UNIVERSIDADE
ESTADUAL DE LONDRINA

ALISSON WILSON DOS SANTOS SANZOVO

ASPECTOS MORFOLÓGICOS DE RAÍZES E PRODUTIVIDADE DE FEIJÃO E SOJA INOCULADOS OU COINOCULADOS COM MICRORGANISMOS PROMOTORES DE CRESCIMENTO DE PLANTAS

Londrina
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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Agronomia da Universidade Estadual de Londrina, como requisito parcial para à obtenção do título de Doutor em Agronomia.

Orientador: Prof. Dr. Marco Antonio Nogueira

Londrina
2023

Ficha de identificação da obra elaborada pelo autor, através do Programa de Geração Automática do Sistema de Bibliotecas da UEL

Sanzovo, Alisson Wilson dos Santos .
Aspectos morfológicos de raízes e produtividade de feijão e soja inoculados ou coinoculados com microrganismos promotores de crescimento de plantas / Alisson Wilson dos Santos Sanzovo. - Londrina, 2023.
86 f.

Orientador: Marco Antonio Nogueira.
Tese (Doutorado em Agronomia) - Universidade Estadual de Londrina, Centro de Ciências Agrárias, Programa de Pós-Graduação em Agronomia, 2023.
Inclui bibliografia.

1. Bactérias Promotoras de Crescimento - Tese. 2. Microalgas - Tese. 3. Cianobatérias - Tese. 4. Actinobactérias - Tese. I. Nogueira, Marco Antonio. II. Universidade Estadual de Londrina. Centro de Ciências Agrárias. Programa de Pós-Graduação em Agronomia. III. Título.

CDU 63

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BANCA EXAMINADORA

Orientador: Prof. Dr. Marco Antonio Nogueira
Universidade Estadual de Londrina

Profª. Drª. Alexandra Scherer
Universidade Norte do Paraná

Profª. Drª. Amanda Letícia Pit Nunes
Santa Clara Agrociência

Prof. Dr. Artur Berbel Lirio Rondina
Rizobacter do Brasil

Prof. Dr. Leonel Vinicius Constantino
Leonel Vinicius Constantino

Londrina, 24 de Fevereiro de 2023.

AGRADECIMENTOS

À minha avó/mãe Irene Gonçalvez dos Santos (*in memorian*) que sempre esteve presente e que tive a honra e tenho um orgulho imensurável de ser seu neto/filho. Obrigado por ser minha bússola, me orientar e me guiar a ser alguém melhor, seu amor me salvou e me manteve forte nos meus objetivos.

Ao meu pai Mauricio Aquino Fernandes (*in memorian*), que foi me apresentado como meu padrastro, se tornou meu pai e partiu como meu herói. Me ensinou que o amor transcede qualquer obstáculo e que às vezes um “estranho” pode ser o maior presente que o destino pode nos oferecer.

À minha vó Maria José Dias Sanzovo (*in memorian*) que sempre me recepcionou com seus abraços, sua voz doce e seu sorriso aberto e será lembrada todos os dias.

Aos meus familiares, Allef Junior Fernandes, Erica Pellizari, Neuci dos Santos Fernandes, Cicero Gonçalvez e Wilson Sanzovo que me apoiam e me fornecem abrigo nos piores e melhores momentos.

À minha esposa Juliana Thais Pavan Sanzovo, por todo auxílio e paciência, que me apoia e incentiva a lutar todos os dias, mesmo quando as probabilidades são minimas de sucesso.

Agradeço aos meus orientadores Prof. Dr. Marco Antonio Nogueira e Profª. Drª. Diva de Souza Andrade não só pela constante orientação neste trabalho, mas sobretudo pela amizade e paciência, por serem exemplos de dedicação profissional.

Aos colegas e amigos do laboratório de Biotecnologia do Solo da Embrapa Soja, principalmente àqueles com que mais convivi: Artur, Jonathan, Gabriel, Thiago, Amanda, Flavia e a todos os profissionais do campo e laboratório que me ajudaram arduamente nas coletas.

À Universidade Estadual de Londrina (UEL) e ao Programa de Pós – Graduação em Agronomia e aos professores que participaram e colaboraram para a minha formação.

À Capes, pela concessão da bolsa de estudos que contribuiu para realização deste trabalho.

A todos aqueles que contribuíram de forma direta ou indireta para a realização do trabalho. Obrigada a todos.

“Mesmo quando tudo parece desabar, cabe a mim decidir entre rir ou chorar, ir ou ficar, desistir ou lutar; porque descobri, no caminho incerto da vida, que o mais importante é o decidir.” (Cora Coralina)

SANZOVO, Alisson Wilson dos Santos. **Aspectos morfológicos de raízes e produtividade de feijão e soja inoculados ou coinoculados com microrganismos promotores de crescimento de plantas**. 2023. 86 f. Tese (Doutorado em Agronomia) – Universidade Estadual de Londrina, Londrina, 2023.

RESUMO

Os custos crescentes e os efeitos negativos do uso demasiado de fertilizantes nitrogenados nos agrossistemas para produção agrícola reforçam a necessidade de alternativas biológicas para a produção agrícola. Essa demanda tem resultado no crescente uso de bactérias promotoras de crescimento de plantas como ferramenta da prática agrícola. O objetivo deste trabalho foi avaliar efeitos da inoculação ou coinoculação de microrganismos promotores de crescimento de plantas em aspectos morfológicos de raízes e produtividade de soja e feijão. Foram realizados quatro experimentos de campo, dois para cada cultura. As plantas foram avaliadas quanto à massa de raízes, comprimento total e específico, volume, diâmetro, densidade tecidual, número de raízes laterais, comprimento e incidência de pelos radiculares, número e massa seca de nódulos, massa seca da parte aérea e teor de N e rendimento de grãos. Houve efeitos da inoculação e coinoculação das bactérias promotoras de crescimento de plantas nas características morfológicas radiculares, atributos da parte área e rendimento de grãos. Sugere-se que as interações entre microrganismos benéficos possuam efeito sinérgico e incrementem características da morfologia das raízes que favoreçam ao aumento do rendimento das culturas.

Palavras-chave: *Azospirillum brasilense*; *Nostoc muscorum*; *Streptomyces* spp. Comprimento de raízes; pelos absorventes; volume de raízes.

SANZOVO, Alisson Wilson dos Santos. **Morphological aspects of roots and productivity of beans and soybeans inoculated or coinoculated with plant growth-promoting microorganisms**. 2023. 86 p.Thesis (PhD in Agronomy) – State University of Londrina, Londrina, 2023.

ABSTRACT

The increasing costs and negative effects of excessive use of nitrogen fertilizers in agrosystems for agricultural production reinforce the need for biological alternatives for agricultural production. This demand has resulted in the increasing use of plant growth-promoting bacteria as a tool in agricultural practice. The aim of this work was to evaluate the effects of inoculation or coinoculation of plant growth-promoting microorganisms on morphological aspects of roots and productivity of soybeans and beans. Four field experiments were carried out, two for each crop. The plants were evaluated for root mass, total and specific length, volume, diameter, tissue density, number of lateral roots, length and incidence of root hairs, number and dry mass of nodules, dry mass of the aerial part and N content. and grain yield. There were effects of inoculation and coinoculation of plant growth-promoting bacteria on root morphological characteristics, area attributes and grain yield. It is suggested that interactions between beneficial microorganisms have a synergistic effect and increase characteristics of root morphology that favor increased crop yield.

Palavras-chave: *Azospirillum brasilense*; *Nostoc muscorum*; *Streptomyces* spp. Root length; root hairs; root volume.

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1 INTRODUÇÃO

O uso de inoculantes com bactérias promotoras de crescimento de plantas tornou-se uma estratégia atraente em sistemas agrícolas sustentáveis, uma vez que contribui para o desenvolvimento e rendimento das plantas, e pode diminuir os custos de produção e o consumo de recursos não renováveis.

O solo é um ambiente diverso e abriga uma grande quantidade de espécies de microrganismos, alguns conhecidos por colonizarem a rizosfera, desempenharem papeis importantes na fertilidade do solo e aumento na produtividade das culturas por meio de diferentes mecanismos. Bactérias Promotoras de Crescimento de Plantas (BPCPs) podem melhorar o desenvolvimento da planta por meio do aumento da disponibilidade de nutrientes, pela produção de fitormônios e até mesmo induzindo resistência sistêmica contra fitopatógenos ou tolerância à seca.

Embora exista um grande volume de informações sobre BPCPs, a maioria dos trabalhos se concentra nos filos Bacteroidetes, Firmicutes e Proteobacteria, dos quais os gêneros mais comumente estudados são *Azospirillum*, *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Enterobacter*, *Gluconacetobacter*, *Paenibacillus*, *Pseudomonas*, *Rhizobium* e *Serratia* (BHATTACHARYYA; JHA, 2012). No entanto, trabalhos sobre a promoção de crescimento vegetal por organismos dos filos Actinobacteria e Cianobacteria são escassos, apesar de serem procariotos amplamente difundidos na natureza e possuírem atributos considerados úteis para agricultura (PRASANNA et al., 2012; FRANCO-CORREA; CHAVARRO-ANZOLA, 2016).

Em ambientes naturais e agrícolas, a capacidade das plantas em adquirir eficientemente os recursos do solo é influenciada pela arquitetura do sistema de raízes, que compreende aspectos morfológicos como a quantidade, comprimento e espessura de raízes, e a incidência e comprimento dos pelos radiculares (JUNGK, 2001; HALING et al. 2016; RONDINA et al. 2020). Sabe-se que BPCPs podem influenciar o sistema de raízes das plantas, no entanto, poucos trabalhos destacam os efeitos sobre os aspectos morfológicos das mesmas, ficando em grande parte, restritos à massa e à razão raiz/parte aérea (HTWE; MOH; YAMAKAWA, 2018).

O objetivo deste trabalho foi avaliar mudanças nos aspectos morfológicos de raízes, nutricionais e produtivos da soja e do feijão inoculados ou coinoculados com microrganismos promotores de crescimento de plantas.

2. REVISÃO DE LITERATURA

2.1 PRODUÇÃO E IMPORTÂNCIA ECONÔMICA DA SOJA

A produção de soja [*Glycine max* (L.) Merrill] é uma atividade de grande relevância econômica no cenário agrícola mundial, com produção estimada de 392,80 milhões de toneladas de grãos na safra 2022/23 (USDA, 2022). A importância desta cultura pode ser atribuída à grande aplicabilidade de seus grãos, que apresentam elevados teores de proteína e óleo, servindo principalmente para nutrição animal, humana e produção de biodiesel (HIRAKURI; LAZZAROTO, 2014).

Atualmente, o Brasil é o maior produtor mundial de soja, produzindo cerca de 123 milhões de toneladas de grãos nas safras 2021/22, enquanto os EUA encontram-se em segundo e a Argentina em terceiro, com uma produção de cerca 121 e 44 milhões de toneladas, respectivamente (USDA, 2022).

A cultura da soja começou a ser cultivada no Brasil em 1882 (SOUSA; VALLE; MORENO, 2000) no Estado da Bahia (SEDIYAMA, 2009), mas não se adaptou ao clima da região. Por volta de 1891, novas tentativas foram realizadas em outros estados, como São Paulo e Rio Grande do Sul, onde a planta apresentou melhor desenvolvimento. A cultura tornou-se economicamente importante a partir de 1970, dando início à sua ascensão na agricultura brasileira e possibilitando a consolidação do país como um dos principais exportadores dessa oleaginosa (DALL'AGNOL et al. 2007; TOLLEFSON, 2010, EMBRAPA SOJA, 2016).

O sucesso do agronegócio brasileiro da soja pode ser atribuído a diversos fatores, dentre os quais o investimento em pesquisas científicas, que possibilitaram o desenvolvimento de cultivares adaptadas às diversas condições climáticas e de solo (ALVES; BODDEY; URQUIAGA, 2003), bem como a seleção de bactérias fixadoras de nitrogênio eficazes nas condições do país, permitindo altas produtividades de soja sem o uso de fertilizantes químicos nitrogenados (HUNGRIA; CAMPO; NOGUEIRA, 2012; SATURNO et al., 2017).

2.2 PRODUÇÃO E IMPORTÂNCIA ECONÔMICA DO FEIJÃO

O feijão-comum (*Phaseolus vulgaris* L.) se caracteriza pelo alto teor de proteína de suas sementes e vagens, sendo um alimento grande importância entre os povos da América Latina e África, onde o acesso à proteína animal é limitado (KOCIRA et al., 2020).

A área mundial dedicada à produção de feijões é de aproximadamente 33 milhões de hectares, e a área cultivada na safra 2021/22 no Brasil foi de 2,8 milhões, de acordo com a Companhia Nacional de Abastecimento (CONAB) (CONAB, 2022, FAOSTAT, 2022). Embora haja uma crescente queda na área nas últimas décadas (CONAB, 2022), ainda é um alimento muito requisitado nos países em desenvolvimento (HUERTAS et al., 2022).

No Brasil, cerca de 60% da área com feijão corresponde ao grupo carioca, enquanto o restante corresponde ao feijão preto (CONAB, 2021). Dentre as diferentes cultivares de feijão carioca plantadas, a BRS Pérola caracteriza-se por elevada qualidade de grãos e por unir alto potencial produtivo e resistência à ferrugem (*Phakopsora pachyrhizi*), ao mosaico-comum (Bean golden mosaic virus), à mancha-angular (*Isariopsis griseola*) e à murcha-de-fusário (*Fusarium oxysporum*) (LANNA; FERREIRA; BARRIGOSSI, 2004). Essa cultivar apresenta arquitetura ereta, com resistência ao acamamento, possui ciclo de 85 a 99 dias da emergência à maturação fisiológica, estabilidade de produção, grãos claros e de boa qualidade comercial (MELO et al., 2011).

Quanto a cultivar BRS Esteio, segundo a Embrapa (2015), apresenta ciclo de 85 a 94 dias da emergência à maturação fisiológica, com hábito de crescimento indeterminado e alto potencial produtivo, podendo atingir 4.700 kg/ha. A arquitetura de plantas é ereta e apresenta boa resistência ao acamamento, estando adaptada à colheita mecânica, inclusive direta.

Embora possua ampla adaptação e capacidade de distribuição geográfica, ainda são os pequenos e médios produtores os responsáveis pela maior parte do cultivo do feijão produzido no Brasil (SILVA; WANDER, 2013; WANDER; SILVA, 2017), mesmo com mudanças na dinâmica da produção nas últimas décadas, com maior concentração da produção a produtores de maior porte (PELEGRINI; CAPANEMA; HASPARYK, 2017). A adoção de melhores práticas agrícolas é necessária devido à dificuldade de acesso e altos custos de aquisição de insumos para esses produtores (SILVA; WANDER, 2013).

2.3 FIXAÇÃO BIOLÓGICA DE NITROGÊNIO (FBN)

O N é o nutriente mais exigido pelas plantas, e está presente na clorofila, aminoácidos, ácidos nucleicos, e outras macromoléculas. Qualquer perturbação no seu metabolismo causa graves consequências no crescimento e desenvolvimento das plantas (FUKUSHIMA; KUSANO, 2014).

