UNIVERSIDADE FEDERAL DE LAVRAS

JOÃO PAULO SANTOS CARVALHO

FEASIBILITY TO BREED FOR BIOLOGICAL NITROGEN FIXATION IN SOYBEAN

LAVRAS – MG
2020

JOÃO PAULO SANTOS CARVALHO

FEASIBILITY TO BREED FOR BIOLOGICAL NITROGEN FIXATION IN SOYBEAN

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

Prof. Dr. Adriano Teodoro Bruzi
Orientador

LAVRAS - MG
2020

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Carvalho, João Paulo Santos.
Feasibility to breed for biological nitrogen fixation in soybean / João Paulo Santos Carvalho. - 2020.
76 p. : il.

Orientador(a): Adriano Teodoro Bruzi.

Tese (doutorado) - Universidade Federal de Lavras, 2020.
Bibliografia.

1. Melhoramento de soja. 2. Dialelo recorrente. 3. Seleção recorrente. I. Bruzi, Adriano Teodoro. II. Título.

JOÃO PAULO SANTOS CARVALHO

FEASIBILITY TO BREED FOR BIOLOGICAL NITROGEN FIXATION IN SOYBEAN

VIABILIDADE DE SELEÇÃO PARA FIXAÇÃO BIOLÓGICA DE NITROGÊNIO EM SOJA

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

APROVADA em 30 de outubro de 2020.

Dr. José Maria Villela Padua	Souza Cruz
Dra. Fatima Maria de Souza Moreira	UFLA
Dr. Carlos Eduardo Pulcinelli	UFLA
Dra. Flávia Barbosa Silva Botelho	UFLA

Prof. Dr. Adriano Teodoro Bruzi
Orientador

LAVRAS - MG
2020

À Deus e à minha família, pelo apoio e presença durante todo o caminho, em especial aos meus pais, Maria Helena e Eleno (in memoriam) pois são os grandes responsáveis por esta conquista.

Dedico

AGRADECIMENTOS

A Deus.

À Universidade Federal de Lavras e ao Departamento de Agricultura, pelo acolhimento e oportunidade concedida para a realização do doutorado.

Aos meus pais, Maria Helena e Eleno (*in memoriam*), por serem exemplos de amor e dedicação, pela confiança, carinho e incentivo.

A toda a minha família e amigos, pelo incentivo, conselhos, paciência e companheirismo em todos os momentos.

Ao Professor Doutor Adriano Teodoro Bruzi, pela orientação, apoio, amizade e por ser um exemplo de professor e pessoa que inspira a todos.

À *Iowa State University* e à Dr. Silvia Cianzio, por abrirem as portas mim, permitindo a realização de um sonho.

À Marli dos Santos Túlio pela paciência, compreensão, conversas, amizade e por ser uma exemplo de compromisso com o trabalho.

Aos amigos do Grupo Pesquisa Soja, pela convivência, amizade, apoio na condução dos experimentos e diversos ensinamentos.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

O presente trabalho foi realizado com apoio do Conselho Nacional de Desenvolvimento Científico e tecnológico (CNPq).

A todos vocês, meu mais sincero MUITO OBRIGADO!

RESUMO

A soja [*Glycine max* (L.) Merril] é uma das mais importantes leguminosas cultivadas em todo o mundo. É a principal fonte de proteína e óleo comestível por possuir alto teor de óleo (20-25%) e proteína (42-45%) nas sementes. Devido ao alto teor de proteína nos grãos, as plantas necessitam de uma grande quantidade de N, cerca de 80 kg para produzir uma tonelada de grãos. No Brasil, devido a seleção de estirpes de *Bradyrhizobium* altamente eficientes na fixação biológica de nitrogênio (FBN), essa demanda pode ser totalmente fornecida via FBN, não necessitando da adubação nitrogenada. A substituição de fertilizantes nitrogenados pela FBN na soja, resulta em uma economia de cerca de 20 bilhões de dólares para a agricultura brasileira anualmente. Como a agricultura está cada vez mais competitiva e buscando ser mais eficiente, reduzindo custos de produção e respeitando o meio ambiente, a FBN vem como uma alternativa viável que une os dois objetivos. Portanto, o desenvolvimento de genótipos de soja cada vez mais eficientes nesse processo é de grande importância. Assim, objetivou-se estudar a capacidade de FBN de progênies de soja e a capacidade de associação simbiótica utilizando diferentes estirpes de *Bradyrhizobium*, bem como selecionar progênies e estirpes mais eficientes para FBN, visando nortear as estratégias de melhoramento para a FBN em soja. Foram conduzidos experimentos em casa de vegetação e em campo. No experimento de casa de vegetação foram utilizadas 24 progênies de soja e cinco estirpes de *Bradyrhizobium* (SEMIA 5079, SEMIA 587, SEMIA 5019, UFLA 06-24 e INPA 03-11B). Os dados foram submetidos a análise de variância e as médias comparadas pelo teste de Scott-knott. Foi realizada a análise dialélica parcial para estimar a capacidade geral e específica de combinação das progênies e estirpes de *Bradyrhizobium*. As melhores progênies e estirpes foram selecionadas considerando suas médias e capacidade geral de combinação para os seguintes caracteres: teor de nitrogênio, teor de clorofila, número de nódulos, peso fresco e seco de nódulos e índice Z. Dez progênies e duas estirpes (SEMIA 587 e INPA 03-11B) foram selecionadas para os experimentos de campo. Observou-se que a FBN é influenciada pelo genótipo, estirpes e pela interação genótipo-estirpe. Há predominância de efeitos aditivos nos caracteres relacionados à FBN, indicando que a seleção recorrente pode ser uma estratégia de melhoramento para esse caráter. As progênies tardias foram superiores para caracteres relacionados à nodulação em campo, porém, sem ganho em produtividade. A progênie EA-8 se destacou como a mais produtiva, com bons resultados nos caracteres relacionados à FBN e de ciclo precoce. A estirpe INPA 03-11B foi a que apresentou o melhor resultado para produtividade, indicando alto potencial para sua utilização como inoculante na cultura da soja.

Palavras-chave: Melhoramento de soja. Dialelo parcial. Seleção recorrente. Genética quantitativa. Simbiose.

ABSTRACT

Soybean [*Glycine max* (L.) Merril] is one of the most important legumes grown worldwide. It is the main source of protein and edible oil because of the high oil content (20-25%) and protein (42-45%) in the seeds. Due to the high protein content in the grains, plants need a large amount of N, about 80 kg to produce a ton of grains. In Brazil, due to the selection of *Bradyrhizobium* strains highly efficient in biological nitrogen fixation (BNF), this demand can be fully supplied from BNF, not requiring nitrogen fertilization. The substitution of nitrogen fertilizers by BNF in soybeans crops results in savings of around 20 billion dollars annually for Brazilian agriculture. As agriculture is increasingly competitiveness and seeking to be more efficient by reducing production costs while respecting the environment. In this context, BNF is a viable alternative serving to meet these objectives. Therefore, the development of soybean genotypes efficient in BNF is important. Thus, the purpose of this work was to study the BNF ability of soybean progenies and their ability for symbioses using different *Bradyrhizobium* strains as well as to select more efficient progenies and strains for BNF, aiming to guide strategies in soybean breeding to BNF. In a greenhouse experiment, were evaluated 24 soybean progenies and five *Bradyrhizobium* strains (SEMIA 5079, SEMIA 587, SEMIA 5019, UFLA 06-24 and INPA 03-11B). For the trial progenies and strains, analysis of variance was conduct and means were compared by Scott-Knot test. Partial diallel analysis was performed to estimate the general and specific combination ability for the progenies and *Bradyrhizobium* strains. The best progenies and strains were selected considering their means and general combining ability for the following traits: nitrogen content, chlorophyll content, number of nodules, fresh and dry weight of nodules and Z index. Ten progenies and two strains (SEMIA 587 and INPA 03-11B) were selected to carry out a field trial experiment. The BNF was influenced by genotype, strain, and genotype-strain interactions. There was a predominance of additive effects on the traits related to BNF, indicating that recurrent selection could be a strategy for improvement of these traits. Late maturity progenies showed higher values for the traits related to field nodulation but did not show gains in grain yield. The EA-8 progeny stood out as having a high yield, with good results in traits related to BNF and early maturity. INPA 03-11B strain showed the best result for grain yield, indicating high potential for its use as an inoculant in soybean crops.

Keyword: Soybean breeding. Partial diallel. Recurrent selection. Quantitative genetics. Symbiosis.

SUMÁRIO

PRIMEIRA PARTE

PRIMEIRA PARTE

1 INTRODUÇÃO

Estima-se que no mundo, o processo de FBN é responsável pelo fornecimento de 200 milhões de toneladas de N anualmente, contribuindo com quase 50% do N total nas áreas cultivadas (PEOPLES, 2009; RAMÍREZ-PUEBLA, 2019). A soja é provavelmente o exemplo mais bem sucedido da utilização da FBN para suprir a necessidade de N da planta. Cerca de 60% do N requerido pela cultura no mundo é fornecido pela FBN (CIAMPITTI; SALVAGIOTTI, 2018; SALVAGIOTTI *et al.*, 2008).

No Brasil, os constantes avanços dos programas de melhoramento, associados à seleção de estirpes de *Bradyrhizobium* adaptadas às condições brasileiras, capazes de substituir o uso de fertilizantes nitrogenados, foram, sem dúvida, fundamentais para a expansão da cadeia produtiva da soja no Brasil (HUNGRIA *et al.*, 2006; ZUFFO *et al.*, 2015). A substituição de fertilizantes nitrogenados pela FBN na soja resulta em uma economia de cerca de 20 bilhões de dólares para a agricultura brasileira anualmente (SANTOS; NOGUEIRA; HUNGRIA, 2019).

Como a maioria dos processos fisiológicos das plantas, a FBN pode sofrer interferências quando submetida a condições adversas. Por exemplo, solos com alto teor de N e aplicação de fertilizante nitrogenado inibe a formação e atividades dos nódulos (STREETER; WONG, 1988; YAMASHITA *et al.*, 2019). A simbiose entre a soja e o rizóbio é altamente influenciada pela especificidade entre os hospedeiros, sendo a eficiência da FBN limitada pelos genótipos de soja e estirpes de *Bradyrhizobium* (OLIVARES; BEDMAR; SANJUÁN, 2013). Os fatores abióticos tais como seca, salinidade, acidez do solo, dentre outros, também limitam a eficiência da fixação de N bem como a sobrevivência da bactéria (SILVA *et al.*, 2017).

Os caracteres fenotípicos normalmente avaliados em estudos de FBN referem-se à nodulação (peso e número de nódulos) e a capacidade de assimilação de N pela planta (N_2 fixado pelo processo biológico). A avaliação desses caracteres é trabalhosa e demorada, portanto, muitas vezes tem sido negligenciada em programas de melhoramento (DWIVEDI *et al.*, 2015).

No Brasil, ao contrário dos EUA, Austrália e da África, apesar de não haver um programa de melhoramento especificamente voltado para a FBN, a maximização do processo foi favorecido pela condução dos programas de melhoramento em solos pobres em N, e pela inoculação das sementes com estirpes eficientes de *Bradyrhizobium*, sem a aplicação de fertilizantes nitrogenados. A FBN em soja, no Brasil, é apontada como um dos fatores responsáveis pelo sucesso do cultivo dessa leguminosa no país (ALVES; BODDEY; URQUIAGA, 2003; HUNGRIA *et al.*, 2006).

Existem relatos de que muito embora o incremento na capacidade de FBN não tenha sido alvo dos melhoristas de soja, a seleção para tamanho das sementes, crescimento e desenvolvimento vegetativo das plantas e produtividade pode ter influenciado indiretamente este caráter, já que estão diretamente relacionados com a demanda de nitrogênio pela planta (BALBOA *et al.*, 2018; TAMAGNO *et al.*, 2017). Munõz *et al.* (2016) compararam a capacidade de FBN entre cultivares comerciais e espécies selvagens, e relatam que os genótipos melhorados apresentaram maior capacidade de fixação biológica de nitrogênio.

Os estudos iniciais envolvendo a FBN, denotam que a eficiência do processo é altamente dependente do genótipo da planta (WILSON *et al.*, 1940). Incrementos na proporção de N proveniente da FBN têm sido alcançados pelo melhoramento e seleção de genótipos, estirpes bacterianas e na interação genótipo-estirpe (TAMAGNO *et al.*, 2018). Contudo, devido à dificuldade de avaliar as características fenotípicas relacionadas à FBN, pesquisadores têm buscado identificar QTLs (*Quantitative Trait Loci*) que controlam a nodulação e FBN em genótipos de soja. Estudos conduzidos com este objetivo, identificaram uma série de QTLs relacionados à nodulação em diferentes estágios de desenvolvimento da soja que podem ser utilizados como uma alternativa para condução de programas de melhoramento (GRUNVALD *et al.*, 2018; HWANG *et al.*, 2014; SANTOS *et al.*, 2013; YANG *et al.*, 2017; YANG *et al.*, 2019).

Diversos estudos mostram que os efeitos aditivos são predominantes na herança da capacidade de fixação de nitrogênio. Em relação às estimativas de herdabilidade, os resultados denotam valores de baixa a alta magnitude (AGOYI *et al.*, 2016a; NICOLÁS; ARRABAL; HUNGRIA, 2002; YANG *et al.*, 2019). Assim, estudos científicos mostram que o próximo passo para elevar a atividade da FBN, consiste na seleção de cultivares de soja, visando possíveis indicações de estratégias para o melhoramento genético (SINCLAIR; NOGUEIRA, 2018; TORRES *et al*., 2015). Contudo, esses resultados ainda são escassos. Portanto, objetivou-se estudar a capacidade de FBN de progênies de soja e a capacidade de associação simbiótica utilizando diferentes estirpes de *Bradyrhizobium*, bem como selecionar progênies e estirpes mais eficientes no processo, visando nortear as estratégias de melhoramento para a FBN em soja, bem como a recomendação de estirpes mais eficientes.

2 REFERENCIAL TEÓRICO

2.1 Fixação Biológica de Nitrogênio (FBN)

O nitrogênio (N) é um dos fatores mais limitantes na produção agrícola. A fim de obter altos rendimentos, os agricultores tendem a utilizar altas dosagens de adubação nitrogenada, o que aumenta os custos de produção e podem ocasionar efeitos adversos ao meio ambiente, como a poluição do ar e da água (SANTOS *et al.*, 2013; LI; ZENG; LIAO, 2016). Por outro lado, as plantas podem obter o N através da decomposição da matéria orgânica, da adubação nitrogenada e fixação biológica de nitrogênio (FBN), sendo as duas últimas fontes, as que mais contribuem para o suprimento de N (HUNGRIA; CAMPO; MENDES, 2007).

A FBN é o processo bioquímico por meio do qual o nitrogênio presente na atmosfera (N_2) é convertido em formas assimiláveis (NH_4^+ ou NH_3^+) pelas plantas. A conversão do N nas leguminosas ocorre nos nódulos, que são estruturas criadas pelas bactérias ao se associarem simbioticamente com as plantas (FOX *et al.*, 2016; YANG *et al.*, 2017).

A simbiose entre leguminosas e rizóbios não é obrigatória. A planta pode se desenvolver e completar seu ciclo de vida sem qualquer associação com os rizóbios, e estes também ocorrem como organismos de vida livre no solo. Entretanto, sob condições limitantes de nitrogênio, os simbiontes procuram uns aos outros por meio de uma troca de sinais. Dessa maneira, existem genes específicos entre a planta hospedeira e organismos simbiontes chamados genes nod, os quais se diferem entre as espécies de rizóbios e determinam a faixa de hospedeiros (TAIZ; ZEIGER, 2013).

Primeiramente, para o estabelecimento da relação simbiótica entre bactéria fixadora de nitrogênio e seu hospedeiro, deve ocorrer a migração da bactéria em direção às raízes da planta. Essa migração é uma resposta mediada por atrativos químicos que são secretados pelas raízes, sendo eles, isoflavonoides e betaínas, responsáveis por ativarem a proteína NodD no rizóbio. Esta irá induzir outros genes nod a codificar as proteínas de nodulação, a qual está envolvida na produção de fatores Nod (TAIZ; ZEIGER, 2013).

A infecção e organogênese do nódulo ocorrem simultaneamente. As bactérias se aderem aos pelos das raízes e induzem o enrolamento das células dos mesmos, devido aos fatores Nod liberados por ela, dando início ao processo de infecção. Posteriormente, ocorre a formação de um canal de infecção e o desenvolvimento do nódulo, onde as bactérias se diferenciam em bacteroides fixadores de nitrogênio (TAIZ; ZEIGER, 2013).

A enzima que catalisa a fixação do N_2 é a nitrogenase, formada por duas unidades proteicas, a Ferro-proteína (Fe-proteína) e a Molibdênio-Ferro-proteína (MoFe-proteína), responsáveis pela fixação de nitrogênio no nódulo. Porém, para que essa fixação ocorra, são necessárias condições anaeróbicas para a atuação da enzima nitrogenase. Para isso, os nódulos possuem heme proteínas, denominadas leghemoglobinas, que apresentam alta afinidade com O_2. Sua função é aumentar a taxa de transporte do oxigênio para a respiração das células bacterianas simbióticas, levando à redução dos níveis de oxigênio na célula infectada. O principal meio de transporte do nitrogênio contido nos nódulos para a parte aérea é na forma de ureídeos (KING; PURCELL, 2005). A atividade da leghemoglobina pode ser observada pela coloração rósea no interior do nódulo, caracterizando-o ativo (TAIZ; ZEIGER, 2013).

Estima-se que no mundo, o processo de FBN é responsável pelo fornecimento de 200 milhões de toneladas de N anualmente, contribuindo com quase 50% do N total nas áreas cultivadas (PEOPLES, 2009; RAMÍREZ-PUEBLA, 2019). Devido a sua contribuição é considerada o segundo mais importante processo biológico do planeta, ficando atrás apenas da fotossíntese (MENDES *et al.*, 2010).

A soja é provavelmente o exemplo mais bem sucedido da utilização da FBN para suprir a necessidade de N da planta. Cerca de 60% do N requerido pela cultura no mundo é fornecido pela FBN (CIAMPITTI; SALVAGIOTTI, 2018; SALVAGIOTTI *et al.*, 2008).

No Brasil, os constantes avanços dos programas de melhoramento, associado à seleção de estirpes de *Bradyrhizobium* adaptadas às condições brasileiras, capazes de substituir o uso de fertilizantes nitrogenados, foram, sem dúvida, fundamentais para a expansão da cadeia produtiva da soja no Brasil (HUNGRIA *et al.*, 2006; ZUFFO *et al.*, 2015).

A substituição de fertilizantes nitrogenados pela FBN na soja resulta em uma economia de cerca de 20 bilhões de dólares para a agricultura brasileira anualmente (SANTOS; NOGUEIRA; HUNGRIA, 2019).

Como a maioria dos processos fisiológicos das plantas, a FBN pode sofrer interferências quando submetida a condições adversas. Por exemplo, solos com alto teor de N e a aplicação de fertilizante nitrogenado, inibem a formação e atividades dos nódulos (STREETER; WONG, 1988; YAMASHITA *et al.*, 2019). A simbiose entre a soja e o rizóbio é altamente influenciada pela especificidade entre os hospedeiros, sendo a eficiência da FBN limitada pelos genótipos de soja e estirpes de *Bradyrhizobium* (OLIVARES; BEDMAR; SANJUÁN, 2013). Os fatores abióticos tais como seca, salinidade, acidez do solo, dentre outros, também limitam a eficiência da fixação de N bem como a sobrevivência da bactéria (SILVA *et al.*, 2017).

Além desses fatores, a nodulação também varia em função do crescimento e desenvolvimento fenológico da planta. No caso da soja, os primeiros nódulos se tornam visíveis a partir de 10 a 15 dias após a emergência das plantas (estádios V1 a V2) (FEHR; CALVINESS, 1977) dependendo da boa especificidade entre o cultivar de soja e a estirpe de bactéria, além de condições ambientais favoráveis e manejo (CÂMARA, 2000).

A partir do 3° ou 4° trifólio (V4 a V5) (FEHR; CALVINESS, 1977), a nodulação aumenta a intensidade, acompanhando o incremento de matéria seca da parte aérea e de raízes, que também se reflete na intensificação do crescimento do número e da weighta seca de nódulos. O primeiro pico desse incremento da nodulação se dá no florescimento pleno das plantas (estádio R2) (FEHR; CALVINESS, 1977) em resposta ao primeiro pico de atividade fotossintética, observado no início do florescimento (DORNHOFF; SHIBLES, 1970; KOLLER; NYQUIST; CHORUSH, 1986).

Devido a esse incremento de fotossíntese, a soja aumenta a taxa de fixação de CO_2, disponibilizando mais carbono para atender a alta demanda energética da carga de flores do estádio R2 e, com isso, há um decréscimo na nodulação em número e peso seca após o florescimento (CÂMARA, 2014).

