta alto teor de AGM e uma quantidade significativa de AGP, principalmente na forma de ácido graxo Omega 3 (ω-3) (ZAMBIAZI et al., 2007) e o ácido alfa-linolênico (ALA) (HE; CHEN, 2013). Esses AGP são essenciais para a nutrição humana e não são sintetizados pelo organismo humano, conforme documentado por Simopoulos, Leaf e Salem Júnior (1999). O consumo regular de alimentos ricos em ω-3 pode proporcionar muitos benefícios à saúde, incluindo a redução de doenças cardiovasculares (HE; CHEN, 2013) e o câncer de mama (MOUROUTI; PANAGIOTAKOS, 2013).

Os alimentos têm como finalidade suprir as necessidades nutricionais, além da prevenção de doenças causadas pelas deficiências nutricionais e à manutenção dos tecidos no ser humano (BASSINELLO; NAVES, 2006). Na literatura, a alimentação balanceada está relacionada à prevenção de doenças, entre elas, doenças cardiovasculares, câncer, osteoporose, artrite e degeneração muscular conforme a idade (COSTA; BORÉM, 2003).

As plantas transgênicas podem desempenhar um papel importante no futuro, como fonte de AGP (ADARME-VEGA; THOMAS-HALL; SCHENK, 2014), principalmente a cultura da soja (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS - FAO (2013) relata que a soja produzida é suficiente para fornecer um terço da necessidade global de proteínas dos alimentos, desde que, seja destinada totalmente para a alimentação humana. Nesse cenário, a soja se apresenta como uma cultura promissora para suprir parte das necessidades básicas da humanidade, devido sua composição química, além de grande potencial na prevenção de doenças crônicas.

2.2 Fixação biológica de nitrogênio

O nitrogênio (N) é classificado como um nutriente essencial às plantas, sendo o elemento mais abundante na atmosfera terrestre (cerca de 78,3% na forma de N_2) (PRADO, 2008). É constituinte de muitos componentes das células, entre eles, aminoácidos, proteínas, ácidos nucleicos e, da molécula de clorofila, a qual é responsável pela fotossíntese, cuja produção de fotoassimilados é de fundamental importância para a manutenção e desenvolvimento celular (TAIZ; ZEIGER, 2013).

Contudo, a planta não absorve o N_2, sendo necessária à transformação do N_2 gasoso para as formas assimiláveis: amônio (NH_4^+) ou nitrato (NO_3^-). Há três formas de transformação: fixação biológica (FBN), industrial e atmosférica (PRADO, 2008). No Brasil, o uso do nitrogênio na cultura da soja é totalmente oriundo da FBN, por se tratar de uma espécie leguminosa, resultando no melhor custo/benefício.

A FBN é um processo significativo no setor agrícola, sendo o processo biológico que contribui com a maior parte do N fixado. Estima-se que fornece cerca de 175 milhões de toneladas de N para a biosfera, ou seja, 65% do total, que o faz ser o segundo processo biológico mais importante do planeta depois da fotossíntese, junto a decomposição orgânica (MOREIRA; SIQUEIRA, 2006).

No Brasil, a FBN é essencial para o cultivo da soja, tornando-se uma das tecnologias mais difundida no cenário agrícola nacional. Na safra 2015/16 proporcionou uma economia de US$ 8,7 bilhões em fertilizantes nitrogenados. Além disso, evita a contaminação de águas subterrâneas com nitrato, oriundo do uso intensivo de fertilizantes nitrogenados na agricultura, pois o íon têm alta taxa de lixiviação no solo (FAGAN et al., 2007). Para Araújo et al. (2011) o nitrato e o nitrito podem reagir com aminas e amidas e formarem agentes cancerígenos a partir de compostos N-nitrosos.

A FBN é um processo no qual o nitrogênio atmosférico (N_2) é convertido em amônia (NH_3), catalisada por organismos vivos. Os organismos responsáveis pela fixação de nitrogênio, denominados de diazotróficos são procariotos e realizam a fixação por meio da enzima conhecida como nitrogenase. Essa enzima é sensível ao oxigênio, que pode destruí-la irreversivelmente. Essa reação é endergônica, isto é, a amônia é mais rica em energia que o nitrogênio atmosférico e, para que a reação ocorra, é necessário fornecimento de energia, armazenada na forma de ATP (SILVEIRA; FREITAS, 2007).

A introdução da FBN foi umas das grandes propulsoras para o cultivo em larga escala da soja no Brasil; as bactérias do gênero *Bradyrhizobium*, ao entrarem em contanto com o sistema radicular, infectam via pelos radiculares, para formar os nódulos (SCHONINGER et al., 2011). Com a finalidade de fixar o N_2 atmosférico, em troca, por ser organismos simbióticos, as plantas fornecem fotoasssimilados para as bácterias, formando-se uma associação planta-bactéria, em que ambas se beneficiam.

Os inoculantes recomendados para a cultura da soja têm a taxonômia das estipes classificadas em duas espécies conforme verificado por Chueire et al. (2003). Esses autores relatam que a SEMIA 587 e SEMIA 5019 (= 29 w), pertencem à espécie *Bradyrhizobium elkanii* e as duas outras, SEMIA 5079 (= CPAC 15) e SEMIA 5080 (= CPAC 7), à espécie *Bradyrhizobium japonicum*.

Estudos apontam para a eficiência da FBN na cultura da soja de acordo com a área de cultivo (FAGAN et al., 2007). Entretanto, há controvérsias sobre a eficiência de bactérias fixadoras em áreas que já são cultivadas com soja. Lima, Lopes e Lemos (1998) relatam que a nodulação promovida pelos rizóbios já estabelecidos no solo foi abundante, sendo que não foram constatadas diferenças expressivas devidas à inoculação.

Campos, Hungria e Tedesco (2001) não obtiveram resposta à prática da reinoculação na cultura da soja em área de plantio direto no Rio Grande do Sul, em razão da eficiência da população de *Bradyrhizobium* naturalizada do solo, que foi capaz de suprir as necessidades de nitrogênio da cultura. Esses autores também verificaram que a aplicação de uma dose elevada de nitrogênio mineral reduziu a massa nodular e não resultou em incrementos no rendimento da cultura.

Por outro lado, de acordo com Pavanelli e Araújo (2009), a inoculação da soja cultivada nos solos sob cultivo de pastagens e culturas anuais, oriundos de sete municípios da região oeste paulista, apresentou incrementos de nodulação e fixação de N. Para Alves et al. (2006) a FBN na cultura da soja, com inoculação de rizóbio e sob plantio direto, proporciona alta produtividade de grãos e balanço positivo de N para o sistema.

2.3 *Azospirillum brasilense*: importância e funções fisiológicas nas plantas

O solo é um ecossistema que apresenta uma grande diversidade de rizobactérias promotoras do crescimento de plantas (RPCPs), que podem ser de vida livre ou associada às raízes das plantas. Para Silveira e Freitas (2007), as RPCPs constituem de quaisquer bactérias que têm efeitos benéficos no crescimento de uma ou mais espécies vegetais, com exceção dos rizóbios que, apesar da relação benéfica ao crescimento vegetal, são resultado de uma relação simbiótica.

Bashan e Holguin (1998) modificaram o termo “rizobactérias” por “bactérias”, logo RPCP foi substituído BPCP, a fim de facilitar a nomenclatura. Esses autores também recomendaram a divisão do grupo BPCP: i) bactérias com efeito direto nos mecanismos fisiológicos ou bioquímicos (BPCB); ii) bactérias que atuam de forma indireta no controle biológico de patógenos (biocontrole-BPCB).

Na literatura, as bactérias diazotróficas mais estudadas como BPCBs associativas, pertencem ao gênero *Azospirillum.* Devido, principalmente, a sua utilização como inoculantes, comercializados no Brasil com recomendação para as culturas do trigo e milho. Hungria (2011) ao utilizar o *Azospirillum*, obteve aumentos de 31 e 26% na produtividade de grãos para as culturas do trigo e milho, respectivamente, porém, com fornecimento de parte do nitrogênio requerido pela planta pelo fertilizante mineral. Cabe salientar, que a bactéria *Azospirillum* é endofítica em gramíneas e associativa em culturas leguminosas (HUNGRIA, 2011). Para Araújo (2008) o *Azospirillum* apresenta as seguintes vantagens: antagonismo a agentes patogênicos; produz fitormônios; não é muito sensível às variações de temperatura e ocorre em todos os tipos de solo e clima.

Diversos trabalhos têm documentado o sucesso da utilização de *Azospirillum* na inoculação de plantas de arroz, trigo, milho (CANGAHUALA-INOCENTE et al., 2013; CASSÁN et al., 2001; HUNGRIA, 2011; HUNGRIA et al., 2010; FERREIRA et al., 2013). Portanto, as bactérias do gênero *Azospirillum* apresentam considerável potencial de aplicação em sistemas agrícolas. Nos relatos existentes na literatura, os autores ressaltam a contribuição do gênero *Azospirillum* quando inoculado em diversas espécies (especialmente gramíneas), para as variáveis de produção dessas culturas.

O *Azospirillum*, quando utilizado isoladamente, têm grande importância, cujos efeitos benéficos têm sido postulados, parcialmente, devido à capacidade de estimular a produção de hormônios vegetais em quantidades expressivas, o que resulta no crescimento das plantas. As pesquisas têm demonstrado a capacidade do *Azospirillum brasilense* em produzir auxinas, giberelinas e citocianinas em condições "*in vitro*" (ARSHAD; FRANKENBERGER

JÚNIOR, 1997; CACCIARI et al., 1989; CROZIER et al., 1988). Nesses estudos tem-se observado que a colonização por essas bactérias tem proporcionado à produção dos fitohormônios que estimulam a formação do sistema radicular, que resulta em uma maior eficiência na absorção dos nutrientes. Consequentemente, aumenta o crescimento, desenvolvimento e rendimento de culturas agronomicamente importantes em todo o mundo (BALDANI; BALDANI; DOBEREINER, 1987; FERREIRA et al., 2013; HUNGRIA et al., 2010).

Thuler et al. (2003) trabalhando com isolados de *Azospirillum spp.* no interior de raiz na cultura da mandioca, em meio "*in vitro*", verificaram a excreção de hormônios como ácido indol-3-acético (AIA) e etileno, além de reguladores de crescimento, como poliaminas, e produção de aminoácidos. Na literatura, há três vias metabólicas para a produção de AIA pelo *Azospirillum*: duas dependentes de triptofano, denominadas indole-3-acetamida (IAM) e indole-3-piruvato (IpyA), e a terceira via independente de presença de triptofano (DOBBELAERE et al., 1999; LAMBRECHT et al., 2000).

O AIA pertence à classe das auxinas, cuja função é atuar em vários processos de crescimento e alongamento, devido ao aumento da extensibilidade da parede, divisão e diferenciação celular. As GAs são fitohormônios que atuam no crescimento e desenvolvimento dos caules e folhas vegetais, atuando na altura das plantas, floração e no retardamento do envelhecimento dos tecidos vegetais (TAIZ; ZEIGER, 2013).

Barassi et al. (2008), ao avaliarem diversos trabalhos sobre as respostas fisiológicas induzidas por *Azospirillum*, destacaram o aumento do teor de clorofila, que reflete diretamente na melhoria dos parâmetros fotossintéticos, condutância estomática, melhoria no potencial hídrico, maior teor de prolina na parte aérea e raízes, incremento no teor de água do apoplasto, maior elasticidade da parede celular, aumento na altura das plantas e na fitomassa.