A atmosfera terrestre consiste em 78% de gás nitrogênio (N_2), mas a disponibilidade de N reativo é limitada em muitos solos, já que nenhuma planta é capaz de o utilizar nessa forma. As principais fontes de nitrogênio para as plantas são a matéria orgânica do solo após mineralização, a fixação não-biológica de N_2, os fertilizantes nitrogenados, e a fixação biológica do nitrogênio (FBN) (GARRATT et al., 2018). A produção de fertilizantes nitrogenados é feita a partir da reação do nitrogênio atmosférico e nafta, com o uso de fontes energéticas não renováveis para que a reação aconteça, formando a amônia (NH_3), que é a base da maioria dos fertilizantes nitrogenados. Entretanto, o N proveniente dos fertilizantes químicos tem baixa eficiência de aproveitamento (cerca de 50%) devido a perdas causadas por desnitrificação, lixiviação e volatilização das formas inorgânicas de N no solo, o que pode ser agravado por práticas inadequadas de manejo (CANTARELLA, 2007).

O N resultante da mineralização da matéria orgânica é limitado, principalmente nos solos tropicais, pois esta fonte, que já é limitada, se esgota após poucos anos de cultivos. Além disso, as práticas de manejo no ambiente tropical devem priorizar a manutenção da matéria orgânica do solo e não sua mineralização. Da mesma forma, a fixação não-biológica, resultante de descargas elétricas, vulcanismo e combustão, contribui apenas com uma pequena parcela do N demandado pelos ecossistemas naturais e agrossistemas anualmente (HUNGRIA; CAMPO; MENDES, 2007).

Entretanto, algumas bactérias, denominadas diazotróficas, reduzem o nitrogênio atmosférico na forma amoniacal que pode ser assimilada pelas plantas. As mais conhecidas dessas bactérias são coletivamente chamadas de rizóbios, que possuem em seu metabolismo a capacidade de sintetizar a enzima nitrogenase, capaz de quebrar a tripla ligação do N_2 e reduzi-lo em amônia (NH_3), processo este denominado FBN (TAIZ; ZEIGER 2013; LOPES; DIAS-FILHO; GURGEL.,2022). A simbiose entre plantas da família Fabaceae e os rizóbios pode resultar no suprimento total ou parcial da demanda de N da planta pelo rizóbio, dependendo do grau de interação planta-bactéria. Esse processo engloba uma intensa troca de sinais moleculares entre os organismos e a expressão de genes resultando na formação de estruturas denominadas nódulos, onde o processo da FBN ocorre (SINCLAIR; VADEZ, 2012; HUNGRIA; NOGUEIRA, 2022).

O uso destas bactérias como inoculantes tornou-se uma ferramenta para para a nutrição nitrogenada de algumas culturas, sobretudo as leguminosas, resultando em uma produção de forma mais sustentável, reduzindo a necessidade de insumos químicos e também proporcionando tolerância a estresses abióticos (HUNGRIA; NOGUEIRA, 2022). No Brasil, inoculantes para a soja contendo *Bradyrhizobium* spp. precisam conter isoladamente ou em combinação as estirpes SEMIA 5079, SEMIA 5080, SEMIA 587 ou SEMIA 5019, autorizadas pelo Ministério da Agricultura, Pecuária e Abastecimento (MAPA) para a produção de inoculantes (ZILLI et al., 2011; DA COSTA et al., 2017).

O uso de inoculantes à base de *Bradyrhizobium* possibilita uma economia anual de aproximadamente US$ 17,88 bilhões em fertilizantes nitrogenados no Brasil (TELLES, NOGUEIRA e HUNGRIA, 2023). Bactérias deste gênero são capazes de suprir a demanda da planta por N, mesmo para altos rendimentos, tornando a adubação nitrogenada desnecessária nessa cultura, o que garante maior competitividade da *commodity* no mercado internacional (MOREIRA; SIQUEIRA, 2006; FAGAN et al., 2007; BOMFETI et al*.,* 2011; HUNGRIA; NOGUEIRA, 2022).

No feijão, várias espécies de bactérias do gênero *Rhizobium* têm sido descritas como capazes de formar nódulos e, na maioria das vezes, fixar N_2 em simbiose com a leguminosa: *R. leguminosarum* bv. *phaseoli* (JORDAN, 1984), *R. tropici* (MARTÍNEZ-ROMERO et al., 1991), *R. etli* bv. *phaseoli* (SEGOVIA et al., 1993), *R. giardinii* (bv. *giardinii* e bv. *phaseoli)* (AMARGER et al., 1997), *R. leucaenae* (RIBEIRO et al., 2012), *R. freirei* (DE AGNOL et al., 2013) e *R. paranaense* (DE AGNOL et al., 2014). Estudos realizados em grandes grupos de cepas de *R. tropici,* incluindo *R. freirei*, *R. leucaenae* e *R. paranaense* mostraram que esta espécie tinha maior estabilidade genética do que outros microssimbiontes de feijão, provavelmente devido a uma única cópia do gene nifH em seu cromossomo (PIHA; MUNNS, 1987; MARTÍNEZ-ROMERO et al., 1991).

Outras bactérias diazotróficas não simbióticas também são empregadas para a produção de inoculantes comerciais no Brasil, a exemplo do

Azospirillum brasilense, o qual também apresenta a capacidade de promover crescimento de plantas pela síntese de fitormônios (SANTOS; NOGUEIRA; HUNGRIA, 2019). As principais estirpes comerciais de *A. brasilense* recomendadas pelo MAPA para a produção de inoculantes são Ab-V5 e Ab-V6 e foram inicialmente autorizadas no Brasil para uso como inoculantes para milho e trigo (*Triticum aestivum* L.) (HUNGRIA et al. 2010; MAPA, 2011).

Embora a FBN tenha se tornado uma grande ferramenta na agricultura, ela pode sofrer interferências de condições adversas, com destaque para os fatores ambientais, tais como: disponibilidade hídrica, salinidade, temperatura elevada, pH, deficiência de nutrientes e luminosidade, que interferem na eficiência simbiótica (FIGUEIREDO et al., 2008; SINCLAIR; VADEZ, 2012; HUNGRIA; KASCHUK, 2013; MUKHTAR et al., 2020).

A disponibilidade de água influencia não só a produção e desenvolvimento das culturas, mas também, impacta na interação microrganismos-plantas (SINCLAIR; VADEZ, 2012; HUNGRIA; KASCHUK, 2013; ENEBE; BABALOLA, 2018; IPEK et al., 2019; DANISH et al., 2020). A seca eleva a temperatura do solo, o que pode inibir a multiplicação e a manutenção dos microrganismos de interesse que foram inoculados. Por sua vez, o excesso de água reduz a disponibilidade de oxigênio no solo, restringindo microrganismos aeróbios obrigatórios (FIGUEIREDO et al., 2008; ENEBE; BABALOLA, 2018; HARTMAN; TRINGE, 2019; IPEK et al., 2019).

O pH do solo também interfere no metabolismo das plantas, e tem reflexo na atividade microbiológica, podendo inibir a atividade dos microrganismos que habitam a rizosfera (SALWAN et al., 2019). A luminosidade pode interferir na interação planta-microorganismos modificando a quantidade e a composição química dos exsudatos radiculares (VENTURI; KEEL, 2016; LOPES et al., 2018), enquanto a temperatura impacta os atributos morfológicos, fisiológicos e bioquímicos das plantas, interferindo na interação planta-microrganismo ao alterar a composição da exsudação radicular (ALI et al., 2011; MEENA et al., 2015; IPEK et al., 2019).

2.4 COINOCULAÇÃO DE BACTÉRIAS PROMOTORAS DE CRESCIMENTO DE PLANTAS

A rizosfera abriga uma comunidade de organismos procariotos benéficos conhecidos como bactérias promotoras de crescimento de plantas

(BPCPs). Esses organismos têm a capacidade de colonizar as raízes das plantas proporcionando benefícios aos seus hospedeiros, modulando os níveis de fitormônios, aumentando a disponibilidade de nutrientes, resistência contra patógenos e diminuindo a necessidade do uso de fertilizantes químicos (ABHILASH et al., 2016; LOPES; DIAS-FILHO; GURGEL, 2021). Os microrganismos benéficos, previamente selecionados e testados, podem ser inoculados em sementes, folhas, raízes de mudas ou diretamente no solo (KUMARI et al., 2019; KHAN et al., 2020; KHOSHRU et al., 2020).

Uma estratégia que tem ganhado destaque nos últimos anos é a de combinar os inoculantes microbianos, conhecida como coinoculação ou inoculação mista, uma técnica que tem a finalidade de produzir um efeito sinérgico, em que se superam os resultados positivos obtidos quando os inoculantes são usados isoladamente (FERLINI, 2006; BÁRBARO et al., 2008).

Uma das coinoculações mais conhecidas é a que envolve o uso de *Bradyrhizobium* spp. e *Azospirillum brasilense*, caracteriza-se por ser uma prática sustentável que tem se mostrado promissora, contando com diversos estudos que têm relatado efeito benéfico na cultura da soja (HUNGRIA; NOGUEIRA; ARAUJO, 2013; CHIBEBA et al., 2015; HUNGRIA; NOGUEIRA; ARAUJO, 2015, CEREZINI et al., 2016; TEIXEIRA FILHO et al., 2017; RONDINA et al., 2020). Isso se deve, entre outros fatores, à capacidade desta combinação em promover estimulo à nodulação inicial e à atividade da FBN, ao crescimento da planta, maior desenvolvimento do sistema radicular, além de amenizar efeitos negativos como a restrição hídrica (MOLLA et al., 2001; CHIBEBA et al., 2015; HUNGRIA; NOGUEIRA; ARAUJO, 2015; CEREZINI et al., 2016; RONDINA et al., 2020).

Assim como *Azospirillum*, outras BPCPs são relatadas na literatura, a exemplo das cianobactérias *Anabaena* e *Nostoc* (PRASANNA et al. 2008; HASHTROUDI et al., 2012; LAHROUNI et al., 2013). Estas cianobactérias filamentosas são fixadoras de nitrogênio em vida livre e em simbiose com plantas dos gêneros *Gunnera* e *Azolla* (SANTI; BOGUSZ; FRANCHE, 2013). Andrade e Colozzi-Filho (2014), em experimento com feijoeiro, obtiveram resultados promissores na produtividade da cultura em função da coinoculação com cianobactérias (IPR7061) e rizóbios (SEMIA 4077), equivalente ao tratamento adubado com nitrogênio mineral.

As microalgas, especialmente da espécie *C. vulgaris*, que produzem fitormônios semelhantes às citocininas, quando testada em coinoculação com outras microalgas como a *Spirulina platensis*, desempenharam um papel na melhoria das plantas de arroz (*Oryza sativa*), em termos de número de folhas, área foliar, peso fresco e seco de parte aérea, disponibilidade de nitrogênio, fósforo e potássio no solo, assim como maior germinação das sementes e produtividade de grãos (DINESHKUMAR et al., 2018).

Outro importante grupo de BPCPs são as actinobactérias como as do gênero *Streptomyces*, com efeitos positivos da coinoculação com rizóbios na nodulação da ervilha (*Pisum sativum*), aumentando a frequência nodular e o vigor dos bacteróides dentro dos nódulos devido à melhora na assimilação nodular do ferro e possivelmente de outros nutrientes (TOKALA et al., 2002). Soe e Yamakawa (2013), avaliando estirpes de *Bradyrhizobium* spp. isoladas de soja em Myanmar e os efeitos da coinoculação com *Streptomyces griseoflavus* P4 sobre a FBN, observaram que algumas variáveis como a massa de nódulos secos e a estimativa da FBN, avaliada pela atividade de redução de acetileno, foram alteradas positivamente pela inoculação mista.

2.5 CIANOBACTÉRIAS

As cianobactérias (anteriormente chamadas algas verde-azuladas) são organismos procariontes pertencentes ao domínio Bacteria, caracterizadas por serem autótrofas capazes de realizar fotossíntese oxigênica e respiração simultaneamente, em um mesmo compartimento celular, participando dos ciclos biogeoquímicos do carbono, oxigênio e nitrogênio (VERMAAS, 2001; MARGESIN et al., 2008).

As cianobactérias possuem uma grande variedade morfológica em uma estrutura filamentosa que combinam várias células. Ocupam diversos habitats, incluindo solo, lagos de água doce e oceanos, bem como ambientes com características extremas, desde lagos glaciais a desertos áridos (HERRERO et al., 2001; GUPTA et al., 2013). Algumas células que conferem a característica filamentosa a estes microrganismos se especializam em estruturas chamadas heterocistos e acinetos (MEEKS et al., 2002).

Os heterocistos têm o papel de proteger a enzima nitrogenase do oxigênio, onde catalisa N_2 em NH_3 e o disponibiliza para o procarioto (FLEMING;

HASELKORN, 1973). Além de ter uma parede celular mais grossa, que dificulta a entrada do O_2, essa estrutura é desprovida do fotossistema II, o que não leva à formação do O_2, o qual é incompatível com a nitrogenase. Já os acinetos são células modificadas capazes de promover resistência a condições ambientais desfavoráveis. Sua função pode ser comparada à de esporos de bactérias e seu protoplasma acumula reservas nutricionais (STAINER; COHEN-BAZIRE, 1977).

Dentro do grupo das cianobactérias, as espécies que habitam o solo têm despertado interesse para uso na agricultura, devido à diversidade de metabólitos sintetizados pelas mesmas e o potencial de uso da sua biomassa como condicionador de solo e/ou fertilizante (SHARIATMADARI et al., 2013). Esses microrganismos podem aumentar a eficiência de uso de fertilizantes químicos nitrogenados e podem aumentar o potencial produtivo das culturas direta e indiretamente (VAISHAMPAYAN et al. 2001; MISHRA e PABBI 2004).

Os gêneros *Anabaena*, *Spirulina* e *Nostoc* são os mais estudados para fins biotecnológicos (WEHR et al*.,* 2015). Dentre as cianobactérias, o gênero *Nostoc* é usado principalmente para aplicações médicas e nutrição humana em países como China, Colômbia, Índia e Japão (LIAO et al*.,* 2015; GUO et al*.,* 2016; VIDAL et al*.,* 2018). Embora a maioria dos estudos com esta cianobactéria ainda esteja voltado para suas aplicações farmacêuticas e nutricionais, o seu uso como inoculante e biofertilizante tem demonstrado resultados benéficos em diferentes culturas, como arroz (*Oryza sativa*), canola (*Brassica napus*), tomate (*Lycopersicon esculentum*), milho (*Zea mays*), trigo (*Triticum* spp.), ervilha (*Pisum sativum*) e aveia (*Avena sativa*) (BRAHMAPRAKASH et al. 2012; ORTIZ-MORENO; SOLARTE-MURILLO; SANDOVAL-PARRA, 2020). Como inoculante, *Nostoc* possui a capacidade de fixar nitrogênio nos heterocistos e pode liberar metabólitos biologicamente ativos na rizosfera que podem induzir respostas sistêmicas na planta que resultam no aumento do crescimento vegetal (PRASANNA et al., 2013). Além disso, também há relatos do seu uso na prevenção de erosão, aumento da fertilidade do solo e produção de biocombustíveis (DHAR et al., 2015; RANJAN et al., 2016; SINGH et al., 2017).

2.6 ACTINOBACTÉRIAS

Actinobactérias, anteriormente denominadas actinomicetos, são bactérias Gram-positivas, filamentosas ou em forma de bastonete, com alto teor de guanina e citosina (C+G) em seu DNA. Os gêneros *Streptomyces*, *Actinoplanes*, *Micromonospora*, *Amycolatopsis* e *Saccharopolyspora* são os maiores produtores de biomoléculas de importância comercial (CHAUDHARY et al., 2013).