Com a evolução da frutificação, incrementa-se a atividade fotossintética e um novo pico é atingido nos estádios R5.1 e R5.2 (FEHR; CALVINESS, 1977), que correspondem ao início da formação das sementes, e, posteriormente, há um decréscimo na nodulação acompanhando a senescência da planta.

2.2 Melhoramento da FBN em Soja

Os caracteres fenotípicos normalmente avaliados em estudos de FBN referem-se à nodulação (peso e número de nódulos) e a capacidade de assimilação de N pela planta (N_2 fixado pelo processo biológico). A avaliação desses caracteres é trabalhosa e demorada, portanto, muitas vezes tem sido negligenciada em programas de melhoramento (DWIVEDI *et al.*, 2015).

Mesmo assim, alguns programas específicos para o melhoramento da FBN em soja foram desenvolvidos na Austrália, na África e nos EUA. O programa australiano explorou a variação natural de tolerância ao nitrato. O programa compreendeu dois ciclos de seleção entre 1986 e 1997. No primeiro ciclo foram feitos cruzamentos entre linhagens coreanas tolerantes ao nitrato (K464, K466, K468 e K469) e cultivares adaptadas (Valder, Reynolds, Forrest e

Bossier). Foram avaliadas 849 plantas F_2 para fixação de N_2 em solos ricos em nitrato, sendo selecionadas as que apresentaram mais de 31% do N oriundo da FBN, para obtenção das gerações $F_{2:6}$ e $F_{2:7}$. Com base nos resultados obtidos foram selecionadas quatro linhagens em que a contribuição do N derivado da FBN foi próximo a 50% e produtividade entre 1,7 a 2,67 t/ha.

No segundo ciclo, as quatro linhagens selecionadas foram cruzadas com cultivares mais produtivos (Lachlan e Valiant) e o método SSD foi utilizado para obtenção de linhagens $F_{2:7}$, $F_{2:8}$ e $F_{2:9}$. Esses materiais foram avaliados em conjunto com as linhas do programa principal e o desempenho dos materiais do programa de FBN, em termos de produtividade superou o dos genitores Lachlan e Valiant, entretanto, os materiais não superaram a produtividade de linhagens do programa principal de melhoramento australiano. Nenhum dos materiais foi lançado como cultivar, pois, apresentaram suscetibilidade à *Phytophthora sojae* e o programa foi encerrado em 1998 (HERIDGE; ROSE, 2000; HERRIDGE; TURPIN; ROBERTSON, 2001).

Sinclair *et al.* (2007) relatam os progressos obtidos em um programa iniciado em 1993, com o objetivo de explorar a característica de fixação de N_2 tolerante à dessecação do solo da cultivar Jackson, visando o desenvolvimento de linhagens mais produtivas em ambientes secos. O programa compreendeu quatro fases: cruzamento e seleção de progênies F_3; *screening* preliminar em casa de vegetação para seleção de linhagens $F_{3:4}$ com maior FBN quando submetidas ao estresse hídrico; avaliações de campo para identificar genótipos com maior produtividade em ambientes pouco produtivos (ambientes secos) e experimentos em casa de vegetação para comprovar a tolerância da FBN ao estresse hídrico nas linhagens mais produtivas. As linhagens R01-416F e R01-581F apresentaram uma atividade de FBN superior (25% e 15%) comparados com o do cultivar genitor KS4895.

Nos Estados Unidos, a existência de estirpes de *Bradyrhizobium* spp. bem estabelecidas nos solos, altamente competitivas, mas pouco eficientes no processo de FBN tem inviabilizado a introdução de novas estirpes mais eficientes. A busca por alternativas que possibilitem a introdução efetiva de estirpes superiores levou à identificação dos genes rj_1, Rj_2, Rj_3 e Rj_4, em alguns materiais americanos, que restringem a nodulação com certos sorogrupos de bactérias (DEVINE; BREITHAUPT, 1980). No entanto, a obtenção de um único genótipo vegetal que combine todos os níveis de restrição da nodulação desejados com as estirpes estabelecidas no solo é difícil, e pode, também, restringir a nodulação com estipes desejáveis. Além disso, a expressão do fenótipo de restrição da nodulação pode ser influenciada por fatores ambientais, como a temperatura (SADOWSKY *et al.*, 1995).

O programa de melhoramento desenvolvido desde o início dos anos de 1980 pelo Instituto Internacional de Agricultura Tropical (IITA), na Nigéria, tem explorado a combinação da capacidade de nodulação promíscua dos materiais locais com as caraterísticas melhoradas dos cultivares americanos, visando obter linhagens que combinem produtividade e nodulação promíscua (HERRIDGE; ROSE, 2000). O programa tem obtido sucesso no desenvolvimento de linhagens mais produtivas e de nodulação promíscua. No entanto, a habilidade de nodulação promíscua dessas linhagens tem se mostrado limitada e dependente da população de rizóbios do solo, apresentando desempenhos melhores em um número limitado de ambientes. Outra estratégia desenvolvida no Zimbabwe e Zâmbia consiste na identificação de materiais superiores selecionados entre as variedades locais, com base no potencial de produção e na habilidade de nodulação promíscua. Esse programa tem tido sucesso em assegurar a nodulação promíscua em diferentes ambientes, variedades locais como Magoy e Hermom 147 foram recomendadas e são cultivadas por pequenos agricultores (MPEPEREKI *et al.*, 2000).

No Brasil, ao contrário dos EUA, Austrália e da África, apesar de não haver um programa de melhoramento especificamente voltado para a FBN, a maximização da FBN foi favorecida pela condução dos programas de melhoramento em solos pobres em N, e pela inoculação das sementes com estirpes eficientes de *Bradyrhizobium*, sem a aplicação de fertilizantes nitrogenados. A FBN em soja no Brasil é apontada como um dos fatores responsáveis pelo sucesso do cultivo dessa leguminosa no País (ALVES; BODDEY; URQUIAGA, 2003; HUNGRIA *et al.*, 2006).

Existem relatos de que muito embora o incremento na capacidade de FBN não tenha sido alvo dos melhoristas de soja, a seleção para tamanho das sementes, crescimento e desenvolvimento vegetativo das plantas e produtividade pode ter influenciado indiretamente este caráter, já que estão diretamente relacionados com a demanda de nitrogênio pela planta (BALBOA *et al.*, 2018; TAMAGNO *et al.*, 2017). Munõz *et al.* (2016) compararam a capacidade de FBN entre cultivares comerciais e espécies selvagens, os autores relatam que os genótipos melhorados apresentaram maior capacidade de fixação biológica de nitrogênio.

Os estudos iniciais envolvendo a FBN, demostram que a eficiência do processo é altamente dependente do genótipo da planta (WILSON *et al.*, 1940). Portanto, diversos trabalhos vêm sendo conduzidos visando avaliar essa variação genética natural em diferentes condições ambientais como excesso de nitrato e estresse hídrico, bem como, buscar interações planta-bactérias mais eficientes. Estes trabalhos visam direcionar possíveis programas de melhoramento para FBN.

Elevados níveis de nitrato no solo na fase inicial do desenvolvimento da planta afetam negativamente a FBN, acarretando na queda de produtividade caso as duas fontes de N (N mineral e a FBN) não forem suficientes para suprir as necessidades da planta. Em alguns países a soja é cultivada em solos que possuem alto teor de N naturalmente, portanto, estudos relacionados à seleção de genótipos com simbiose tolerante ao nitrato e super nodulação foram conduzidos por diversos pesquisadores. (AKAO; KOUCHI, 1992; CARROLL; MCNEIL; GRESSHOFF, 1985; GREMAUD; HARPER, 1989; HARPER; GIBSON, 1984; HERIDGE; ROSE, 2000; HERRIDGE; TURPIN; ROBERTSON, 2001; LOHRKE; ORF; SADOWSKY, 1996).

Outro fator ambiental que afeta sensivelmente a FBN é o estresse hídrico. Leguminosas como a soja, nas quais o N_2 fixado é translocado para a parte aérea na forma de ureídos (alantoina e ácido alantoico) são particularmente sensíveis a níveis moderados de dessecação do solo (BARAL; SILVA; IZAGUIRRE-MAYORA, 2016). Sendo assim, a seleção genótipos que têm bom rendimento mesmo em situações de estresse hídrico devido a manutenção da FBN, tem sido alvos de diversos estudos (CHEN *et al.*, 2007; CEREZINI *et al.*, 2017). Em estudo mais recente Cerezini *et al.* (2019), avaliaram seis genótipos quanto aos parâmetros fisiológicos e à fixação biológica de nitrogênio, em condições de restrição hídrica, e sua capacidade de recuperação após reidratação, com base em componentes de produção. Concluíram que os genótipos BRB14-207526, BRB14-207527 e R02-1325 apresentam melhores desempenhos fisiológicos e menor acúmulo de ureídos sob seca, além de maior produção de grãos após a sua reidratação. As progênies BRB14-207526 e BRB14-207527 apresentam características de tolerância à seca, enquanto R02-1325 é uma fonte promissora de tolerância à seca para o melhoramento da soja.

Incrementos na proporção de N proveniente da FBN têm sido alcançados pelo melhoramento e seleção de genótipos, estirpes bacterianas e na interação genótipo-estirpe (TAMAGNO *et al.*, 2018). Com o objetivo de detectar cultivares de soja com maior capacidade de nodulação e fixação biológica de nitrogênio, Borher e Hungria (1998) avaliaram 152 cultivares inoculadas com três estirpes de *Bradyrhizobium*. Concluíram que as cultivares Bossier, BR-29, J-200 e Ivaí apresentaram melhor desempenho simbiótico com todas as estirpes e acumularam 30% a mais de N que a média geral. Em estudo de Agoyi (2016), com o objetivo de identificar genótipos com nodulação promíscua para serem usados como parentais para iniciar um programa de melhoramento com foco nesse caráter, conclui que os genótipos NamII, WonderSoya, Bulindi 48C, NamSoy 4M, MakSoy 3N, NamSoy 3, K-Local, Kabanyolo 1, UG 5, Soprano, MakSoy 2N, e MakSoy 5N se destacam por apresentarem melhor resultados e poderiam ser indicados como possíveis parentais.

A interação entre estirpes de *Bradyrhizobium* x genótipos de soja têm sido relatada em diversos estudos (AGOYI *et al.*, 2016b; AGOYI *et al.*, 2017; ARGAW, 2014; KIERS; RATCLIFF; DENISON, 2013; TAMAGNO *et al.*, 2018). Portanto, o estudo dessa interação se faz necessário para otimizar a FBN indicando inoculantes e genótipos que possuem uma interação positiva para FBN

Devido a dificuldade de avaliar as características fenotípicas relacionadas com a FBN, pesquisadores têm buscado identificar QTLs (*Quantitative Trait Loci*) que controlam a nodulação e FBN em genótipos de soja. Nicolás, Hungria e Arias (2006) conduziram um dos primeiros estudos no Brasil visando identificar QTLs relacionados a FBN e nodulação em progênies de soja. Utilizaram 160 progênies F_2, oriundas de cruzamento de duas cultivares brasileiras (Embrapa 20 e BRS 133) (NICOLÁS; ARIAS; HUNGRIA, 2002). O mapeamento foi realizado utilizando 45 marcadores SSR para nodulação (número de nódulos e peso seco de nódulos) e crescimento da planta (peso seco da raiz). Identificaram 21 marcadores SSR associados aos caracteres avaliados, indicando que a seleção assistida com uso de marcadores SSR pode uma alternativa viável para melhorar características relacionadas à FBN em programas de melhoramento.

Desde então, vários estudos vêm sendo conduzidos com este objetivo, sendo identificados uma série de QTLs relacionados à nodulação em diferentes estágios de desenvolvimento da soja que podem ser utilizados como uma alternativa para condução de programas de melhoramento (GRUNVALD *et al.*, 2018; HWANG *et al.*, 2014; SANTOS *et al.*, 2013; YANG *et al.*, 2017; YANG *et al.*, 2019).

Assim, estudos científicos mostram que o próximo passo para elevar a atividade da FBN, consiste na seleção de cultivares de soja, visando possíveis indicações de estratégias para o melhoramento genético (SINCLAIR; NOGUEIRA, 2018; TORRES *et al*., 2015).

2.4 Estimativas de parâmetros genéticos e fenotípicos

O conhecimento do tipo de ação gênica que predomina no controle genético de um caráter é um fator importante, principalmente para a condução eficiente de um programa de melhoramento (ACQUAAH, 2012; BERNARDO, 2014; NADEEM; AZHAR, 2004). Quando o controle genético é complexo, envolvendo muitos *locus*, ou então quando a influência de fatores ambientais sobre a expressão do caráter for pronunciada, há uma maior dificuldade em se conhecer com detalhes a natureza de ação gênica presente (VENCOVSKY; BARRIGA, 1992).

As estimativas de parâmetros genéticos e fenotípicos, como variância, herdabilidade e ganho de seleção, têm grande importância nos programas de melhoramento, uma vez que possibilitam a tomada de decisões relacionadas à escolha do método de melhoramento mais apropriado, bem como aos caracteres a serem selecionados nas etapas iniciais e avançadas do programa de melhoramento (ACQUAAH, 2012; BERNARDO, 2014; CRUZ, 2005).

Como o fenótipo de um indivíduo está ligado a fatores genéticos e ambientais, as variações do fenótipos são resultantes das variações decorrentes de ambos fatores, genótipo e ambiente, assim, têm-se que a variância fenotípica ($\sigma^2{}_F$) é igual a soma das variâncias genótipicas ($\sigma^2{}_G$) e ambiental ($\sigma^2{}_E$) (RAMALHO *et al.*, 2012)

Uma vez que a variância ambiental não é um fator controlável, esta é considerada como um erro experimental (BORÉM, 2009), portanto, cabe ao melhorista a sensibilidade de identificar e selecionar os melhores genótipos.

Bernardo (2010) afirma que a variância aditiva mede a variação dos efeitos que são transmitidos de uma geração para outra, assim, a variância aditiva apresenta importância fundamental em predizer as trocas na média da população, devido a seleção.

De acordo com Fehr e Calviness (1977), a estimação da herdabilidade é de grande valia para o melhorista, pois, para caracteres com alta herdabilidade, a seleção nas gerações iniciais de autofecundação se torna bastante eficaz. Entretanto, caracteres com baixa herdabilidade, a seleção deve ser praticada em gerações mais avançadas, uma vez que o aumento da homozigose, decorrente do processo de endogamia, resulta em um aumento da herdabilidade no sentido restrito.

Diversos estudos mostram que os efeitos aditivos são predominantes na herança da capacidade de fixação de nitrogênio. Em relação às estimativas de herdabilidade, os resultados denotam valores de baixa a alta magnitude (TABELA 1) (AGOYI *et al.*, 2016a; NICOLÁS; ARRABAL; HUNGRIA, 2002; YANG *et al.*, 2019).

Tabela 1 - Herdabilidade e *Quantivative trait loci* (QTL) associados com a fixação biológica de N em soja (Continua).

Cruzamento e população	**Estirpes utilizadas**	**Caracteres avaliados**	**Herdabilidade**	**Resumo dos QTLs associados as características relacionadas à FBN**	**Referência**
168 $F_{9:11}$ linhagens endogâmicas recombinantes, derivadas do cruzamento dos genótipos FC1 (alta capacidade de FBN) x FC2 (baixa capacidade de FBN).	Três estirpes de *B. elkani* BXYD3 BXBL9 e BDYD1.	- Nº, weighta e tamanho de nódulos; - Weighta seca de plantas; -Teor de N total.	A herdabilidade no sentido amplo para todos os caracteres variou de 0,48 a 0,87. Sugerindo que a variação nas características de FBN foi determinado principalmente pelo genótipo.	Foi identificado dois QTLs ligados a FBN. O qBNF-16, onde explicou de 15,9 a 59,0% da variação fenotípica encontrada. Enquanto o qBNF-17 explicou de 12,6 a 18,6% da variação observada.	YANG, *et al.*, 2019
113 $F_{7:8}$ linhagens endogâmicas recombinantes, derivadas do cruzamento das cultivares ‘Bossier’ (alta capacidade de FBN) e ‘Embrapa 20’ (média capacidade de FBN).	*B. japonicum* SEMIA 5079 *B. elkani* SEMIA 587	- Nº de nódulos (NN); - Weighta seca de nódulos (MSN); - Relação entre NN/MSN (MSN/NN); -Weighta seca da parte aérea (MSPA).	A herdabilidade estimada para NN, MSN, MSN/NN e MSPA foi de 0,41; 0,30; 0,30 e 0,49, respectivamente	Identificados dois QTLs para MSN/NN no cromossomo 13 a 67cM. O efeito aditivo desse loco foi 0,31 mg.nodulo^{-1} e explicou 18,13% da variação fenotípica. Outro QTL para MSN foi identificado no cromossomo 19 a 24 cM. O efeito aditivo desse loco foi de 0,57 g.planta^{-1} e explicou 14,93% da variação fenotípica.	GRUNVALD, *et al.*, 2018
157 $F_{2:7}$ linhagens endogâmicas recombinantes, derivadas do cruzamento das cultivares ‘Bossier’ (alta capacidade de FBN) e ‘Embrapa 20’ (média capacidade de FBN).	*B. japonicum* SEMIA 5079 *B. elkani* SEMIA 587	- Nº de nódulos (NN); - Weighta seca de nódulos (MSN); - Relação entre NN/MSN (MSN/NN); -Weighta seca da parte aérea (MSPA).	Os valores de herdabilidade foram: 0,33 (NN); 0,33 (MSN); 0,27 (MSN/NN) e 0,49 (MSPA).	Foram identificados dois QTLs para MSPA nos GLs E e L, três QTLs para NN nos GLs B1, E, e I, e um QTL para a MSN/NN no GL I, explicando 15,4%, 13,8% e 6,5% variação fenotípica, respectivamente.	SANTOS *et al.*, 2013

Tabela 1 - Herdabilidade e *Quantivative trait loci* (QTL) associados com a fixação biológica de N em soja (Conclusão).

Cruzamento e população	**Estirpes utilizadas**	**Caracteres avaliados**	**Herdabilidade**	**Resumo dos QTLs associados as características à FBN**	**Referência**
160 $F_{2:3}$ linhagens endogâmicas recombinantes, derivadas do cruzamento das cultivares 'Embrapa 20' (média capacidade de FBN) e 'BRS 133' (baixa capacidade de FBN)	*B. japonicum* SEMIA 566 e *B. elkani* SEMIA 587	- Nº de nódulos (NN); - Weighta seca de nódulos (MSN); - Relação entre MSN/NN (MSN/NN); -Weighta seca da parte aérea (MSPA).	Os valores de herdabilidade foram: 0,13 (NN); 0,26 (MSN); 0,20 (MSN/NN) e 0,61 (MSPA).	Observaram duas regiões genômicas associadas com NN e MSN, contribuindo com 7% e 10% variação fenotípica, respectivamente. Houve efeito aditivo para os caracteres avaliados. Encontraram seis interações epistáticas entre QTLs não ligados a NN, MSN e MSPA, explicando 15% da variação dos caracteres e aumentando a expressão fenotípica de 8 a 28%.	NICOLÁS *et al.*, 2006
157 $F_{2:7}$ linhagens endogâmicas recombinantes, derivadas do cruzamento das cultivares 'Bossier' (alta capacidade de FBN) e 'Embrapa 20' (média capacidade de FBN)	*B. japonicum* SEMIA 566 e *B. elkani* SEMIA 587	- Nº de nódulos (NN); - Weighta seca de nódulos (MSN); - Relação entre MSN/NN (MSN/NN).	Os valores de herdabilidade foram: 0,30 (NN); 0,27 (MSN); 0,27 (MSN/NN) e 0,49 (MSPA).	Foi relatado doze associações significativas em quatro GLs (B1, C2, D1b e H), sendo três MSPA, quatro para NN, duas para MSN e três para MSN/NN. Todos os QTLs foram de efeitos menores e apresentaram efeitos aditivos.	SANTOS *et al.*, 2006
136 F_2 linhagens endogâmicas recombinantes, derivadas do cruzamento das cultivares 'SJ2' e 'Suwon'	*B. japonicum* DASA 01026	- Nº de nódulos (NN); - Weighta fresca de nódulos (MFN); - Weighta seca de nódulos (MSN); - Weighta seca da parte aérea (MSPA); - Atividade de redução de acetileno (ARA).	Os valores de herdabilidade foram: 0,78 (NN); 0,72 (MFN); 0,55 (MSN); 0,66 (MSPA) e 0,50 (ARA).	Foram identificados 5 QTLs associados com NN, 3 com MSN, 4 com MSPA e 2 com ARA, sendo a maioria com pouca contribuição na variação fenotípica, exceto para o QTL no GL O contribuindo com variação de 17% para NN (Sat_038), 18% para MSN (Sat_274), 14% para MSPA (Sat _274), e 12% para ARA (Sat_274).	TANYA *et al.*, 2005

Fonte: Do autor (2020).