Bashan et al. (2005), trabalhando com o *Azospirillum brasilense* na cultura do trigo, verificaram aumento significativo na quantidade de vários pigmentos fotossintéticos, tais como clorofilas "a" e "b", e nos pigmentos fotoprotetivos auxiliares, como zeaxantina, ateroxantina, luteína, neoxantina e beta-caroteno, que resultariam em plantas mais verdes e sem estresse hídrico.

Diante do exposto, a aplicação de *Azospirillum brasilense* poderá estimular a produção de fitohormônios na cultura da soja, além de incrementar o aparato fotossintético. Todos esses fatores abordados poderão promover uma melhor fotossíntese nas plantas, resultando em uma maior produtividade da cultura. Empresas do ramo de fertilizante/inoculantes já relatam o ganho produtivo do produto, porém, o *Azospirillum brasilense* ainda não é registrado no Ministério da Agricultura e Abastecimento (MAPA) para a cultura da soja.

2.4 Coinoculação *Bradyrhizobium japonicum* e *Azospirillum brasilense*

Os trabalhos com a bactéria *Azospirillum brasilense* em leguminosas ainda são muito incipientes. Entretanto, as pesquisas já realizadas abordam resultados benéficos da combinação da bactéria *Azospirillum brasilense* com a *Bradyrhizobium japonicum* utilizadas na coinoculação das sementes conforme verificado por vários autores (CASSÁN et al., 2009; GROPPA; ZAWOZINIK; TOMARO, 1998; IRUTHAYATHAS; GUNASEKASAN; VLASSAK, 1983; JUGE et al., 2012). Para Bárbaro et al. (2011), a coinoculação ou inoculação mista consiste no uso de combinações de diferentes microrganismos, os quais produzem um efeito múltiplo, superando os resultados obtidos com os mesmos, quando utilizados na forma isolada.

Benintende et al. (2010), comparando o efeito dessa coinoculação, verificaram estimulação no crescimento, nodulação e acúmulo de nitrogênio. A coinoculação das bactérias nas sementes tem promovido incremento na produção vegetal, devido a maior fixação de nitrogênio pelos microrganismos.

Ao utilizar o *Azospirillum brasilense*, vem sendo observado o efeito benéfico da associação entre os microrganismos, principalmente pela capacidade que as bactérias desse genêro têm de produzir fitohormônios que promovem um maior desenvolvivento do sistema radicular e, portanto, a possibilidade de explorar um volume mais amplo de solo (BÁRBARO et al., 2008).

De modo geral, ocorre a potencialização da nodulação e maior crescimento radicular, em resposta à interação positiva entre as bactérias simbióticas (*Bradyrhizobium*) e as bactérias diazotróficas, em especial as pertencentes ao gênero *Azospirillum* (BÁRBARO et al., 2011). No entanto, os resultados obtidos com a inoculação combinada em leguminosas pode apresentar respostas contraditórias, ou seja, tanto estimular como inibir a formação de nódulos e o crescimento radicular em um sistema simbiótico, variando em função do nível de concentração do inóculo e do tipo de inoculação (BÁRBARO et al., 2008), sendo necessária a realização de novos testes para comprovar a viabilidade da coinoculação.

A estimulação da nodulação pela inoculação de leguminosas com *A. brasilense* pode estar relacionada com o incremento na indução da expressão de genes *Nod*, responsáveis pelo incremento de raízes laterais, da densidade de pelos radiculares e das ramificações dos seus pelos. Foi verificado em vários estudos aumento na produtividade de leguminosas com a utilização de *A. brasilense,* incorporado na coinoculação apresenta valores superiores à inoculação de *Bradyrhizobium* (BURDMANN; HAMAQUI; OKON, 2000 apud BÁRBARO et al., 2009).

As interações biológicas de *Bradyrhizobium* com outras bactérias do solo têm sido objeto de interesse, pela sua evidente repercussão econômica, pois melhora a nodulação e maior crescimento, em resposta à interação positiva entre as bactérias simbióticas (no caso *Bradyrhizobium*) e as bactérias diazotróficas, em especial as pertencentes ao gênero *Azospirillum* (BÁRBARO et al., 2008).

3 CONCLUSÃO

Os resultados do presente trabalho permitem concluir que:

Artigo 1:

- A inoculação com as bactérias *Bradyrhizobium japonicum* influência todos os parâmetros estudados. Observou-se um melhor desempenho morfosiológico e maior nodulação da soja.

- O uso de *Azospirillum brasilense* isolado ou com coinoculação com as *Bradyrhizobium japonicum* não promove influência nas variáveis avaliadas.

Artigo 2:

- A inoculação ou não de *Azospirillum brasilense* associado à *Bradyrhizobium japonicum* não afeta os caracteres agronômicos e a produtividade dos grãos em cultivares de soja RR®.

Artigo 3:

- A pulverização foliar de doses de *Azospirillum brasilense* não afeta os caracteres agronômicos e a produtividade dos grãos em cultivares de soja RR®.

Artigo 4:

- Independente da cultivar de soja, a aplicação de até 700 mL por ha do inoculante com bactérias de *Azospirillum brasilense* aplicado no estádio V_3 das plantas, não afeta a produtividade, o potencial fisiológico e a incidência de danos das sementes.

Portanto, o uso *Azospirillum brasilense* não promove benefícios nos caracteres morfoagronômicos em cultivares de soja RR®. Além disso, estes microrganismos não são registrados para esta cultura, conforme constatado em abril de 2016 no Ministério da Agricultura, Pecuária e Abastecimento (MAPA). Dessa forma, deve-se realizar novos estudos como o objetivo de selecionar estirpes eficientes para a cultura da soja.

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SEGUNDA PARTE - ARTIGOS

ARTIGO 1 Co-inoculation of *Bradyrhizobium japonicum* and *Azospirillum brasilense* in the soybean crop[1]

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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 Universidade Federal de Lavras (UFLA), no setor de Grandes Culturas do Departamento de Agricultura, 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 seeds) and two application rates of *Bradyrhizobium japonicum* (0 and 3.0 mL kg^{-1} of seeds), 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 seeds 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 effect on the parameters evaluated.

Keywords: *Glycine max* (L.) Merrill, 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 fitohormônios. 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 casa de vegetação 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 sementes) e duas doses de *Bradyrhizobium japonicum* (0 e 3,0 mL kg^{-1} de sementes), 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 trifólios, 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 sementes, proporciona melhor desempenho morfofisiológico e maior nodulação na cultura da soja cultivada em vasos. A utilização de *Azospirillum brasilense* isoladamente e em coinoculação com *Bradyrhizobium japonicum* não promove efeitos nos parâmetros avaliados.

Palavras-chave: *Glycine max* (L.) Merrill, fitohormônios, 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 than 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 increased 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 or 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). Thus they 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 Universidade Federal de Lavras (UFLA), no setor de Grandes Culturas do Departamento de Agricultura, 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 dystrophic red latosol (Embrapa, 2013), 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.

Table 1 - Chemical composition of the soil dystrophic red latosol (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	0.3	0.1	0.1	2.9	0.5	3.4	0.8	20	2.4	14.0

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

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 seeds - strains AbV$_5$ and AbV$_6$), which is respectively, 0,2 x 10^4, 4 x 10^4, 6 x 10^4 and 8 x 10^4 CFU/seed and, two application rates of *Bradyrhizobium japonicum* (0 and 3.0 mL kg^{-1} of seeds), with 0 and 1.8 x 10^6 CFU/seed. *Azospirillum brasilense* strains were derived from the inoculant Azo® (1 x 10^8 CFU/mL) and *Bradyrhizobium japonicum* inoculant from Nitragin Cell Tech HC® (3 x 10^9 CFU/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 (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.

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
	cm	unit	g		
B. japonicum (B)					
P (Value) (B)	<0.01**	<0.01**	<0.01**	<0.01**	<0.01**
A. brasilense (A) (mL kg^{-1})					
0	55.37	16.12	7.85	3.79	0.97
0.5	54.75	16.62	7.83	3.86	0.90
1.0	53.62	16.25	7.99	3.50	0.83
1.5	53.37	15.75	6.82	3.06	0.78
2.0	53.75	15.25	6.71	3.40	0.75
Average	54.17	16.00	7.44	3.52	0.85
P (Value) (A)	0.8461^{ns}	0.8215^{ns}	0.1766^{ns}	0.3074^{ns}	0.1221^{ns}
B x A	0.9904^{ns}	0.6959^{ns}	0.6375^{ns}	0.9269^{ns}	0.1519^{ns}
CV (%)	7.58	15.01	18.03	23.06	21.34

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

Continued **Table 2**.

Sources of variation	RV	CC	LNC
	cm^3	-	g kg
B. japonicum (B)			
P (Value) (B)	<0.01**	<0.01**	<0.01**
A. brasilense (A) (mL kg^{-1})			
0	28.75	35.30	23.97
0.5	30.25	35.70	23.46
1.0	26.62	35.56	23.27
1.5	25.12	35.42	22.55
2.0	24.87	34.20	22.41
Average	27.12	35.24	23.13
P (Value) (A)	0.2220^{ns}	0.9457^{ns}	0.8827^{ns}
B x A	0.1122^{ns}	0.5712^{ns}	0.9981^{ns}
CV (%)	19.65	11.27	14.82

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

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. Thus it is possible that there was competition between the bacteria of the genus *Bradyrhizobium* or even of native species, limiting 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 recommended 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).