As actinobactérias comumente habitam a rizosfera, sendo um componente importante desse ambiente devido às suas interações com as plantas. Estes microrganismos exibem ampla morfologia, desde a formas hifais até cocóides (VENTURA et al., 2007). Apresentam ainda diferentes propriedades fisiológicas e metabólicas, tais como a produção de enzimas extracelulares e uma grande variedade de metabólitos secundários, inclusive com propriedades antimicrobianas (RAJU et al., 2010; OLANREWAJU; BABALOLA, 2019).

Esta plasticidade metabólica lhes permite utilizar diferentes fontes de energia e carbono para crescimento, nos mais diversos ecossistemas (BHAT et al., 2013), como sedimentos marinhos e de lagos, ambientes extremos como solos de desertos e da Antártica, até mesmo trato gastrointestinal e em simbiose com plantas da família Fabaceae (VENTURA et al., 2007; CARRO et al., 2012; ENCHEVA-MALINOVA et al., 2014).

Devido a estas potencialidades, a triagem, isolamento e caracterização destas bactérias têm sido áreas de intenso estudo no mundo inteiro nas últimas décadas, resultando na descoberta de novas espécies e novas moléculas (RIFAAT, 2003; NINGTHOUJA; SANASAM; NIMAICHAND, 2009; RIZVI; KAMBLE; KADAM, 2012). Historicamente, o gênero *Streptomyces* está entre os mais comumente descritos (FRANCO-CORREA; CHAVARRO-ANZOLA, 2016). e tem grande importância econômica na produção de substâncias biologicamente ativas, sendo responsável por cerca de 75% dos antibióticos naturais obtidos de actinobactérias (NEWMAN; CRAGG; SNADER, 2003; JIMÉNEZ-ESQUILÍN; ROANE, 2005).

Embora a maioria dos estudos com estas bactérias esteja voltada à produção de substâncias biologicamente ativas, como antibióticos, enzimas e vitaminas (WIETSE de et al., 2005; TYC et al. 2017), as actinobactérias também possuem mecanismos diretos e indiretos que influenciam no crescimento e na proteção das plantas, assim como outras BPCPs (FRANCO-CORREA; CHAVARRO-ANZOLA, 2016). Os mecanismos diretos envolvem estímulo à fixação biológica de nitrogênio (SATHYA; VIJAYABHARATHI; GOPALAKRISHNAN, 2017), hormônios de crescimento (JOG; NARESHKUMAR; RAJKUMAR, 2016), solubilização de fosfato e aquisição de ferro (JOG et al., 2014).

Os efeitos indiretos estão relacionados ao controle ou mitigação de efeitos deletérios de estresses externos de fontes bióticas ou abióticas por meio da produção de substâncias de baixo peso molecular (álcoois, amônia, cetonas, cianogênios, sulfetos, enzimas que degradam a parede celular de fungos fitopatogênicos e metabólitos secundários com propriedades biocidas) (GLICK, 2012; BOUIZGARNE, 2013; DEY; PAL; TILAK, 2014).

Em leguminosas, estudos com microscopia eletrônica de varredura (MEV) indicam que há interação entre *Streptomyces* e a nodulação de ervilha (*Pisum sativum*) (TOKALA et al., 2002). Segundo Tokala et al. (2002), a espécie *S. lydicus* cepa WYEC 108 coloniza as superfícies dos nódulos e raízes da ervilha, aumentando o número e tamanho médio dos nódulos. Os autores atribuem essa melhora ao aumento no vigor dos bacteroides de rizóbios fixadores de N_2 colonizando as células internas dos nódulos, aumentando a assimilação nodular de ferro e, possivelmente, outros nutrientes.

Outros atributos como o teor de N e massa das raízes secas também aumentaram, indicando seu efeito promotor e influência na simbiose rizóbio-leguminosa de uma maneira não específica, mesmo em condições de campo. Gopalakrishnan et al. (2015), avaliando cinco estirpes de *Streptomyces* sp. (CAI-17, CAI-68, CAI-78, KAI-26 e KAI-27) quanto às suas propriedades de promover crescimento em grão-de-bico (*Cicer arietinum*) nas estações pós-chuvas, observaram que todos as estirpes testadas aumentaram o número e massa dos nódulos, peso da raiz e peso da parte aérea 30 dias após a semeadura.

Estas investigações reforçam a capacidade dos *Streptomyces* sp. atuarem como produtores de substâncias biologicamente ativas que lhes confere propriedades como BPCP.

2.7 MICROALGAS

As microalgas são classificadas como seres microscópicos e unicelulares, eucariontes e fotossintetizantes, presentes nos mais diversos ambientes (aquáticos/úmidos), com vantagens únicas, incluindo altas taxas de crescimento, facilidade de cultivo, baixo custo de crescimento e grande capacidade de adaptação, o que permite que seu cultivo seja estabelecido em pequenas áreas (WANG et al., 2014; PEREZ-GARCIA et al., 2011; ODJADJARE et al., 2017).

Esses microrganismos versáteis, ainda pouco explorados e diversos, possuem várias características que ainda precisam ser descobertas e exploradas (ODJADJARE et al., 2017; RIZWAN et al., 2018). Na agricultura, tem se utilizado como condicionadores de solo e biofertilizantes. Nessa função, eles têm capacidades de influenciar o crescimento e o desenvolvimento das plantas melhorando significativamente a germinação, o volume das raízes, a formação de clorofila, o acúmulo de carotenoides, a biomassa da parte aérea, potencial de redução de nitrato e o rendimento de grãos de soja e feijão (TRIPATHI et al., 2008; IYOVO; DU; CHEN., 2010; LIN et al., 2013; ODJADJARE et al., 2017).

Entre as microalgas, o gênero *Chlorella* tem sido amplamente utilizado para biofertilização, principalmente por fornecer grandes quantidades de micro e macronutrientes, metabólitos e hormônios promotores de crescimento de plantas, como as citocininas (WIJFFELS, 2013; ELARROUSSIA et al., 2016; KHOLSSI et al., 2018). Devido a essas características e o potencial biotecnológico, várias pesquisas foram feitas. Shaaban (2001) observou que o extrato aquoso de *C. vulgaris* aplicado via pulverização foliar aumentou a absorção de nutrientes, crescimento e rendimento de trigo (*Triticum aestivum* L. var. Giz 69). Este estudo constatou que uma concentração de 50% (v/v) de extrato de algas via foliar (25 dias após a semeadura) aumentou o crescimento e o ganho de massa em 140% e 40%, respectivamente.

Faheed e Fattah (2008), avaliando o efeito de *C. vulgaris* em alface (*Lactuca sativa*), encontraram aumento de até 186% em diversas características como crescimento, biomassa e teor de pigmentos em mudas adubadas com a microalga. Em 2016, Özdemir et al. (2016), utilizaram esta mesma espécie como biofertilizante na produção de tomate (*Lycopersicon esculentum*) em estufas, obtendo um aumento no crescimento das plantas e em algumas qualidades de frutos como peso seco, sólidos solúveis totais, vitamina C e rendimento. Schreiber et al. (2018) descreveram o potencial de *C. vulgaris* para acumular fósforo e fertilizar substratos pobres em nutrientes.

Refaay et al. (2021) avaliaram o impacto da pulverização foliar com suspensões de *C. vulgaris* como bioestimulantes nas características de crescimento e produção de *Phaseolus vulgaris* e observaram que os tratamentos com as suspensões da microalga tiveram aumentos na altura total das plantas (26,9%), biomassa seca (37,3%), teor de proteínas, carboidratos totais, bem como massa de vagens, número de vagens por planta e número de sementes/vagem. *C. vulgaris* também foi coinoculada em sementes de trevo (*Trifolium* spp.) com diferentes BPCPs, como *Pseudomonas putida*, *Stenotrophomonas maltophilia* e *Serratia proteomaculans*, resultando em raízes mais longas na coinoculação com *S. proteomaculans*, enquanto a coinoculação com *S. maltophilia* resultou em plântulas com maior biomassa seca de raízes e parte aérea (RAPOSO; DE MORAIS, 2011).

2.8 MORFOLOGIA DO SISTEMA RADICULAR

As raízes representam cerca 16 a 63% da biomassa total das plantas e desempenham funções essenciais, como a aquisição de água e nutrientes, armazenamento de recursos, ancoragem das plantas e fonte de C e nutrientes às comunidades microbianas, especialmente na rizosfera (POORTER et al., 2012; ERKTAN; MCCORMACK; ROUMET, 2018).

Em ambientes naturais e agrícolas, a capacidade das plantas em adquirir eficientemente os recursos do solo determina seu sucesso competitivo e o potencial produtivo. Devido à baixa mobilidade de alguns nutrientes e a limitada deposição de carbono no solo de alguns ambientes, as plantas desenvolveram diferentes estratégias de enraizamento para obter os recursos necessários e lidar com a alta heterogeneidade espacial e temporal na disponibilidade de água e nutrientes no solo (JUNGK, 2001).

A capacidade das plantas de explorar o solo em busca de água e nutrientes é definida pela arquitetura do sistema radicular, que compreende aspectos da morfológicos como a quantidade, comprimento, espessura, distribuição das raízes e a incidência e comprimento dos pelos radiculares (JUNGK, 2001; HALING et al. 2016; RONDINA et al. 2020). Em geral, plantas que apresentem raízes com um conjunto de características morfológicas favoráveis, como maior comprimento, baixa densidade de tecidos, alta relação superfície/massa, menor diâmetro e alta incidência de pelos radiculares longos, são mais eficientes na aquisição de recursos do solo, o que contribui para maior eficiência no uso de fotoassimilados para o desenvolvimento e produção (ERKTAN et al., 2018; RONDINA et al., 2019).

As raízes da maioria das espécies de plantas são capazes de produzir pelos radiculares, que são considerados como um subsistema de pequena escala para aquisição de nutrientes (JUNK, 2001). Os pelos radiculares são altamente variáveis em número, comprimento e longevidade. Devido às suas características morfológicas conseguem aumentar a área de exploração das raízes, por permitir contato maior e mais eficaz entre estas e o solo, o que é particularmente importante quando a concentração de nutrientes próxima à superfície da raiz é baixa (JUNK, 2001; HALING et al. 2013).

A fim de melhorar a compreensão das relações estrutura-função das raízes, alguns estudos morfológicos permitem distinguir a função pelo diâmetro. Raízes grossas (diâmetro > 2 mm) são responsáveis pelo transporte e armazenamento de água, nutrientes e compostos orgânicos, e pela ancoragem e estabilização das plantas (FITTER, 2002; ESHEL,2013; MCCORMACK et al., 2015). Raízes finas (diâmetro ≤ 2 mm) são partes mais distais, plásticas e efêmeras das raízes (FITTER, 2002; MCCORMACK et al., 2015) e são as principais responsáveis pela absorção de água e nutrientes (COMAS et al., 2002).

Além da morfologia, a taxa de absorção de água e nutrientes por unidade de massa ou comprimento de raízes também sofre influência de microrganismos, especialmente aqueles que habitam a rizosfera (KOWALCHUK et al., 2002). Os principais microrganismos empregados como bioinsumos na agricultura pertencem aos gêneros *Azospirillum, Bacillus, Beauveria, Burkholderia, Clostridium, Enterobacter, Flavobacterium, Klebsiella, Pseudomonas, Rhizobium, Frankia, Serratia* e *Streptomyces* e *Trichoderma* (ABHILASH et al., 2016; VAN OOSTEN et al., 2017; GOUDA et al., 2018).

Alguns desses microrganismos são capazes de modular fitormônios, como as auxinas, que atuam na regulação da divisão celular, diferenciação do tecido vascular, formação de raízes adventícias e laterais, alongamento e aumento da área de superfície das raízes, e a citocinina, que resulta em aumento do desenvolvimento das raízes, atividade do câmbio vascular, divisão e diferenciação celular (GOUDA et al., 2018; KHAN et al., 2020).

Estudos recentes como de Rondina et al. (2019, 2020) fornecem evidências experimentais de que as interações entre microrganismos benéficos e as raízes podem incrementar características do sistema de raízes e determinar parcialmente o sucesso da planta no ambiente. Rondina et al. (2020) observaram que a coinoculação da soja com *Bradyrhizobium* e *A. brasilense* melhorou várias características morfológicas das raízes, aumentando a capacidade da planta em enfrentar episódios de seca moderada, resultando em maiores produtividades quando comparadas com plantas inoculadas apenas com *Bradyrhizobium*.

Trabalhos como o de Gang et al. (2018) também fornecem relatos positivos de microrganismos benéficos sobre o crescimento e desenvolvimento de características morfológicas das raízes. Foi identificado que a rizobactéria *Klebsiella* SGM 81 produz ácido indol-3-acético nas proximidades das raízes de *Dianthus caryophyllus*, o que foi identificado como o principal mecanismo que explica o aumento da produção de pelos radiculares e crescimento da planta.

Embora as raízes expressem uma pluralidade de morfologias e funções, por estarem escondidas na matriz do solo são difíceis de se estudar. Poucos trabalhos são dedicados ao estudo das características morfológicas das raízes e as implicações que isso traz para o desempenho das plantas. Por isso, avanços nessa área podem contribuir para compreender e melhorar o desenvolvimento de diferentes espécies de plantas, incluindo as comerciais.

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3. ARTICLE A: COMMON BEAN CO-INOCULATION WITH *RHIZOBIUM* AND *STREPTOMYCES*: ROOT MORPHOLOGICAL TRAITS

ABSTRACT

Nitrogen (N) is one of the most required nutrients for common bean (*Phaseolus vulgaris* L.) but its supply via biological nitrogen fixation (BNF) has been little explored. We evaluated the effects of co-inoculation of *Rhizobium* spp. with *Azospirillum brasilense* or five *Streptomyces* sp. strains on germination, root morphological traits and nodulation of common bean, as well as on shoot development and grain yield. Four experiments were carried out: one on *Germitest* paper in the laboratory, another on sterile substrate in the greenhouse, and two under field conditions. The treatments in the laboratory were: (1) non-inoculated control; (2) inoculation with *Rhizobium tropici* CIAT899 + *R. freireii* PRF81 (R); (3) co-inoculation with R + *Azospirillum brasilense* (Ab-V5+Ab-V6); (4) R + *Streptomyces* BTM 287; (5) R + *Streptomyces* BTM 289; (6) R + *Streptomyces* BTM 298; (7) R + *Streptomyces* BTM 290; (8) R + *Streptomyces* BTM 304. In the greenhouse, besides the laboratory treatments, a non-inoculated treatment with mineral N-fertilization was added. In the field experiments, treatments 1 to 3 were maintained, and a control with N-fertilization and the treatments 4 and 7, which were promising in the previous experiments were assessed. In the laboratory, the number of normal, abnormal, and dead seedlings, initial seedling growth (radicle length, shoot length, and number of branches of the root system) were evaluated. In the other trials, plants were evaluated for root dry weight, total length, volume, diameter, root length density, number of branches per meter, number and dry matter of nodules, shoot dry matter and N content, and grain yield. Co-inoculations with *Rhizobium* spp. and isolates BTM290 and BTM287 of *Streptomyces* sp. performed best both in the laboratory (greater seedling viability, increase of root length, and number of branches), and in the greenhouse (smaller root diameters, and higher number and dry mass of nodules). In the field, co-inoculated plants with R + BTM287 and R + BTM290 promoted higher root length densities in the soil and smaller root diameters, while R + *A. brasilense* and R + *Streptomyces* BTM287 increased the volume of roots in the soil. The isolates of *Streptomyces* sp. improved root morphological traits of common bean and showed to be promising for stimulation of root system.