REFERÊNCIAS

AGOYI, E.E. *et al*. Mode of inheritance of promiscuous nodulation and combining abilities in soybean genotypes. **International Journal of Agronomy and Agricultural Research** (IJAAR), [*S.l.*], v. 9, n. 1, p. 73-82, 2016a.

AGOYI, E.E. *et al*. Screening soybeans genotypes for promiscuous symbiotic association with *Bradyrhizobium* strains. **African Crop Science Journ**al, [*S.l.*], v. 24, n. 1, p. 49–59, 2016b.

AGOYI, E.E. *et al*. Ureide Essay to Assess N_2-fixation Abilities of Soybean (Glycine Max) Genotypes under Different Bradyrhizobium Strains. **J. Crop Sci. Biotech**., [*S.l.*], v. 20, n. 2, p. 65-72, 2017.

AKAO, S.; KOUCHI, H. Light microscopic observation of root hair curling of soybean induced by Rhizobium infection. **J. Soil Sci. Plant Nutr.**, [*S.l.*], v. 60, p. 53-55, 1989.

ALVES, B.J.R.; BODDEY, R.M.; URQUIAGA, S. The success of BNF in soybean in Brazil. **Plant and Soil,** v. 252, n. 1, p. 1–9, 2003.

ACQUAAH, G. **Principles of Plant Genetics and Breeding**. 2nd Edition. John Wiley & Sons Ltda., 2012. 740 p.

ARGAW, A. Symbiotic effectiveness of inoculation with Bradyrhizobium isolates on soybean [*Glycine max* (L.) Merrill] genotypes with different maturities. **Springer Plus,** [*S.l.*], v. 3, p. 753, 2014.

BALBOA, G.R., SADRAS, V.O., CIAMPITTI, I.A. Shifts in soybean yield, nutrient uptake, and nutrient stoichiometry: A historical synthesis analysis. **Crop Sci.,** [*S.l.*], v. 58, p. 43–54, 2018.

BARAL, B.; SILVA, J.A.T. da; IZAGUIRRE-MAYORAL, M.L. Early signaling, synthesis, transport and metabolism of ureides. J**ournal of Plant Physiology,** [*S.l.*], v. 193, p. 97-109, 2016.

BERNARDO, R. **Breeding for Quantitative Traits in Plants.** Woodbury, Minnesota: Stemma Press, 2010.

BERNARDO, R. **Essentials of plant breeding**. Woodbury: Stemma Press, 2014. 252 p.

BOHRER, T.R.J.; HUNGRIA, M. Avaliação de cultivares de soja quanto à fixação biológica do nitrogênio. **Pesquisa Agropecuária Brasileira**, [*S.l.*], v. 33, n. 6, p. 937–952, 1998.

BORÉM, A. **Hibridação Artificial de Plantas**. 2. ed. Viçosa: UFV, 2009.

CALDWELL, B.E. Inheritance of a strain-specific ineffective nodulation in soybean. **Crop Sci.,** [*S.l.*], v. 6, p. 427-428, 1966.

CÂMARA, G.M.S. Fixação biológica de nitrogênio em soja. **Informações agronômicas** (IPNI), [*S.l.*], n. 147, 2014.

CÂMARA, G.M.S. Nitrogênio e produtividade da soja. *In*: **Soja**: Tecnologia da Produção II. Piracicaba: ESALQ/USP, 2000. p. 295–339.

CARROLL, B.J.; MCNEIL, D.L.; GRESSHOFF, P.M.A supernodulation and nitrate tolerant symbiotic (nts) soybean mutant. **Plant Physiology,** [*S.l.*], v. 78, p. 34–40, 1985.

CEREZINI, P. *et al.* Physiological and N_2-fixation-related traits for tolerance to drought in soybean progenies. **Pesquisa Agropecuária barsileira,** [*S.l.*], v. 54, p. 1-10, 2019.

CEREZINI, P. *et al.* Water restriction and physiological traits in soybean genotypes contrasting for nitrogen fixation drought tolerance. **Scientia Agricola**, [*S.l.*], v. 74, p. 110-117, 2017.

CHEN, P. *et al.* Registration of soybean germplasm lines R01-416F and R01-581F for improved yield and nitrogen fixation under drought stress. **Journal of Plant Registrations**, [*S.l.*], v.1, p.166-167, 2007.

CIAMPITTI, I.A.; SALVAGIOTTI, F. New insights into soybean biological nitrogen fixation. **Agronomy Journal, Geneva**, [*S.l.*], v. 110, n. 4, p. 1185–1196, 2018.

CRUZ, C.D. **Princípios de genética quantitativa.** 2. ed. Viçosa: UFV, 2005.

DEVINE, T.E.; BREITHAUPT, B.H. Significance of Incompatibility Reactions of *Rhizobium japonicum* Strains with Soybean Host Genotypes1. **Crop Science,** [*S.l.*], v. 20, n. 2, p. 269, 1980.

DORNHOFF, G.M.; SHIBLES, R.M. Varietal Differences in Net Photosynthesis of Soybean Leaves1. **Crop Science,** [*S.l.*], v. 10, n. 1, p. 42, 1970.

DWIVEDI, S.L. *et al.* Advances in host plant and *Rhizobium* genomics to enhance symbiotic nitrogen fixation in grain legumes. **Adv. Agron.,** [*S.l.*], v. 129, 1–116 p., 2015.

FEHR, W.R.; CAVINESS, C.E. Stages of Soybean Development. **Special Report,** [*S.l.*], v. 80, n. March, p. 11, 1977.

FOX, A. R. *et al.* Major cereal crops benefit from biological nitrogen fixation when inoculated with the nitrogen-fixing bacterium Pseudomonas protegens Pf-5X940. Environ. **Microbiol.,** [*S.l.*], v. 18, p. 3522–3534, 2016.

GREMAUD, M.F.; HARPER, J.E. Selection and initial characterization of partially nitrate tolerant nodulation mutants of soybean. **Plant Physiology,** [*S.l.*], v. 89, p. 169-173, 1989.

GRUNVALD, A.K. *et al.* Identification of QTLs Associated with Biological Nitrogen Fixation Traits in Soybean Using a Genotyping-by-Sequencing Approach. **Crop Science,** [*S.l.*], v. 58, p. 2522-2532, 2018.

HARPER, J. E.; GIBSON, A. H. Differential nodulation tolerance to nitrate among legume species. **Crop Sci,** [*S.l.*], v. 24, p. 173–179, 1984.

HARPER, J.E.; NICKELL, C.D. Genetic analysis of non-nodulating soybean mutants in a hypernodulating background. **Soybean Genet. Newslett.,** [*S.l.*], v. 22, p. 185 – 190, 1995.

HERRIDGE, D.; ROSE, I. Breeding for enhanced nitrogen fixation in crop legumes. **Field Crops Research**, [*S.l.*], v. 65, n. 2–3, p. 229–248, 2000.

HERRIDGE D.F.;TURPIN J.E. ROBERTSON, M.J. Improving nitrogen fixation of crop legumes through breeding and agronomic management: analysis with simulation modelling. **Australian Journal of Experimental Agriculture,** [*S.l.*], v. 41, p. 391-401, 2001.

HUNGRIA, M. *et al.* Nitrogen nutrition of soybean in Brazil: Contributions of biological N 2 fixation and N fertilizer to grain yield. **Canadian Journal of Plant Science,** [*S.l.*], v. 86, n. 4, p. 927 939, 2006.

HUNGRIA, M.; CAMPO, R.J.; MENDES, I.C. **A importância do processo de fixação biológica do N para a cultura da soja:** componente essencial para a competitividade do produto brasileiro. Londrina - PR: Embrapa Soja, 2007. 80 p.

HWANG, S. *et al.* Genetics and mapping of quantitative traits for nodule number, weight, and size in soybean (Glycine max L.[Merr.]). **Euphytica,** [*S.l.*], v. 195, p. 419-434, 2014.

KIERS, E.T.; RATCLIF, W.C.; DENISON, R.F. Single-strain inoculation may create spurious correlations between legume fitness and rhizobial fitness. **New Phytol.,** [*S.l.*], v. 198, p. 4–6, 2013.

KING, C.A.; PURCELL, L.C. Inhibition of N2 fixation in soybean is associated with elevated ureides and amino acids. Plant Physiology, v. 137, n. 4, p. 1389–1396, 2005.

KOLLER, H.R.; NYQUIST, W.F.; CHORUSH, I.S. Growth analysis of soybean community. **Crop Science,** [*S.l.*], v. 10, p. 407-412, 1986.

LI, X.; ZENG, R.; LIAO, H. Improving crop nutrient efficiency through root architecture modifications. **J. Integr. Plant Biol.,** [*S.l.*], v. 58, p. 193–202, 2016.

LOHRKE, S.; ORF, J.; SADOWSKY, M. Inheritance of host-controlled restriction of nodulation by Bradyrhizobium japonicum strain USDA 110. **Crop Sci,** [*S.l.*], v. 36, p. 1271–1276 1996

MENDES, I. *et al.* **Fixação biológica de nitrogênio na soja:** bom para a qualidade do solo, para o bolso do agricultor e para o Brasil. Disponível em: http://www.diadecampo.com.br. Acesso em: 15 jan. 2020.

MPEPEREKI, S. *et al.* Soybean and sustainable agriculture. Promiscuous soybeans in Southern Africa. **Field Crops Research,** Amsterdam, v. 65, n. 2, p. 137-150, 2000.

MUÑOZ, N. *et al.* Improvement in nitrogen fixation capacity could be part f the domestication process in soybean. **Heredity,** [*S.l.*], v. 117, p. 84-93, 2016.

NADEEM, K.; AZHAR, F.M. Genetic analysis of seed cotton yield and its components in *Gossypium hirsutum* L. **International Journal of Agriculture and Biology,** [*S.l.*], v. 6, p. 865-868, 2004.

NICOLÁS, M.F.; ARIAS, C.A.A.; HUNGRIA, M. Genetics of nodulation and nitrogen fixation in Brazilian soybean cultivars. **Biology and Fertility of Soils,** [*S.l.*], v. 36, n. 2, p. 109–117, 2002.

NICOLÁS, M.F.; HUNGRIA, M.; ARIAS, C.A.A. Identification of quantitative trait loci controlling nodulation and shoot weight in progenies from two Brazilian soybean cultivars. **Field Crops Research,** [*S.l.*], v. 95, p. 355-366, 2006.

OLIVARES, J.; BEDMAR, E.J.; SANJUÁN, J. Biological nitrogen fixation in the context of global change. **Mol. Plant Microbe Interact.**, [*S.l.*], v. 26, p. 486–494, 2013.

PEOPLES, M.B. *et al.* The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. **Symbiosis.,** [*S.l.*], v. 48, p. 1-17, 2009.

RAMALHO, M.A.P. *et al.* A**plicações da genética quantitativa no melhoramento de plantas autógamas**. Lavras: UFLA, 2012.

RAMÍREZ-PUEBLA, S.T. *et al.* La diversidad de rizobios nativos de México a la luz de la genómica. **Rev. Mex. Biodivers.**, [*S.l.*], v. 90, p. 1-10, 2019.

SADOWSKY, M.J. *et al.* Restriction of nodulation by *Bradyrhizobium japonicum*is mediated by factors present in roots of *Glycine max*. **Applied and Environmetal Microbiology,** Washington, v. 61, n. 2, p. 832-836, 1995.

SALVAGIOTTI, F. *et al.* Nitrogen uptake, fixation and response to fertilizer N in soybeans: a review. **Field Crop Research,** Amsterdam, v. 108, n. 1, p. 1–13, 2008.

SANTOS, M.A. *et al*. Mapping of QTLs associated with biological nitrogen fixation traits in soybean. **Hereditas,** [*S.l.*], v. 150, p. 17–25, 2013.

SANTOS, M.A. dos. **Mapeamento de QTLs para caracteres relacionados com a fixação biológica de nitrogênio (FBN) em soja.** 2009. 101 p. Tese (Doutorado em Genética e melhoramento de plantas) - Escola Superior de Agricultura "Luiz de Queiroz" - USP, Piracicaba, 2009.

SANTOS, M.S.; NOGUEIRA, M.A.; HUNGRIA, M. Microbial inoculants: reviewing the past, discussing the present and previewing na outstanding future for the use of beneficial bacteria in agriculture. **AMB Express**, [*S.l.*], v. 9, n. 205, p. 1-22, 2019.

SILVA, J.R. *et al.* Can stress response genes be used to improve the symbiotic performance of rhizobia? **AIMS Microbiology**, [*S.l.*], v. 3, p. 365–382, 2017.

SINCLAIR, T.R. *et al.* Drought tolerance and yield increase of soybean resulting from improved symbiotic N2 fixation. **Field Crops Research,** [*S.l.*], v. 101, n. 1, p. 68–71, 2007.

SINCLAIR, T.R.; NOGUEIRA, M.A. Selection of host-plant genotype: the next step to increase grain legume N2 fixation activity. **Journal of Experimental Botany,** [*S.l.*], v. 69, p. 3523-3530, 2018.

STREETER, J.; WONG, P.P. Inhibition of legume nodule formation and N2 fixation by nitrate. **Crit. Rev. Plant Sci.**, [*S.l.*], v. 7, p. 1–23, 1988.

TAIZ, L.; ZIEGER, E. **Fisiologia vegetal.** (Trad). *In*: SANTARÉM, E.R. *et al.* 5. ed. Porto Alegre: Artemed, 2013. 919 p.

TAMAGNO, S. *et al.* Interplay between nitrogen fertilizer and biological nitrogen fixation in soybean: implications on seed yield and bioweight allocation. **Scientific Reports,** London, v. 8, p. 1-11, 2018.

TAMAGNO, S. *et al.* Nutrient partitioning and stoichiometry in soybean: A synthesis-analysis. **Field Crops Res.**, [*S.l.*], v. 200, p. 18–27, 2017.

TANYA, P. *et al.* Identification of SSR markers associated with N2-fixation components in soybean [*Glycine max* (L.) Merr.]. **Korean J. Genet.,** [*S.l.*], v. 27, p. 351–359, 2005.

TORRES, A.R. *et al*. Genetic structure and diversity of a soybean germplasm considering biological nitrogen fixation and protein content. **Scientia agrícola,** [*S.l.*], v. 72, p. 47-52, 2015

VENCOVSKY, R.; BARRIGA, P. **Genetica biométrica no fitomelhoramento**. Ribeirão Preto: Sbg, 1992.

VEST, G. Rj3 a gene controlling ineffective nodulation in soybean. **Crop Sci.**, [*S.l.*], v. 10, p. 34-35, 1970.

VEST, G.; CALDWELL, B.E. Rj4 a gene controlling ineffective nodulation in soybean. **Crop Sci**., [*S.l.*], v. 12, p. 692 – 693, 1972.

VUONG, T.D.; NICKELL, C.D.; HARPER, J.E. Genetic and allelism analyses of hypernodulation soybean mutants from two genetic backgrounds. **Crop Sci.,** [*S.l.*], v. 36, p. 1153– 1158, 1996.

YAMASHITA, N. *et al.* Effects of different chemical forms of nitrogen on the quick and reversible inhibition of soybean nodule growth and nitrogen fixation activity. **Front. Plant Sci.**, [*S.l.*], v. 10, p. 131, 2019.

YANG, Y. *et al.* Characterization of genetic basis on synergistic interactions between root architecture and biological nitrogen fixation in soybean. **Front. Plant Sci.,** [*S.l.*], v. 8, p. 1466, 2017.

YANG, Y. *et al.* Genetic Analysis and Mapping of QTLs for Soybean Biological Nitrogen Fixation Traits Under Varied Field Conditions. **Front. Plant Sci.,** [*S.l.*], v. 10, n. 75, p. 1-11, 2019.

WILLIAMS, L.F.; LYNCH, D.L. Inheritance of a nonnodulating character in the soybean. **Agronomy Journal,** [*S.l.*], v. 46, p. 28–29, 1954.

WILSON, P.W. **Biochemistry of symbiotic Nitrogen fixation.** University of Wisconsin Press: Madison, 1940. 130 p.

ZUFFO, A.M. *et al.* Co-inoculation of *Bradyrhizobium japonicum* and *Azospirillum brasiliense* in the soybean crop. **Revista de Ciências Agrárias**, [*S.l.*], v. 38, n. 1, p. 87-93, 2015.

SEGUNDA PARTE – ARTIGO*

ARTIGO - COMBINING ABILITIES OF SOYBEAN PROGENIES AND BRADYRHIZOBIUM STRAINS FOR BIOLOGICAL NITROGEN FIXATION

ABSTRACT

Agriculture is increasingly competitiveness and seeking to be more efficient by reducing production costs while respecting the environment. In this context, BNF is a viable alternative serving to meet these objectives. Therefore, the development of soybean genotypes efficient in BNF is important. Thus, the purpose of this work was to study the BNF ability of soybean progenies and their ability for symbioses using different *Bradyrhizobium* strains as well as to select more efficient progenies and strains for BNF, aiming to guide strategies in soybean breeding to BNF. In a greenhouse experiment, were evaluated 24 soybean progenies and five *Bradyrhizobium* strains (SEMIA 5079, SEMIA 587, SEMIA 5019, UFLA 06-24 and INPA 03-11B). For the trial progenies and strains, analysis of variance was conduct and means were compared by Scott-Knot test. Partial diallel analysis was performed to estimate the general and specific combination ability for the progenies and *Bradyrhizobium* strains. The best progenies and strains were selected considering their means and general combining ability for the following traits: nitrogen content, chlorophyll content, number of nodules, fresh and dry weight of nodules and Z index. Ten progenies and two strains (SEMIA 587 and INPA 03-11B) were selected to carry out a field trial experiment. The BNF was influenced by genotype, strain, and genotype-strain interactions. There was a predominance of additive effects on the traits related to BNF, indicating that recurrent selection could be a strategy for improvement of these traits. Group II progenies showed higher values for the traits related to field nodulation but did not show gains in grain yield. The EA-8 progeny stood out as having a high yield, with good results in traits related to BNF and early maturity. INPA 03-11B strain showed the best result for grain yield, indicating high potential for its use as an inoculant in soybean crops.

Keyword: Soybean breeding. Partial diallel. Recurrent Selection. Quantitative genetics. Symbiosis.

1 INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is one of the most important oil crops worldwide. It is the main source of protein and edible oil for humans due to the high oil (20-25%) and protein (42-45%) in seeds (AZIZ *et al*., 2016). Currently, Brazil is the largest producer and exporter of soybean. Approximately 36.9 million hectares were grown in the 2019/20 cropping season, resulting in a production of 120.88 million metric tons of grains (CONAB, 2020).

Many factors contributed to the success of soybean in Brazil, such as advances in disease control, soil management techniques, and the constant advances of breeding programs. The launch of high yield cultivars resistant to diseases and pests associated with *Bradyrhizobium* strain selection adapted to Brazilian conditions capable of replacing the use of nitrogen fertilizers, was undoubtedly fundamental for the expansion of the soybean production chain in Brazil (HUNGRIA *et al.*, 2006; ZUFFO *et al*., 2015).

Nitrogen is one of the most critical nutrients required by cultivated plants, and it is essential for the success of any crop. Due to the high protein content in soybean grains, these plants require a large amount of N (RODRIGUES-NAVARRO *et al.,* 2011; KINUGASA *et al.,* 2012), approximately 80 kg per metric ton of grains. In Brazil, the inoculation of soybean with elite *Bradyrhizobium* spp. strains can fully supply the crop's demand for N, negating the need for N fertilizers. Likely because of breeding for BNF, soybean symbioses are very sensitive to N fertilization, which drastically reduces nodulation (HUNGRIA *et al.*, 2007; HUNGRIA; MENDES, 2015; SANTOS; NOGUERIA; HUNGRIA, 2019). Brasil is the global leader in the use of inoculants for soybean crops (HUNGRIA; MENDES, 2015; OKON *et al.*, 2015; ANPII, 2016), where approximately 78% of the cropping area are inoculated yearly (ANPII, 2018).

The replacement of N fertilizers with BNF in soybeans crops generates an annual economic benefit of approximately 20 billion dollars for Brazilian agriculture (SANTOS; NOGUEIRA; HUNGRIA, 2019). In addition to reducing costs, environmental benefits are achieved because the substitution of N minerals reduces the pollution of water resources that occurs due to the eutrophication of lakes and rivers. It also reduces atmospheric pollution and avoids the high energy expenditure related to the production of N fertilizers (BRACINNI *et al.*, 2016; ZANINI *et al.*, 2012).

In Brazil, research with inoculants has been highly advanced, through genetic control and selection of microorganisms with good efficiency. However, since the initial studies involving BNF, it has been observed that the process efficiency is highly dependent on the plant genotype (WILSON, 1940). Several studies have shown differences between genotypes (AGOYI *et al.*, 2016b), strains (SOLOMON; PANT; ANGAW, 2012; ZIMER *et al.*, 2016) and genotype-strain interactions (ARGAW, 2014) for traits related to BNF.