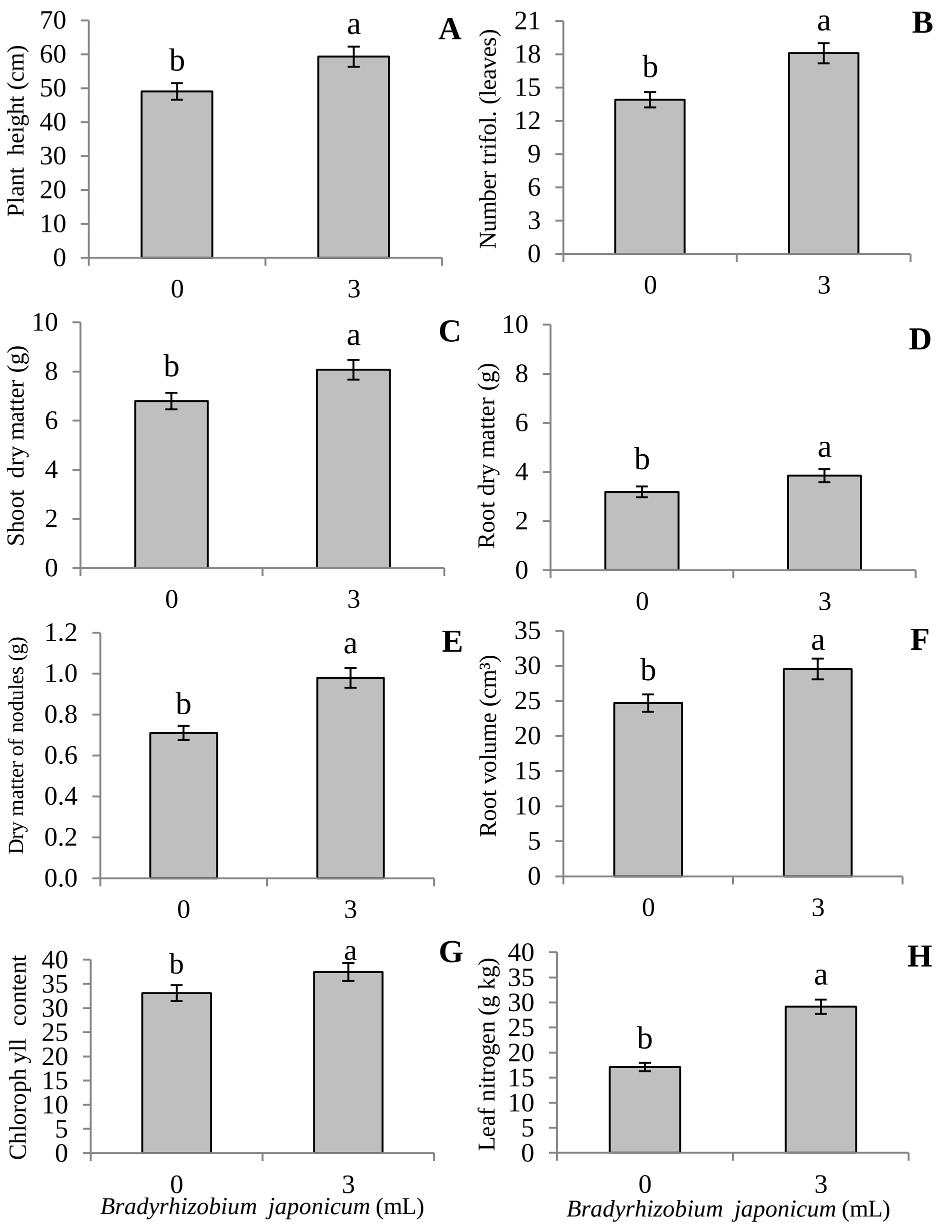

Figure 1 - Plant height, shoot dry matter, root dry matter, dry matter of nodules, root volume, chlorophyll content, leaf nitrogen, subjected to application of *Bradyrhizobium japonicum* in soybean. Lavras, MG, Brazil, 2013/2014 crop season. Mean values followed by the same lowercase letter do not differ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

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, consequently, 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 (OM) 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 may be the consequence of 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* affects all the variables studied, and a better morphophysiological performance; a greater nodulation of soybean also was observed. The use of *Azospirillum brasilense* alone or in co-inoculation with *Bradyrhizobium japonicum* does not have a effect on the variables evaluated.

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ARTIGO 2 Morphoagronomic and yield traits of RR® soybean due to inoculation via *Azospirillum brasilense* groove[2]

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ABSTRACT: In the last decades, the Brazilian soybean productive chain has passed through a transformation process in which the yield, efficiency, profitability, economic and environmental sustainability are very important issues. In this context, the introduction of microorganisms has provided an increase in grains yield. The objective of this study was to evaluate the inoculation of *Azospirillum brasilense* associated with *Bradyrhizobium japonicum* in agronomic traits and the soybean productivity. The experiment was conducted in randomized blocks in 4 x 2 factorial, with four cultivars (Anta 82 RR®, BRS Favorita RR®, BRSMG 780RR®, BRSMG 820RR®) and two treatments with *Azospirillum brasilense* (inoculated and non-inoculated) with three replications in two growing seasons. The following traits were evaluated: plant height, shoot dry biomass, chlorophyll content, leaf nitrogen content at flowering; and at harvest, there were evaluated the plant height, the insertion of the first pod, number of pods per plant, number of grains per pod, thousand-grain weight, grain yield and grain harvest index. There was a significant effect of growing seasons and cultivars in an isolated way, and interaction of them in most traits. The conditions in which the study was conducted, with or without inoculation of *Azospirillum brasilense* associated with *Bradyrhizobium japonicum* do not affect the agronomic traits and grain yield in RR® soybean cultivars.

Key words: *Glycine Max* (L.) Merrill, growth promoting bacteria, *Rhyzobium.*

Introduction

Soybean [*Glycine max* L. Merrill] is an oilseed of great economic importance in the national and international market due to the high levels of protein and oil in their seeds/grains (Lima et al., 2015). The cultivation has been widely studied due to its high nutritional value and great consumer market. FAO (2013) reports that the soybean produced is enough to provide one-third of the global need of food proteins, since it is completely intended for human consumption.

In this scenario, Brazil is the second largest world producer and exporter of soybeans, with 31,902,400 ha^{-1} of sown area, with an average grain yield of 3011 kg ha^{-1} in the 2014/2015 harvest (Conab, 2015). The Brazilian soybean productive chain has undergone modernization processes, which provide the increase in grain yield (Zuffo et al., 2015a).

The introduction of *Bradyrhizobium*, which perform the biological nitrogen fixation (BNF), was one of the major drivers for large-scale soybean cultivation in Brazil (Zuffo et al., 2015b). Therefore, the technological advances in soil microbiology area are important for the viability of soybean cultivation. Besides these bacteria, the soil is an ecosystem that has a great variety of plant growth promoting bacteria (PGPBs), that can be free-living or associated with plant roots.

For Silveira and Freitas (2007), PGPBs constitute of any bacteria that have beneficial effects on the growth of one or more vegetal species, except for the rhizobia that despite the beneficial relation to vegetal growth, is the result of a symbiotic relationship. In the literature, the most studied diazotrophs bacteria as associative PGPBs, belong to the genus *Azospirillum*. Mainly due to its use as inoculants, commercialized in Brazil with a recommendation for the grasses. Hungria (2011), while using the *Azospirillum,* noted increases of 31 and 26% for grain yield in wheat and corn crops, respectively, but with supply of part of the nitrogen required by the plant by the mineral fertilizer.

For Araújo (2008), the *Azospirillum* has the following advantages: the bacteria is endophytic, i.e., penetrates the roots of plants; presents antagonism to pathogens; produces phytohormones; it is not very sensitive to temperature variations and occurs in all kinds of soil and climate. Among the plant hormones, research has demonstrated the ability of *Azospirillum brasilense* in producing auxin, gibberellins, cytokinins under "in vitro" conditions (Masciarelli et al., 2013).

The use of inoculation with *Azospirillum brasilense* in leguminous plants has been studied, however the effects are still contradictory. Reports presented by Barbaro et al. (2009), Hungria et al. (2013), Hungria et al. (2015) show influences on the agronomic traits of soybean crop, but the results checked by Gitti et al. (2012), Zuffo et al. (2015a), do not support the authors mentioned above.

Therefore, the objective was to evaluate the inoculation via *Azospirillum brasilense* groove and its influence on agronomic traits and soybean yield.

Material e Methods

The experiment was conducted in the 2013/14 and 2014/15 growing seasons, in Lavras - MG, no Centro de Desenvolvimento Científico e Tecnológico em Agropecuária - Fazenda Muquém, located at latitude 21°12'S, 44°58'W longitude and altitude of 918 m in soil classified as dystrophic red latosol (Emprapa, 2013), with clayey texture, with the following textural values: clay: 640 g kg^{-1}; silt: 200 g kg^{-1}; sand: 160 g kg^{-1}. The chemical composition of the experimental area soil is shown in Table 1.

Table 1. Chemical composition of a dystrophic red latosol (0.0-0.2m) before the experiment installation, Lavras-MG, Brazil, for the 2013/14 and 2014/15 growing seasons.

Growing Season	pH	Ca^{2+}	Mg^{2+}	Al^{3+}	H^++Al^{3+}	SB	P	K	OM	V
	H_2O	------------ $cmol_c$ dm^{-3}				------------------	- mg dm^{-3} -		dag kg^{-1}	%
2013/14	6.4	5.0	1.4	0	2.9	6.7	11.4	118	3.4	69.8
2014/15	6.2	3.8	0.8	0	0.9	4.8	20.8	92	2.2	83.5

H + Al: potential acidity; SB: sum of bases; MO: organic matter; V: base saturation

The climate is Cwa according to the Köppen classification, with average annual temperature of 19.3ºC and normal annual rainfall of 1,530 mm (Dantas et al., 2007). Climatic data during the experiments were collected at the weather station of the National Institute of Meteorology (INMET) located at the Federal University of Lavras-UFLA and are presented in Figure 1.

The experimental design was a randomized block, arranged in a 4 x 2 factorial, with four cultivars (Anta 82 RR®, BRS Favorita RR®, BRSMG 780RR®, BRSMG 820RR®) and two treatments with *Azospirillum brasilense* (inoculated and non-inoculated) with three replications. Each plot consisted of four sowing rows of 5 m in length spaced in 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 the useful area.

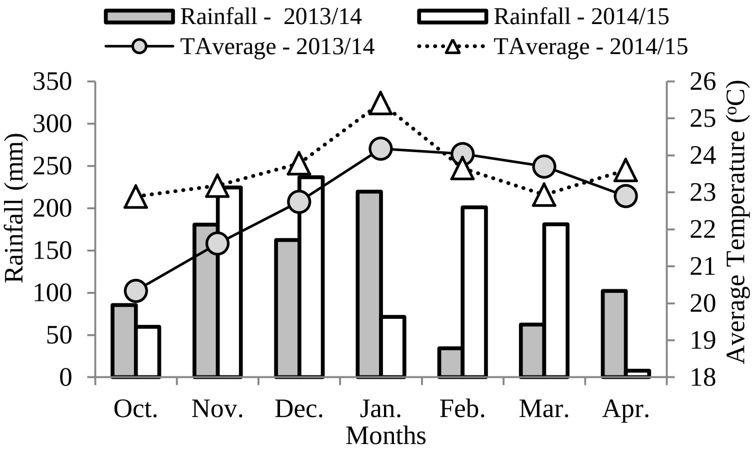

Figure 1. Monthly means for rainfall and air temperature in Lavras-MG, Brazil, for 2013/14 and 2014/15 growing seasons, during the experiment evaluations. Source: National Institute of Meteorology (INMET).

The sowing was carried in November of each growing season. Fertilization consisted of 350 kg ha^{-1} of the N-P_2O_5-K_2O (02-30-20) formulated, applied via groove. *Bradyrhizobium japonicum* (Brad) and *Azospirillum brasilense* (Azos) bacteria were inoculated via groove after soybean sowing. The dosage of *Bradyrhizobium japonicum* was 18 ml kg^{-1} of seed - SEMIA 5079 and 5080 strains, containing 10.8x10^{6} CFU/seed of the inoculant Nitragin Cell Tech HC® (3x10^{9} CFU/ml). For *Azospirillum brasilense* was used the dosage of 6 ml kg^{-1} of seed - AbV5 AbV6 strains, containing 24x10^{4} CFU/seed of the inoculant Azo® (1x10^{8} CFU/ml).

The microorganisms inoculation was carried out using a motorized backpack sprayer, coupled to the bar with four spray tips XR 110.02, applying spray volume equivalent to 150 L ha^{-1}. First, there was inoculated *Bradyrhizobium japonicum*, and then the *Azospirillum brasilense*.