Keywords: Actinobacteria; Bioinputs; *Phaseolus vulgaris*; Root architecture; PGPB.

3.1 INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the main crops produced in Brazil, with almost 3 million tons of grains in the 2021/22 crop season in 2.85 million hectares (CONAB, 2022). Its importance goes beyond the economic feature, being relevant for food safety as important source of energy and proteins in the population diet of several countries, in addition to cultural features since beans are employed in the cuisine of several regional dishes. Common bean has been cultivated from small to large farm areas in all Brazilian regions in diversified agricultural systems (OLIVEIRA et al., 2017).

The intrinsically low fertility of cultivated Brazilian soils, especially poor in nitrogen (N), and low-technological levels are limiting for common-bean yields (PELEGRINI *et al.*, 2009). Estimates are that only 20% of the production comes from technified systems, reaching over 3,500 kg ha^{-1} of grains (EMBRAPA, 2015; OLIVEIRA et al., 2017), whereas the national average yield was 1.050 kg ha^{-1} in the 2021/22 season (CONAB, 2022). Common bean can establish symbiosis with N-fixing bacteria of the *Rhizobium* genus, which colonize the root and form nodules where the biological nitrogen fixation (BNF) process takes place. However, most of N-nutrition in the production systems, especially in the more technified ones, has been based on N-fertilizers. The N-fixing rhizobial strains CIAT 899 (*Rhizobium tropici*) and PRF 81 (*R. freirei*) have been used in commercial inoculants for common bean in Brazil (DALL'AGNOL *et al.*, 2013). However, sometimes the fixed N is not enough to reach high yield levels, being necessary supplemental application with N-fertilizer. This has been attributed to the low nodulation capacity of some genotypes (FONSECA *et al.*, 2013) or due to the high competitivity in the soil with native rhizobia little efficient in N-fixation (RAPOSEIRAS et al., 2006).

The high cost of N-fertilizers and the need for a more sustainable agriculture open opportunity for technologies based on bioinputs to improve plant nutrition and biostimulation for a more efficient fit to the production system. Therefore, technologies for improvement of BNF rates and plant nutrition may contribute to improve crop yield in a more sustainable way. There are evidences that other beneficial bacteria can positively affect the symbiotic performance between *Rhizobium* and the host plant (SATHYA, 2017). Strategies that help the establishment and maintenance of common bean-rhizobia symbiosis and a more effective BNF process, such as co-inoculation with other N-fixing (DA CONCEIÇÃO

JESUS *et al*., 2018) or plant growth-promoting bacteria (PGPB) (HUNGRIA et al., 2013;) have been explored especially in tropical regions to increase the nodulation and the BNF rate in common bean.

Some PGPB as *Azospirillum brasilense* have been widely used as commercial inoculants, including co-inoculation with rhizobia in common bean and soybean [*Glycine max* (L.) Merrill] (SANTOS; NOGUEIRA; HUNGRIA, 2019). Although there is an emerging consensus that *A. brasilense* improves root morphological traits due to synthesis and secretion of phytohormones (RONDINA et al., 2020), most of the studies on co-inoculation of common bean with rhizobia and *A. brasilense* demonstrate a general improvement of the root system (mass and volume) and nodulation (MALINICH; BAUER, 2018).

The actinobacteria belonging to the genus *Streptomyces* are also promising PGPB for co-inoculation with rhizobia in common bean (NONTHAKAEW et al., 2022), but despite popular as biocontrol agents, their use as inoculant is still low (OLANREWAJU; BABALOLA, 2019). Most of *Streptomyces* species efficiently colonizes the rhizoplane and the rhizosphere and can promote plant growth by phytohormones production, such auxins, cytokinins, and gibberellins (SADEGHI *et al*., 2012; JOG; RAKJUMAR, 2016). Although actinobacteria may improve some traits in common bean, most of the plant growth-promoting species have antibacterial activity (ADEGBOYE; BABALOLA, 2012; JOG; RAKJUMAR, 2016), and little is known on how they may affect the symbiotic rhizobia.

Scientific researches on PGPB have focused mostly on the shoot growth and development, whereas roots are have generally been assessed only for mass and root/shoot ratio (HTWE; MOH; YAMAKAWA, 2018). However, morphological traits related to the acquisition of soil resources, as well as effects on seeds germination, are seldom investigated.

Germination and emergence of seedlings are also traits of great importance for the crop development potential. As this is a critical phase, in which the seedlings are more vulnerable to environmental stresses (DUTRA et al., 2016), the increase of the plantlet initial vigor can improve the crop establishment, resulting in greater yield potential. *Streptomyces* spp. can stimulate or inhibit seed germination, depending on the bacterial strain and the plant species (WIDMER; GUERMACHE, 2004; OKAZAWA et al., 2021).

New combinations of PGPB may represent a biotechnological strategy for growing common bean, resulting in faster germination and plants with improved root architecture, and thus improving the crop efficiency and yield potential. The aim of this study was to evaluate germination, shoot attributes, root morphological traists and yield of common bean inoculated with *Rhizobium* spp. or co-inoculated with *A. brasilense* or *Streptomyces* sp.

3.2 MATERIAL AND METHODS

Laboratorial experiments

The strains of *Rhizobium* spp. (CIAT 899 and PRF 81) and *A. brasilense* (Ab-V5 and Ab-V6) were obtained from the “Diazotrophic and Plant Growth-Promoting Bacteria Culture Collection of Embrapa Soja” (WFCC Collection # 1213, WDCM Collection # 1054), in Londrina - PR, Brazil. Inoculants containing *A. brasilense* were prepared in DYGS broth medium (FUKAMI *et al*., 2018) without L-tryptophan, incubated at 28 °C at 120 rpm for 120 h. The concentration of viable cells was adjusted to 2.56×10^8 colony forming units (CFU) mL^{-1}. *Rhizobium* spp. CIAT 899 and PRF 81 were grown separately in modified yeast-mannitol-agar (YMA) medium (HUNGRIA et al., 2016) at 28 °C at 120 rpm for 48 h. Then, both bacterial suspensions were merged and the final viable cells concentration was adjusted to 6.02×10^8 mL^{-1}.

Streptomyces sp. strains were obtained from the “Laboratory for Microbial Genetics Culture Collection, Universidade Estadual de Londrina - UEL”. Compatibility experiments with *Rhizobium* spp. (CIAT 899 and PRF 81) were performed for the strains of *Streptomyces* sp. Previous tests were made in mineral medium (SAMBROOK et al., 1989) by the cross-streaking method (BALOUIRI; SADIKI; IBNSOUDA, 2016). Five *Streptomyces* strains were selected among 20 isolates, which were able to grow in co-culture with *Rhizobium* sp. without impair its development.

The selected strains BTM 287, BTM 289, BTM 290, BTM298, and BTM 304 were cultivated in ISP-2 broth medium (PRIDHAM et al., 1957) at 28 °C, 120 rpm for 7 days. The inoculums were standardized by absorbance at 600 nm and plated on medium ISP-2 for CFU counting, which resulted in 1.44×10^7; 4.17×10^6; 6.61×10^6; 3.77×10^7 and 2.78×10^6 CFU mL^{-1} of broth, respectively.

The germination experiment was performed in the Laboratory for Microbial Genetics at the Dept. of General Biology, Londrina State University, Londrina – PR, Brazil. Treatments were: 1) no-inoculation (Control); 2) inoculated with *Rhizobium* spp. (*R. tropici* CIAT 899 + *R. freirei* PRF 81) (R); 3) R + *Azospirillum brasilense* Ab-V5 + Ab-V6 (R + AZO); 4) R + *Streptomyces* BTM 287 (R + BTM 287); 5) R + *Streptomyces* BTM 289 (R + BTM 289); 6) R + *Streptomyces* BTM 290 (R + BTM 290); 7) R + *Streptomyces* BTM 298 (R + BTM 298); 8) R + *Streptomyces* BTM 304 (R + BTM 304). The experimental design was entirely randomized with four replications.

For each replication, 50 common bean seeds cv. BRS Estilo were surface-disinfested with 70% ethanol for 3 min and 4% of commercial sodium hypochlorite solution for 4 min, followed by 10 rinses with deionized water (ANDRADE; HAMAKAWA, 1994). One milliliter of each inoculant was added to the seeds, according to the treatment. The seeds were disposed on *Germitest®* paper moisturized with deionized water (2.5 times the dry paper weight), rolled, and incubated at 22 °C under 12/12 h of light/dark. The counting of germinated seedlings was made at the fifth and ninth day after incubation. The percentages of dead seeds, normal and abnormal seedlings were recorded (BRASIL, 2009). The seed viability was calculated by the (G – I) expression, where G represents the total germinated seeds and I the germinated seeds that resulted in inviable seedlings (BRASIL, 2009).

Shoot and primary root lengths of normal plantlets and the numbers of primary root ramifications were also recorded.

Greenhouse experiment

The experiment was performed in the greenhouse of the Soil Biotechnology Laboratory of Embrapa Soja, Londrina – PR, Brazil, in modified Leonard jars (VINCENT, 1970), containing sterile substrate composed of washed sand and crushed coal (3:1, v:v), receiving N-free sterile nutrient solution (BROUGHTON and DILWORTH, 1971).

Treatments were similar to the laboratory experiment, in addition to a non-inoculated, N-fertilized control (Control + N), to provide 80 mg L^{-1} of N in the nutrient solution as KNO_3. In each pot, five seeds of common bean cultivar BRS Estilo previously surface-disinfested (ANDRADE; HAMAKAWA, 1994) and inoculated according to the respective treatment were sown. Five days after emergence (DAE) plantlets were thinned to one per pot. At 30 DAE plants were removed from the pots and roots were separated from shoots. The roots were washed in tap water and the nodules collected, counted, and dried at 60 °C until constant mass to assess the nodule dry weight (NDW).

Total root length (TRL) was determined by the line intersection method in Petri's dishes (TENNANT, 1975). Root volume (RV) was determined by the water displacement in graduated cylinder. Average root diameter (RD) was calculated by the expression $2\ (V\ /\ L\pi)^{0.5}$, where V and L represents the root system volume and length, respectively (RONDINA et al., 2020).

Root (RDW) and shoot (SDW) dry weights were taken after drying at 60 °C until constant weight. Dry shoots were ground in a mill (18 mesh) and the N concentration was determined by the Berthelot method (WILLIS et al., 1996). Root specific length (RSL) was obtained as the ratio between total length and its respective dry mass. Root tissue density (RTD) was determined by the ratio between dry mass and its respective volume.

Field experiments

Two field experiments were performed in the 2020 crop season in Arapoti, PR (24° 09' 28'' S, 49° 49' 37'' W, 860 m altitude a.s.l.). The local climate is classified as Cfa (wet subtropical) according to Köppen-Geiger's classification (CAVIGLIONE et al., 2000). The soil is classified as Typic Acrustox according to Soil Survey Staff (2014) (or "Latossolo Vermelho escuro", according to the Brazillian classification – Santos et al., 2018) and with loamy loan texture.

Experiment 1 was carried out with common bean cv. BRS Esteio, whereas experiment 2 used cv. BRS Pérola. Both cultivars present indeterminate growth type, with closed branches (type II) and opened branches (type III), respectively (RODRIGUES et al., 2005; PEREIRA et al., 2013).

Six treatments were defined for each cultivar: (1) non-inoculated control (Control); (2) non-inoculated, N-fertilized (80 kg ha^{-1} of N as urea in topdressing) (Control + N); (3) inoculated with *Rhizobium tropici* CIAT899; (3) R + *A. brasilense* Ab-V5 + Ab-V6 (R + Azo); (4) R + *Streptomyces* sp. BTM 287 (R + BTM 287); (5) R + *Streptomyces* sp. BTM 290 (R + BTM 290). The experimental design was in randomized blocks with five replications.

Inoculation with *Rhizobium tropici* CIAT 899 (R) and *Azospirillum* *brasilense* (Ab-V5 + Ab-V6) were made with peat-based commercial inoculants. Before use, an analysis of each $^{-1}$ of *R. Tropici*, both registered in the Ministry of Agriculture, Livestock and Food Supply of Brazil as inoculants for common bean. The *Streptomyces* sp. BTM287 and BTM 290 were selected based on the results inoculant revealed cell concentration of $1{,}9 \times 10^9$ CFU g^{-1} of *Azospirillum* and $3{,}8 \times 10^8$ CFU g of the greenhouse experiment and grown in the ISP-2 medium up to cell concentration of 1.44×10^7 CFU mL^{-1} (PRIDHAM et al., 1957). The inoculants were applied to provide 1.2×10^6, 2.0×10^5, 6.0×10^6, and 2.0×10^6 CFU $seed^{-1}$ of *Rhizobium* spp., *A. brasilense*, BTM287, and BTM290, respectively.

The plots were composed by six lines of 6 m length spaced 0.4 m between lines, totaling 16.2 m^2 (2.7 m x 6.0 m). The sowing density was 12 seeds per linear meter to allow 10 plants per meter; simultaneously to sowing, 56 kg ha^{-1} of P_2O_5 (PEREIRA, 2013) were applied in furrow. N-fertilizer was applied only in the treatment 2 (positive control with N), with N fertilizer (Super N - urea) to provide 80 kg ha^{-1} of N, distributed between sowing (40 kg ha^{-1}) and topdressing after 15 DAS (40 kg ha^{-1}).

Evaluations were made in the four central lines, disregarding 0.50 m of each edge. In both experiments, three plants were randomly selected in each plot for assessment of root morphological traits at the R6 stage (FERNANDEZ *et al.*, 1982). In each plot, three undeformed subsamples were taken perpendicularly to the sowing line using a metallic cylinder of 192.3 cm^3 (5 cm height and 7 cm of diameter), at 5 cm from the selected plant.

Each sample was immersed in water for 15 min to make easier the detachment of the adhering soil from roots. Then, the subsamples were pooled and subjected to wet sieving (0.50 mm mesh) to manually separate living roots from the dead ones based on their tissue consistency, color, and elasticity (GOWER, 1987). Root length (RL) was assessed by the gridline intersection method (TENNANT, 1975) and used to calculate the root length density (RD) in the soil (root length per soil volume). The number of ramifications per meter of root was calculated as the ratio between the number of ramifications and the root length. The specific root length, average diameter, dry weight, and root volume were assessed as described for the greenhouse experiment.

The harvest occurred in July 12, 2020, at the full maturation stage (R9). Grain yield was determined in the central useful area of each plot (5,4 m^2), disregarding 0.50 m of each edge of the plot. The grains were cleaned, weighted, and the mass adjusted to 13% of moisture based on a grain moisture meter (Gehaka Agri G800 model, São Paulo, Brazil).

Data analysis

The dataset was evaluated for normality of errors by Shapiro-Wilk and homoscedasticity by Bartlett's tests previously to ANOVA. Percentage of germination and seed viability were subjected to transformation to arc-sine $(x/100)^{0.5}$, whereas the number of nodules and nodules dry weight were transformed to log (x + 1.5) to meet the assumptions of the parametric test. The dataset was analyzed by one-way ANOVA, following the Skott Knott's test ($\alpha = 5\%$) using the R Studio (R Core Team, 2019) software.