Although increasing BNF capacity has not been the target of soybean breeders, the selection for seed size, plant growth and vegetative development, as well as grain yield may have indirectly influenced this trait, since these factors are directly related to the plant nitrogen demand (BALBOA; SADRAS; CIAMPITTI, 2018; TAMAGNO *et al.*, 2017). Munõz *et al.* (2016) compared the BNF ability between commercial cultivars and wild species, and the authors reported that the improved genotypes showed higher biological nitrogen fixation ability.

Even though there is evidence that BNF is involved in the soybean plants domestication, this trait has not been commonly evaluated in breeding programs in Brazil, or in other countries. Reports show that additive effects are predominant in the inheritance of nitrogen fixation ability. Regarding heritability estimates, the results denote values from low to high magnitude (AGOYI *et al.*, 2016a; NICOLÁS; ARRABAL; HUNGRIA, 2002; YANG *et al.*, 2019).

Thus, scientific studies show that the next step to increase BNF is to select more efficient soybean genotypes for this characteristic, to possibly improve breeding strategies (SINCLAIR; NOGUEIRA, 2018; TORRES *et al.*, 2015). However, these results are still scarce. The purpose of this study was to evaluate the BNF ability of soybean progenies and their symbiotic association capacities with different *Bradyrhizobium* strains, as well as to select more efficient progenies and strains for BNF, aiming to guide breeding strategies for BNF in soybean.

2 MATERIALS AND METHODS

2.1 Greenhouse experiment

The experiment was carried out in a greenhouse in the Department of Soil Science of the *Universidade Federal de Lavras* (UFLA), in 2018. The experiment was conducted in an RCB (randomized complete block) design with four replications in a factorial scheme. The progenies evaluated came from the Soybean Breeding Program of the UFLA.

Twelve progenies were selected from the recurrent selection program for early maturity (Group I) (RIBEIRO, 2018) and 12 progenies were selected from the recurrent selection program for grain yield (Group II) (SOARES, 2018). The description and origin of the progenies are described in Table 2.

Table 2 - Progenies genealogy from recurrent selection program.

Group I

Progenies	Origin	Population	Generation
EA - 1	Soybean Research - UFLA	C-0 SR EA	$S_{0:5}$
EA - 2	Soybean Research - UFLA	C-0 SR EA	$S_{0:5}$
EA - 3	Soybean Research - UFLA	C-0 SR EA	$S_{0:5}$
EA - 4	Soybean Research - UFLA	C-0 SR EA	$S_{0:5}$
EA - 5	Soybean Research - UFLA	C-0 SR EA	$S_{0:5}$
EA - 6	Soybean Research - UFLA	C-0 SR EA	$S_{0:5}$
EA - 7	Soybean Research - UFLA	C-0 SR EA	$S_{0:5}$
EA - 8	Soybean Research - UFLA	C-0 SR EA	$S_{0:5}$
EA - 9	Soybean Research - UFLA	C-0 SR EA	$S_{0:5}$
EA - 10	Soybean Research - UFLA	C-0 SR EA	$S_{0:5}$
EA - 11	Soybean Research - UFLA	C-0 SR EA	$S_{0:5}$
EA - 12	Soybean Research - UFLA	C-0 SR EA	$S_{0:5}$

Group II

Progenies	Origin	Population	Generation
YD - 1	Soybean Research - UFLA	C-0 SR YD	$S_{0:5}$
YD - 2	Soybean Research - UFLA	C-0 SR YD	$S_{0:5}$
YD - 3	Soybean Research - UFLA	C-0 SR YD	$S_{0:5}$
YD - 4	Soybean Research - UFLA	C-0 SR YD	$S_{0:5}$
YD - 5	Soybean Research - UFLA	C-0 SR YD	$S_{0:5}$
YD - 6	Soybean Research - UFLA	C-0 SR YD	$S_{0:5}$
YD - 7	Soybean Research - UFLA	C-0 SR YD	$S_{0:5}$
YD - 8	Soybean Research - UFLA	C-0 SR YD	$S_{0:5}$
YD - 9	Soybean Research - UFLA	C-0 SR YD	$S_{0:5}$
YD - 10	Soybean Research - UFLA	C-0 SR YD	$S_{0:5}$
YD - 11	Soybean Research - UFLA	C-0 SR YD	$S_{0:5}$
YD - 12	Soybean Research - UFLA	C-0 SR YD	$S_{0:5}$

Source: The author (2020).

Five *Bradyrhizobium* strains were tested, three of which have been approved by the Ministry of Agriculture, Livestock and Supply (MAPA) for use as inoculant in soybean, and two were selected at soil microbiology laboratory of UFLA (TABLE 3). In addition, to controls were used: I - no inoculation and no N fertilizers; II - no inoculation with N fertilizer.

Table 3 - *Bradyrhizobium* strains description.

Genus/Species	**Original designation**	**Institution**
B. japonicum	SEMIA 5079	*Embrapa Cerrados*
B. elkani	SEMIA 587	*FEPAGRO/UFRGS/Embrapa Cerrados*
B. elkani	SEMIA 5019	*Embrapa Agrobiologia/ Embrapa Cerrados/ FEPAGRO/UFRGS*
Bradyrhizobium spp.	UFLA 06-24	*Universidade Federal de Lavras*
B. elkani	INPA 03-11B	*Universidade Federal de Lavras*

Source: The author (2020).

The substrate was composed of a dystrophic red latosol (EMBRAPA, 2013) with a very clayey texture and washed sand at a proportion of 2:1 v/v. The soil used in the experiment was collected from the 0-0.20 m layer in an area that does not have a history of agricultural use.

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

Sowing was carried out in January 2018. Soybean seeds were previously disinfested by immersion in 70% alcohol for three minutes and then in sodium hypochlorite solution (3%) diluted to 20% for two minutes. After that, the seeds were washed in distilled water. Six seeds were sown per pot (PL 24 with a volume of 5 liters) and after fifteen days, thinning was performed leaving two plants. The inoculation was performed during the sowing with 1 ml of inoculum (10,8 $x10^6$ CFU) per seed. In the N fertilizer treatment was applied 700 mg of N partitioned weekly, following the INSTRUÇÃO NORMATIVA SDA Nº 13 recommendation (BRASIL, 2011).

Irrigation was performed manually twice a day (morning and afternoon), with distilled water to the field capacity. When the plants reached the R2 stage (FEHR; CALVINESS, 1977) were evaluated the following traits:

- Plant height: Distance from the plant root collar to the tip of the main stem, in centimeters;
- Number of trifoliate leaves: The number of trifoliate leaves per plant was counted;

- Chlorophyll content: Using the SPAD, the chlorophyll content was measured in a trifoliate leaf from the middle part of the plant;
- Nitrogen content: The plant was cut in the cotyledons insertion and these were oven dried at 65ºC until they reached constant weight and ground to perform the analysis of the N content, following the methodology proposed by Malavolta *et al.* (1997);
- Fresh weight of shoots: Obtained by weighing the shoots of the two plants per plot;
- Dry weight of shoots: Obtained by weighing the two plants of the plot, after drying in an oven at 65ºC;
- Number of nodules: It was performed counting the nodules from the two plants per plots;
- Fresh weight of nodules: The nodules were detached, and the weighing was subsequently performed;
- Dry weight of nodules: obtained by weighing the nodules, after drying in an oven at 65°C;
- Root volume: Determined by immersing the root in a graduated beaker with water and measuring the remaining volume.

For the number of nodules, fresh and dry weight of nodules due to does not have a normal distribution it was necessary to transform the data using the equation $\sqrt{x} + 1$. Subsequently, with the tests of the ANOVA assumptions, the data were submitted to analysis of variance and the means were grouped by Scott - Knott test (1974), at 5% probability, using the software R Core Team (2016).

The data were submitted to the analysis of variance according to the statistical model presented below:

$$y_{ijk} = \mu + p_i + t_j + b_k + pt_{ij} + e_{ijk} \tag{1}$$

where:

y_{ijk}: observed value referring to the plot that received progeny *i*, the N source *j*, in block *k*;

μ: constant associated with all observations;

p_i: effect of progeny *i*;

t_j: effect of N source *j*;

b_k: effect of block *k*;

pt_{ij}: effect of progeny *i* with N source *j* interaction;

e_{ijk}: random experimental error associated with observation y_{ijk}.

Experimental precision was evaluated by estimating accuracy (RESENDE; DUARTE, 2007), according to the model:

$$r = \sqrt{1 - \frac{1}{F_c}}\ x\ 100 \tag{2}$$

where:

r: accuracy expressed as a percentage;

F_c: calculated F value (Progenies).

The coefficient of variation was obtained through the following estimator:

$$CV = \frac{\sqrt{MSE}}{\bar{x}} \tag{3}$$

where:

MSE: mean square of error;

$\bar{x}$: overall mean.

For the standardized variables index (Z index), the observations of chlorophyll content, nitrogen content, dry weight of nodules and number of nodules were standardized to make them directly comparable, according to the model proposed by Mendes, Ramalho and Abreu (2009).

$$z_{ijkl} = \frac{y_{ijkl} - \bar{y}_{..kl}}{s_{..jk}} \tag{4}$$

where:

z_{ijk}: standardized phenotypic value of the plot that received progeny i, the N source j in block k for the trait l;

y_{ijkl}: value observed in the plot that received progeny i, N source j in block k for the trait l;

$\bar{y}_{..kl}$: phenotypic mean of block k for trait l;

$s_{..jk}$: phenotypic standard deviation associated with block k for trait l.

Because the Z variable can assume negative or positive values, the constant four was added to make them positive. After standardization, the Z sum was obtained for each plot, which can be calculated by the following expression:

$$z_{ijkl} = \sum_{k=1}^{n} z_{ijkl} = z_{ijk1} + z_{ijk2} + .. + z_{ijkn} \tag{5}$$

Subsequently, statistical analysis was conventionally performed. Using the 24 progenies and the five strains mentioned above, a general and specific combining ability study was performed according to the method proposed by Melo and Santos (1999). For the diallel analysis, the following traits were considered: nitrogen content, chlorophyll content, number of nodules, fresh weight of nodules and dry weight of nodules.

A modified version of the Griffing IV method (1956) was used in a partial diallel arrangement proposed by Geraldi and Miranda Filho (1988). One group was the progenies and the other the strains. In progeny x strain interactions, the general combining ability corresponds to the mean combining ability of progenies and strains. The specific combining ability corresponds to the capacity to match each progeny with each strain.

The mathematical model proposed for analysis of progeny x strain interactions by means of a partial diallel was:

$$y_{ij} = \mu + g_i + g_j + s_{ij} + \bar{e}_{ij} \quad (6)$$

where:

y_{ij}: mean value of the diallelic combination between progeny *i* and strain *j*;

μ: constant, which in this case represents the general mean of the diallel treatments;

g_i: effect of the general combining ability of progeny *i*;

g_j: effect of the general combining ability of strain *j*;

s_{ij}: effect of the specific combining ability between progeny *i* and strain *j*;

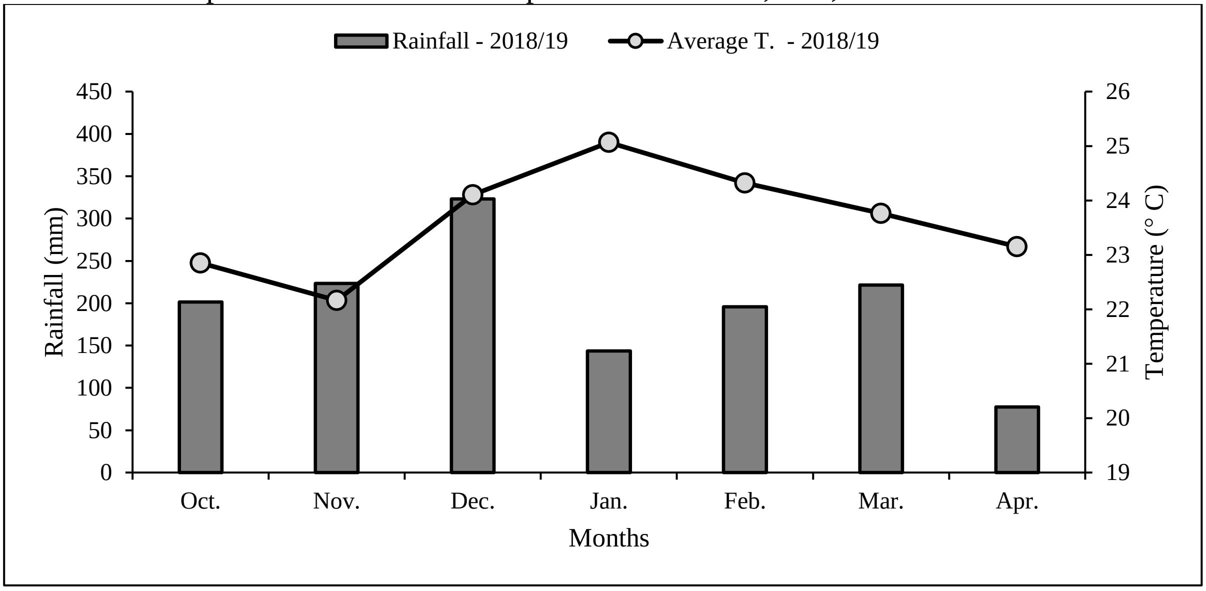
$\bar{e}_{ij}$: experimental error mean.

Considering the results of the analysis of variance and diallel analysis, the progenies and strains that presented the highest positive value for general combining ability and the higher phenotypic mean were selected.

2.2 Field experiment

Two experiments were carried out with progenies from group I and two with progenies from group II, that were previously selected. Two distinct areas were used to conduct the experiments in which there was no previous report of soybean cultivation at *Centro de Desenvolvimento Científico e Tecnológico em Agropecuária - Fazenda Muquém*, located in Lavras, with a mean altitude of 918 m, 21°12' S and 45º00' W. The annual average rainfall is 1,530 mm and the average temperature is 19.3°C. Figure 1 shows the climatic data during the field trial.

Figure 1 - Monthly variations of rainfall and average temperature in the period from October to April of the 2018/2019 crop season in Lavras, MG, Brazil.



Source: The author (2020), adapted from Instituto Nacional de Meteorologia - INMET (2020).

Fertilization was performed according to Sediyama, Silva and Borém (2015). The soil composition from the environments are presented in Table 4.

Table 4 - Soil Chemical composition from layer 0-20 cm.

Chemical properties	Unit	Environments	
		1	2
pH	H_2O	5.5	6.1
K	mg/dm³	272.19	213.30
P		42.54	27.60
Na		-	-
Ca^{2+}	$cmol_c/dm^3$	3.43	3.53
Mg^{2+}		1.23	1.16
Al^{3+}		0.10	0.10
H+Al		3.00	1.90
SB		5.36	5.24
CTC (t)		5.46	5.34
CTC (T)		8.36	7.14
V	%	64.09	73.35
m		1.83	1.87
MO	dag/kg	2.22	2.37
P_{rem}	$cmol_c/dm^3$	25.80	29.60

Source: The author (2020).

The experiment was conducted in a completely randomized block design with four replications in a factorial scheme (5 progenies x 4 treatments). Five progenies from group I were used (Progenies EA-1, EA-2, EA-6, EA-7 and EA-8) and five from group II (Progenies

YD-2, YD-4, YD-5, YD-9 and YD-12), previously selected in the greenhouse stage. The strains SEMIA 587 and INPA 03-11B presented the best results in the greenhouse stage, so they were used in the field experiment. The following N sources were evaluated: I- inoculated with SEMIA 587; II- inoculated with INPA 03-11B; III- without inoculation, with N fertilizer; VI- no inoculation and no N fertilizer. The plots consisted of four rows that were five meters in length, with rows spaced at 0.5 m. The two central rows were considered as working area.

Sowing was performed manually in a no-tillage area in November 2018 and harvest in March 2019. The inoculation with *Bradyrhizobium* strains was performed by spraying the inoculant on the furrow after sowing. A dose of 18 mL p. c. kg^{-1} of seed (SEMIA 587 and INPA 03-11B strains) containing 10,8 x10^{6} CFU/seed of the inoculant was applied. For application, a motorized costal spray unit was used, coupled to a bar with four XR 11002 spray nozzles, spraying a volume equivalent to 150 L.ha^{-1}. In the N fertilizer treatment was applied 200 kg of N/ha, being 50% after sowing and 50% 35 days after sowing, following the recommendations from INSTRUÇÃO NORMATIVA SDA Nº 13 (BRASIL, 2011).

Pest control was performed according to the need with insecticides from chemical group of Neonicotinoid, Pyrethroid and Chlorpyrifos. Post-emergence weed control was performed with glyphosate at 2 L.ha^{-1} dose.

Diseases control were performed with fungicide Fox® (trifloxistrobin; protioconazole) at 0.4 L p.c. ha^{-1} dose recommended per package leaflet. For application, a motorized costal spray was used, coupled to a bar with four XR 11002 spray nozzles, spaced 50 cm and calibrated for flow of 150 L.ha^{-1}.

When the plants reached the R2 stage (FEHR; CALVINESS, 1977) five random plants per plot were selected to evaluate the following traits:

- Days to flowering: 50% of the plants of the plot in full bloom;
- Chlorophyll content: Using the SPAD, the chlorophyll content was measured in a trifoliate leaf from the middle part of the plant;
- Leaf nitrogen content: One trifoliate leaf per plant was collected from the middle part of the plant. The trifoliate leaves were dried in an oven at 65ºC until they reached constant weight and ground to perform the N content analysis, following the methodology proposed by Malavolta *et al.* (1997);
- Fresh weight of shoots: Obtained by collecting and weighing the plant shoots;
- Dry weight of shoots: Obtained by weighing the plant shoots, after drying in an oven at 65ºC;

- Fresh weight of nodules: The nodules of the plants were highlighted and subsequently weighed;
- Dry weight of nodules: Obtained by weighing the nodules, after drying in an oven at 65°C;
- Number of nodules: Performed by counting the plant nodules;

When the plants reached the R8 stage (FEHR; CALVINESS, 1977) the following traits were evaluated:

- Lodging score: Evaluated according to Bernard *et al.* (1965) with the following scores:
  1 - For all upright plants;
  2 - For some leaning or slightly lodged plants;
  3 - For all plants moderately leaning or 25 to 50% lodged;
  4 - For all plants severely leaning or 50 to 80% lodged;
  5 - For more than 80% of the plants lodged.
- Plant height: Distance from the plant root collar to the tip of the main stem, in centimeters, measured in 5 plants selected at random;
- First pod insertion: Distance from the soil to the first pod of the plant, in centimeters, measured in 5 plants selected at random;
- Days to full maturity: 90% of the plants of the plot in the R8 stage (full maturity);
- Grain yield: determined from harvest of two 5 meters rows from each plot. The weight of grains obtained in each plot was corrected for moisture of 13%, obtaining, yield in bags.ha^{-1}.

For the number of nodules, fresh and dry weight of nodules due to does not have a normal distribution it was necessary to transform the data using the equation $\sqrt{x} + 1$. Subsequently, with the tests of the ANOVA assumptions, the data were submitted to analysis of variance and the means were grouped by Scott - Knott test (1974), at 5% probability, using the software R Core Team (2016). Estimates of coefficient of variation and accuracy were used to measure precision, described in the equations (1) and (2).

The individual analyses per experiment, for all traits, were performed using the statistical model:

$$y_{ijk} = \mu + p_i + t_j + b_k + pt_{ij} + e_{ijk} \tag{7}$$

where:

y_{ijk}: observed value referring to the plot that received progeny i, the N sources j, in block k;

μ: constant associated with all observations;

p_i: effect of progeny i;

t_j: effect of N source j;

b_k: effect of block k;

pt_{ij}: effect of progeny i with N source j interaction;

e_{ijk}: random experimental error associated with observation y_{ijk}.

The analysis involving all experiments followed the statistical model:

$$y_{ijkl} = \mu + p_i + t_j + a_k + b_{l(k)} + (pt)_{ij} + (pa)_{ik} + (ta)_{jk} + (pta)_{ijk} + e_{ijk(l)} \quad (8)$$

where:

y_{ijkl}: observed value referring to the plot that received progeny i, N source j, in environment k, in block l;

μ: constant associated with all observations;

p_i: effect of progeny i;

t_j: effect of N source j;

a_k: effect of the environment k;

$b_{l(k)}$: effect of block l within the environment k;

pt_{ij}: effect of progeny i with N source j interaction;

pa_{ik}: effect of progeny i with environment k interaction;

tajk: effect of N source j with environment k interaction;

pta_{ijk}: effect of progeny i with N source j in environment k interaction;

$and_{ijk(l)}$: random experimental error associated with observation y_{ijk}.