At the beginning of flowering (R_1) was determined: plant height - with assistance of a millimeter rule; shoot dry biomass - using a forced air circulation oven at 60 °C for 72 hours until constant weight, with posterior plant residues weighting. It was held also the collection of leaves (third trifoliate from top to bottom) then washed in deionized water and placed with the shoot dry biomass for drying. The dried leaves were ground in a Wiley mill. Chemical analysis of leaf tissue of macro and micronutrients were conducted according to the methodology described by Sarruge and Haag (1974); leaf chlorophyll content - using chlorophyll portable model SPAD 502 Plus® measuring 3 points in each trifoliate leaf in different parts on the same leave, always in the leaf blade between the nerves in the third trefoil from top to bottom.

At harvest the following traits were obtained: plant height and insertion of the first pod - with a millimeter ruler assistance. Later, was held the collection of five plants per plot to assess the number of pods per plant and number of grains per pod - through manual counting; thousand grain weight - according to the methodology described in Brasil (2009); grain yield - standardized to grain moisture of 13% in kg ha^{-1}. It was also determined the grain harvest index (GHI) in the following way: GHI = grain yield/grains productivity + straw.

Individual and joint variances analyses were performed adopting the statistical model and an analysis procedure similar to that presented by Ramalho et al. (2012). The means were grouped by the Scott-Knott test (1974). Statistical analysis was performed with assistance of statistical package SISVAR® (Ferreira, 2011).

Results and Discussion

Except for plant height on flowering, the nitrogen amount on leaf tissues and the height of insertion of the first pod, it was found that the growing season can have a significant ($P \leq 0.01$) influence on the studied variables (Table 2 and 3). This results are in accordance with Felisberto et al. (2015), that also observed differences on soybean agronomic variables according to the crop year.

The number of grain per pod and the grain harvest index were not significantly influencied by cultivars (Table 2). Soares et al. (2015a) and Felisberto et al. (2015) observed an effect of cultivars on the agronomic traits evaluated. The authors affirm that it was expected, since cultivars have different genetic background, growth habit, maturation group and other characteristics, providing variations.

Regarding the inoculation with *Azospirillum brasilense*, no statistical differences on the evaluated characteristics were observed. Zuffo et al. (2015b) also observed no differences with the inoculation of soybean seeds with only *Azospirillum brasilense* or co-inoculated with *Bradyrhizobium japonicum* on the plant height, number of trefoil, shoot dry biomass and roots, dry mass of nodules, root volume, chlorophyll content and leaf nitrogen, in accordance with the results of this study.

When the interaction between cultivar x growing season was studied, statistical differences were observed for the first pod insertion height, yield and grain production components (Table 2 and 3). The interaction between cultivar x growing season was also verified by Soares (2015b), indicating that the responses, considering the environmental variations, were not the same for the evaluated cultivars. It was expected, since the growing seasons presented differences for rainfall occurrence and temperatures, variations that are unpredictable and the researcher do not have control.

Table 2. Analysis of variance and means for plant height at flowering (PHF), shoot dry biomass (SDB), chlorophyll content (CLC) and N (NC) in leaves tissues of RR® soybean on flowering stage (R_1), obtained with inoculation and non-inoculation of *Azospirillum brasilense* with *Bradyrhizobium japonicum* in RR® soybean cultivars, to 2013/14 and 2014/15 growing seasons. Lavras-MG, Brazil.

Sources of variation	DF	ANOVA (QM)[1]			
		PHF	SDB	CLC	NC
		- - cm - -	- - g - -	-	- g kg -
Year (Yr)	1	9.72^{ns}	15330.20**	171.25**	10.45^{ns}
Cultivar (C)	3	255.90**	166.77*	19.19*	49.88**
Azospirillum (Az)	1	9.01^{ns}	121.28^{ns}	0.14^{ns}	7.36^{ns}
Yr x C	3	36.72^{ns}	95.70^{ns}	3.48^{ns}	19.26^{ns}
Yr x Az	1	1.47^{ns}	68.45^{ns}	6.35^{ns}	20.28^{ns}
C x Az	3	45.59^{ns}	81.31^{ns}	2.03^{ns}	5.33^{ns}
Yr x C X Az	3	5.04^{ns}	112.54^{ns}	2.07^{ns}	0.38^{ns}
Error	28	16.12	36.76	5.50	7.29
Mean	-	53.47	57.88	39.54	43.82
CV (%)	-	7.51	10.47	5.93	5.98
Factors		Means			
Growing Season[2]					
2013/14		53.02 a	75.76 a	41.43 a	43.35 a
2014/15		53.92 a	40.01 b	37.65 b	44.29 a
Azospirillum[2]					
Presence		53.04 a	59.47 a	39.48 a	44.21 a
Absence		53.90 a	56.29 a	39.60 a	43.43 a
Cultivars[3]					
Anta 82 RR®		49.23 c	62.75 a	38.17 b	44.37 a
BRS Favorita RR®		50.23 b	55.01 b	40.69 a	44.65 a
BRSMG 820RR®		49.23 c	54.92 b	38.76 b	40.84 b
BRSMG 780RR®		59.15 a	58.86 a	40.53 a	45.43 a

[1] ** and * significant for 1 and 5% of probability, respectively, for F test; ^{ns} – non-significant; MS – Means Square; DF – degree of freedom; CV – coefficient of variation.

[2] means followed by the same letter have no difference, according to F test.

[3] means followed by the same lowercase in the column are from the same group, according to Scott Knott (1974) test at 5% of probability.

Table 3. Variance analysis and means for plant height at harvest (PHH), insertion of the first pod (IFP), number of pods per plant (NPP), number of grains per pod (NGP), thousand-grain weight (TGW), grain yield (GY) and grain harvest index (GHI) on RR® soybean cultivars at maturation (R_8), with inoculation and non-inoculation of *Azospirillum brasilense* with *Bradyrhizobium japonicum* on RR® soybean cultivars, to 2013/14 and 2014/15 growing seasons. Lavras-MG, Brazil.

Sources of variation	GL	ANOVA (QM)[1]			
		PHH	IFP	NPP	NGP
		- - - - - cm - - - - -		- - - - - unity- - - -	
Year (Yr)	1	3898.80**	0.27^{ns}	3560.40**	0.66**
Cultivar (C)	3	81.48*	34.20**	137.92*	0.07^{ns}
Azospirillum (Az)	1	18.00^{ns}	0.12^{ns}	65.80^{ns}	0.16^{ns}
Yr x C	3	38.32^{ns}	15.38**	251.19**	0.27*
Yr x Az	1	7.52^{ns}	0.96^{ns}	11.40^{ns}	0.04^{ns}
C x Az	3	19.20^{ns}	3.91^{ns}	97.42^{ns}	0.04^{ns}
Yr x C X Az	3	22.28^{ns}	7.53^{ns}	52.88^{ns}	0.21^{ns}
Error	28	19.75	2.63	53.69	0.08
Mean	-	70.77	13.55	59.10	2.07
CV (%)	-	6.28	11.67	12.40	13.77
Factors		Means			
Growing Season[2]					
2013/14		79.78 a	13.47 a	67.71 a	2.19 a
2014/15		61.75 b	13.62 a	50.49 b	1.95 b
Azospirillum[2]					
Presence		71.38 a	13.50 a	60.27 a	2.01 a
Absence		70.15 a	13.60 a	57.93 a	2.13 a
Cultivars[3]					
Anta 82 RR®		72.35 a	11.13 b	57.36 b	2.06 a
BRS Favorita RR®		66.88 b	13.65 a	55.18 b	2.08 a
BRSMG 820RR®		72.15 a	14.80 a	62.13 a	2.16 a
BRSMG 780RR®		71.70 a	14.61 a	61.73 a	1.96 a

[1] ** and * significant for 1 and 5% of probability, respectively, for F test; ^{ns} – non-significant; MS – Means Square; DF – degree of freedom; CV – coefficient of variation.

[2] means followed by the same letter have no difference, according to F test.

[3] means followed by the same lowercase in the column are from the same group, according to Scott Knott (1974) test at 5% of probability.

Continued **Table 3**.

Sources of variation	GL	ANOVA (QM)¹		
		TGW	GY	GHI
		- - - g - - -	kg ha^{-1}	-
Year (Yr)	1	722.68**	8037206.39**	0.1435**
Cultivar (C)	3	4049.74**	1218618.91**	0.0080^{ns}
Azospirillum (Az)	1	10.87^{ns}	9157.97^{ns}	0.0002^{ns}
Yr x C	3	720.77**	543960.63**	0.0061^{ns}
Yr x Az	1	196.62^{ns}	35154.75^{ns}	0.0009^{ns}
C x Az	3	10.46^{ns}	200718.36^{ns}	0.0040^{ns}
Yr x C X Az	3	50.54^{ns}	258302.26^{ns}	0.0012^{ns}
Error	28	87.90	144445.51	0.0028
Mean	-	158.04	3583.63	0.50
CV (%)	-	5.93	10.61	10.95
Factors				
Growing Season²				
2013/14		154.16 b	3993 a	0.56 a
2014/15		161.92 a	3175 b	0.45 b
*Azospirillum*²				
Presence		157.57 a	3597 a	0.50 a
Absence		158.52 a	3570 a	0.50 a
Cultivars³				
Anta 82 RR®		133.77 c	3532 b	0.50 a
BRS Favorita RR®		177.99 a	3229 c	0.53 a
BRSMG 820RR®		163.03 b	4003 a	0.47 a
BRSMG 780RR®		157.37 b	3571 b	0.50 a

[1] ** and * significant for 1 and 5% of probability, respectively, for F test; ^{ns} – non-significant; MS – Means Square; DF – degree of freedom; CV – coefficient of variation.

[2] means followed by the same letter have no difference, according to F test.

[3] means followed by the same lowercase in the column are from the same group, according to Scott Knott (1974) test at 5% of probability.

For the interactions between growing season x *Azospirillum brasilense*, cultivar x *Azospirillum brasilense* and growing season x cultivar x *Azospirillum brasilense,* no statistical differences were observed (Table 2 and 3). Therefore, it can be inferred that the inoculation with *Azospirillum brasilense* have no relation with cultivar and growing season. This fact can be explained by the absence of variability of the “variation cause” (*Azospirillum brasilense*).

The highest values for shoot dry biomass, chlorophyll content, plant height on harvest, number of pods per plant, number of grains per pod, grain yield and harvest index were obtained during the 2013/14 growing season. It was due to the climatic conditions (Figure 1), mainly during January flowering, when a high pluviometric index was observed for the 2013/14 growing season.

The highest values for number of pods per plant and number of grains per pod affected directly on grain yield and higher harvest index. On the other hand, the thousand-grain weight was lower for this growing season, presumably by the increase of drain (number of pods and number of grain per pod), with less amount of photoassimilates for each grain.

The efficiency of *Azospirillum brasilense* soil inoculation on soybean culture can be related to the low competition between the soil microflora and the native bacteria from *Azospirillum* genus (Didonet et al., 2000). The authors also conclude that besides the inoculant quality, the inoculation process is crucial to achieve a higher number of viable bacteria. Therefore, it is possible that a competition with *Bradyrhizobium* or native bacteria prevented the beneficial effect of *Azospirillum brasilense* on the development of agronomic traits and the soybean grain yield on these study conditions.