3.3 RESULTS AND DISCUSSION

The treatments did not affect germination, however the viability of seeds increased when co-inoculated with *Rhizobium* spp. and some *Streptomyces* spp. strains (R + BTM287, R + BTM290, and R + BTM298) (Table 1). Although the objective of our work was not to evaluate the production of phytohormones, some actinobacteria, especially belonging to the genus *Streptomyces*, displays plant growth-promoting capacity due to production of phytohormones, especially auxins, gibberellins, and cytokinins groups, like indoleacetic acid (IAA), gibberellic acid (GA3), and zeatin and kinetin, respectively (ALDESUQUY; MANSOUR; ABO-HAMED, 1998; SOLANS et al., 2011).

Auxins and cytokinins promote seed germination by upregulation of expression and transcription of genes related to cyclins, which belong to a family of proteins that control the progression of the cell cycle (XUE *et al.*, 2021). IAA can increase the number of lateral roots on seedlings by either exogenous application or by bacterial production (KOLB; MARTIN, 1985; ZELENA; KUTACEK; CERMAK, 1988; CASSÁN et al., 2009).

In our study the seedlings originated from seeds co-inoculated with R + BTM 287, R + BTM 290, R + BTM 298, and R + BTM 304 presented higher root lengths, while the number of root branches increased with R + BTM 287 and R +

BTM 290 (Table 1). Faster root development, by either primary root elongation or emergence of secondary lateral roots, allows the young seedlings to have more and earlier access to nutrients and water (PATTEN; GLICK, 2002).

Sreevidya et al. (2016) evaluating four *Streptomyces* sp. SAI-13 and SAI-29 (isolated from chickpea rhizosphere), VAI-7 (from *Annona squamosa* L. vermicompost) and VAI-40 (from *Jatropha curcas* L. vermicompost) inoculated in *Cicer arietinum* L. observed a 17% increase in radicle length compared with the uninoculated control. In the present study, the increase in radicle length was up to 33.6% in seeds co-inoculated with R + BTM 304. The shoots of plants inoculated with R, or co-inoculated with R + AZO, R + BTM 290, R + BTM 298, and R + BTM 304 were taller than those co-inoculated with R + BMT 287 and R + BTM 289 (Table 1).

Due to its high capacity to produce metabolites with biopesticide capacity, most of the studies on the genus *Streptomyces* have focused on their biocontrol activities, but its plant growth-promotion ability has also been surveyed (NASSAR et al., 2003; EL-TARABILY 2008; OLANREWAJU; BABALOLA, 2018). Sadeghi et al. (2012) showed that a *Streptomyces* isolate (C), originally designated for biocontrol, also had plant growth-promoting capacity due to production of IAA and siderophores, phosphate solubilization, and improvement of plant tolerance to saline stress. Results included increases in N, P, Fe, and Mn shoot contents, germination rates, height and dry weight of shoots. Most of *Streptomyces* are efficient colonizers of the rhizosphere and rhizoplane. Some *Streptomyces* isolates increased the shoot N content, heigh, and biomass of several crops.

.In the greenhouse experiment, the plants co-inoculated with R+ Azo, R+ 287, R+289 or R+290 presented the highest root dry mass (Figure 1D). The root length and volume of plants co-inoculated with R + BTM 287 or R + BTM 290 differed from the single inoculation with *Rhizobium* spp., resulting in an amazing increase by 135% and 106% for root length, and by 36% and 32% for root volume, respectively (Figure 1A and 1B).

The positive response on plant root growth due to co-inoculation with R + BTM287 and R + BTM290, in both laboratory and greenhouse experiments, indicates positive effects of beneficial microorganisms, promoting improvements in the architecture of the root system that make the plant more efficient in exploring the soil for water and nutrients and then support greater and more stable yield (DA SILVA et al., 2004; HALING et al., 2013; ERKTAN; MCCORMACK; ROUMET, 2018).

In addition to length, thinner root diameter (≤ 2 mm) make the root system more effective in reaching water and nutrients (ROSOLEM, 1995; RYLTER, 1997). The influx, especially for nutrients of low mobility in the soil, increases with the decrease in the fine root diameter (EISSENSTAT, 1992).

The roots of plants co-inoculated with R + BTM 287, R + BTM 290, R + BTM 298, and R + BTM 304 had the lowest root diameters (Figure 1C). Thinner roots proliferate more quickly in the soil and are more efficient than thicker root systems in the acquisition of resources (EISSENSTAT, 1992).

Co-inoculation with R + BTM 287, R + 289, and R+ BTM 290 also increased nodulation and nodule dry weight of common-bean (Figure 1F). Similarly, the inoculation with *Streptomyces lydicus* strain WYEC108 increased the size of nodules of *Pisum sativum* L. (TOKALA *et al.*, 2002).

Positive effects of co-inoculation with *Streptomyces* in the nodulation were also observed in *Cicer arietinum* (SREEVIDYA *et al.*, 2016) and *Glycine max* (HTWE; MOH; YAMANAKA, 2018). It is worth mentioning that in our study we observed improvement of root morphological traits in addition to an increase in nodulation. A more ramified and longer root system can result in more root nodulation due to greater probability of interception of rhizobial cells and more infection sites (RONDINA *et al.*, 2020).

Finally, plants co-inoculated with R + 290, R + 289, R + 287, and R + Azo presented more shoot dry weight (Figure 1G) and total N accumulated in plants (Figure 1H).

In the field, co-inoculation of common bean genotype BRS Esteio with R + AZO, R + BTM 287, and R + BTM 290 increased the root length density in the soil. The co-inoculations R + BTM 287 and R + BTM 290 decreased root diameter, whereas R + AZO and R + BTM 287 increased the root volume (Table 2). Co-inoculation with R + BTM 287 improved three root morphological traits, i.e., increased root length and volume, and decreased the root diameter. These traits are associated with higher plant capacity for acquisition of resources from the soil.

As for BRS Esteio, BRS Pérola plants co-inoculated with R + BTM 287 also had improvement in the root morphological traits. The root length density in the soil increased whereas the root diameter decreased compared with the non-inoculated controls (Table 2). For this genotype, the co-inoculation with both *Streptomyces* strains decreased the number of root ramifications.

The treatments did not affect grain yield in both common bean genotypes, even the N-fertilized control (Table 2). The average yield is considered high compared with the national average (CONAB, 2022) and suggests ideal conditions during the plant development. However, under adverse conditions like short periods of drought, improvements in root morphological traits promoted by co-inoculation can be crucial for a greater stability of grain yield (HUNGRIA *et al.*, 2015; SILVA *et al.*, 2019).

3.4 CONCLUSION

The co-inoculations of *Rhizobium* spp. and *Streptomyces* (strains BTM 287 and BTM 290) improved the root morphological traits and nodulation of common bean in greenhouse, as well as increased the viability of seedlings and radicle ramification in the laboratory experiments.

In the field, the co-inoculation of common bean genotypes BRS Pérola and BRS Esteio with R + BTM 287 improved the root morphological traits that can improve the plant capacity to explore the soil, evidencing the biotechnological potential of this combination of beneficial microorganisms.

Table 1. Seed germination, viability of seedlings, shoot height, radicle length, and number of lateral roots at nine days after inoculation/co-inoculation with *Rhizobium* spp., *Azospirillum brasilense* or *Streptomyces* sp. isolates in the laboratory experiment.

Treatment [a]	Germination	Viability	Shoot height	Radicle length	lateral roots
	(%)	(%)	(cm)	(cm)	(number per plant)
Control	78 a	79 b	10.2 a	9.45 b	15.2 d
R	82 a	84 b	9.7 a	8.57 b	13.2 d
R + AZO	80 a	85 b	10.5 a	9.05 b	14.7 d
R + BTM287	79 a	93 a	8.7 b	9.65 a	38.5 a
R + BTM289	89 a	88 b	7.4 b	7.30 c	31.2 b
R + BTM290	77 a	96 a	9.6 a	10.52 a	41.0 a
R + BTM298	76 a	93 a	10.6 a	10.45 a	33.5 b
R + BTM304	78 a	79 b	9.9 a	11.45 a	22.0 c
p-value	0.031	<0.001	<0.001	<0.001	<0.001
C.V. (%)	9.45	10	9.75	5.78	6.47

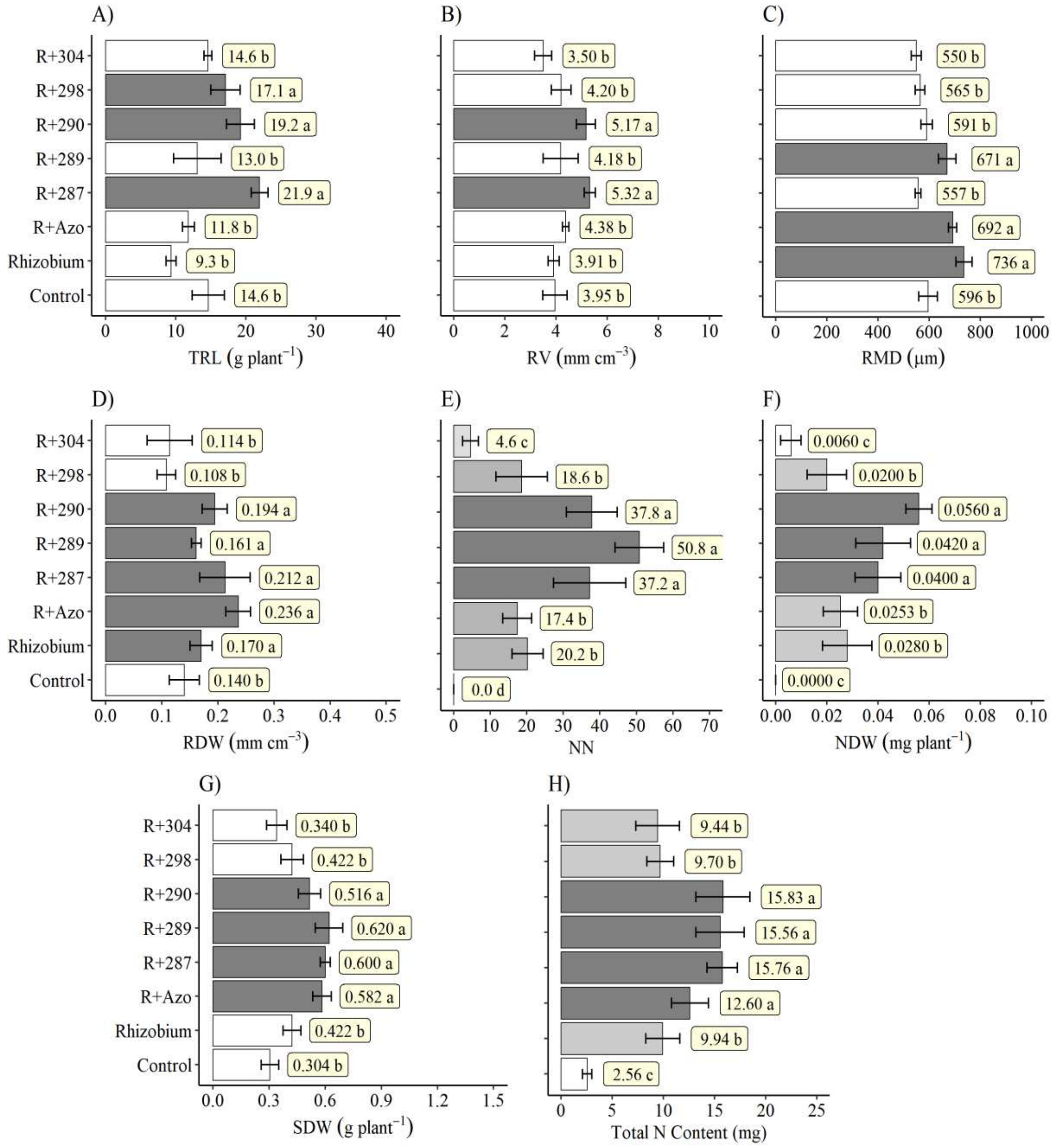
[a] Control = non inoculated; R = inoculation with *Rhizobium* spp. CIAT 899 and PRF 81; R + Azo = co-inoculation with *Rhizobium* spp. CIAT 899 and PRF 81 and *Azospirillum brasilense* Ab-V5 and Ab-V6; R + BTM 287 = co-inoculation with R and *Streptomyces* BTM 287; R + BTM 289 = co-inoculation with R and *Streptomyces* BTM 289; R + BTM 290 = co-inoculation with R and *Streptomyces* BTM 290; R + BTM 298 = co-inoculation with R and *Streptomyces* BTM 298; R + BTM 304 = co-inoculation with R and *Streptomyces* BTM 304. Data represent the means of four replicates. Means followed by different letters differ from each other by the Scott-Knott test at p ≤ 0.05.

Table 2. Root morphological traits and grain yield of two common bean cultivars (BRS Esteio and BRS Pérola) with different inoculation/co-inoculation with *Rhizobium* spp., *Azospirillum brasilense* or *Streptomyces* sp. isolates in field experiments performed in Arapoti, PR, in the 2019/20 season.

Treatments[a]	Root length density in the soil	Average root diameter	Root volume	Root ramifications	Grain yield
	(mm cm^{-3} soil)	(µm)	(mm^3 cm^{-3})	(n° m^{-1})	(kg ha^{-1})
			BRS Esteio		
Control	1.55 b	445 a	0.24 b	284	2959
Control + N	3.59 b	385 a	0.40 b	311	3389
R	3.79 b	401 a	0.39 b	276	3023
R + AZO	7.05 a	402 a	0.79 a	303	3061
R + BTM287	11.18 a	325 b	0.91 a	263	2892
R + BTM290	8.13 a	283 b	0.37 b	285	2867
p-value	0.015	0.017	<0.001	0.769	0.560
C.V. (%)	59.85	15.93	32.57	17.35	13.9
			BRS Pérola		
Control	3.48 b	441 a	0.50	251 a	2555
Control + N	5.60 b	445 a	0.85	220 b	2848
R	8.02 b	369 b	0.85	286 a	2613
R + AZO	7.15 b	3301 c	0.62	274 a	2936
R + BTM287	18.13 a	312 c	0.81	219 b	2737
R + BTM290	10.26 b	243 d	0.74	230 b	2506
p-value	<0.001	<0.001	0.290	0.006	0.712
C.V. (%)	40.62	8.92	32.60	10.30	16.50

[a] Control = non inoculated; Control + N = non inoculated and N-fertilized (80 kg ha^{-1} of N as urea); R = inoculation with *Rhizobium* spp. CIAT 899 and PRF 81; R + Azo = co-inoculation with *Rhizobium* spp. CIAT 899 and PRF 81 and *Azospirillum brasilense* Ab-V5 and Ab-V6; R + BTM 287 = co-inoculation with R and *Streptomyces* BTM 287; R + BTM 290 = co-inoculation with R and *Streptomyces* BTM 290. Data represent the mean of four replicates. Means followed by different letters differ from each other by the Scott-Knott test at p ≤ 0.05.