For the standardized variables index (Z index), the observations of the trait's chlorophyll content, days to full maturity, chlorophyll content, leaf nitrogen content, dry weight of nodules, number of nodules and grain yield were standardized to make them directly comparable, according to the model proposed by Mendes, Ramalho and Abreu (2009).

Because the Z variable can assume negative or positive values, the constant four was added to make them positive. The use of this multiple selection index of the six traits assumes that the higher the Z value, better is the selection. However, for the trait days to full maturity, lower values of the trait better are the progeny. Thus, to make the six traits working in the same direction, it was necessary to multiply by -1 the Z values for days to full maturity. After standardization, the Z sum was obtained for each plot. Subsequently, statistical analysis was conventionally performed.

3 RESULTS AND DISCUSSIONS

The comparison of genotypes in experiments is a common activity in plant breeding, aiming to identify differences and select superior individuals. Therefore, to be successful in selection, it is necessary to perform precise experiments ensuring more accurate estimates and consequently more assertive recommendations (FILHO; JUNIOR; LÚCIO, 2012). In this study, the coefficient of variation and selective accuracy were used to measure experimental precision. The highest magnitude of the coefficient of variation was observed in the dry weight of shoots, at 31.48%. Accuracy ranged from 68.14% for the fresh weight of nodules to 98.32% for plant height (TABLE 5).

According to Pimentel Gomes (2009), the coefficient of variation is classified as low (<10%), medium (10%-20%), high (20%-30%) and very high (>30%). On the other hand, accuracy estimates are considered of great magnitude when higher than 70%, medium between 30% and 70% and low accurate when below 30% (RESENDE; DUARTE, 2007).

For the number of trifoliate leaves, fresh weight of shoots, chlorophyll content, fresh weight of nodules and root volume, the CV was classified as high and for dry weight of shoots was classified as very high however, when the accuracy of each trait was estimated, it was noticed that the values showed a large magnitude. This fact can be explained because the accuracy considers the Fc value (QMT/QME), in addiction to considering the variation between progenies not only the experimental error as is done in the calculation of the CV. Thus, with higher Fc values of the progeny, more accurate values will be obtained. Coefficients of variation above 20% for these traits were also reported in studies conducted by Bohrer and Hungria (1998); Brandelero, Peixoto and Ralish (2009); Pelaquim *et al.* (2016).

The existence of genetic variability is essential for the success of plant breeding programs (BERNARDO, 2020; RAMALHO *et al.*, 2012). In the summary of variance analysis (TABLE 5) there was a significant difference in the source of variation progenies for all evaluated traits, indicating that there are genetic differences in the evaluated progenies. In the decomposition of the source of variation group I, it was observed that there were no significant differences in the nitrogen content, chlorophyll content, number of nodules, fresh weight of nodules and Z index. For group II, significant differences were observed for all traits.

In the contrast group I vs II, significant differences were observed for most traits, except for the number of trifoliate leaves, fresh weight of shoots, fresh weight of nodules and dry weight of nodules. For the N sources, a significant difference was detected for all characteristics. The PROG x N interaction was significant for plant height, nitrogen content, dry weight of nodules and Z index.

In the literature, it is possible to find studies that highlight differences between soybean genotypes in relation to their BNF ability. In a study conducted by Agoyi *et al.* (2016b), with the objective of identifying genotypes with greater potential for BNF, the authors concluded that the cultivars NamII, WonderSoya, Bulindi 48C, NamSoy 4M, MakSoy 3N, NamSoy 3, K-Local, Kabanyolo 1, UG 5, Soprano, MakSoy 2N, and MakSoy 5N presented promising results and could be used to initiate a breeding program aimed at BNF.

When evaluating progenies from group I and II individually, it was observed that in group I, there was no significant difference for most traits related to BNF. For group II, differences were observed for all traits. It is possible to infer that for group II progenies there is a difference between them in relation to their ability to fix nitrogen.

With the N source, significant differences were observed for all traits, indicating that there were differences between *Bradyrhizobium* strains in relation to BNF efficiency. Solomon, Pant and Angaw (2012) when studying the effects of two Bradyrhizobium japonicum strains on soybean cultivars, highlighted that the TAL 379 strain presented superior results for the components linked to BNF. In a study conducted in Germany, the authors tested the effect of four Bradyrhizobium strains with different soybean cultivars. Differences between the strains for the evaluated traits were observed (ZIMER *et al*., 2016). This finding corroborates with the results obtained in the present study.

Table 5 - Summary of variance analysis related to evaluation of progenies and N sources.

SV	DF	p - value										
		PH	**TRI**	**FWS**	**DWS**	**NC**	**CC**	**NN**	**FWN**	**DWN**	**RV**	**Z**
Progenies (PROG)	23	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0089	0.0000	0.0000	0.0000
Group I (I)	11	0.0000	0.0000	0.0000	0.0001	n.s.	n.s.	n.s.	n.s.	0.0116	0.0000	n.s.
Group II (II)	11	0.0000	0.0000	0.0000	0.0001	0.0003	0.0000	0.0019	0.0000	0.0000	0.0070	0.0034
I vs II	1	0.0000	n.s.	n.s.	0.0000	0.0000	0.0000	0.0000	n.s.	n.s.	0.0000	0.0000
N sources (N)	6	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PROG x N	138	0.0064	n.s.	n.s.	n.s.	0.0000	n.s.	0.0002	n.s.	0.0467	n.s.	0.0157
Fc (Progenies)		30.09	3.23	7.76	5.68	7.03	4.97	2.13	1.86	3.72	10.48	3.14
Fc (N sources)		28.01	10.03	62.26	68.72	32.30	80.34	844.44	71.80	433.35	39.95	167.17
Accuracy (Progenies)		98.32	83.13	93.33	90.78	92.62	89.37	72.92	68.14	85.51	95.1	82.60
CV (%)		13.25	23.07	28.93	31.48	13.29	20.59	17.25	26.31	7.17	26.07	9.74
Overall mean		23.37	5.14	16.33	3.47	3.14	25.91	152.17	1.84	0.33	24.06	16.00

SV: Source of variation; DF: Degrees of freedom; Fc: F calculated; CV: Coefficient of variation; PH: Plant height; TRI: Number of trifoliate leaves; FMS: Fresh weight of shoots; DMS: Dry weight of shoots; NC: Nitrogen content; CC: Chlorophyll content; NN: Number of nodules; FMN: Fresh weight of nodules; DMN: Dry weight of nodules; RV: Root volume; Z: Z index.

Source: The author (2020).

When there is interaction, there are two options, either to consider the results for each environment separately or to consider the means of the environments. Considering that the greatest challenge to the breeder is to identify superior cultivars for planting in farmers' fields (EEUWIJK; BUSTOS-KORTS; MALOSETTI, 2016), as the future climatic conditions are unknown the best option is to consider the means of the environments so there is a better chance of representing the future cropping locations. Furthermore, when dealing with breeding, it can be impossible to develop and recommend cultivars for specific locations. Therefore, the above-mentioned strategy has been frequently used (DUDLEY, 1997; FERREIRA *et al.*, 2015). Thus, Table 6 shows the phenotypic means for each progenies group. Among group I, the plant height ranged from 19.96 to 22.72 centimeters. The range of variation in the number of trifoliate leaves was 0.98. The fresh weight of shoots varied between 15.00 and 20.80 grams. For the dry weight of shoots the highest mean was 4.38 grams, which was 25% higher than the lowest estimated mean. The amplitude between the highest and lowest estimates for dry weight of nodules was 0.12 grams. The root volume varied from 21.71 to 31.29 ml.

For the group II, the total variation in plant height was 10.32 cm. The number of trifoliate leaves ranged from 4.91 to 6.37 trifoliate leaves per plant. For the fresh weight of shoots, the highest estimated mean was 35.72% higher than the lowest one. The variation in dry weight of shoots was 1.2 grams. The nitrogen content ranged from 3.09 to 3.58%. For the chlorophyll content, the highest mean was 31.25 and the lowest was 21.57 SPAD units. The variation in number of nodules was 34.15 nodules per plant. For the fresh weight of nodules, the highest mean was 10% higher than the lowest. The fresh weight of nodules ranged from 0.94 to 1.41 grams. The highest mean for the root volume was 24.45% higher than the lowest mean. For the Z index the variation was 1.77.

Table 7 shows the results of comparing the phenotypic means of progenies between the groups. When analyzing the dry weight of nodules, the group I progenies EA-7, EA-8, EA-9, EA-10, EA-11 and EA-12, as well as the group II progenies YD-2, YD-4, YD-8 and YD-9 presented higher phenotypic means. However, for the dry weight of shoots, the group I progenies, including EA-7, EA-8, EA-9, EA-10, EA-11 and EA-12, mostly presented higher means, in addition to the group II progenies YD-4 and YD-8. The ability of early maturing progenies to convert available nitrogen into dry matter more quickly may be a possible explanation for this result. Divito *et al.* (2016) showed that early maturing cultivars had a higher proportion of dry weight of shoots during the seed formation period than late maturing cultivars, inferring that early cultivars may fix nitrogen and convert dry matter faster than late cultivars.

Santachiaria, Borrás and Rotundo (2018) observed that cultivars of maturity group III had a faster N absorption rate than cultivars of maturity group IV, from R1 to R5.

The nitrogen content was higher for 11 group I progenies. Only progeny YD-12 did not present a higher mean than the others. The EA-2 progeny was the only progeny with the highest means among group II.

Table 6 - Phenotypic means of soybean progenies evaluated under different N sources, within each group (I and II).

Progenies	PH	TRI	FWS	DWS	NC	CC	NN	FWN	DWN	RV	Z
Group I											
EA - 1	20.66 b	4.84 b	16.44 c	3.57 b	2.86 a	27.10 a	121.44 a	1.21 a	0.24 b	23.85 b	15.64 a
EA - 2	19.96 b	4.77 b	15.16 c	3.29 b	3.18 a	27.17 a	131.79 a	1.37 a	0.25 b	21.71 b	16.35 a
EA - 3	20.68 b	4.71 b	16.00 c	3.45 b	3.13 a	27.78 a	122.54 a	1.49 a	0.29 a	25.53 b	16.43 a
EA - 4	21.48 a	4.75 b	15.86 c	3.47 b	2.98 a	26.72 a	116.21 a	1.23 a	0.23 b	25.03 b	15.74 a
EA - 5	20.36 b	4.68 b	16.49 c	3.47 b	2.98 a	25.61 a	120.34 a	1.37 a	0.27 b	23.57 b	15.78 a
EA - 6	20.31 b	4.73 b	15.00 c	3.34 b	2.91 a	25.78 a	127.46 a	1.32 a	0.25 b	24.28 b	15.66 a
EA - 7	22.72 a	5.21 a	19.17 b	4.01 a	2.97 a	26.21 a	134.56 a	1.42 a	0.31 a	31.39 a	16.09 a
EA - 8	22.09 a	5.16 a	18.29 b	3.90 a	2.95 a	27.36 a	138.30 a	1.44 a	0.30 a	28.94 a	16.18 a
EA - 9	22.35 a	5.14 a	18.49 b	3.95 a	2.88 a	27.41 a	124.55 a	1.42 a	0.29 a	29.07 a	15.94 a
EA - 10	20.98 b	5.32 a	20.77 a	4.31 a	2.85 a	27.42 a	113.21 a	2.96 a	0.36 a	30.71 a	16.04 a
EA - 11	20.27 b	5.39 a	20.65 a	4.15 a	3.00 a	28.03 a	113.42 a	1.54 a	0.31 a	28.93 a	16.26 a
EA - 12	22.48 a	5.66 a	20.80 a	4.38 a	2.93 a	26.65 a	112.36 a	1.30 a	0.28 a	29.82 a	15.83 a
Group II											
YD - 1	23.60 d	4.77 c	13.86 b	2.92 b	3.39 a	21.57 d	97.61 b	1.00 b	0.22 b	21.19 a	16.62 a
YD - 2	22.94 d	5.00 c	15.86 a	3.35 b	3.40 a	25.42 c	126.11 a	1.34 a	0.29 a	23.39 a	16.10 a
YD - 3	24.18 d	5.31 c	13.48 b	2.70 b	3.40 a	22.43 d	104.65 b	1.02 b	0.21 b	20.35 b	16.41 a
YD - 4	33.26 a	6.37 a	18.00 a	3.90 a	3.30 b	28.22 b	126.11 a	1.32 a	0.32 a	22.60 a	16.37 a
YD - 5	24.65 d	5.39 c	14.27 b	3.13 b	3.33 b	23.02 d	113.84 a	1.21 a	0.25 b	21.32 a	16.18 a
YD - 6	24.10 d	4.91 c	14.23 b	3.10 b	3.29 b	25.47 c	105.67 b	1.16 a	0.24 b	19.32 b	16.12 a
YD - 7	23.58 d	4.94 c	15.17 b	2.97 b	3.26 b	22.39 d	91.96 b	1.02 b	0.21 b	21.78 a	15.95 a
YD - 8	27.24 c	5.73 b	18.36 a	3.90 a	3.58 a	31.25 a	117.50 a	1.41 a	0.31 a	22.96 a	16.15 a
YD - 9	23.62 d	4.92 c	11.57 b	2.75 b	3.19 b	25.17 c	116.42 a	0.94 b	0.23 b	17.67 b	15.60 b
YD - 10	23.79 d	4.91 c	14.37 b	3.06 b	3.31 b	23.16 d	96.82 b	0.94 b	0.19 b	20.07 b	16.57 a
YD - 11	30.46 b	5.21 c	13.88 b	2.85 b	3.27 b	26.08 c	103.22 b	1.14 a	0.24 b	21.62 a	15.02 b
YD - 12	25.07 d	5.46 c	15.78 a	3.33 b	3.09 b	24.28 c	119.46 a	1.18 a	0.27 a	22.39 a	14.85 b

The means followed by the same letter in the columns belong to the same group by the Scott-Knott test at 95% reliability. PH: Plant height (cm); TRI: Number of trifoliate leaves (unit/plant); FMS: Fresh weight of shoots (grams/plant); DMS: Dry weight of shoots (grams/plant); NC: Nitrogen content (%); CC: Chlorophyll content (SPAD unit); NN: Number of nodules (unit/plant); FMN: Fresh weight of nodules (grams/plant); DMN: Dry weight of nodules (grams/plant); VR: Root volume (ml/plant); Z: Z index.

Source: The author (2020).

Table 7 - Phenotypic means of soybean progenies evaluated under different N sources, between groups (I and II).

Progenies	PH	TRI	FWS	DWS	NC	CC	NN	FWN	DWN	RV	Z
Group I											
EA - 1	20.66 f	4.84 c	16.44 c	3.57 b	2.86 b	27.10 a	121.44 a	1.21 b	0.24 b	23.85 b	15.56 c
EA - 2	19.96 f	4.77 c	15.16 c	3.29 b	3.18 a	27.17 a	131.79 a	1.37 b	0.25 b	21.71 c	16.29 c
EA - 3	20.68 f	4.71 c	16.00 c	3.45 b	3.13 b	27.78 a	122.54 a	1.49 b	0.29 a	25.53 b	16.36 c
EA - 4	21.48 f	4.75 c	15.86 c	3.47 b	2.98 b	26.72 a	116.21 a	1.23 b	0.23 b	25.03 b	15.66 c
EA - 5	20.36 f	4.68 c	16.49 c	3.47 b	2.98 b	25.61 a	120.34 a	1.37 b	0.27 a	23.57 b	15.79 c
EA - 6	20.31 f	4.73 c	15.00 c	3.34 b	2.91 b	25.78 a	127.46 a	1.32 b	0.25 b	24.28 b	15.58 c
EA - 7	22.72 e	5.21 c	19.17 b	4.01 a	2.97 b	26.21 a	134.56 a	1.42 b	0.31 a	31.39 a	16.01 c
EA - 8	22.09 f	5.16 c	18.29 b	3.90 a	2.95 b	27.36 a	138.30 a	1.44 b	0.30 a	28.94 a	16.11 c
EA - 9	22.35 e	5.14 c	18.49 b	3.95 a	2.88 b	27.41 a	124.55 a	1.42 b	0.29 a	29.07 a	15.86 c
EA - 10	20.98 f	5.32 b	20.77 a	4.31 a	2.85 b	27.42 a	113.21 a	2.96 a	0.36 a	30.71 a	15.97 c
EA - 11	20.27 f	5.39 b	20.65 a	4.15 a	3.00 b	28.03 a	113.42 a	1.54 b	0.31 a	28.93 a	16.19 c
EA - 12	22.48 e	5.66 b	20.80 a	4.38 a	2.93 b	26.65 a	112.36 a	1.30 b	0.28 a	29.82 a	15.75 c
Group II											
YD - 1	23.60 d	4.77 c	13.86 d	2.92 c	3.39 a	21.57 b	97.61 b	1.00 b	0.22 b	21.19 c	15.53 c
YD - 2	22.94 e	5.00 c	15.86 c	3.35 b	3.40 a	25.42 a	126.11 a	1.34 b	0.29 a	23.39 b	16.58 b
YD - 3	24.18 d	5.31 b	13.48 d	2.70 c	3.40 a	22.43 b	104.65 b	1.02 b	0.21 b	20.35 c	15.70 c
YD - 4	33.26 a	6.37 a	18.00 b	3.90 a	3.30 a	28.22 a	126.11 a	1.32 b	0.32 a	22.60 b	16.91 b
YD - 5	24.65 d	5.39 b	14.27 d	3.13 c	3.33 a	23.02 b	113.84 a	1.21 b	0.25 b	21.32 c	15.83 c
YD - 6	24.10 d	4.91 c	14.23 d	3.10 c	3.29 a	25.47 a	105.67 b	1.16 b	0.24 b	19.32 c	16.04 c
YD - 7	23.58 d	4.94 c	15.17 c	2.97 c	3.26 a	22.39 b	91.96 b	1.02 b	0.21 b	21.78 c	15.31 c
YD - 8	27.24 c	5.73 b	18.36 b	3.90 a	3.58 a	31.25 a	117.50 a	1.41 b	0.31 a	22.96 b	17.74 a
YD - 9	23.62 d	4.92 c	11.57 d	2.75 c	3.19 a	25.17 a	116.42 a	0.94 b	0.23 b	17.67 c	15.85 c
YD - 10	23.79 d	4.91 c	14.37 d	3.06 c	3.31 a	23.16 b	96.82 b	0.94 b	0.19 b	20.07 c	15.50 c
YD - 11	30.46 b	5.21 c	13.88 d	2.85 c	3.27 a	26.08 a	103.22 b	1.14 b	0.24 b	21.62 c	16.06 c
YD - 12	25.07 d	5.46 b	15.78 c	3.33 b	3.09 b	24.28 b	119.46 a	1.18 b	0.27 a	22.39 b	15.74 c

The means followed by the same letter in the columns belong to the same group by the Scott-Knott test at 95% reliability. PH: Plant height (cm); TRI: Number of trifoliate leaves (unit/plant); FMS: Fresh weight of shoots (grams/plant); DMS: Dry weight of shoots (grams/plant); NC: Nitrogen content (%); CC: Chlorophyll content (SPAD unit); NN: Number of nodules (unit/plant); FMN: Fresh weight of nodules (grams/plant); DMN: Dry weight of nodules (grams/plant); VR: Root volume (ml/plant); Z: Z index.

Source: The author (2020).

The phenotypic means of the N sources are presented in Table 8. Plant height ranged from 18.85 to 22.29 cm. For the number of trifoliate leaves, the highest mean was 22.14% higher than the lowest mean. The range of variation for fresh weight of shoots was 10.5 grams. The highest mean for dry weight of shoots was 5.17 grams, 54.54% higher than the lowest mean. The nitrogen content ranged from 3.35 to 2.44%. The variation in the chlorophyll content was 16.68 SPAD units. The number of nodules per plants ranged from 0.86 to 281.56. The fresh weight of nodules varied by 2.48 grams between treatments. For the dry weight of nodules, the highest mean was 0.46 and the lowest was 0.00 grams. For root volume, the highest mean was 37.61% higher than the lowest mean. The highest mean value for the Z index was 17.41 and the lowest was 12.39.

The N fertilizer treatment showed higher phenotypic means for the traits PH, TRI, FWS, DWS, NC and RV. A possible explanation for this fact would be based on the possibility that nitrogen is more readily available with N fertilization than with inoculation. According to Hungria, Campos and Mendes (2007), nitrogen fertilizer is in readily available to plants, so it is more easily absorbed by soybeans. Nogueira, Júnior and Ragagnin (2010), when evaluating the effect of different doses of nitrogen fertilization on the nodulation of soybean plants, verified that the vegetative development of plants is positively affected by N fertilizers, corroborating the results obtained in the present study.