Studies that showed benefits in using *Azospirillum brasilense* in leguminous crops (Hungria et al., 2013; Hungria et al. 2015) did not showed benefits of the leguminous inoculation with *Bradyrhizobium japonicum* and *Azospirillum*, possible because of an increase in the nodulation and N_2 fixation or even due to indirect factors that can be involved. The same authors describe that the resistance to water deficit can be increased and Yadegari et al. (2008) affirm that this is due to the involved bacteria. Otherwise, even with a low rainfall index observed on February and March of 2013/14 (Figure 1), in the present study these benefits were not observed.

In a general manner, cultivars presented satisfactory agronomic characteristics (Table 2). On the economic point of view, the grain yield is more important than the other components, and all cultivars presented values above average for the growing season in Minas Gerais state - 2658 kg ha^{-1}, achieved during 2014/2015 growing season (CONAB, 2015). The BRSMG 820RR® reached the grain yield of 4003 kg ha^{-1}, 50% more than the grain yield for the 2014/15 growing season. However, it should be noticed that BRSMG 820RR® is the latest cultivar (RM 8.2) when compared to the others.

For the interaction between cultivar x growing season, it was observed that the first pod insertion height for the cultivar Anta 82 RR® was lower, mainly during the 2014/15 growing season (Figure 2A). For the number of pods per plant, BRSMG 820RR® presented the higher mean for the 20013/14 growing season (Figure 2B). For the number of grains per pod during the 2013/14 growing season, no statistical differences were observed between cultivars, otherwise, when the subsequent growing season were evaluated, it was observed that BRSMG 820RR® presented the higher value (Figure 2C). For thousand-grain weight, BRS Favorita RR® presented the highest mean during the two evaluated years, but during the 2014/15 crop year it was not different from BRSMG 820RR® cultivar (Figure 2D). For the grain yield, the cultivars BRSMG 820RR® and BRSMG 780RR® presented better performance for the 3013/14 growing season and the cultivars Anta 82 and BRSMG 820RR® during the 2014/15 growing season, showing the plasticity of the BRSMG 820RR® cultivar (Figure 2E).

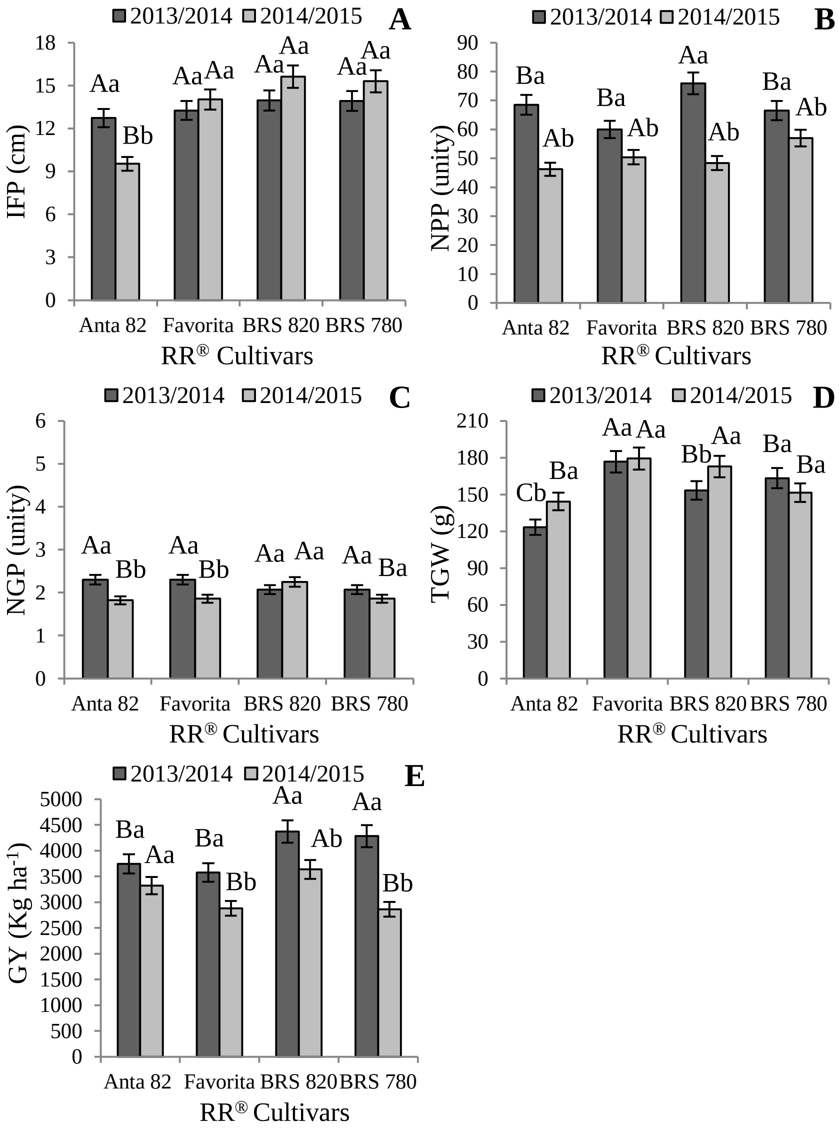

Figure 2. Insertion of the first pod – IFP (A), number of pods per plant – NPP (B), number of grains per pod – NGP (C), thousand-grain weight – TGW (D), grain yield – PG (E) of RR® soybean cultivars for the 2013/2014 and 2014/2015 growing seasons, in Lavras, MG, Brazil. Means followed by the same letter, upper case on the same growing season and lower case for the same cultivar, are from the same group, according to Scott Knott (1974) test at 5% of probability.

As previously reported, the interaction between growing season x cultivar is an expected fact and, in addition, each cultivar can present intrinsic characteristics an according to the environmental conditions, the agronomic and productive characteristics can be influenced. Thus, characteristics like plasticity of a cultivar are very important, so it can have the ability to modify its morphology depending on environmental conditions.

According to the results and taking into consideration the conditions that this study was conducted, the use or not of *Azospirillum brasilense* inoculation, did not affect the evaluated agronomic variables and the grain yield in RR® soybean cultivars.

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ARTIGO 3 Soybean cultivars agronomic performance and yield according to doses of *Azospirillum brasilense* applied to leaves[3]

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Abstract

Alternative methods of inoculation such as the application of the bacteria *Azospirillum brasilense* via spray in an advanced stage of the crop can increase the soybean performance. The aim of this study was to evaluate the agronomic traits and grain yield in soybean crops through *Azospirillum brasilense* doses applied by spray coverage. The experiment was conducted in randomized blocks in a 4 × 6 factorial, with four cultivars (Anta 82 RR®, BRS Favorita RR®, BRSMG 780RR®, BRSMG 820RR®) and six doses of *Azospirillum brasilense* (0, 300, 400, 500, 600, 700 mL ha^{-1}) with three replications in two crop years. It was evaluated the characters plant height, shoot dry mass, chlorophyll content and leaf nitrogen content at flowering. At harvest the evaluated characters were plant height, height of the first pod insertion, number of pods per plant, number of grains per pod, thousand grain mass, grain yield and grain harvest index. For the conditions that this study was carried out and according to the results, the spray with different *Azospirillum brasilense* doses did not affect the agronomic variables studied and the grain yield in RR® soybean cultivars.

Key words: agronomic traits; growth promoting bacteria; *Glycine max* (L.) Merrill

Abbreviations: BNF_ Biological nitrogen fixation; RR_Roundup Ready; PHF _Plant height on flowering; SDM_Shoot dry mass; CC_chlorophyll content; NC_N content; PHH_Plant height at harvest; IFP_height of the first pod insertion; NPP_Number of pods per plant; NGP_Number of grains per pod; TGM_Thousand grain mass; GY_Grain yield; GHI_ Grain harvest index. RGM_ Relative maturity group; CFU_ Colony Forming Unit; R_1_ Phenological Stage – flowering; SPAD_Chlorophyllmeter; GHI_Grain harvest index.

Introduction

Soybean [*Glycine max* (L.) Merrill] is one of the most important crops in Brazil and worldwide. According to the Brazilian National Company of Food and Supply - Conab (2015), in 2014/15 crop year, the soybean area under cultivation in Brazil reached approximately 31,902,400 ha, with an average grain yield of 3011 kg ha^{-1}.

From the perspective of nitrogen fertilization on soybeans, the use of *Bradyrhizobium*, which performs the biological nitrogen fixation (BNF), was one of the major driving for large-scale cultivation in Brazil (Zuffo et al., 2015a). However, when considering the current limitations (high temperatures, water stress, nutritional factors and the seed treatments with fungicides and insecticides) and potential benefits of BNF on soybeans and also the promoted benefits on different crops with the inoculation of *Azospirillum brasilense*, it can be inferred that these bacteria could increase the soybean crop performance (Hungria et al., 2007).

Bacteria of the genus *Azospirillum* have the advantages of being endophytic or can penetrate plant root, presents antagonism to pathogens, are able to produce phytohormones, are not very sensitive to temperature variations and can be present in all soil and climate types (Araujo, 2008). Among the plant hormones, research has demonstrated the ability of *Azospirillum brasilense* to produce auxin, gibberellins and cytokinins under "in vitro" conditions (Masciarelli et al., 2013).

It is possible to find on literature the positive effects of *Azospirillum brasilense* use on legume crops (Hungria et al., 2013; Hungria et al. 2015), however, it is questionable if the benefits are only due to increased nodulation and nitrogen fixation, or if other indirect factors are involved. For Yadegari et al. (2008), these bacteria improve the drought resistance.

The traditional inoculation of nitrogen fixing bacteria has been done on seeds, however, according to Campo et al. (2009) and Zilli et al. (2009), the success of the inoculation depends on environmental conditions and management practices before inoculation, such as seed chemical treatments with insecticides and/or fungicides. Thus, alternative methods of inoculation, as the spray bacteria application in crops in advanced stages, can provide interesting results. In addition, different doses of inoculants can be a viable strategy to determine the amount to be used in new areas without inoculation history or in areas with high soil acidity, where the recommendation is the application of double dose (Chueiri et al., 2005).

Therefore, all those management practices are due to accomplish the modern requirements of agriculture, seeking the economic, social and environmental sustainability (Chaparro et al., 2012). Thus, the aim of this study was to evaluate the agronomic treatments and grain yield in soybeans according to *Azospirillum brasilense* doses applied by spray coverage.

Material e Methods

The experiment was performed during the 2013/14 and 2014/15 crop years, in Lavras - MG, Brazil, no Centro de Desenvolvimento Científico e Tecnológico em Agropecuária - Fazenda Muquém, located at 21°14'S latitude, 45°00'W longitude and altitude of 918 m in a soil classified as dystrophic red latosol (Embrapa, 2013), with clayey, with the following textural values: clay: 640 g kg^{-1}; silt: 200 g kg^{-1}; sand: 160 g kg^{-1}. The chemical composition of the experimental area soil is shown on Table 1.

Table 1. Chemical composition of a dystrophic red latosol (0.0-0.2m) before the experiment installation, Lavras-MG, Brazil, for the 2013/14 and 2014/15 growing seasons.