Figure 1. Common bean root morphological traits in different inoculation/co-inoculation treatments in the greenhouse experiment. Error bars indicate standard deviation (n = 5). Means followed by different letters differ from each other by the Scott-Knott test at $p \leq 0.05$. Control = non inoculated; Control + N = non inoculated and N-fertilized; R = inoculation with *Rhizobium* spp. CIAT 899 and PRF 81; R + Azo = co-inoculation with *Rhizobium* spp. CIAT 899 and PRF 81 and *Azospirillum brasilense* Ab-V5 and Ab-V6; R + BTM 287 = co-inoculation with R and *Streptomyces* BTM 287; R + BTM 289 = co-inoculation with R and *Streptomyce*s BTM 289; R + BTM 290 = co-inoculation with R and *Streptomyces* BTM 290; R + BTM 298 = co-inoculation with R and *Streptomyces* BTM 298; R + BTM 304 = co-inoculation with R and *Streptomyces* BTM 304. TRL = Total root length (A); RV = Root system volume (B); RD = Root average diameter (C); RDW = Root dry weight (D); NN = Number of nodules (E); NDW = Nodule dry weight (F); SDW = Shoot dry weight (G); Total N content = Total Nitrogen content (H).


A)
R+304
R+298
R+290
R+289
R+287
R+Azo
Rhizobium
Control
14.6 b
17.1 a
19.2 a
13.0 b
21.9 a
11.8 b
9.3 b
14.6 b
0
10
20
30
40
TRL (g plant^{-1})
B)
3.50 b
4.20 b
5.17 a
4.18 b
5.32 a
4.38 b
3.91 b
3.95 b
0
2
4
6
8
10
RV (mm cm^{-3})
C)
550 b
565 b
591 b
671 a
557 b
692 a
736 a
596 b
0
200
400
600
800
1000
RMD (μm)
D)
R+304
R+298
R+290
R+289
R+287
R+Azo
Rhizobium
Control
0.114 b
0.108 b
0.194 a
0.161 a
0.212 a
0.236 a
0.170 a
0.140 b
0.0
0.1
0.2
0.3
0.4
0.5
RDW (mm cm^{-3})
E)
4.6 c
18.6 b
37.8 a
50.8 a
37.2 a
17.4 b
20.2 b
0.0 d
0
10
20
30
40
50
60
70
NN
F)
0.0060 c
0.0200 b
0.0560 a
0.0420 a
0.0400 a
0.0253 b
0.0280 b
0.0000 c
0.00
0.02
0.04
0.06
0.08
0.10
NDW (mg plant^{-1})
G)
R+304
R+298
R+290
R+289
R+287
R+Azo
Rhizobium
Control
0.340 b
0.422 b
0.516 a
0.620 a
0.600 a
0.582 a
0.422 b
0.304 b
0.0
0.3
0.6
0.9
1.2
1.5
SDW (g plant^{-1})
H)
9.44 b
9.70 b
15.83 a
15.56 a
15.76 a
12.60 a
9.94 b
2.56 c
0
5
10
15
20
25
Total N Content (mg)

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4. ARTICLE B: CO-INOCULATION OF *BRADYRHIZOBIUM* SPP. WITH GROWTH-PROMOTING MICROORGANISMS CHANGES ROOT MORPHOLOGICAL TRAITS AND IMPROVES SOYBEAN YIELD

ABSTRACT

We evaluated the effects of co-inoculation of soybean with *Bradyrhizobium japonicum* and *B. diazoefficiens* and four microorganisms on root morphological traits and nodulation, as well as on shoot development and grain yield. Experiments were carried out: in a sterile substrate in a greenhouse and two in field conditions (Lutecia and Paranavai). The greenhouse treatments were: non-inoculated (Control); inoculated with *Bradyrhizobium* spp. SEMIA 5079 and SEMIA 5080 (Brady); co-inoculation with Brady and *Azospirillum brasilense* Ab-V5 and Ab-V6 (Brady + Azos); co-inoculation with Brady and *Chlorella vulgaris* (Brad + Chlo); co-inoculation with Brady and *Nostoc muscorum* (Brady + Nost); co-inoculation with Brady and *Streptomyces* sp. BTM 289 (Brady + Stre); Brady + Strep + foliar spray with *Streptomyces* sp. BTM 289 at V3 stage (Brady + Stre + Stre(f)). In the field experiments, a non-inoculated treatment with N-mineral fertilization (Control + N) was added. Plants were evaluated for root dry mass, total length and length by diameter class, volume, diameter, root length density, number of branches per meter, number and dry matter of nodules, shoot dry matter, and N content and yield in the field. The Brad + Azos and Brad + Nost treatments had the longest root lengths and lowest root tissue densities among the inoculated groups. In the field, in Lutécia, the co-inoculation Brad + Nost improved root dry mass, length density, root volume, number of nodules and nodule dry mass, while in Paranavai, co-inoculation Brady + Azo presented greater root length density in the soil, and number and mass of dry nodules were higher for inoculated and co-inoculated treatments compared with the controls. The highest grain yield and N concentration in Lutecia were reached in the Control + N and Brady + Azo treatments, whereas in Paranavai the Control + N, but immediately followed by the co-inoculated treatments. Coinoculation of BPCPs and *Bradyrhizobium* is presented as a tool to maximize attributes of the root system and yield.

Keywords: Actinobacteria; *Glycine max*; Root architecture; PGPB.

4.1 INTRODUCTION

Biological nitrogen fixation (BNF) is particularly important for soybean [*Glycine max* (L.) Merill] crop, since N-fixing bacteria of the genus *Bradyrhizobium* establish symbiotic relationship with the plant for nitrogen (N) supply (ORMEÑO-ORRILLO; HUNGRIA; MARTINEZ-ROMERO, 2013; HUNGRIA; MENDES, 2015). Moreover, N is the most required nutrient by soybean, about 80 kg of N for each 1,000 kg of grains (HUNGRIA; MENDES 2015; HUNGRIA; NOGUEIRA 2019). The use of BNF instead of N-fertilizers has been an environmentally-friendly approach for supply the crop requirements for N, since N-fertilizers production and application have several implications on environmental impacts and economic costs (HUNGRIA et al. 2006; HUNGRIA; MENDES, 2015).

Brazil has been the world's leader on soybean production and exportation (USDA, 2021). One of the reasons for this leadership is the use inoculation with elite *Bradyrhizobium* strains to fulfill the soybean N needs (HUNGRIA et al. 2006, 2020; HUNGRIA; MENDES 2015; HUNGRIA; NOGUEIRA, 2019), providing high grain yield at low cost. The commercialization of inoculants in Brazil reached about 70 million doses in 2019, mostly for soybean (SANTOS; NOGUEIRA; HUNGRIA, 2019). It is noteworthy that the replacement of N-fertilizer by the BNF saves over U$ 17 billion per year in Brazil (TELLES et al., 2023).

Co-inoculation of *Bradyrhizobium* spp. and *Azospirillum brasilense*, a plant growth-promoting bacteria (PGPB), has shown to be beneficial, stimulating early nodulation, root development, and BNF in soybean, resulting in yield gains (HUNGRIA; NOGUEIRA; ARAUJO, 2013; CHIBEBA et al., 2015; HUNGRIA; NOGUEIRA; ARAUJO, 2015; CEREZINI et al., 2016; TEIXEIRA FILHO et al., 2017; RONDINA et al., 2020). Besides *Azospirillum*, other bacteria have also been described as PGPB, such as cyanobacteria and actinobacteria (TOKALA et al., 2002; PRASANNA et al., 2012; HASHTROUDI et al., 2012; LAHROUNI et al., 2013).

Nostoc muscorum is a filamentous cyanobacterium that can fix N as a free-living or in symbiosis with plants of the genus *Gunnera* and *Azolla* (SANTI; BOGUSZ; FRANCHE, 2013). In this cyanobacterium, N is fixed in an specialized structure named heterocyst, in addition to release of several biologically active metabolites, such as phytohormones and hygroscopic polysaccharides that lead to systemic responses and improve plant growth (PRASANNA et al., 2012).

Streptomyces sp. is an Actinobacteria that can benefit the plant by several mechanisms like stimulus to BNF (SATHYA; VIJAYABHARATHI; GOPALAKRISHNAN, 2017), production of phytohormones (JOG; NARESHKUMAR; RAJKUMAR, 2016), phosphate solubilization, and iron uptake (JOG et al., 2014). *Streptomyces* sp. can also relieve plant biotic and abiotic stresses (GLICK, 2012; BOUIZGARNE, 2013; DEY; PAL; TILAK, 2014).

Green microalgae have been applied in the production of biofuel, human nutrition, animal feed, wastewater treatment, and agriculture (SARFI et al., 2014). *Chlorella vulgaris* is one of the most remarkable green microalgae (SARFI et al., 2014) and its use as biofertilizer has been promising, since it can improve growth and nutrition of several plant species (KIM et al., 2014; OZDEMIR; SUKATAR; OZTEKIN, 2016; DINESHKUMAR et al., 2020; AL DAYEL; EL SHERIF, 2021). However, little is known about microalgae effects on soybean development.

The ability of a plant to exploit the soil for water and nutrients is in part defined by the architecture of its root system, including quantity, length, thickness, and distribution, and root hair incidence and length (JUNGK, 2001; HALING et al. 2016; RONDINA et al. 2020). However, most studies have not focused on investigating beneficial changes on root morphological traits caused by PGPB. The aim of this study was to evaluate changes in shoot attributes, root morphological traits and soybean yield due to co-inoculation with *Bradyrhizobium* spp. and different plant growth-promoting microorganisms.

4.2 MATERIAL AND METHODS

Greenhouse experiment

The experiment was performed in a greenhouse at Embrapa Soja, Londrina - PR, Brazil (23° 11 '30.7" S, 51°11' 00.8" W). Soybean plants (cv. BRS 1010 IPRO) were cultivated in 500 cm³ modified Leonard jars (YATES et al. 2016) containing sterilized substrate composed by sand and crushed coal (3:1, v/v) supplied with sterile nutrient solution without N, pH 6.6-6.8 (BROUGHTON; DILWORTH, 1971). The treatments consisted of: (1) non-inoculated (Control); (2) inoculated only with *B. japonicum* SEMIA 5079 and *B. diazoefficiens* SEMIA 5080 (Brad); (3) co-inoculated with Brad and *A. brasilense* Ab-V5 and Ab-V6 (Brad +

Azos); (4) co-inoculated with Brad and *Chlorella vulgaris* (Brad + Chlo); (5) co-inoculated with Brad and *Nostoc muscorum* (Brad + Nost); (6) co-inoculated with Brad and *Streptomyces* sp. BTM 289 (Brad + Stre); (7) co-inoculation with Brad and Stre + leaf spray of *Streptomyces* sp. BTM 289 at the V3 stage (Brad + Stre + Stre (f)). The experimental design was completely randomized with six replications for each treatment.

Production of inoculum and experimental setup

The *Streptomyces* sp. strain BTM 289 was obtained from the "Laboratory for Microbial Genetics and Culture Collection of the State University of Londrina – UEL", Londrina PR Brazil. The strain was cultivated in ISP-2 broth (PRIDHAM et al., 1957), at 28 °C, and shaking at 120 rpm for 120 h. The concentration of viable cells was adjusted based on the absorbance (O. D. 600 nm) to 3.67×10^{7} colony forming units (CFU) mL^{-1}.

The cyanobacterium, *Nostoc muscorum* strain 027, was provided by the Laboratory and Culture Collection of Cyanobacteria and Phycotoxins at the Federal University of Rio Grande (FURG), Rio Grande do Sul, Brazil. The inoculant was prepared cultivating *N. muscorum* in BG11 medium (STANIER et al., 1971) without N for 30 days in a growth chamber with 12 h of light/dark at 30 °C and 22 °C, respectively. After growing, *N. muscorum* cells were centrifuged (4,000 × *g*, 10 min, 25 °C) (centrifuge Z383 HERMLE K model) and the pellet was resuspended in the same growth medium to reach the cell concentration $2{,}70 \times 10^{8}$ CFU mL^{-1} (GAVILANES et al., 2020).

The *Chlorella vulgaris* strain was obtained from the IPR microalgae culture collection of the Rural Development Institute – RDI, Londrina PR Brazil. The inoculant was prepared by cultivating the microalgae in Bold Basal Medium (BBM) (STANIER et al., 1971) for 30 days in a growth chamber with 12 h light/dark at 30 °C and 22 °C, respectively. After growth, the *C. vulgaris* cells were centrifuged (4,000 × *g*, 10 min, 25 °C) (model Z383 HERMLE K centrifuge) and the pellet was resuspended in BBM to reach a cell concentration of 1.50×10^{8} CFU mL^{-1} (GAVILANES et al., 2020).

The strains of *A. brasilense* and *Bradyrhizobium* spp. were obtained from the “Diazotrophic and Plant Growth-Promoting Bacteria Culture Collection of Embrapa Soja” (WFCC Collection # 1213, WDCM Collection # 1054), Londrina PR Brazil. The *A. brasilense* strains Ab-V5 (= CNPSo 2083) and Ab-V6 (= CNPSo 2084) were selected from a program for effective growth promotion of maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) (HUNGRIA et al., 2010). The inoculants containing *A. brasilense* were prepared in DYGS broth medium (FUKAMI et al., 2018) without L-tryptophan and, after growth for 120 h at 28 ºC and 120 rpm, the concentration of viable cells was adjusted to 2.56×10^5 CFU mL^{-1}. The *Bradyrhizobium* spp. strains SEMIA 5079 and SEMIA 5080 were cultivated in YM broth medium (HUNGRIA et al., 2016) at 28 ºC and 120 rpm for 72 h. The concentration of viable cells was adjusted to 6.02×10^9 CFU mL^{-1} (O.D. 660 nm).

Before sowing, soybean seeds were surface-disinfected by immersion in 70% ethanol for 1 min, followed by 10% commercial sodium hypochlorite (2-3%) for 3 min, and then rinsed six times in sterile distilled water (YATES et al., 2016). Five seeds were sown per pot, then each seed was inoculated with 1.0 mL of inoculant according to the respective treatment. After the emergence, plantles were thinned to one per pot. The leaf application of *Streptomyces* sp. was made at V3 stage using a manual sprayer to apply 1.0 mL per plant.

Plant sampling, and shoots and roots analysis

Thirty days after emergence (DAE), plants were collected, and shoots and roots were separated. Shoots were dried at 60 °C until constant mass. The N shoot content was determined by the Green Salicylate method (WILLIS et al., 1996) in sulfuric extract.

The root system was washed in tap water and its volume was determined by water displacement in a graduated cylinder. Total root length was measured by the line intersection method (TENNANT, 1975). Root diameter was calculated by the formula $2(V/L\pi)^{0,5}$, where V and L correspond to the volume and the root length, respectively (RONDINA et al., 2020).

A root system sample was separated, dyed with methylene blue 1%, carefully scattered on glass trays containing a few amount of water, and scanned in a table scanner at 600 dpi resolution for image analysis with the Safira® software (JORGE et al., 2010) to assess the roots length by class of diameter (< 0.50 mm;

between 0.50 and 1.00 mm; between 1.01 and 2.00 mm; > 2.00 mm) (RONDINA et al., 2020).

Approximately 150 mg of thin fresh roots from each plant was maintained in FAA solution (5% formaldehyde, 5% acetic acid, and 90% ethanol-70%) for counting of lateral roots and analysis of root hair incidence and length. Randomly, 100 root hairs were selected for measurement of length under an optical microscope (×100 magnification) with micrometric ocular (ZANGARO et al., 2005).