On the other hand, for the characteristics referring to nodulation (NN, FWN and DWN) the treatment with N fertilizer presented the lowest means, even when compared to the treatment without inoculation and N fertilizer. This fact is due to the reduction in the bacterial efficiency of atmospheric N fixation when using nitrogen fertilizers. A similar result was reported by Zuffo *et al.* (2019), where evaluated doses and time of N fertilizer associated with inoculation of soybean cultivars and concluded that the number and dry weight of nodules were negatively influenced by N fertilization. Saturno *et al.* (2017), when evaluating the effect of N fertilizer on biological nitrogen fixation in soybean, also reported that nitrogen fertilization reduced the means of traits linked to nodulation (NN and DWN). Hungria *et al.* (2006) also showed a drastic reduction in the number and dry weight of nodules by applying 50 to 100 kg. ha^{-1} of N in soybean.

Among the strains evaluated, treatments using *B. elkani* strains (SEMIA 587, SEMIA 5019 and INPA 03-11B) presented higher phenotypic means for the NN, FWN and DWN than treatments using SEMIA 5079 (*B. japonicum*) and UFLA 06-24 (*Bradyrhizobium* spp.). In this sense, Bizarro *et al.* (2011), found that *B. elkani* strains predominantly occupied nodules when compared with *B. japonicum*, indicating greater competitiveness of *B. elkani*. Corroborating the results obtained in this study. Ribeiro *et al.* (2015) evaluated the symbiotic capacity and genetic diversity of 46 *Bradyrhizobium* spp. strains, which were isolated from soils from different regions of Brazil. Here, phylogenetic analysis indicated that the UFLA 06-24 strain did not group with any other strain and may represent a new species. It was also observed that this strain provided a higher DMS mean than SEMIA-5019, similar to the findings in this study.

Table 8 - Phenotypic means for inoculated treatments, with N fertilizer (Ad. N) and without inoculation and N fertilizer (No N/I) for the evaluated traits.

N sources	PH	TRI	FWS	DWS	NC	CC	NN	FWN	DWN	RV	Z
SEMIA 5079	21.62 a	5.06 b	18.39 b	3.95 c	3.08 b	30.89 a	135.25 d	1.56 b	0.36 b	26.14 b	17.18 a
SEMIA 587	21.88 a	4.96 b	17.02 c	3.48 d	2.86 c	24.64 c	275.22 a	2.49 a	0.46 a	24.96 b	17.06 a
SEMIA 5019	21.47 a	4.95 b	17.56 c	3.72 c	3.14 b	28.19 b	203.63 b	2.13 a	0.47 a	25.09 b	17.41 a
UFLA 06-24	22.29 a	5.11 b	18.82 b	4.40 b	3.05 b	31.22 a	175.29 c	1.71 b	0.38 b	26.25 b	17.24 a
INPA 03-11B	20.56 b	4.85 b	16.65 c	3.35 d	2.85 c	24.78 c	281.56 a	2.49 a	0.43 a	25.41 b	16.99 a
Ad. N	21.70 a	5.78 a	23.19 a	5.17 a	3.35 a	32.76 a	0.86 f	0.01 c	0.00 d	37.27 a	13.69 b
No N/I	18.85 c	4.5 c	12.69 d	2.35 e	2.44 d	16.08 d	17.38 e	1.48 b	0.17 c	23.25 b	12.39 c

The means followed by the same letter in the columns belong to the same group by the Scott-Knott test at 95% reliability. PH: Plant height (cm); TRI: Number of trifoliate leaves (unit/plant); FMS: Fresh weight of shoots (grams/plant); DMS: Dry weight of shoots (grams/plant); NC: Nitrogen content (%); CC: Chlorophyll content (SPAD unit); NN: Number of nodules (unit/plant); FMN: Fresh weight of nodules (grams/plant); DMN: Dry weight of nodules (grams/plant); VR: Root volume (ml/plant); Z: Z index.

Source: The author (2020).

When we aim to study the interaction between treatment groups and genotypes, partial diallel analysis can be applied. Diverse studies conducted to evaluate the efficiency of BNF are focused on characteristics related to the N assimilation capacity by the plant and to the components of nodulation (ARACHCHIGE *et al.*, 2020; GRUNVALD *et al.*, 2018; HWANG *et al.*, 2014; NICOLÁS; ARIAS; HUNGRIA, 2002; NICOLÁS; HUNGRIA; ARIAS, 2006; SANTOS *et al.* 2013; YANG *et al.*, 2019). Thus, to perform the partial diallel study, the traits NC, CC, NN, DWS and DWN as well as the Z index were considered.

In this sense, this analysis was carried out considering the progenies and the strains. The summary of the diallel analysis is presented in Table 9. There was effect of GCA for both groups (progenies and strains). There was an effect of SCA only for NC and NN.

Table 9 - Summary of partial diallel analysis for different progenies and inoculated treatments using Griffing's model IV, for the nitrogen content (NC), chlorophyll content (CC), number of nodules (NN), fresh weight of nodules (FWN), dry weight of nodules (DWN) and Z index (Z).

		p-value					
SV	**DF**	**NC**	**CC**	**NN**	**FWN**	**DWN**	**Z**
Combinations	119	0.0000	0.0000	0.0000	0.0000	0.0000	0.0061
G.C.A. Prog.	23	0.0000	0.0000	0.0002	0.0000	0.0000	0.0000
G.C.A. Str.	4	0.0005	0.0000	0.0000	0.0000	0.0000	0.0134
S.C.A.	92	0.0000	n.s.	n.s.	n.s.	n.s.	n.s.
Error	357						

SF: source of variation; G.C.A. Prog.: General combining ability of progenies; G.C.A. Str.: General combining ability of strains; S.C.A.: Specific combining ability.

Source: The author (2020).

Estimates of general and specific abilities and the relative contributions of GCA and SCA to the best combinations, i.e., progenies vs strains are presented in Table 10. It should be emphasized that for almost all the evaluated characteristics (66.67%) there was a major predominance of GCA, thus denoting a greater contribution of additive effects to the expression of the evaluated traits.

The higher magnitudes of GCA (g_i or gj) indicate greater association ability and consequently better biological nitrogen fixation. When evaluating the progenies group, a higher g_i indicates that a genotype has greater plasticity, that is, regardless of the strain it is expected that the result of biological fixation is positive, achieving higher results for the attributes correlated to BNF. In this sense, the progenies EA-11 and EA-7, were among the five best progenies for three evaluated traits (TABLE 1, APPENDIX).

In turn, when assessing GCA strain, it is possible to infer that the association plasticity of the strain independent of the soybean genotype. The strains SEMIA 587 and INPA 03-11B presented higher positive values of g_j for most traits (TABLE 1, APPENDIX).

The efficacy of diallel analysis has been proven in several studies related to resistance to disease in plants (SILVA *et al.*, 2014; PEREIRA *et al.*, 2015; VALDO *et al.*, 2016; LEITE *et al.*, 2017, LIMA, 2019), however, it has not yet been used to evaluate the relationship between soybean progenies and *Bradyrhizobium* strains. According to the results, a predominance of GCA was observed, thus showing a greater contribution of additive effects to the expression of the traits. Similar results were reported by Agoyi *et al.* (2016a), Nícolas, Arrabal and Hungria (2002) and Yang *et al.* (2019), in which the authors concluded that for most traits related to BNF there was a greater contribution of additive effects.

In this context, considering that there was a predominance of additive effects, a strategy that could be used in soybean breeding programs to increase biological nitrogen fixation ability would be recurrent selection. This breeding strategy was proposed for allogamous plants (HALLAUER; MIRANDA FILHO, 1988), however in recent years it has been used in autogamous plant breeding to increase the frequency of favourable alleles in bean (SILVA *et al.*, 2010; JUNIOR MENEZES *et al.*, 2013; PIRES *et al.*, 2014; LEITE *et al.*, 2016; LOPES *et al.*, 2019), soybean (POSADAS; ESKRIDGE; GRAEF, 2014) and rice (MORAES JÚNIOR *et al.*, 2015). Considering the improvement in biological nitrogen fixation, the best progenies could be intercrossed with elite genotypes to increase the frequency of favorable alleles for this trait in soybean crops.

Table 10 - Means of the 5 best combinations (Progeny x Strain) for the nitrogen content, chlorophyll content, number of nodules, fresh weight of nodules and dry weight of nodules and respective general combining ability for progenies ($\hat{g}i$) and strains ($\hat{g}j$), as well as the specific combining ability ($\hat{s}ij$). Data obtained in the partial diallel adopting the Griffing model IV. (To be continued)

Nitrogen content

Combinations	Mean	$\hat{g}_i$	$\hat{g}_j$	$\hat{s}_{ij}$	% GCA^1	% SCA^2
YD 3 x SEMIA 5079	3.90	0.3071	0.0974	0.3058	56.94	43.06
YD 8 x SEMIA 587	3.90	0.4053	-0.0722	0.3761	46.97	53.03
YD 10 x SEMIA 5079	3.85	0.1970	0.0974	0.3691	44.36	55.64
YD 1 x UFLA 06-24	3.84	0.1765	0.0640	0.4090	37.02	62.98
YD 11 x INPA 03-11B	3.83	0.3383	-0.1172	0.4226	34.35	65.65
				Mean:	43.93	56.07

Chlorophyll contente

Combinations	Mean	$\hat{g}_i$	$\hat{g}_j$	$\hat{s}_{ij}$	% GCA^1	% SCA^2
EA 1 x UFLA 06-24	34.82	1.8062	2.6090	4.1060	51.81	48.19
EA 4 x UFLA 06-24	34.35	1.9562	2.6090	3.4810	56.74	43.26
EA 2 x UFLA 06-24	34.25	1.1412	2.6090	4.1960	47.19	52.81
EA 4 x SEMIA 5079	34.20	1.9562	2.8534	3.0866	60.91	39.09
YD 8 x SEMIA 587	33.20	5.5062	-2.6990	4.0890	40.71	59.29
				Mean:	51.47	48.53

Number of nodules

Combinations	Mean	$\hat{g}_i$	$\hat{g}_j$	$\hat{s}_{ij}$	% GCA^1	% SCA^2
EA 7 x INPA 03-11B	352.69	1.2531	2.0951	1.2898	72.19	27.81
EA 2 x INPA 03-11B	338.56	1.0021	2.0951	1.1571	72.80	27.20
EA 9 x SEMIA 587	327.25	0.7994	1.5523	1.5944	59.60	40.40
EA 6 x INPA 03-11B	319.34	0.9274	2.0951	0.6997	81.20	18.80
EA 4 x INPA 03-11B	318.27	0.4709	2.0951	1.1280	69.47	30.53
				Mean:	71.05	28.95

Fresh weight of nodules

Combinations	Mean	$\hat{g}_i$	$\hat{g}_j$	$\hat{s}_{ij}$	% GCA^1	% SCA^2
EA 7 x INPA 03-11B	3.06	0.1284	0.1003	0.1531	59.90	40.10
EA 11 x SEMIA 587	2.99	0.1304	0.1219	0.1090	69.83	30.17
EA 9 x SEMIA 587	2.82	0.0924	0.1219	0.0927	69.79	30.21
EA 7 x SEMIA 587	2.79	0.1284	0.1219	0.0544	82.13	17.87
EA 4 x INPA 03-11B	2.76	0.0805	0.1003	0.1065	62.93	37.07
				Mean:	68.92	31.08

Dry weight of nodules

Combinations	Mean	$\hat{g}_i$	$\hat{g}_j$	$\hat{s}_{ij}$	% GCA^1	% SCA^2
YD 4 x SEMIA 5019	0.64	0.0422	0.0473	0.0771	53.73	46.27
EA 9 x SEMIA 5019	0.59	0.0383	0.0473	0.0553	60.75	39.25
EA 7 x INPA 03-11B	0.59	0.0742	0.0165	0.0471	65.84	34.16
YD 8 x SEMIA 587	0.58	0.0522	0.0299	0.0507	61.86	38.14
EA 12 x INPA 03-11B	0.56	0.0243	0.0165	0.0823	33.14	66.86
				Mean:	55.06	44.94

Table 10 - Means of the 5 best combinations (Progeny x Strain) for the nitrogen content, chlorophyll content, number of nodules, fresh weight of nodules and dry weight of nodules and respective general combining ability for progenies (ĝi) and strains (ĝj), as well as the specific combining ability (ŝij). Data obtained in the partial diallel adopting the Griffing model IV. (Conclusion)

Z index						
Combinations	**Mean**	**$\hat{g}_i$**	**$\hat{g}_j$**	**$\hat{s}_{ij}$**	**% GCA[1]**	**% SCA[2]**
YD 8 x SEMIA 587	20.20	2.1444	0.2405	1.8243	56.66	43.34
YD 8 x INPA 03-11B	19.18	2.1444	0.1966	0.8401	73.59	26.41
EA 9 x SEMIA 5019	18.75	0.3514	0.4357	1.9655	28.60	71.40
YD 2 x SEMIA 5079	18.60	1.2056	-0.5489	1.9488	25.21	74.79
YD 4 x SEMIA 5019	18.42	1.0473	0.4357	0.9467	61.04	38.96
				Mean:	49.02	50.98

[1] % GCA= (ĝi + ĝj) / (ĝi + ĝj + ŝij) x 100; [2] % SCA=ŝij / / (ĝi + ĝj + ŝij) x 100.

Source: The author (2020).

To conduct a breeding program for biological nitrogen fixation, an alternative is to identify progenies that associates higher phenotypic means and higher magnitudes of GCA. Therefore, the best progenies and strains were selected considering their means and general combining ability for the following traits: nitrogen content, chlorophyll content, number of nodules, fresh and dry weight of nodules and Z index. Thus, the following progenies were selected: Group I - EA-1, EA-2, EA-6, EA-7, EA-8; Group II - YD-2, YD-4, YD-5, YD-9, YD-12. For the strains, SEMIA 587 and INPA 03-11B were noteworthy.

The selected progenies, and the best strains were evaluated in field trials in two environments. A summary of the analysis of variance of the evaluated traits is presented in Table 11. For the progenies, significant differences were observed for most traits, except for the chlorophyll content and dry weight of nodules. In the decomposition of the group I progenies there were no significant differences in plant height, chlorophyll content, fresh and dry weight of nodules and Z index. The contrast I vs II was significant for most traits except to the chlorophyll content, dry weight of nodules and grain yield.

The accuracy ranged from 68.86 (dry weight of shoots) to 99.90% (days to flowering). For the coefficient of variation, the values were 23.94 for fresh weight of shoots to 1.74% for days to flowering. In the N sources, significant differences were observed for all traits. There was no effect of the environment on the lodging score or plant height. In turn, the interaction PROG x N x ENV was significant for lodging score, days to flowering and full maturity, nitrogen content, fresh and dry weight of shoots, number of nodules, grain yield and Z index, showing the non-coincidental behavior of progenies under the different N sources, as well as in the environments.

Since there were significant differences for almost all evaluated traits, except for chlorophyll content and dry weight of shoots, it is evident that there are genetic differences between progenies in relation to BNF. For the N sources, there were significant differences for all traits. The progeny x strain interaction was significant for most of the evaluated traits. These results corroborate a study conducted by Argaw (2014), where three *Bradyrhizobium* strains and six soybean genotypes with different maturation groups were tested and there were significant effects of genotype, strain, and genotype x strain interaction.

Table 11 - Summary of variance analysis related to the evaluation of progenies and N sources conducted in Lavras - MG.

SV	**DF**	**p - value**													
		R2 evaluated traits								**R8 evaluated traits**					
		DTF	**CC**	**LNC**	**FWS**	**DWS**	**FWN**	**DWN**	**NN**	**Lodg.**	**PH**	**INS**	**DFM**	**Yield**	**Z**
Progenies (PROG)	9	0.0000	n.s.	0.0000	0.0000	0.0000	0.0000	n.s.	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Group I	4	0.0000	n.s.	0.0016	0.0016	0.0077	n.s.	n.s.	0.0002	0.0006	n.s.	0.0015	0.0000	0.0000	n.s.
Group II	4	0.0000	n.s.	0.0014	0.0014	n.s.	n.s.	n.s.	0.0001	0.0000	0.0000	0.0000	0.0000	n.s.	0.0000
I vs II	1	0.0000	n.s.	0.0000	0.0000	0.0000	0.0000	n.s.	0.0000	0.0000	0.0000	0.0000	0.0000	n.s.	0.0000
N source (N)	3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0358	0.0000	0.0005	0.0000	0.0002	0.0000
Environment (ENV)	1	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	n.s.	0.0149	0.0000	0.0000	0.0000	0.0000
PROG x N	27	0.0000	n.s.	0.0000	0.0000	0.0000	0.0651	n.s.	0.0000	0.0000	n.s.	0.0032	0.0000	0.0027	n.s.
PROG x ENV	9	0.0000	n.s.	0.0000	0.0000	0.0000	n.s.	n.s.	0.0058	n.s.	n.s.	n.s.	0.0000	n.s.	n.s.
N x ENV	3	0.0000	0.0440	n.s.	n.s.	0.0107	0.0000	0.0000	0.0000	n.s.	n.s.	n.s.	0.0000	n.s.	0.0000
PROG x N x ENV	27	0.0000	n.s.	0.0000	0.0000	0.0000	n.s.	n.s.	0.0000	0.0086	n.s.	n.s.	0.0000	0.0038	0.0087
Fc (Progenies)		521.486	3.873	0.954	42.251	50.608	4.474	1.902	9.989	13.104	8.373	25.999	435.191	6.507	9.178
Fc (N source)		238.588	3.506	8.096	28.020	35.576	70.883	78.963	104.257	2.898	9.922	6.173	33.541	6.956	27.884
Accuracy (Progenies)		99.90	86.13	-	98.81	99.01	88.12	68.86	94.86	96.11	93.84	98.06	99.89	92.00	94.40
CV (%)		2.16	8.69	9.98	23.94	21.07	17.96	11.65	27.91	21.59	11.19	18.21	1.74	21.00	12.20
Overall mean		50.95	39.40	5.39	209.42	47.57	0.53	0.19	44.52	1.07	80.46	12.64	120.06	46.00	16.00

SV: Source of variation; DF: Degrees of freedom; Fc: F calculated; CV: Coefficient of variation; Lodg.: Lodging score; PH: Plant height; INS: First pod insertion; DTF: Days to flowering; DTM: Days to full maturity; CC: Chlorophyll content; LNC: Leaf nitrogen content; FMS: Fresh weight of shoots; DMS: Dry weight of shoots; MFN: Fresh weight of nodules; MSN: Dry weight of nodules; NN: Number of nodules; Yield: Grain yield; Z: Z index.

Source: The author (2020).

The phenotypic means within each group of progenies are presented in Table 12. For the group I progenies there were no differences between the phenotypic means for plant height, chlorophyll content, nitrogen content, dry weight of nodules and Z index. The variation for days to full maturity was six days, with the mean cycle of the earliest progenies being 111 days. The number of nodules ranged from 14.52 to 32.83 nodules per plant. The grain yield highest mean was 56.01 bags.ha^{-1}, with an amplitude of 16.28 bags.ha^{-1}.

For the group II progenies, the plant height varied by 12.47 cm. For days to full maturity, the variation was 10 days. The chlorophyll content ranged from 36.67 to 39.40 SPAD units. The highest mean for number of nodules was 45.86% higher than the lowest mean.

When comparing phenotypic means between the groups of progenies (TABLE 13), the group II presented a longer cycle 7 to 34 days of difference in relation to the group I. These group II also presented higher phenotypic means for FWS, DWS, FWN, DWN and NN. However, when comparing the traits CC and LNC, the progenies showed similar means. These differences can be attributed largely to the greater biomass acquired by the plants (BUSHBY; LAWN, 1992) and longer duration of growth and development in the field (EAGLESHAM *et al.* 1982). Similar results were also obtained by Argaw (2014), who concluded that late genotypes had better phenotypic means for DWS, DWN and NN, but did not differ for LNC when testing three *Bradyrhizobium* strains and six soybean genotypes with different maturation groups. Sogut (2006) observed that inoculation with *Bradyrhizobium* strains was more effective in late genotypes than in medium and early genotypes.

The grain yield mean between the two groups was similar. The EA-8 progeny stood out as having the highest yield, with good results in traits related to BNF and early maturity. Santachiara, Borrás and Rotundo (2017) and Santachiara *et al.* (2017) concluded that cultivars of different maturation groups may present similar yield results, despite differences in the capture and use of resources. A similar result was reported by Hungria *et al.* (2006) in which the authors conducted several field experiments and obtained similar yields for early and medium cultivars. On the other hand, Argaw (2014) reported that late genotypes were more efficient in BNF, than other maturity groups.

The N sources means are presented in Table 14. For plant height, the inoculated treatments were superior to the others. The N fertilizer treatment presented the lowest mean for first pod insertion. For leaf nitrogen content, the best treatments were INPA 03-11B and N fertilizer. The N fertilizer treatment showed a higher DMS mean. For fresh and dry weight of nodules, number of nodules and Z index, inoculated treatments had higher means. For grain yield, inoculation with INPA 03-11B presented the best mean (50.16 bags.ha^{-1}), indicating high potential of this strain as an inoculant in soybean crops. This strain is approved by MAPA for use as an inoculant in cowpea. Several studies have shown its efficiency in BNF for this crop and in promoting gains in grain yield (JÚNIOR *et al.*, 2018; OLIVEIRA *et al.*, 2020; SANTOS *et al.*, 2019; XAVIER *et al.*, 2017). However, studies using this strain in soybean crops are scarce. One of the few reports in the literature, Miguel and Moreira (2001) comparing strains recommended for soybean (Br 29 and SEMIA 587) with INPA03-11B, concluded that the strain proved effective with a number of nodules and Nase activity similar to the strains recommended for soybean and suggested that this strain be subjected to field efficiency trials.