Growing Season	pH	Ca^{2+}	Mg^{2+}	Al^{3+}	$H^{+}+Al^{3+}$	SB	P	K	OM	V
	H_2O	------------ $cmol_c$ dm^{-3}			------------------		- mg dm^{-3} -		dag kg^{-1}	%
2013/14	6.4	5.0	1.4	0	2.9	6.7	11.4	118	3.4	69.8
2014/15	6.2	3.8	0.8	0	0.9	4.8	20.8	92	2.2	83.5

H + Al: potential acidity; SB: sum of bases; MO: organic matter; V: base saturation

The region climate is Cwa, according to the Köppen classification, with annual average temperature of 19.3ºC and normal annual rainfall of 1,530 mm (Dantas et al., 2007). The climatic data during the experiments were collected at the meteorological station of the National Institute of Meteorology (INMET) located at the Federal University of Lavras-UFLA, Brazil, and are presented on Figure 1.

The experiment was conducted in randomized blocks in a 4 x 6 factorial, with four cultivars (Anta 82 RR®, BRS Favorita RR®, BRSMG 780RR®, BRSMG 820RR®) and six doses of *Azospirillum brasilense* (0, 300 , 400, 500, 600, 700 mL ha^{-1}) with three replications. Each plot was consisted of four sowing rows of 5 m in length, spaced with 0.50 m, and the area of each plot was 10 m^2 (5 m x 2 m), being considered the area of the two central rows.

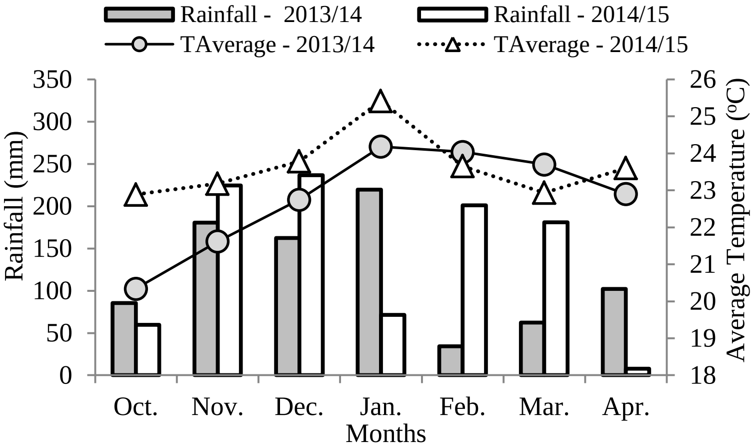

Figure 1. Monthly means for rainfall and air temperature in Lavras-MG, Brazil, for 2013/14 and 2014/15 growing seasons, during the experiment evaluations. Source: National Institute of Meteorology (INMET).

The sowing was held in November of each crop year and the fertilization consisted of 350 kg ha^{-1} made of N-P_2O_5-K_2O (02-30-20), applied via groove. The *Bradyrhizobium japonicum* bacteria were inoculated via groove after soybean sowing. The *Bradyrhizobium japonicum* dose was 18 mL p. c. kg^{-1} of seeds - strains SEMIA 5079 and 5080, containing 10.8 x 10^6 CFU/seed inoculant Nitragin Cell Tech HC® (3 x 10^9 CFU/mL). The *Azospirillum brasilense* was applied in V_3 stage (open of the second trefoil), using the Azo® inoculant (1 x 10^8 CFU/mL) with AbV_5 and AbV_6 strains. The microorganisms application was performed using a motorized backpack sprayer coupled to a bar with four spray nozzles XR 110.02, applying a water volume equivalent to 150 L ha^{-1}. The cultural trais (weeds, insects and plant diseases control) applied before and after sowing were the ones recommended for the region, when necessary.

At the beginning of flowering (R_1) the following variables was determined on five plants per plot: plant height - with a millimeter ruler, and shoot dry mass - using a forced air circulation oven at 60°C for 72 hours until constant weight, with subsequent weighing of the plant residues. It was also held the collection of leaves (third trifoliate from top to bottom) then they were washed with deionized water and placed to dry in order to calculate shoot dry mass. The dried leaves were ground in a Wiley mill. Chemical analysis of nitrogen content on leaf tissue of were conducted according to the methodology described by Sarruge & Haag (1974) and the leaf chlorophyll content was carried out using a chlorophyllmeter SPAD 502 Plus®, with three measurements on different parts of the trifoliate leaves, at the leaf blade, between the third trifoliate ribs, from top to bottom. At harvest the following characteristics were obtained: plant height and height of the first pod insertion - with the aid of a millimeter ruler.

Later, five plants per plot were collected in order to assess the number of pods per plant and number of grains per pod - manually counted; the one thousand grain mass - according to the methodology described in Brasil (2009); the grain yield - standardized for grain moisture of 13% in kg ha^{-1}. It was also determined the grain harvest index (GHI) using the formula GHI = grain yield / grain yield + straw.

The individual and joint variance analyses were performed adopting the statistical model and similar analysis procedure provided by Ramalho et al. (2012). The means were aggregated by Scott-Knott test (1974) and the statistical analysis was performed using the statistical package SISVAR® (Ferreira, 2011).

Results and Discussion

For all evaluated variables, significant influence ($p \leq 0.01$) of the crop year was observed (Tables 2 and 3). Soares et al. (2015a) also observed the influence of this source of variation in soybean agronomic characteristics.

Except for the number of grains per pod and the grains index of harvest, the cultivars promoted a significant influence ($p \leq 0.01$) on other studied variables (Tables 2 and 3). This fact was already expected due to the cultivar different genetic background, growth habit, maturation group and other atributes that promoted these kind of variation (Soares et al., 2015a; Felisberto et al., 2015).

For the doses of *Azospirillum brasilense* source of variation, no significant effect was observed for the evaluated variables (Tables 2 and 3). The lack of benefitial effects with the use of *Azospirillum brasilense* inoculation was also verified by Gitti et al. (2012), Bassini et al. (2015), Zuffo et al. (2015a) and Zuffo et al. (2015b). The authors used the *Azospirillum* application in the seed or in the planting furrow and did not observed advantages in the use of the bacteria on soybean agronomic variables.

For the cultivar and crop year interaction, statistical differences was observed for plant dry mass, nitrogen content, height of the first pod insertion, number of pods per plant, thousand grain weight and grain yield (Tables 2 and 3). Soares et al. (2015b) also verified the interaction of cultivar x crop year on the soybean, showing that the response to environmental variations was not the same for the evaluated cultivars. To other interactions, no statistical difference (Tables 2 and 3) was observed. Thus, the variation of these factors were not the same.

Table 2. Variance analysis and means for plant height on flowering (PHF), shoot dry mass (SDM), chlorophyll content (CC) and N content (NC) on leaf tissues of RR® soybean at full flowering (R_1), obtained during the experiment with doses of *Azospirillum brasilense* applyed in V_3 stage leaves in RR® soybean cultivars, during the 2013/14 and 2014/15 crop years. Lavras, MG, Brazil.

Sources of variation	DF	ANOVA (MS)[1]			
		PHF	SDM	CC	NC
		- - cm - -	- - g - -	-	- g kg -
Year (Y)	1	638.40**	14351.24**	187.15**	1164.51**
Cultivar (C)	3	768.78**	178.83*	61.70**	33.45*
Dose (D)	5	3.79^{ns}	98.01^{ns}	8.20^{ns}	6.11^{ns}
Y x C	3	18.13^{ns}	208.63*	19.27^{ns}	30.82*
Y x D	5	18.27^{ns}	75.19^{ns}	3.95^{ns}	5.91^{ns}
C x D	15	25.07^{ns}	48.65^{ns}	6.39^{ns}	9.55^{ns}
Y x C x D	15	27.82^{ns}	98.19^{ns}	6.59^{ns}	5.45^{ns}
Error	92	23.16	63.77	10.55	9.30
Mean	-	54.36	45.82	40.22	42.18
CV (%)	-	8.85	17.43	8.08	7.25
Factors		Means[2]			
Crop year[2]					
2013/14		52.26 b	55.80 a	41.36 a	39.34 b
2014/15		56.47 a	35.81 b	39.08 b	45.02 a
Azos® Doses (mL)					
0		54.60	43.25	39.45	41.81
300		54.81	44.77	40.41	42.22
400		54.60	44.74	39.70	41.93
500		53.91	48.20	41.03	43.09
600		54.38	48.23	40.22	41.70
700		53.86	45.73	40.56	42.35
Cultivars[3]					
Anta 82 RR®		48.72 c	44.46 b	38.78 b	41.79 b
BRS Favorita RR®		53.72 b	47.12 a	41.06 a	42.41 a
BRSMG 820RR®		55.71 b	43.47 b	39.49 b	41.12 b
BRSMG 780RR®		59.57 a	48.23 a	41.57 a	43.39 a

[1] ** and * significant at 1 and 5% of probability, according to F test, respectivelly. ^{ns} – not significant; MS – Mean Square; DF – degree of freedom; CV – coefficient of variation.

[2] means followed by the same letter are not different according to F test.

[3] 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.

Table 3. Variance analysis and mean values for plant height at harvest (PHH), insertion of the first pod (IFP), number of pods per plant (NPP), number of grains per pod (NGP), thousand grain mass (TGM), grain yield (GY) and grain harvest index (GHI) in RR® soybean at full maturation stage (R_8) obtained in the experiment with doses of *Azospirillum brasilense* applied on V_3 stage leaves in RR® soybean cultivars during the 2013/14 and 2014/15 crop years. Lavras – MG, Brazil.

Sources of variation	GL	ANOVA (MS)¹			
		PHH	IFP	NPP	NGP
		- - - - - cm - - - - -		- - - - - unity - - -	
Year (Y)	1	750.76**	307.12**	902.00**	1.71**
Cultivar (C)	3	588.85**	225.03**	978.58**	0.07^{ns}
Dose (D)	5	26.58^{ns}	0.86^{ns}	12.70^{ns}	0.04^{ns}
Y x C	3	61.60^{ns}	60.67**	458.11**	0.14^{ns}
Y x D	5	27.96^{ns}	2.14^{ns}	74.07^{ns}	0.02^{ns}
C x D	15	26.31^{ns}	3.15^{ns}	48.04^{ns}	0.05^{ns}
Y x C x D	15	311.20^{ns}	4.38^{ns}	68.13^{ns}	0.18^{ns}
Error	92	32.41	4.22	72.73	0.08
Mean	-	70.01	14.91	57.04	1.99
CV (%)	-	8.13	13.78	14.95	14.66
Factors		Means²			
Crop year²					
2013/14		72.30 a	13.42 b	59.55 a	2.10 a
2014/15		67.73 b	16.37 a	54.54 b	1.88 b
Azos® Doses (mL)					
0		68.62	14.79	57.04	1.94
300		69.78	14.98	57.25	2.04
400		69.10	14.92	56.28	2.01
500		71.32	14.94	56.30	1.93
600		70.98	14.63	58.24	2.01
700		70.29	15.19	57.09	1.99
Cultivars³					
Anta 82 RR®		67.82 b	11.17 b	54.19 b	2.01 a
BRS Favorita RR®		65.41 b	16.12 a	51.35 b	1.92 a
BRSMG 820RR®		73.41 a	15.95 a	59.83 a	1.99 a
BRSMG 780RR®		73.41 a	16.40 a	62.80 a	2.02 a

[1] ** and * significant at 1 and 5% of probability, according to F test, respectivelly. ^{ns} – not significant; MS – Mean Square; DF – degree of freedom; CV – coefficient of variation.

[2] means followed by the same letter are not different according to F test.

[3] 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.

Continued **Table 3**.