To estimate the number of lateral roots, the number of tips in the root sample was obtained under a stereomicroscope at ×30 magnification. After analysis, the root system was dried at 60° until constant mass for weighting. Tissue density was calculated as the ratio between root dry weight and its respective volume. specific root tissue density was calculated as the ratio between total length and its respective dry weight. Finally, the root/shoot ratio between root dry weight and shoot dry weight was calculated.

Field experiments

Experimental sites, design, treatments, and management

Two field experiments were performed in the 2018/2019 seasons, in Lutécia, SP (22º 12 '23.1” S, 50º 25' 59.5” W, 460 m altitude) and in Paranavaí, PR (22º 57 '29.9 ”S, 52º 27' 88.3” W, 405 m altitude). The soils in both sites are classified as “Neossolo Quazarênico” by the Brazilian System of Soil Classification (SANTOS et al., 2018), corresponding to Quartzipsamments (SOIL SURVEY STAFF, 2014), with very sandy texture (<10% of clay). The climate at both sites is classified as Cfa (mesothermal, humid subtropical), according to Köppen-Geiger (CAVIGLIONE et al., 2000).

Sowings were performed on October 23 and 30, 2018, in Local 1 and in Local 2, respectively. Before sowing, soil samples were taken at 0-20 cm of depth for estimating the established population of soybean-nodulating Bradyrhizobia, and chemical and granulometric analyses at Embrapa Soja laboratories. *Bradyrhizobium* population was estimated by the most probable number (MPN) method (HUNGRIA et al., 2006), using soybean cv. BRS 1010 IPRO as trap plant (Table 1).

The temperature, rainfall, and potential evapotranspiration in Lutécia and Paranavaí are shown in Figure 1. Data were collected from the Agrometeorological Information Integrated Center (http://www.ciiagro.sp.gov.br/ciiagroonline/) and from the agrometeorological station of the Agronomic Institute of Paraná (www.iapar.br), for Lutécia and Paranavaí, respectively. The soybean season in both sites was atypical with severe occurrence of drought, characterized by potential evapotranspiration higher than rainfall between November and January in both sites, extending until February in Paranavaí (Figure 1).

Soybean cv. BRS 1010 IPRO was sown in both experiments, without seed treatments with fungicide, insecticide, or micronutrients. The treatments in both sites were similar to the greenhouse treatments: non inoculated control (Ctrl); non inoculated control + N-fertilizer (100 kg ha^{-1} at the sowing and 100 kg ha^{-1} at the R1 flowering stage, as urea) (Ctrl + N); inoculated with *Bradyrhizobium* spp. SEMIA 5079 and SEMIA 5080 (Brad); co-inoculation with Brad and *A. brasilense* Ab-V5 and Ab-V6 (Brad + Azos); co-inoculation with Brad and *C. vulgaris* (Brad + Chlo); co-inoculation with Brad and *N. muscorum* (Brady + Nost); co-inoculation with Brad and *Streptomyces* sp. BTM 289 (Brady + Stre); Brady + Strep + leaf spray with *Streptomyces* sp. BTM 289 at the V3 stage (Brady + Stre + Stre(f)).

The treatments with *Bradyrhizobium* spp. received a commercial peaty inoculant at a rate to supply 1.2 × 10^{6} cells per seed, while the co-inoculated ones received a liquid *A. brasilense* commercial inoculant to provide 2.0 × 10^{5} cells per seed. In Lutécia, plots had seven lines of 7.5 m of length, spaced 0.45 m apart, while in Paranavaí plots consisted of eight lines of 6.5 m of length and 0.45 m apart, both at a density of 330,000 plants ha^{-1}. The experiments were performed in randomized blocks design with six replications.

Simultaneously to the sowing, 300 kg ha^{-1} of N-P-K (0-20-20) were applied in furrow. The sowing was performed in a no-till system on straw of *Brachiaria* spp., previously desiccated with Glyphosate (5 L ha^{-1} of commercial product). N-fertilizer was not applied, except in the Ctrl + N treatment. At V4 stage (Ritchie et al., 1994), plants were leaf-sprayed with 20 g ha^{-1} of Mo ($Na_2MoO_4.2H_2O$) and 2,5 g ha^{-1} of Co ($CoCl_2.6H_2O$). In the plots intended to study the effects of leaf supplemental spray of *Streptomyces* sp. BTM 289, the plants were sprayed at 30 days after emergence, using a backpack sprayer pressurized with CO_2 at a rate of 150 L ha^{-1} of water containing 300 mL of the corresponding inoculant.

Plants sampling, analysis, and grain yield

At both sites, four plants at R4 stage were randomly selected in each plot as reference for sampling soil with roots. Samples were taken at 5 cm from the reference plant, using a steel cylinder with 192.3 cm^3 (5 cm of height and 7 cm of diameter). In the laboratory, each sample was placed in 10 L buckets containing water for 5 min to facilitate the soil detachment from roots. Then the samples were gently washed in tap water and root fragments were separated on a 0.50 mm mesh sieve.

As in the greenhouse experiment, roots were disposed on trays containing shallow water, and living roots were separated from dead ones based on color, elasticity, and tissue cohesion. Root length was evaluated by the modified line intersection method (TENNANT, 1975) and used to estimate the density of root length in the soil (the ratio between root length and the sampled soil volume). Root specific length, average diameter, dry weight, volume, and root hair incidence were determined as described for the greenhouse experiment.

The four-plant used as reference for soil sampling were carefully collected with a cutting shovel, and roots and shoots were separated. Shoots were dried at 60 ºC until constant mass. Shoot N concentration and total N content were determined in ground tissues (< 1 mm) after sulfuric digestion by colorimetric quantification based on the green salicylate method (WILLIS et al., 1996). Roots were washed and nodules were detached, counted, and dried until constant mass before weighting.

The harvest was performed at soybean R8 stage, on February 18, 2019 in Paranavaí, and on March 7, 2019, in Lutécia. Plants were harvested in the central area of each plot, being 9.00 m^2 in Paranavaí (four central lines with 5.0 m length) and 7.42 m^2 in Lutécia (three central lines with 5.5 m length). The grains were cleaned, weighed and, after determination of moisture in a grain moisture analyzer (Gehaka, model AGRI G800), the mass was corrected to 13% of moisture.

Statistical analysis

The dataset was tested for normality by Shapiro-Wilk's test and homoscedasticity by Bartlett's test. When necessary, data were log (x + 0.5)–transformed to meet the assumptions of parametric tests. Root-hair incidence and the percentage of root length by root diameter class were transformed to arcsine $(x/100)^{0.5}$ before analysis. Means were analyzed by one-way ANOVA and Tukey's test ($\alpha = 5\%$) using the software R Studio (R Core Team, 2019).

4.3 RESULTS

Greenhouse experiments

The control plants showed the lowest shoot and root dry biomasses, differing from the inoculated ones, which did not differ among them for these attributes (Figures 2G and 2F). Total N in shoots did not differ among treatments (Figure 2H).

Although the inoculated plants showed more root dry weight than the control ones, co-inoculation with Brady + Chlo, and Brady + Strep + Strep(f) had denser root tissues than other (co-)inoculated plants (Figure 2C). The control plants had the longest specific root length (Figure 2B), however, the higher volumes of the root system were found in the single inoculation of Brad and in the co-inoculations Brad + Stre, Brad + Nost, and Brad + Azos (Figure 2D). There was neither effect of treatments on the root medium diameter (Figure 2D) nor in the root length classified by root diameter (Table 2). The Brad, Brad + Nost, and Brad + Azos plants showed greater root lengths (Figure 2A).

Field experiments

In Lutécia there was no effect of treatments on shoot dry weight(Table 3). However, in Paranavaí the *Bradyrhizobium* spp. co-inoculated with *A. brasilense*, *N. muscorum*, or *Streptomyces* sp. with aditional leaf spray increased the shoot dry weights. The root length density in the soil of plants co-inoculated with Brad + Azos was higher than the non-inoculate control in Lutécia (Table 3). However, in Paranavaí, in addition to Brad + Azos, the plants co-inoculated with Brad + Nost also presented higher root length density. The remaining treatments did not affect the root length density in the soil compared with the control plants.

In both sites, there were no differences for specific root length and root average diameter among the inoculated or co-inoculated plants, but in Lutécia plants coinoculated with Brad + Azos presented higher root specific length than the non-inoculated control (Table 3). In Paranavaí, the co-inoculation of *Bradyrhizobium* spp. And *N. muscorum* significantly increased the root volume in the soil compared with the single inoculation with Brad and the non-inoculated control plants.

In Lutécia, nodulation (number and mass of nodules) did not differ among inoculated and co-inoculated plants (Table 3). However, in Paranavaí, the number of nodules increased in the Brad + Azos, and Brad + Nost plants compared with the sole Brad-inoculated ones. Moreover, the co-inoculations Brad + Chlo, and Brad + Stre + Stre(f) increased nodule dry weight compared with the sole inoculation with *Bradyrhizobium* spp. In both sites, for all variables, the co-inoculated Brad + Stre plants did not differ significantly from the sole Brad inoculated ones (Table 3).

Treatments did not affect shoot dry weight in both sites, except the Brad + Stre + Stre (f) that significantly decreased this variable in Lutécia compared with the +N control. Among co-inoculated plants, shoot N concentration was higher in the Brad + Azos co-inoculation in both sites, and was equivalent to the N-fertilized control and the Brad + Stre + Stre (f) plants in Lutécia. None of the co-inoculated treatments, in both sites, significantly increased the shoot N concentration above the sole inoculation with *Bradyrhizobium* spp. A similar pattern was observed for total N content in shoots. Compared with the non-inoculated control, higher grain yields were recorded in plants co-inoculated with Brad + Azos in Lutécia , whereas all co-inoculated plants in Paranavaí had superior yields than the non-inoculated control and the sole inoculation with *Bradyrhizobium* spp.

4.4 DISCUSSION

The effects of PGPR on soybean root volume, tissue density, and length allowed us to identify combinations of microorganisms that possibly affected cell division and differentiation, leading to changes in the architecture of the root system and positively affected soybean grain yield, even under adverse conditions due to occurrence of drought at the critical reproductive stage.

Results from the greenhouse trial demonstrated combinations that synergistically improved the average root length, root volume, and root tissue density. The co-inoculations Brad + Azos and Brad + Nost highlighted for increasing root lengths and decreasing the root tissue densities. Longer root length is one of the traits more directly related to the root-soil contact, providing more effective area for soil exploration and improvement of water and nutrient uptake (HODGE 2004; COMAS et al., 2012; MORETTI et al., 2019). In addition, lower root tissue density indicates lower metabolic costs for construction of roots, and more effective acquisition of nutrients, especially the ones with low mobility in the soil (LYNCH; HO, 2005; DA ROCHA, MICHEL CASTTELANI et al., 2010).

The co-inoculation of soybean with *Bradyrhizobium* and *Azospirillum brasilense* usually leads to yield increase, which is in part attributed to significant improvement of the root system architecture, especially by stimulating the formation of lateral roots, increasing the total root length, root hair incidence, and the number and mass of nodules (CHIBEBA et al., 2015; RONDINA et al., 2020). In both greenhouse and field experiments, treatments did not affect the percentage of root length by class of diameter or average rood diameter, respectively. However, Rondina et al. (2020) demonstrated that soybean co-inoculation with *Bradyrhizobium* and *Azospirillum* increased the amount of roots thinner than 0.50 mm of diameter compared with either the uninoculated control plants or sole inoculation with *Bradyrhizobium*, in a greenhouse experiment. Nevertheless, in the field experiments, these authors also did not find differences among treatments for root diameters. Several reasons could be raised to explain these different results, including plant genotypes and environmental conditions.

Little is known about the effect of cyanobacteria of the genus *Nostoc* on soybean. Our work is the first one to demonstrate beneficial effects of co-inoculation of *Nostoc muscorum* and *Bradyrhizobium* spp. in soybean. Sholkamy et al. (2015) demonstrated positive effects of sole inoculation with *N. muscorum* or with *N. rivulare* in soybean treated or not with N fertilizer. Increases in shoot size, shoot dry mass, leaf area, and pods fresh weigh were observed, but an in-depth analysis of the roots was not carried out. Further studies with *Nostoc* confirm its positive effects on root development in several plants. In rice (*Oryza sativa*), root colonization by

Nostoc sp. influences lateral root formation, elongation, and root hair incidence (SALAMAH et al., 2019). Growth-promoting substances extracted from *N. calicole* have potential to increase root length and benefit the development of several plants such as *Paspalum scrobiculatum*, *Vigna unguiculata*, *Sorghum bicolor*, *Oryza sativa*, and *Zea mays* (SURESH et al., 2019).

Although the actinobacteria *Streptomyces* sp. has been more studied for biocontrol effect in agricultural systems, they can also help to promote plant growth. Our results demonstrated that Brad + Stre co-inoculation can result in positive effects on soybean, although this co-inoculation had few differences from the sole inoculation with *Bradyrhizobium*, or co-inoculation with *Azospirillum*.

Some studies have demonstrated the synergistic effect between *Streptomyces griseoflavus* P4 and different strains of *Bradyrhizobium* in soybean (Htwe, 2019a; 2019b; 2018a; 2018b; SOE; YAMAKAWA, 2013). In these studies, although co-inoculations of *S. griseoflavus* and *Bradyrhizobium* sp. proved promising, few differences were found between co-inoculated plants and sole inoculated with *Bradyrhizobium*. These works used soybean varieties and bacterial strains from Japan and Myanmar. Our work, however, co-inoculations improved soybean yields in the trial under severe drouth in Paranavaí. These findings stimulate further works to better understand the effect of *Bradyrhizobium*-*Streptomyces* co-inoculation, especially under drought.