The effects of nitrogen fertilization on nodulation are well documented in the literature. Several studies show that soybean yield is dependent on BNF and even in highly productive environments and soybean yields under inoculation may be similar or higher than those with N fertilization (HUNGRIA *et al.*, 2006; HUNGRIA; CAMPO; MENDES, 2007; KASCHUK, *et al.*, 2016; DE LUCA; NOGUEIRA; HUNGRIA, 2014) but with lower costs and environmental impacts. Saturno *et al.* (2017) reported that nitrogen fertilization has negative effects on BNF and does not improve grain yield. Therefore, nitrogen fertilization in soybean should be avoided, since inoculation with efficient *Bradyrhizobium* strains provides the N required by soybean even in cultivars with high yields.

Thus, we can infer that for the progenies tested, inoculation was able to supply the nitrogen needs of plants and improve grain yield. The results reinforce the recommendations for soybean crops in Brazil, using only inoculation as a source of N (HUNGRIA; MENDES, 2015). The replacement of nitrogen fertilizers by BNF in soybeans results in savings of approximately US$20 billion for Brazilian agriculture annually (SANTOS, NOGUEIRA; HUNGRIA, 2019). In addition to the reduction in costs, socioenvironmental benefits are found because the substitution of mineral N reduces the pollution of water resources that occurs due to the eutrophication of lakes and rivers. It also reduces atmosphere pollution and avoids the high energy expenditure related to the production of nitrogen fertilizers since, on average, six barrels of oil are spent for the synthesis of 1 ton of ammonia (BRACINNI *et al.*, 2016; ZANINI *et al.*, 2012). Therefore, it is essential to develop cultivars more efficient in BNF to seek more profitable agriculture with fewer environmental impacts.

Table 13 - Phenotypic means of soybean progenies evaluated under different N sources, within each group (I and II) in Lavras - MG.

Progenies	**R2 evaluated traits**								**R8 evaluated traits**					
	DTF	**CC**	**LNC**	**FWS**	**DWS**	**FWN**	**DWN**	**NN**	**Lodg.**	**PH**	**INS**	**DFM**	**Yield**	**Z**
Group I														
EA-1	46.00 b	40.86 a	5.32 a	161.58 a	35.47 b	0.18 b	0.09 a	14.52 b	1.00 b	76.73 a	10.86 a	111.00 b	47.30 b	16.23 a
EA-2	46.00 b	40.37 a	5.38 a	173.25 a	37.60 a	0.22 b	0.11 a	20.61 b	1.00 b	78.39 a	11.65 a	111.00 b	44.92 b	16.29 a
EA-6	46.00 b	40.44 a	5.48 a	143.07 b	34.56 b	0.21 b	0.09 a	18.84 b	1.01 b	75.58 a	11.34 a	111.00 b	42.62 c	16.16 a
EA-7	48.00 a	40.10 a	5.58 a	171.12 a	38.93 a	0.30 a	0.13 a	32.83 a	1.09 a	76.24 a	9.44 b	117.00 a	39.73 c	15.44 a
EA-8	48.00 a	39.18 a	5.24 a	138.62 b	32.60 b	0.34 a	0.15 a	31.36 a	1.01 b	80.99 a	11.09 a	117.00 a	56.01 a	15.83 a
Group II														
YD-2	54.00 b	38.77 a	5.38 a	271.49 a	59.18 a	0.44 a	0.14 a	43.43 a	1.01 b	85.86 a	14.18 a	125.00 b	47.65 a	16.60 a
YD-4	54.00 c	38.93 a	5.35 a	264.43 a	61.38 a	0.46 a	0.14 a	50.13 a	1.03 b	83.83 a	12.72 b	124.00 b	45.41 a	16.57 a
YD-5	54.00 b	39.40 a	5.40 a	265.04 a	57.11 a	0.44 a	0.13 a	34.93 b	1.00 b	87.92 a	14.49 a	124.00 b	47.70 a	16.65 a
YD-9	54.00 b	39.25 a	5.33 a	223.98 b	56.33 a	0.48 a	0.16 a	30.91 b	1.06 b	83.57 a	14.91 a	124.00 b	45.94 a	16.40 a
YD-12	57.00 a	36.67 b	5.42 a	281.60 a	62.52 a	0.49 a	0.16 a	27.14 b	1.48 a	75.45 b	15.68 a	134.15 a	42.67 a	13.75 b

The means followed by the same letter in the columns belong to the same group by the Scott-Knott test at 95% reliability. Lodg.: Lodging score (notes 1-5); PH: Plant height (cm); INS: First pod insertion (cm); DTF: Days to flowering (days); DFM: Days to full maturity (days); CC: Chlorophyll content (SPAD unit); LNC: Leaf nitrogen content (%); FWS: Fresh weight of shoots (grams/plant); DWS: Dry weight of shoots (grams/plant); FWN: Fresh weight of nodules (grams/plant); DWN: Dry weight of nodules (grams/plant); NN: Number of nodules (unit/plant); Yield: Grain yield (bags.ha^{-1}); Z: Z index.

Source: The author (2020).

Table 14 - Phenotypic means of soybean progenies evaluated under different N sources, between each group (I and II) in Lavras - MG.

Progenies	**R2 evaluated traits**								**R8 evaluated traits**					
	DTF	**CC**	**LNC**	**FWS**	**DWS**	**FWN**	**DWN**	**NN**	**Lodg.**	**PH**	**INS**	**DFM**	**Yield**	**Z**
Group I														
EA-1	46.00 e	40.86 a	5.32 a	161.58 c	35.47 b	0.18 b	0.09 b	14.52 c	1.00 b	76.73 c	10.86 c	111.00 d	47.30 b	16.79 a
EA-2	46.00 e	40.37 a	5.38 a	173.25 c	37.60 b	0.22 b	0.11 b	20.61 c	1.00 b	78.39 c	11.65 c	111.00 d	44.92 b	16.86 a
EA-6	46.00 e	40.44 a	5.48 a	143.07 d	34.56 b	0.21 b	0.09 b	18.84 c	1.01 b	75.58 c	11.34 c	111.00 d	42.62 c	16.73 a
EA-7	48.00 d	40.10 a	5.58 a	171.12 c	38.93 b	0.30 b	0.13 a	32.83 b	1.09 b	76.24 c	9.44 d	117.00 c	39.73 c	16.51 a
EA-8	48.00 d	39.18 a	5.24 a	138.62 d	32.60 b	0.34 a	0.15 a	31.36 b	1.01 b	80.99 b	11.09 c	117.00 c	56.01 a	16.91 a
Group II														
YD-2	54.00 b	38.77 a	5.38 a	271.49 a	59.18 a	0.44 a	0.14 a	43.43 a	1.01 b	85.86 a	14.18 a	125.00 b	47.65 b	15.73 b
YD-4	54.00 c	38.93 a	5.35 a	264.43 a	61.38 a	0.46 a	0.14 a	50.13 a	1.03 b	83.83 b	12.72 b	124.00 b	45.41 b	15.71 b
YD-5	54.00 b	39.40 a	5.40 a	265.04 a	57.11 a	0.44 a	0.13 a	34.93 b	1.00 b	87.92 a	14.49 a	124.00 b	47.70 b	15.74 b
YD-9	54.00 b	39.25 a	5.33 a	223.98 b	56.33 a	0.48 a	0.16 a	30.91 b	1.06 b	83.57 b	14.91 a	124.00 b	45.94 b	15.51 b
YD-12	57.00 a	36.67 b	5.42 a	281.60 a	62.52 a	0.49 a	0.16 a	27.14 b	1.48 a	75.45 c	15.68 a	134.15 a	42.67 c	13.47 b

The means followed by the same letter in the columns belong to the same group by the Scott-Knott test at 95% reliability. Lodg.: Lodging score (notes 1-5); PH: Plant height (cm); INS: First pod insertion (cm); DTF: Days to flowering (days); DFM: Days to full maturity (days); CC: Chlorophyll content (SPAD unit); LNC: Leaf nitrogen content (%); FWS: Fresh weight of shoots (grams/plant); DWS: Dry weight of shoots (grams/plant); FWN: Fresh weight of nodules (grams/plant); DWN: Dry weight of nodules (grams/plant); NN: Number of nodules (unit/plant); Yield: Grain yield (bags.ha^{-1}); Z: Z index.

Source: The author (2020).

Table 15 - Phenotypic means of inoculated treatments, with N fertilization (Ad. N) and without inoculation and without N fertilization (No N/I) for the evaluated traits.

N sources	**R2 evaluated traits**								**R8 evaluated traits**					
	DTF	**CC**	**LNC**	**FWS**	**DWS**	**FWN**	**DWN**	**NN**	**Lodg.**	**PH**	**INS**	**DFM**	**Yield**	**Z**
SEMIA 587	49.00 b	38.89 b	5.18 b	231.30	49.07 c	0.66 a	0.24 a	55.20 a	1.01 b	82.71 a	12.82 a	119.00	45.47 b	16.66 a
INPA 03-11B	49.00 b	38.68 b	5.48 a	191.95	40.35 d	0.64 a	0.23 a	46.51 b	1.11 a	83.65 a	13.23 a	118.00	50.16 a	17.26 a
Ad. N	52.00 a	40.13 a	5.57 a	237.61	56.05 a	0.07 c	0.03 c	9.12 d	1.07 a	77.90 b	11.73 b	120.00	44.32 b	14.83 b
No N/I	52.00 a	39.88 a	5.32 b	176.81	44.80 b	0.22 b	0.10 b	20.07 c	1.08 a	77.56 b	12.78 a	121.00	44.03 b	15.23 b

The means followed by the same letter in the columns belong to the same group by the Scott-Knott test at 95% reliability. Lodg.: Lodging score (notes 1-5); PH: Plant height (cm); INS: First pod insertion (cm); DTF: Days to flowering (days); DFM: Days to full maturity (days); CC: Chlorophyll content (SPAD unit); LNC: Leaf nitrogen content (%); FMS: Fresh weight of shoots (grams/plant); DMS: Dry weight of shoots (grams/plant); FMN: Fresh weight of nodules (grams/plant); DMN: Dry weight of nodules (grams/plant); NN: Number of nodules (unit/plant); Yield: Grain yield (bags.ha^{-1}); Z: Z index.

Source: The author (2020).

4 CONCLUSIONS

There were differences in the effects of soybean genotypes, *Bradyrhizobium* strains and the interactions between these factors on BNF.

The diallel analysis between soybean progenies and *Bradyrhizobium* strains revealed that there are both additive and nonadditive effects on BNF related traits. However, additive effects were predominant for most of the evaluated traits, so the recurrent selection could be an alternative for conducting breeding programs aimed at that trait.

There is variability among soybean progenies indicating potential genetic gain in selection to increase the biological nitrogen fixation ability.

The EA-8 progeny stood out as having the highest yield, with good results in the traits related to BNF and early maturity. The INPA 03-11B strain presented the best results for grain yield, indicating high potential for use as an inoculant in soybean crops.

REFERENCES

AGOYI, E.E. *et al*. Mode of inheritance of promiscuous nodulation and combining abilities in soybean genotypes. **International Journal of Agronomy and Agricultural Research (IJAAR)**, v. 9, n. 1, p. 73-82, 2016a.

AGOYI, E.E. *et al*. Screening soybeans genotypes for promiscuous symbiotic association with *Bradyrhizobium* strains. **African Crop Science Journal,** [*S.l.*], v. 24, n. 1, p. 49–59, 2016b.

ANPII. Associação Nacional dos Produtores e Importadores de Inoculantes. *In*: CONGRESSO DEBATE MICRORGANISMOS NO FUTURO DA LAVOURA. 2016. Disponível em: http://www.anpii.org.br/congresso-debat e-microrganismos-no-futuro-dalavoura. Acesso em: 15 jan. 2020.

______. Associação Nacional dos Produtores e Importadores de Inoculantes. Levantamento do uso de Inoculantes no Brasil. *In*: CONGRESSO BRASILEIRO DE SOJA, 8., Goiânia, **Anais...** Goiânia, 2018.

ARACHCHIGE, P.S.P. *et al.* Temporal biological nitrogen fixation pattern in soybean inoculated with *Bradyrhizobium*. **Agrosystems, Geosciences & Environment**, [*S.l.*], v. 3, n. 1, 2020.

ARGAW, A. Symbiotic effectiveness of inoculation with Bradyrhizobium isolates on soybean [*Glycine max* (L.) Merrill] genotypes with different maturities. **Springer Plus,** [*S.l.*], v. 3, p. 753, 2014.

AZIZ, A.L.A. *et al*. Contributions of Rhizobium inoculants and phosphorus fertilizer to biological nitrogen fixation, growth and grain yield of three soybean varieties on a Fluvic Luvisol. **American Journal of experimental Agriculture**, [*S.l.*], v. 10, n. 2, p. 1-11, 2016.

BALBOA, G.R.; SADRAS, V.O.; CIAMPITTI, I.A. Shifts in soybean yield, nutrient uptake, and nutrient stoichiometry: A historical synthesis analysis. **Crop Sci.**, [*S.l.*], v. 58, p. 43–54, 2018.

BERNARD, R.L. *et al*. **Results of the cooperative uniform soybeans tests**. Washington: USDA, 134 p., 1965.

BERNARDO, R. **Breeding for quantitative traits in plants.** 3 ed. Woodbury, Minnesota: Stemma Press, 2020. 422 p.

BIZARRO, M.J. *et al.* Genetic variability of soybean bradyrhizobia populations under different soil managements. **Biol. Fertil. Soils**, [*S.l.*], v. 47, p. 357-362, 2011.

BOHRER, T.R.J.; HUNGRIA, M. Avaliação de cultivares de soja quanto à fixação biológica do nitrogênio. **Pesquisa Agropecuaria Brasileira**, [*S.l.*], v. 33, n. 6, p. 937–952, 1998.

BUSHBY, H.V.; LAWN, R.J. Accumulation and partitioning of nitrogen and dry matter by contrasting genotypes of mungbean (*Vigna radiata* (L.) Wilczek). **Aust J Agric Res**, [*S.l.*], v. 43, p. 1609–1628, 1992.

BRACCINI, A.L. *et al*. Co-inoculação e modos de aplicação de Bradyrhizobium japonicum e Azospirillum brasilense e adubação nitrogenada na nodulação das plantas e rendimento da cultura da soja. S**cientia Agraria Paranaensis**, [*S.l.*], v. 15, p. 27-35, 2016.

BRANDELERO, E.M.; PEIXOTO, C.P.; RALISCH, R. Nodulação de cultivares de soja e seus efeitos no rendimento de grãos. **Semina:** Ciências Agrárias, [*S.l.*], v. 30, n. 3, p. 581-588, 2009.

BRASIL. Diário Oficial da União. **Instrução Normativa SDA nº 13**, de 24 de março de 2011. 24 p.

CONAB. Companhia Nacional de Abastecimento. **Acompanhamento da Safra Brasileira de Grãos**. Brasília, 2020. Disponível em: http://www.conab.gov.br . Acesso em: 06 jul. 2020.

DE LUCA, M.J.; NOGUEIRA, M.A.; HUNGRIA, M. Feasibility of lowering soybean planting density without compromising nitrogen fixation and yield. **Agron. J.**, [*S.l.*], v. 106, p. 2118–2124, 2014.

DIVITO, G.A. *et al.* Soybean shows an attenuated nitrogen dilution curve irrespective of maturity group and sowing date. **Field Crops Res.**, [*S.l.*], v. 186,p. 1–9, 2016.

DUDLEY, J.W. Quantitative genetics and plant breeding. **Adv. Agron.,** [*S.l.*], v. 59. n. 1–23, 1997.

EAGLESHAM, A.R.J. *et al.* Mineral N effects on cowpea and soybean crops in a Nigerian soil. II. Amounts of N fixed and accrual to the soil. **Plant Soil**, [*S.l.*], v. 68, p. 183–192, 1982.

EEUWIJK, F.A.V.; BUSTOS-KORTS, D.V.; MALOSETTI, M. What Should Students in Plant Breeding Know About the Statistical Aspects of Genotype × Environment Interactions? **Crop Science**, [*S.l.*], v. 56, n. 5, p. 2119-2140, 2016.

EMBRAPA. Empresa Brasileira de Pesquisa Agropecuária. Centro Nacional de Pesquisa de Solos. **Sistema brasileiro de classificação de solos**. 3. ed. Brasília: Embrapa Solos: Embrapa, 2013.

FEHR, W.R.; CAVINESS, C.E. Stages of Soybean Development. **Special Report**, [*S.l.*], v. 80, n. 3, p. 11, 1977.

FERREIRA, R.A.D.C. *et al.* Implications of the number of years assessment on recommendation of common bean cultivars. **Plant Breeding**, [*S.l.*], v. 134, p. 599-604, 2015.

FILHO, A.C.; JUNIOR, R.L. do C.B.; LÚCIO, A.D.C. Medidas de precisão experimental e número de repetições em ensaios de genótipos de arroz irrigado. **Pesquisa Agropecuaria Brasileira**, [*S.l.*], v. 47, n. 10, p. 1413–1421, 2012.

GERALDI, I.O.; MIRANDA-FILHO, J. Adapted models for the analysis of combining ability of varieties in partial diallel crosses. **Brazilian Journal of Genetics**, [*S.l.*], v. 11, n. 2, p. 419–430, 1988.

GRIFFING, B. Concept of General and Specific Combining Ability in Relation to Diallel Crossing Systems. **Australian Journal of Biological Sciences**, [*S.l.*], v. 9, n. 4, p. 463, 1956.

GRUNVALD, A.K. *et al.* Identification of QTLs Associated with Biological Nitrogen Fixation Traits in Soybean Using a Genotyping-by-Sequencing Approach. **Crop Science**, [*S.l.*], v. 58, p. 2522-2532, 2018.

HALLAUER, A.R.; MIRANDA FILHO, J.B. **Quantitative genetics in maize breeding.** 2nd ed. Ames: Iowa State University Press, 1988. 468 p.

HUNGRIA, M. *et al*. Nitrogen nutrition of soybean in Brazil: contribuitions of biological N_2 fixation and N fertilizer to grain yield. **Canadian Journal of Plant Science**, [*S.l.*], v. 86, n. 4, p. 927-939, 2006.

HUNGRIA, M.; CAMPO, R.J.; MENDES, I.C. **A importância do processo de fixação biológica do N para a cultura da soja: componente essencial para a competitividade do produto brasileiro**. Londrina - PR: Embrapa Soja, 2007. 80 p.

HUNGRIA, M.; MENDES, L.C. Nitrogen fixation with soybean: the perfect symbiosis? *In*: DE BRUIJN, F.J (Ed) **Biological Nitrogen Fixation.** New Jersey: Hoboken, 2015. p. 1009-1023.

HWANG, S. *et al.* Genetics and mapping of quantitative traits for nodule number, weight, and size in soybean (Glycine max L.[Merr.]). **Euphytica**, [*S.l.*], v. 195, p. 419-434, 2014.

INMET. Instituto Nacional de Meteorologia. **Banco de dados meteorológicos para ensino e pesquisa.** Disponível em: http://www.inmet.gov.br/portal/index.php?r=bdmep/bdme. Acesso em: 10 jun. 2019.

JÚNIOR E.B. da S. *et al.* Rhizobium Inoculation of Cowpea in Brazilian Cerrado Increases Yields and Nitrogen Fixation. **Agronomy Journal**, [*S.l.*], v. 110, p. 722-727, 2018.

KASCHUK, G. *et al.* Response of determinate and indeterminate soybean cultivars to basal and topdressing N fertilization compared to sole inoculation with Bradyrhizobium. **Field Crop Research**, [*S.l.*], [*S.v.*], [*S.n.*], p. 1-7, 2016.

KINUGASA, T. *et al.* Demand and supply of N in seed production of soybean (Glycine max) at different N fertilization levels after flowering. **Journal of Plant Research**, [*S.l.*], v. 125, p. 275–281, 2012.

LEITE, M.E. *et al.* Increasing the resistance of common bean to white mold through recurrent selection. **Scientia Agricola**, [*S.l.*], v. 73, n. 1, p. 71–78, 2016.

LEITE, M.E. *et al.* Reaction of common bean lines derived from recurrent selection for white mold resistance and aggressiveness of *Sclerotinia sclerotirum* isolates. **Bioscience Journal**, [*S.l.*], v. 33, n. 5, 2017.