Sources of variation	GL	ANOVA (MS)[1]		
		TGM	GY	GHI
		- - - g - - -	kg ha^{-1}	-
Year (Y)	1	2967.07**	892422.78**	0.4722**
Cultivar (C)	3	6660.63**	4149758.20**	0.0026^{ns}
Dose (D)	5	343.30^{ns}	170814.04^{ns}	0.0011^{ns}
Y x C	3	694.40*	1073466.54**	0.0002^{ns}
Y x D	5	67.80^{ns}	39668.14^{ns}	0.0002^{ns}
C x D	15	224.27^{ns}	135165.25^{ns}	0.0018^{ns}
Y x C x D	15	286.48^{ns}	181375.82^{ns}	0.0002^{ns}
Error	92	185.87	120523.19	0.0012
Mean	-	161.20	3614.92	0.45
CV (%)	-	8.46	9.60	7.92
Factors			Means[2]	
Crop year[2]				
2013/14		156.66 b	3693.65 a	0.50 a
2014/15		165.74 a	3536.20 b	0.39 b
Azos® Doses (mL)				
0		162.65	3593.76	0.45
300		154.82	3528.22	0.46
400		158.97	3601.64	0.44
500		161.59	3546.00	0.44
600		164.02	3758.08	0.45
700		165.15	3661.84	0.45
Cultivars[3]				
Anta 82 RR®		141.59 c	3691.24 b	0.46 a
BRS Favorita RR®		172.15 a	3335.57 c	0.44 a
BRSMG 820RR®		168.09 a	4063.82 a	0.45 a
BRSMG 780RR®		162.97 b	3369.07 c	0.44 a

[1] ** and * significant at 1 and 5% of probability, according to F test, respectivelly. ^{ns} – not significant; MS – Mean Square; DF – degree of freedom; CV – coefficient of variation.

[2] means followed by the same letter are not different according to F test.

[3] 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.

The higher values for shoot dry mass, chlorophyll content, plant height on harvest, number of pods per plant, number of grains per pod, grain yield and harvest index were observed during the 2013/14 crop year. This is related to climatic conditions (Figure 1) mainly during January flowering, with a higher rainfall index for the 2013/14 crop year. Except for thousand grain mass, the higher values for other characters reflected on grain yield and on higher harvest index.

Although it is reported on literature that bacteria that promotes plant growth, like the ones from *Azospirillum* gender, can also promote plant growth, plant hormones production, increase the shoot and root dry mass and increase water deficit resistance (Bashan and Bashan, 2005), this benefits were not observed in this study.

The efficiency lack of *Azospirillum brasilense* inoculation doses on soybean is probably due to the low capacity of competition between the native *Azospirillum diazotrophic* bacteria and soil microflora (Didonet et al., 2000). Therefore, the competition between bacteria may have inhibited the effect of *Azospirillum brasilense*.

BRSMG 820RR® presented grain yield of 4063 kg ha^{-1} and is the latest cultivar (RMG 8.2), when compared to others, evaluated in the present study. For the crop year and cultivar interaction, the cultivar BRSMG 820RR® presented the lowest mean for dry mass (Figure 2A) during the 2013/14 crop year and for N content in leaf tissues (Figure 2B) during the 2014/15 crop year. For height of the first pod insertion, the cultivar Anta 82 RR® presented the lower mean during the two crop years but it is above the minimum height (10 cm) (Figure 2C). The number of pods of BRS Favorita RR® cultivar was the lowest during both evaluated crop years, but during the 2014/15 crop year it was not statistically different from Anta 82 RR® (Figure 2D). For thousand grain mass, the cultivars BRS Favorita RR® and BRSMG 820RR® presented the higher mean during both evaluated crop years, but during the 2013/14 crop year it was not statistically different from BRSMG 780RR® (Figure 2E). For grain yield the cultivar BRSMG 820RR® presented the best yield performance for both evaluated crop years (Figure 2F). Thus, the plasticity of BRSMG 820RR$^{®}$ cultivar was clear.

According to the conditions that this study was carried out and the results, the spray with different *Azospirillum brasilense* doses did not affect the agronomic variables studied and the grain yield in RR$^{®}$ soybean cultivars.

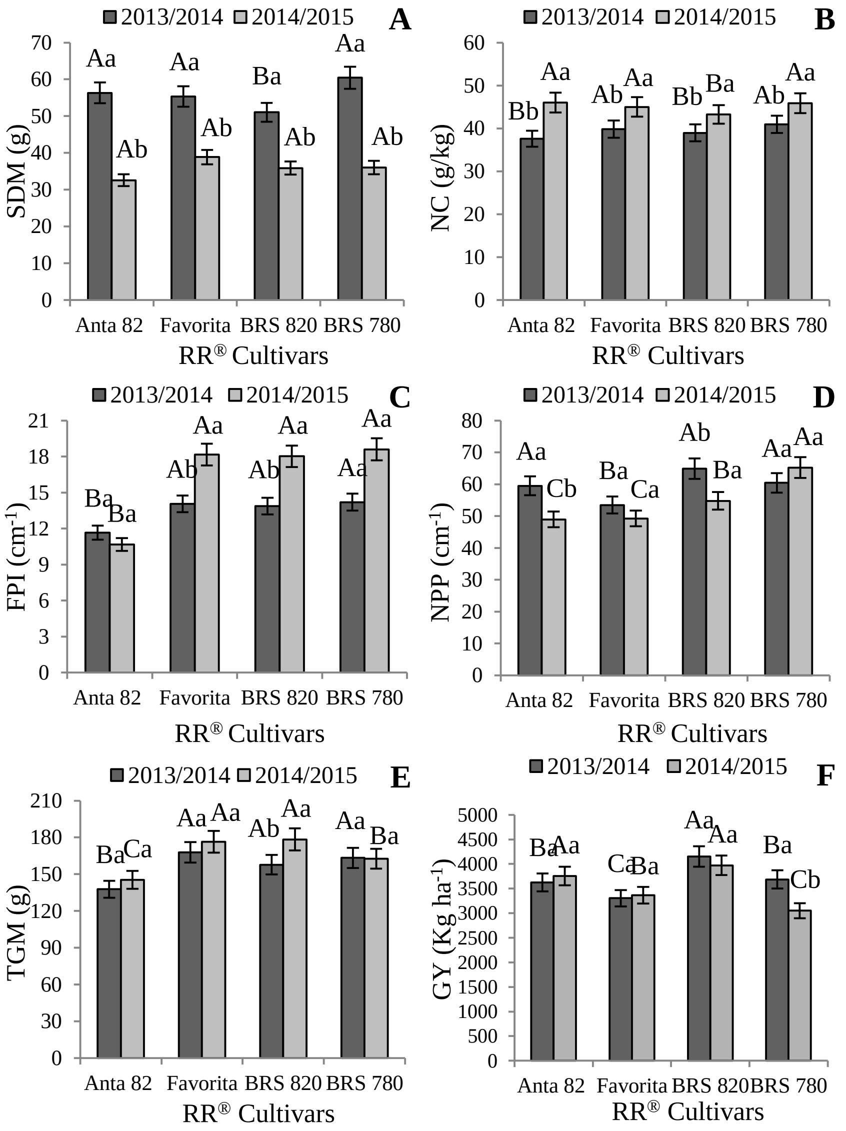

Figure 2. Shoot dry mass - SDM (A), N content in leaf tissues – NC (B), insertion of the first pod – FPI (C), number of pods per plant – NPP (D), thousand grain mass – TGM (E), grain yield – GY (F) in RR® soybean cultivars during the 2013/14 and 2014/15 crop years in Lavras, MG, Brazil. Means followed by the same capital letter in the same crop year and the same lower case for the same cultivar are not different according to Scott Knott (1974) test at 5% of probability.

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ARTIGO 4 Foliar application of *Azospirillum brasilense* in soybean seed physiological quality[4]

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ABSTRACT: 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 the 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®, BRSMG 780RR®, BRSMG 820RR®) were produced in the 2013/2014 crop year in Lavras, Minas Gerais, Brazil, with application of six doses of inoculants based on *Azospirillum brasilense* (0, 300, 400, 500, 600 and 700 mL ha^{-1}) of the strains (AbV_5 and AbV_6). Besides of yield, it was evaluated 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 evaluation by tetrazolium and by the hypochlorite test. Regardless the soybean cultivar, the application of up to 700 mL ha^{-1} of inoculant with *Azospirillum brasilense* applied on V_3 stage of the plants, does not affect yield, physiological potential, and damage incidence of seeds.

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 been 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 crop 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, the inoculation with *Bradyrhizobium japonicum* bacteria is common among producers, making unnecessary the use of nitrogen fertilization due to the efficiency of biological nitrogen fixation (BNF) performed by those bacteria. 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, among them, 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 crops [Zea mays L.] and soybean crops, the treatment of seeds with *Azospirillum brasilense* strains led to significant increase in germination and vigor of the seeds. In wheat crop [*Triticum aestivum* L.] Brzezinski et al. (2014) found that the sanitary quality of wheat seed is not influenced by *Azospirillum brasilense* inoculation. The inoculation of wheat seeds with Azospirillum brasilense favors the vigor (accelerated aging) and seedlings shoot mass.

According to Azevedo et al. (2007) little has been studied about the effects of cultural practices on the physiological quality of soybean seeds. 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 *Azospirillum brasilense* bacteria inoculation applied to the leaves.

Material and Methods

The seeds were produced in 2013/2014 crop year, in Lavras - MG, Brazil, no Centro de Desenvolvimento Científico e Tecnológico em Agropecuária - Fazenda Muquém, located at 21^{o}12'S latitude, 44^{o}58'W longitude and altitude of 918 m in soil classified as dystrophic red latosol (Embrapa, 2013).

The climate is Cwa, according to the Köppen classification, with an average annual temperature of 19.3ºC and normal annual rainfall of 1,530 mm (Dantas et al., 2007). During the seed production process, the climatic data were 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.

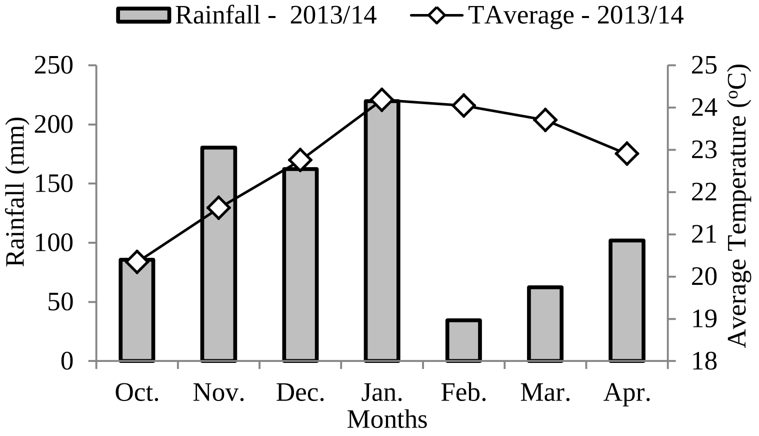

Figure 1. Monthly means for rainfall and air temperature in Lavras, MG, Brazil, in 2013/2014 crop year during soybean production. Source: National Meteorology Institute (INMET).