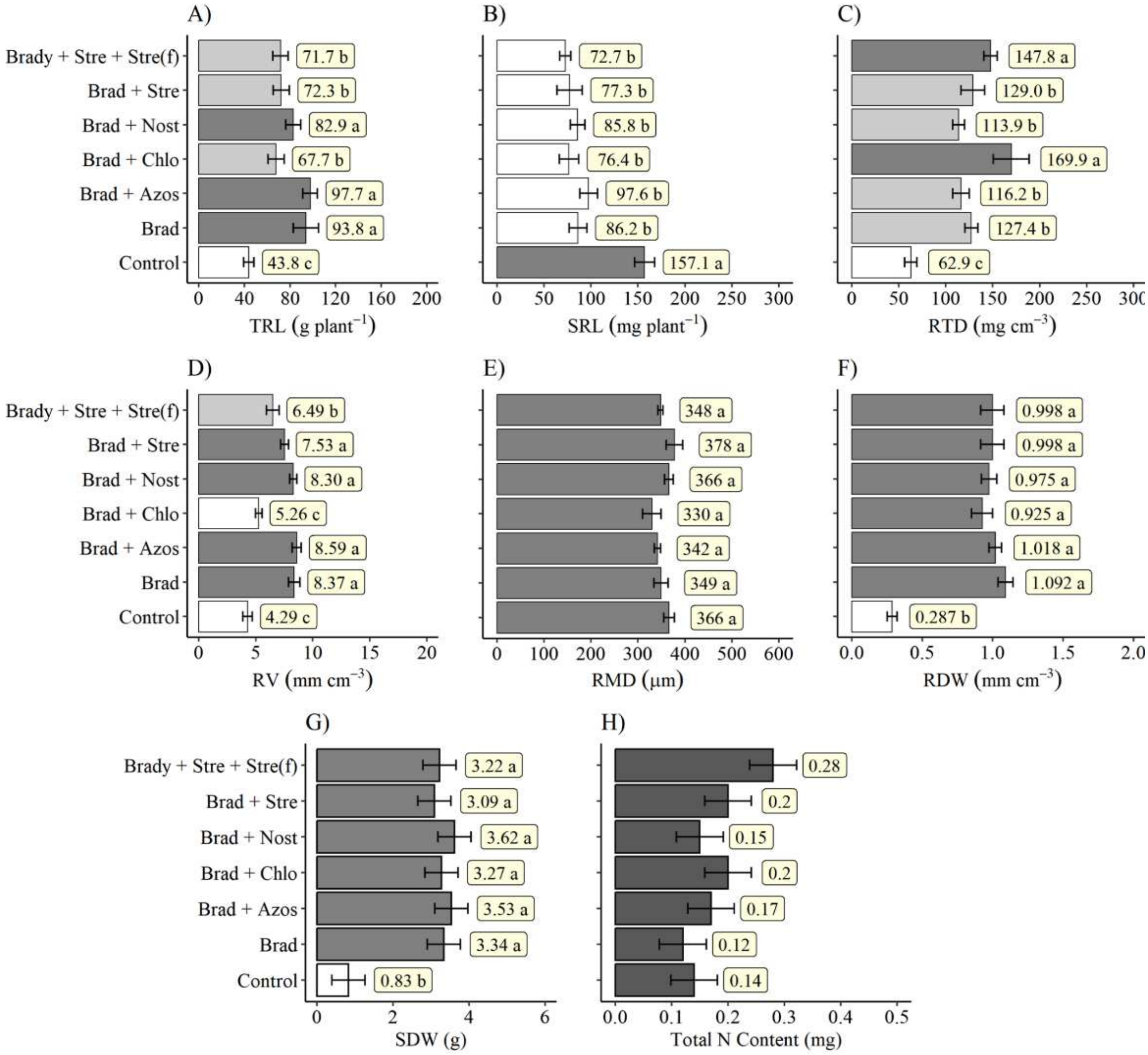
Due to the increasing demand for food production and current challenges of low-carbon agriculture, improving the crop yields without harming the environment is imperative. Increasing the efficiency of soybean inoculation with rhizobia has been a strategy to overcome these challenges. Co-inoculation of rhizobia with other BPCP has proven an effective approach, especially the co-inoculation with *Azospirillum brasilense* (HUNGRIA et al., 2013). Our study highlights other promising microbial isolates for increasing soybean yield in co-inoculation with *Bradyrhizobium* spp. and improving root morphological traits that can help the plant to better explore the soil for water and nutrients. We therefore present possible tools that can be used to improve soybean yield without the need to increase the input of not environmentally friendly inputs.

Table 1. Chemical attributes, granulometric fractions and number of most probable number (MPN) of soybean-nodulating rhizobia at the 0-20 cm layer of soil in the experimental fields before sowing.

City	pH	P	Al	H + Al	Ca	Mg	K	SB	CEC	V	C	Clay	Silt	Sand	Rhizobia
		mg dm^{-3}	--------------------------- cmol$_c$ dm^{-3} ---------------------------							%	g dm^{-3}	------- mg dm^{-3} --------			MPN g^{-1}
Lutécia	5.1	2.93	0.0	1.99	0.91	0.72	0.13	1.75	3.74	47	6.31	10.3	3.10	86.6	zero
Paranavaí	5.1	2.61	0.0	1.88	0.73	0.41	0.14	1.28	3.16	41	5.42	6.55	2.85	90.6	zero

Extractors: Mehlich-1 (P, K$^+$, B, Cu, Fe, Mn, Zn); KCl 1M (Ca^{+2}, Mg^{+2}, Al^{+3}); SMP (H + Al) ; CEC: Cation exchange capacity at pH 7,0; V: base saturation; MPN = most probable number.

Figure 2. Root morphological traits, shoot dry weight, and N concentration and accumulated in soybean shoots at different inoculation/coinoculation treatments in the greenhouse experiment. Error bars indicate standard deviation (n = 5).



[a] Control = non-inoculated; Brad = *Bradyrhizobium* spp. SEMIA 5079 and SEMIA 5080; Azos = *Azospirillum brasilense* Ab-V5 e Ab-V6; Chlo = *Chlorella vulgaris*; Nost = *Nostoc muscorum*. Stre = *Streptomyces* sp. Stre(f) = foliar spray of *Streptomyces* sp.TRL = Total root length; SRL= Specific root length; RTD = Root tissue density; RV = Root system volume; RMD = Root diameter; SDW = Shoot dry weight. Data represent the mean of six repetitions. Means followed by different letters differ from each other by the Tukey test ($p < 0.05$).

Table 2. Percentage of root by class of root diameter of soybean plants that received different co-inoculation treatments with *Bradyrhizobium* spp. and PGPB. Plants cultivated in the greenhouse and harvested 30 days after emergence.

Treatments [a]	Root diameter class (mm)			
	< 0.50	0.50 – 1.0	1.1 – 2.0	> 2.0
		%		
Non-Inoculated Control	68.10	26.20	5.52	0.18
Brad	72.24	22.62	5.03	0.11
Brad + Azos	73.73	20.94	4.96	0.37
Brad + Chlo	73.79	21.52	4.54	0.15
Brad + Nost	71.47	23.04	5.24	0.25
Brad + Stre	68.98	23.76	6.12	1.14
Brad + Stre + Stre(f)	72.80	22.60	4.41	0.20
P-value	0.376	0.239	0.839	0.42
CV (%)	5.65	8.25	20.1	33.2

[a] Control = non-inoculated; Brad = *Bradyrhizobium* spp. SEMIA 5079 and SEMIA 5080; Azos = *Azospirillum brasilense* Ab-V5 e Ab-V6; Chlo = *Chlorella vulgaris*; Nost = *Nostoc muscorum*. Stre = *Streptomyces* sp. Stre(f) = foliar spray of *Streptomyces* sp. Data represent the mean of six repetitions. Means followed by different letters differ from each other by the Tukey test (p <0.05).

Table 3. Soybean root morphological traits and nodulation at the R4 developmental stage receiving different treatments of co-inoculation with *Bradyrhizobium* spp. and PGPB in field experiments performed in Lutécia and Paranavaí in the 2018/19 crop season.

Treatments [a]	Root dry weight	Root length density in the soil	Specific root length	Root volume in the soil	Root average diameter	Number of nodules	Nodules dry weight
	(mg cm^{-3} soil)	(cm cm^{-3} soil)	(cm g^{-1} root)	(mm^{3} cm^{-3} soil)	(µm)	(no. Plant^{-1})	(mg plant^{-1})
				Lutécia			
Non-inoculated Control	3.89	0.80 b	133.92 b	1.34	352.96	0 b	2.06 b
+N Control	4.33	1.31 ab	181.91 ab	1.21	342.27	1.3 b	5.49 b
Brad	5.17	1.46 ab	174.43 ab	1.33	342.90	10.8 a	81.88 a
Brad + Azos	4.73	1.99 a	321.63 a	1.85	343.64	14.4 a	95.18 a
Brad + Chlo	3.90	1.66 ab	226.70 ab	1.46	323.03	12.4 a	96.98 a
Brad + Nost	4.91	1.54 ab	231.63 ab	1.28	335.60	15.4 a	89.94 a
Brad + Stre	4.24	1.51 ab	212.47 ab	1.32	335.73	22 a	109.35 a
Brad + Stre + Stre(f)	4.44	1.65 ab	200.96 ab	1.47	334.54	18.8 a	90.44 a
P-value	0.899	0.034	0.039	0.619	0.355	<0.001	<0.001
CV (%)	26	27.1	37.5	37.7	5.93	33.3	22.7
				Paranavaí			
Non-inoculated Control	0.98 b	1.57 c	959.14	4.23 b	584.94	0 c	0 d
+N Control	2.12 ab	2.42 abc	712.28	6.93 ab	597.99	0 c	0 d
Brad	1.79 ab	1.87 bc	673.61	5.04 b	585.51	2.5 bc	17.34 c
Brad + Azos	2.23 a	2.84 ab	773.40	7.06 ab	560.95	11.67 a	97.74 a
Brad + Chlo	1.91 ab	2.55 abc	987.49	6.88 ab	584.12	7.33 ab	57.58 ab
Brad + Nost	2.41 a	3.10 a	613.07	8.33 a	585.74	9.16 a	89.09 a
Brad + Stre	1.98 ab	2.24 abc	707.82	6.18 ab	592.69	7.16 ab	47.8 bc
Brad + Stre + Stre(f)	2.50 a	2.55 abc	599.91	6.59 ab	580.03	6.16 ab	69.80 ab
P-value	0.002	<0.001	0.529	0.003	0.437	<0.001	<0.001
CV (%)	24.9	21.0	21.3	22.1	4.11	34.4	22.7

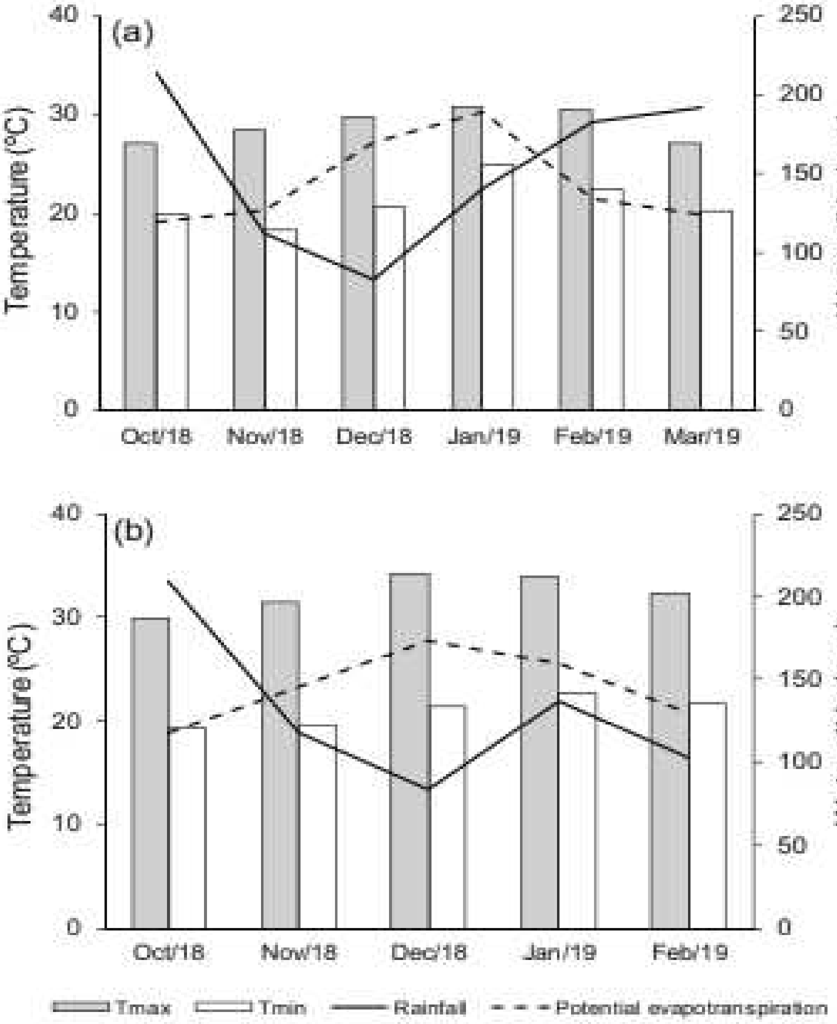
[a] Control = non-inoculated; Control + N = N-fertilized.and non-inoculated; Brad = *Bradyrhizobium* spp. SEMIA 5079 and SEMIA 5080; Azos = *Azospirillum brasilense* Ab-V5 and Ab-V6; Chlo = *Chlorella vulgaris*; Nost = *Nostoc muscorum*. Stre = *Streptomyces* sp. Stre(f) = foliar spray of *Streptomyces* sp. Data represent the mean of six repetitions. Means followed by different letters differ from each other by the Tukey test (p <0.05).

Table 4. Soybean shoot attributes at the R4 developmental stage receiving different treatments of co-inoculation with *Bradyrhizobium* spp. and PGPB in field experiments performed in Lutécia and Paranavaí in the 2018/19 crop season.

Treatments [a]	Shoot dry weight	Shoot N concentration	Total shoot N content	Grain yield
	(g $plant^{-1}$)	(g kg^{-1})	(g $plant^{-1}$)	(kg ha^{-1})
		Lutécia		
Non-Inoculated Control	5.97 ab	19.54 ab	117.55 ab	2319.52 b
+N Control	7.29 a	22.48 a	162.70 a	3060.94 a
Brad	5.08 ab	19.16 ab	98.59 b	2728.58 ab
Brad + Azos	5.05 ab	23.18 a	116.94 ab	3040.01 a
Brad + Chlo	5.59 ab	17.58 b	98.49 b	2909.78 ab
Brad + Nost	5.07 ab	17.16 b	86.94 b	2778.31 ab
Brad + Stre	5.91 ab	15.94 b	92.16 b	2810.74 ab
Brad + Stre + Stre(f)	4.59 b	19.28 ab	87.76 b	2927.80 ab
P-value	<0.001	<0.001	0.005	0.023
CV(%)	21.6	19.8	21.6	11.11
		Paranavaí		
Non-inoculated Control	2.85 b	17.54 de	47,50 c	1051.22 d
+N Control	5.40 a	30.44 a	162,18 a	2658.94 a
Brad	2.45 b	20.84 bcd	52,50 c	1518.66 c
Brad + Azos	3.56 b	24.62 b	87,33 b	2139.84 b
Brad + Chlo	3.42 b	18.32 de	59,67 bc	1929.3b
Brad + Nost	2.49 b	15.05 e	39,17 c	2125.84 b
Brad + Stre	3.31 b	21.76 bc	68,00 bc	1990.46 b
Brad + Stre + Stre(f)	2.99 b	18.2 cde	52,83 c	1988.54 b
P-value	<0.001	<0.001	<0.001	<0.001
CV (%)	18.1	10.5	10.8	9.74

[a] Control = non-inoculated; Control + N = N-fertilized. and non-inoculated; Brad = *Bradyrhizobium* spp. SEMIA 5079 and SEMIA 5080; Azos = *Azospirillum brasilense* Ab-V5 and Ab-V6; Chlo = *Chlorella vulgaris*; Nost = *Nostoc muscorum*. Stre = *Streptomyces* sp. Stre(f) = foliar spray of *Streptomyces* sp. Data represent the mean of six repetitions. Means followed by different letters differ from each other by the Tukey test (p <0.05).

Figure 1. Monthly means of rainfall, potential evapotranspiration, and maximum and minimum air temperatures corresponding to the 2018/19 soybean cropping season in Lutécia (a), and in Paranavaí (b).



Source: Adapted from Rondina et al. (2020)

5. CONCLUSÃO GERAL

A inoculação e coinoculação das bactérias promotoras de crescimento de plantas tem efeito nas características morfológicas radiculares, atributos da parte área e rendimento de grãos.

A coinoculação de BPCPs e *Bradyrhizobium* apresenta-se como uma ferramenta para maximizar atributos do sistema radicular e produtividade. Os isolados de *Streptomyces* sp. melhorou as características morfológicas das raízes do feijoeiro e mostrou-se promissor para estimulação do sistema radicular.

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