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®, BRSMG 780RR®, BRSMG 820RR®) with the application of six doses of the inoculant based on *Azospirillum 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 of 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 $N\text{-}P_2O_5\text{-}K_2O$ (02-30-20), applied via groove. The *Bradyrhizobium japonicum* bacteria were inoculated via groove after soybean seeding. The dose of *Bradyrhizobium japonicum* was 18 mL p. c. kg^{-1} of seeds – strains SEMIA 5079, containing 10.8 x 10^6 CFU/seed of Nitragin Cell Tech HC® (3 x 10^9 CFU/mL). It was used the inoculant Azo® (1 x 10^8 CFU/mL) strains estirpes AbV_5 e AbV_6. 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 experiment harvest was done manually and the legumes threshing was performed by stationary threshing in order to simulate the mechanical harvesting held by the harvester. It was determined the grain yield, being standardized for grain moisture of 13% kg ha^{-1}. Subsequently, the seeds were separated from impurities with the assistance of sieves, being packaged in 'Kraft' model paper bags. Seeds with moisture content above 13% were placed in shade to slow drying. After determination of the adequate moisture content, it was performed the mixing of samples and separation in sieves. For performing the laboratory tests, the seeds classified in the 6.00 mm diameter sieve were used.

Laboratory evaluations were performed using a completely randomized design, all 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 performed using the following determinations:

Thousand seeds mass and moisture content: as recommended by Brasil (2009).

Germination test: in the paper roll form with a water volume in the amount of 2.5 times the dry mass of the substrate, at 25 °C. The evaluations were performed on the 8th day after sowing, according to the criteria established by the Rules for Seed (BRASIL, 2009).

Emergency test under controlled conditions: the substrate used was composed from soil and sand (2:1). After sowing, the trains were kept in a greenhouse at 25ºC and relative air humidity of ±70%. After the emergency 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 emergency index (SEI) calculation, the Maguire (1962) formula was adopted.

Accelerating aging test: realized according to Marcos Filho (1999) recommendations, with 42 grams of seeds per treatment on an adapted stainless steel grille in *gerbox* boxes, with 40 mL of water in the bottom, maintained at 41ºC for 72 hours in a BOD and were evaluated at the germination.

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

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

The variance analysis were 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 were realized with SISVAR® statistical package (Ferreira, 2011).

Results and Discussion

The inoculation doses with isolated *Azospirillum brasilense* bacterias and the interaction of cultivars (C) and bacteria (A) did not lead to a significative difference on the studied tests (Table 1). According to this fact, can be inferred that the physiological quality of the soybean seeds, on average, do not depend on the *Azospirillum 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 observed the *Azospirillum brasilense* bacteria on the soybean agronomical characters.

A possible explanation for the fact that the used doses did not promoted any effect on the physiological quality is because of the absence of effect on agronomical characters, as verified by Gitti et al. (2012), Zuffo et al. (2015a) and Zuffo et al. (2015b). Thus, with no influence on agronomical characteristics on the field, the plant produced seeds with a similar physiological potential.

On the other hand, when the 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 187.2 grams to 127.7 grams for BRS Favorita RR® and Anta 82 RR®, respectively (Table 1). However, all cultivars presented satisfactory productive performance, with results above the average mean for the crop in Minas Gerais state – 2687 kg ha^{-1} – achieved during the 2013/14 crop year (CONAB, 2014).

Table 1. Mean values for yield, thousand seeds mass (TSM), moisture content (MC), germination (GERM), emergency 7 days after emergency – DAE (E7DAE), emergency 15 DAE (E15DAE), speed emergency 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	TSM	MC	GERM	E7DAE	E15DAE
	kg ha^{-1}	g	%	-------------- % -----------------		
Cultivar (C)						
Anta 82 RR®	3262 c	127.77 c	8.27 a	62 a	90 a	93 a
BRS Favorita RR®	4202 a	187.22 a	8.43 a	65 a	86 b	88 b
BRSMG 780RR®	3814 b	168.88 b	8,39 a	59 b	92 a	94 a
BRSMG 820RR®	4385 a	164.88 b	8.58 a	59 b	88 b	89 b
P (Value)	<0.01**	<0.01**	0.10^{ns}	<0.01**	<0.01**	<0.01**
Azospirillum brasilense (A) (mL ha^{-1})						
0	4037	160.00	8.46	63	88	90
300	3784	161.66	8.54	60	89	91
400	4003	165.00	8.39	58	87	91
500	3903	157.50	8.37	61	91	92
600	3969	170.00	8.37	62	90	91
700	3800	165.00	8.38	60	90	92
P (Value)	0.30^{ns}	0.66^{ns}	0.87^{ns}	0.12^{ns}	0.32^{ns}	0.86^{ns}
C x A	0.11^{ns}	0.30^{ns}	0.44^{ns}	0.07^{ns}	0.61^{ns}	0.52^{ns}
CV (%)	8.39	11.65	4.49	7.05	5.27	4.98

** 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.

Continued **Table 1**.

Source of variation	SEI	AAG	Tz$_{(1-3)}$	Tz$_{(1-5)}$	HYPO
	-		%		
Cultivar (C)					
Anta 82 RR®	72.61 a	79.38 a	93.03 a	96.93 a	7.00 b
BRS Favorita RR®	69.32 b	61.38 c	88.47 a	95.56 a	11.22 a
BRSMG 780RR®	75.69 a	72.00 b	92.55 a	96.81 a	6.98 b
BRSMG 820RR®	70.54 b	44.16 d	89.51 a	95.26 a	10.94 a
P (Value)	<0.01**	<0.01**	0.12^{ns}	0.53^{ns}	<0.01**
Azospirillum brasilense (A) (mL ha^{-1})					
0	71.91	63.25	90.53	97.52	8.87
300	71.88	66.66	88.09	94.91	8.91
400	69.16	64.33	93.22	97.82	8.87
500	73.25	59.83	91.98	95.80	9.00
600	71.98	65.41	90.64	94.90	9.41
700	74.06	65.91	90.86	95.89	9.14
P (Value)	0.18^{ns}	0.24^{ns}	0.56^{ns}	0.38^{ns}	0.83^{ns}
C x A	0.64^{ns}	0.06^{ns}	0.88^{ns}	0.77^{ns}	0.79^{ns}
CV (%)	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.

The moisture content on 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 compared to the required standard to commercialize seeds in Brazil, which is 80%, established by the normative instruction nº45 (BRASIL, 2013). Seeds with low or medium germination can generate less competitive seedlings in the field (França 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). To Binotti et al. (2008), the seed pathogens can increase the deterioration and reduce the vigor and germination of the seeds.

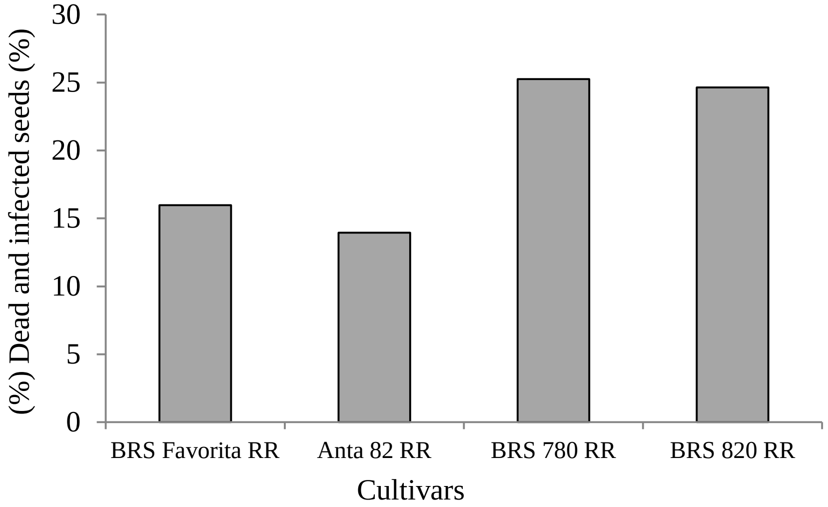

Figure 2. Total percentage of dead and infected seeds during germination test for each cultivar during the 2013/2014 crop year. Lavras. MG. Brazil.

During the emergency tests, 7 and 15 days after emergency, higher means were observed when compared to germination. The cultivars Anta 82 RR® and BRSMG 780RR® demonstrated more emergency percentage. The higher means from emergency test, when compared to germination test have already been mentioned on literature by Henning and França Neto (1980), Bizzeto and Homechin (1997) and Zambiazzi et al. (2014). The authors related that the seedlings, on emergency, releases the infected tegument on the soil, but during the germination test, on the paper, the tegument remains associated to the cotyledons and the fungus promote the seed deterioration.

For the speed emergency index, the same trend related to the emergency test was observed. This fact was expected because of the seedlings that emerge first can grow more and have more biomass because of the photosynthesis in the first growth stages. Besides this, in field conditions, the seedlings from seeds with a higher speed emergency index promote a faster closure between the field lines, leading to a better weed control (França et al. 2010).

For the accelerating aging test, the cultivar BRSMG 820RR® 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 statistical differences between cultivars was observed. For the mechanical damages, moisture deterioration and stink bug damage, that are the main problems for the soybean, different damages was 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.

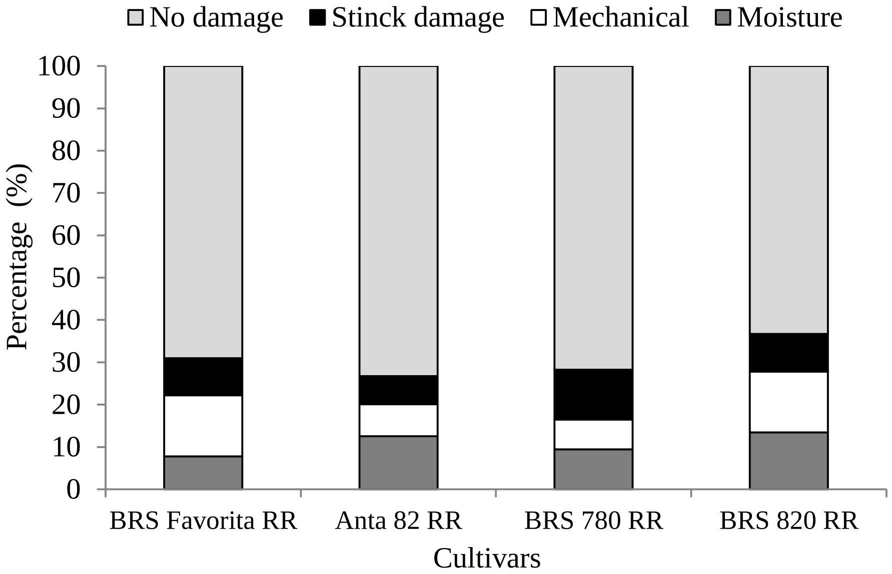

Figure 3. Damages and their respective percentages (%) observed during tetrazolium test for each cultivar during the 2013/2014 crop year. Lavras. MG. Brazil.

For the damage percentage by hypochlorite, Anta 82 RR® and BRSMG 780RR® 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 *Azospirillum brasilense* on the physical and chemical seed quality.

According to the conditions that this study was carried out and the results, despite the soybean cultivar, the inoculant application with *Azospirillum brasilense* bacteria up to 700 mL ha^{-1} applied on plants at V_3 stage do not affect the yield, physiological potential and seed damage incidence.

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