LIMA, J.G. **Soybean breeding strategies for resistence to *Sclerotinia sclerotiorum*.** 2019. 87 p. Tese (Doutorado em Genética e Melhoramento de Plantas) - Universidade Federal de Lavras, Lavras, 2019.

LOPES, F.S. *et al.* Recurrent selection in common bean aiming at resistance to white mold in a greenhouse. **Crop Breed. Appl. Biotechnol.**, [*S.l.*], v. 19, n. 1, p. 95–101, 2019.

MALAVOLTA, E. **Elementos de Nutrição Mineral de Plantas**. São Paulo: Ceres, 1980. 251 p.

MALAVOLTA, E.; VITTI, G.C.; OLIVEIRA, S.A. **Avaliação do estado nutricional das plantas: princípios e aplicações.** 2. ed. Piracicaba: Potafos, 1997. 319 p.

MELO, L.C.; DOS SANTOS, J.B. Identification of resistant genotypes considering polygenic systems in host-pathogen interaction. **Genetics and Molecular Biology**, [*S.l.*], v. 22, n. 4, p. 601–608, 1999.

MENDES, F.F.; RAMALHO, M.A.P.; ABREU, A.F.B. Selection index for choosing segregating populations in common bean. **Pesquisa Agropecuária Brasileira**, [*S.l.*], v. 44, n. 10, p. 1312-1318, 2009.

MENEZES JÚNIOR, J.A.N. *et al.* Two cycles of recurrent selection in red bean breeding. **Crop Breed. Appl. Biotechnol.**, [*S.l.*], v. 13, n. 1, p. 41–48, 2013.

MIGUEL, D.L.; MOREIRA, F.M.S. Influência do ph do meio de cultivo e da turfa no comportamento de estirpes de *Bradyrhizobium*. **Revista Brasileira de ciências do Solo**, [*S.l.*], v. 25, p.873-883, 2001.

MORAIS JÚNIOR, O.P. de. *et al.* Genetic progress after cycles of upland rice recurrent selection. **Sci. Agric.**, [*S.l.*], v. 72, n. 4, p. 297–305, 2015.

MUÑOZ, N. *et al.* Improvement in nitrogen fixation capacity could be part f the domestication process in soybean. **Heredity**, [*S.l.*], v. 117, p. 84-93, 2016.

NICOLÁS, M.F.; ARRABAL ARIAS, C.A.; HUNGRIA, M. Genetics of nodulation and nitrogen fixation in Brazilian soybean cultivars. **Biology and Fertility of Soils**, [*S.l.*], v. 36, n. 2, p. 109–117, 2002.

NICOLÁS, M.F.; HUNGRIA, M.; ARIAS, C.A.A. Identification of quantitative trait loci controlling nodulation and shoot weight in progenies from two Brazilian soybean cultivars. **Field Crops Research**, [*S.l.*], v. 95, p. 355-366, 2006.

NOGUEIRA, P.D.M.; JÚNIOR, D.G.S.; RAGAGNIN, V.A. Clorofila foliar e nodulação em soja adubada com nitrogênio em cobertura. **Global Science and Technology**, [*S.l.*], v. 3, n. 2, p. 117 - 124, 2010.

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

OKON, Y. *et al.* Agronomic applications of Azospirillum and other PGPR. In: De Bruijn FJ (ed) **Biological Nitrogen Fixation**, Wiley, Hoboken, v. 2, [*S.n.*], p. 925–936, 2015.

OLIVEIRA, D.P. *et al.* Selection of elite Bradyrhizobium strains by biometric techniques for inoculation in cowpea. **Soil Sci. Soc. Am. J.**, [*S.l.*], [*S.v.*] [*S.n.*], p. 1-14, 2020.

PELAQUIM, J.A.P. *et al.* Avaliação da fixação biológica de nitrogênio no processo de seleção de linhagens de soja. *In*: JORNADA ACADÊMICA DA EMBRAPA SOJA, 11., 2016, Londrina. **Resumos expandidos...** Londrina: Embrapa Soja, 2016. p. 75-18.

PEREIRA, R. *et al.* Aggressiveness of *Pseudocercospora griseola* strains in common bean genotypes and implications for genetic improvement. **Genet Mol Res**, [*S.l.*], v. 14, [*S.n.*], p. 5044-5053, 2015.

PIMENTEL GOMES, F. **Curso de estatística experimental**. 15. ed. Piracicaba: ESALQ, 2009. 451 p.

PIRES, L.P.M. *et al.* Recurrent weight selection for upright plant architecture in common bean. **Scientia agrícola**, [*S.l.*], v. 71, n. 3, p. 240–243, 2014.

POSADAS, L.G.; ESKRIDGE, K.M.; GRAEF, G.L.. Elite performance for grain yield from unadapted exotic soybean germplasm in three cycles of a recurrent selection experiment. **Crop Sci.**, [*S.l.*], v. 54, n. 6, p. 2536–2546, 2014.

R CORE TEAM. **R:** A Language and Environment for Statistical Computing. R Foundation for statistical Computinh, Vienna, Austria, 2016.

RAMALHO, M.A.P. *et al.* **Aplicações da genética quantitativa no melhoramento de plantas autógamas.** Lavras: UFLA, 2012.

RESENDE, M.D.V. de; DUARTE, J.B. Precisão E Controle De Qualidade Em Experimentos De Avaliação De Cultivares. **Pesquisa Agropecuária Tropical**, [*S.l.*], v. 37, n. 3, p. 182–194, 2007.

RIBEIRO, F. de O. **Seleção genotípica visando à precocidade em soja.** 2018. 60 p. Dissertação (Mestrado em Genética e Melhoramento de Plantas) - Universidade Federal de Lavras, Lavras, 2018.

RIBEIRO, P.R. de *et al.* Symbiotic efficiency and genetic diversity of soybean bradyrhizobia in Brazilian soils. **Agriculture, Ecosystems and Enviroment**, [*S.l.*], v. 212, p. 85-93, 2015.

RODRIGUES-NAVARRO, D.N.I.M. *et al.* Soybean interactions with soil microbes, agronomical and molecular aspects. **Agronomy for Sustainable Development**, [*S.l.*], v. 31, p.173–190, 2011.

SANTOS, M.A. *et al.* Mapping of QTLs associated with biological nitrogen fixation traits in soybean. **Hereditas**, [*S.l.*], v. 150, p. 17–25, 2013.

SANTOS, M.M. dos *et al.* Produtividade de feijoeiro-caupi cultivado com estirpes rizobianas e doses de fósforo. **Scientia Agraria Paranaensis**, [*S.l.*], v. 18, n. 3, p. 209–217, jul./set. 2019.

SANTOS, M.S.; NOGUEIRA, M.A.; HUNGRIA, M. Microbial inoculants: reviewing the past, discussing the present and previewing na outstanding future for the use of beneficial bacteria in agriculture **AMB Express**, [*S.l.*], v. 9, n. 205, p. 1-22, 2019.

SANTACHIARA, G.; BORRÁS, L.; ROTUNDO, J.L. Physiological processes leading to similar yield in contrasting soybean maturity groups. **Agronomy Journal**, [*S.l.*], v. 109, p. 158–167, 2017.

SANTACHIARA, G. *et al.* Relative importance of biological nitrogen fixation and mineral uptake in high yielding soybean cultivars. **Plant Soil**, [*S.l.*], v. 481, p. 191–203, 2017.

SATURNO, D.F. *et al.* Mineral nitrogen impairs the biological nitrogen fixation in soybean of determinate and indeterminate growth types. **Journal of Plant Nutrition**, [*S.l.*], v. 40, n. 12, p. 1690-1701, 2017.

SEDIYAMA, T.; SILVA, F.; BORÉM, A. **Soja do Plantio à Colheita.** Viçosa: UFV, 2015.

SILVA, G.S. *et al.* Estimation of genetic progress after eight cycles of recurrent selection for common bean grain yield. **Crop Breeding and Applied Biotechnology**, [*S.l.*], v. 10, n. 4, p. 351–356, 2010.

SILVA, P.H. *et al.* Reaction of common bean lines and aggressiveness of *Sclerotinia sclerotiorum* isolates. **Genetic and Molecular Research**, [*S.l.*], v. 13, n. 4, p. 9138-9151, 2014.

SINCLAIR, T.R.; NOGUEIRA, M.A. Selection of host-plant genotype: the next step to increase grain legume N2 fixation activity. **Journal of Experimental Botany**, [*S.l.*], v. 69, p. 3523-3530, 2018.

SCOTT, A.; KNOTT, M. Cluster-analysis method for grouping means in analysis of variance. **Biometrics**, [*S.l.*], v. 30, p. 507-512, 1974.

SOARES, I.O. **Potential of recurrent selection for soybean improvement.** 2018. 64 p. Tese (Doutorado em Agronomia/Fitotecnia) - Universidade Federal de Lavras, Lavras, 2018.

SOGUT, T. Rhizobium inoculation improves yield and nitrogen accumulation in soybean (Glycine max) cultivars better than fertilizer. **N Z J Crop Hortic Sci**, [*S.l.*], v. 34, p. 115–120, 2006.

SOLOMON, T.; PANT, L.M.; ANGAW, T. Effects of inoculation by *Bradyrhizobium japonicum* strains on nodulation, nitrogen fixation, and yield of soybean (Glycine max L. Merril) varietes on Nitisols of Bako, Western Ethiopia. **ISRN Agronomy**, [*S.l.*], v. 2012, [*S.n.*], p. 1 - 8, 2012.

TAMAGNO, S. *et al*. Nutrient partitioning and stoichiometry in soybean: A synthesis-analysis. **Field Crops Res.,** [*S.l.*], v. 200, [*S.n.*], p. 18–27, 2017.

TORRES, A.R. *et al*. Genetic structure and diversity of a soybean germplasm considering biological nitrogen fixation and protein content. **Scientia agrícola,** [*S.l.*], v. 72, [*S.n.*], p. 47-52, 2015.

VALDO, S.C.D. *et al.* Differential interactions between *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* and common bean. **Genet. Mol. Res.,** [*S.l.*], v. 15, n. 4, 2016.

XAVIER, G.R. *et al.* Agronomic effectiveness of rhizobia strains on cowpea in two consecutive years. **Australian Journal of Crop Science**, [*S.l.*], v. 11, n. 09, p. 1154-1160, 2017.

WILSON, P.W. **Biochemistry of symbiotic Nitrogen fixation**. Madison: University of Wisconsin Press, 1940. 130 p.

YANG, Q. *et al.* Genetic analysis and mapping of QTLs for soybean biological nitrogen fixation traits under varied field conditions. **Front. Plant Sci.**, [*S.l.*], v. 10, p. 1-75, 2019.

ZANINI, H.L.H.T. *et al*. Caracterização da água da microbacia do Córrego Rico avaliada pelo índice de qualidade de água e de estado trófico. **Engenharia Agrícola,** [*S.l.*], v. 30, p. 732-741, 2012.

ZIMMER, S. *et al*. Effects of soybean variety and Bradyrhizobium strains on yield, protein content and biological nitrogen fixation under cool growing conditions in Germany. **European Journal of Agronomy**, [*S.l.*], v. 72, p. 38 – 46, 2016.

ZUFFO, A.M. *et al*. Adubação nitrogenada na soja inibe a nodulação e não melhora o crescimento inicial das plantas. **Revista em Agronegócio e Meio Ambiente**, [*S.l.*], v. 12, n. 2, p. 333-349, abr./jun. 2019.

ZUFFO, A.M. *et al*. Co-inoculation of *Bradyrhizobium japonicum* and *Azospirillum brasiliense* in the soybean crop. **Revista de Ciências Agrárias**, [*S.l.*], v. 38, n. 1, p. 87-93, 2015.

APPENDIX

Table 1 - Estimation of the general combining ability (GCA) of progenies with different *Bradyrhizobium* strains, GCA for *Bradyrhizobium* strains inoculated in different progenies and specific combining ability (SCA) to match progenies and strains for nitrogen content, chlorophyll content, number of nodules, fresh weight of nodules, dry weight of nodules. (continued)

Prog. / Strains	**SCA**					
	SEMIA 5079	**SEMIA 587**	**SEMIA 5019**	**UFLA 06-24**	**INPA 03-11B**	**GCA**
			Nitrogen content			
EA-1	0.1169^{ns}	-0.1185^{ns}	0.1150^{ns}	0.1981^{ns}	-0.3115*	-0.3096**
EA-2	-0.1038^{ns}	-0.0295^{ns}	0.2830*	-0.1656^{ns}	0.0158^{ns}	-0.0414*
EA-3	0.0424^{ns}	-0.2008^{ns}	0.1037^{ns}	0.0918^{ns}	-0.0370^{ns}	-0.0603**
EA-4	0.5042*	-0.2390^{ns}	0.2165^{ns}	-0.3434*	-0.1384^{ns}	-0.1491**
EA-5	0.2598^{ns}	-0.0229^{ns}	-0.2187^{ns}	0.1504^{ns}	-0.1686^{ns}	-0.2382**
EA-6	-0.1402^{ns}	-0.2407^{ns}	0.5006*	0.1474^{ns}	-0.2671^{ns}	-0.2589**
EA-7	-0.0133^{ns}	-0.1932^{ns}	0.2308^{ns}	-0.0194^{ns}	-0.0049^{ns}	-0.1636**
EA-8	-0.1260^{ns}	-0.1467^{ns}	0.0626^{ns}	-0.0688^{ns}	0.2789*	-0.3287**
EA-9	0.0108^{ns}	0.0451^{ns}	0.3418*	-0.2181^{ns}	-0.1796^{ns}	-0.2747**
EA-10	-0.4182*	-0.0264^{ns}	0.1594^{ns}	0.1235^{ns}	0.1617^{ns}	-0.2350**
EA-11	-0.6056**	0.1277^{ns}	0.0515^{ns}	0.2058^{ns}	0.2205^{ns}	-0.0793**
EA-12	0.2774*	0.2882*	-0.4078*	-0.2767*	0.1190^{ns}	-0.1683**
YD-1	0.0214^{ns}	-0.2541^{ns}	-0.0721^{ns}	0.4090*	-0.1043^{ns}	0.1765**
YD-2	0.0666^{ns}	0.1221^{ns}	-0.1239^{ns}	0.1525^{ns}	-0.2173^{ns}	0.3593**
YD-3	0.3058*	-0.1647^{ns}	0.0696^{ns}	-0.1221^{ns}	-0.0886^{ns}	0.3071**
YD-4	-0.2313^{ns}	0.4727*	-0.0873^{ns}	-0.1594^{ns}	0.0053^{ns}	0.1432**
YD-5	-0.0860^{ns}	-0.0642^{ns}	0.1033^{ns}	-0.0196^{ns}	0.0666^{ns}	0.1771**
YD-6	0.0673^{ns}	-0.0251^{ns}	0.0189^{ns}	-0.1165^{ns}	0.0554^{ns}	0.2078**
YD-7	0.1546^{ns}	-0.3083*	0.0072^{ns}	0.1728^{ns}	-0.0263^{ns}	0.0732**

YD-8	-0.5210*	0.3761*	-0.0369^{ns}	0.0747^{ns}	0.1071^{ns}	0.4053**
YD-9	0.0249^{ns}	0.4177*	-0.0638^{ns}	-0.0275^{ns}	-0.3513*	0.0360^{ns}
YD-10	0.3691*	-0.0166^{ns}	-0.7593*	0.1678^{ns}	0.2390^{ns}	0.1970**
YD-11	0.1758^{ns}	0.2543^{ns}	-0.2332^{ns}	-0.6196*	0.4226*	0.3383**
YD-12	-0.1520^{ns}	-0.0535^{ns}	-0.2607^{ns}	0.2626^{ns}	0.2036^{ns}	-0.1139**
GCA	0.0974**	-0.0722**	0.0281**	0.064**	-0.1172**	
Chlorophyll content						
EA-1	0.5366^{ns}	-1.0360^{ns}	-1.1523^{ns}	4.106*	-2.4543^{ns}	1.8062**
EA-2	2.2766^{ns}	-1.5960^{ns}	0.4377^{ns}	4.196*	-5.3143*	1.1412**
EA-3	-0.1834^{ns}	-3.0310^{ns}	2.0027^{ns}	1.3360^{ns}	-0.1243^{ns}	2.2512**
EA-4	3.0866^{ns}	-2.4860^{ns}	-2.2523^{ns}	3.4810^{ns}	-1.8293^{ns}	1.9562**
EA-5	-0.3084^{ns}	2.1690^{ns}	-1.6723^{ns}	1.6610^{ns}	-1.8493^{ns}	0.0012^{ns}
EA-6	-1.2434^{ns}	-0.2410^{ns}	2.6427^{ns}	1.3010^{ns}	-2.4593^{ns}	0.2112^{ns}
EA-7	0.7116^{ns}	-2.5110^{ns}	0.8477^{ns}	-0.6940^{ns}	1.6457^{ns}	1.2562**
EA-8	-0.8584^{ns}	-2.206^{ns}	0.8277^{ns}	2.1860^{ns}	0.0507^{ns}	0.7262*
EA-9	0.3866^{ns}	0.4890^{ns}	4.0727*	-5.0690*	0.1207^{ns}	1.9812**
EA-10	0.2266^{ns}	-0.3210^{ns}	-0.0123^{ns}	-4.4540*	4.5607*	3.3162**
EA-11	-3.0384^{ns}	0.5640^{ns}	-0.8273^{ns}	0.3810^{ns}	2.9207^{ns}	2.6562**
EA-12	-0.5284^{ns}	2.9740^{ns}	-3.0423^{ns}	-0.4340^{ns}	1.0307^{ns}	2.3962**
YD-1	1.0801^{ns}	0.9575^{ns}	4.2162*	-3.0930^{ns}	-3.1608^{ns}	-4.8873**
Prog. / Strains	**SCA**					
	SEMIA 5079	**SEMIA 587**	**SEMIA 5019**	**UFLA 06-24**	**INPA 03-11B**	**GCA**
Chlorophyll content						
YD-2	2.9466^{ns}	-0.4260^{ns}	-4.2173*	1.9410^{ns}	-0.2443^{ns}	-0.2788^{ns}
YD-3	-2.0534^{ns}	-3.1010^{ns}	3.8577*	0.3410^{ns}	0.9557^{ns}	-4.1788**
YD-4	-4.4934*	3.4340^{ns}	1.0677^{ns}	-0.3740^{ns}	0.3657^{ns}	1.2362**
YD-5	2.0051^{ns}	-1.0175^{ns}	-2.2763^{ns}	2.3245^{ns}	-1.0358^{ns}	-2.8873**

YD-6	0.3036^{ns}	3.1560^{ns}	0.8147^{ns}	-2.8020^{ns}	-1.4723^{ns}	-1.1108**
YD-7	5.9014*	-1.2787^{ns}	-3.0451^{ns}	0.2382^{ns}	-1.8158^{ns}	-4.7261**
YD-8	-4.1134*	4.089*	-0.1523^{ns}	-1.7940^{ns}	1.9707^{ns}	5.5062**
YD-9	-1.6530^{ns}	1.6094^{ns}	-4.382*	-0.0987^{ns}	4.5243*	-2.4392**
YD-10	1.4916^{ns}	0.3440^{ns}	-0.2723^{ns}	-0.6140^{ns}	-0.9493^{ns}	-3.7238**
YD-11	-1.8234^{ns}	1.4040^{ns}	1.8627^{ns}	-4.404*	2.9607^{ns}	-0.3088^{ns}
YD-12	-0.6564^{ns}	-1.9390^{ns}	0.6547^{ns}	0.3380^{ns}	1.6027^{ns}	-1.9008**
GCA	2.8534**	-2.699**	0.0923ns	2.609**	-2.8557**	
Number of nodules						
EA-1	-1.2974^{ns}	0.7910^{ns}	-1.1264^{ns}	1.9084*	-0.2756^{ns}	0.5837**
EA-2	-0.1670^{ns}	0.1689^{ns}	-0.6317^{ns}	-0.5272^{ns}	1.1571^{ns}	1.0021**
EA-3	0.0313^{ns}	-0.6568^{ns}	0.8246^{ns}	0.1734^{ns}	-0.3726^{ns}	0.3925**
EA-4	-1.3069^{ns}	1.1878^{ns}	-0.6493^{ns}	-0.3595^{ns}	1.1280^{ns}	0.4709**
EA-5	-0.8923^{ns}	1.2229^{ns}	-0.9175^{ns}	0.3021^{ns}	0.2848^{ns}	0.3263**
EA-6	-0.4127^{ns}	-0.3745^{ns}	-0.2669^{ns}	0.1267^{ns}	0.9274^{ns}	0.6997**
EA-7	-1.6635*	-0.0804^{ns}	0.9653^{ns}	-0.5112^{ns}	1.2898^{ns}	1.2531**
EA-8	-1.1876^{ns}	-0.6512^{ns}	0.3372^{ns}	0.7910^{ns}	0.7107^{ns}	0.2232*
EA-9	-0.5303^{ns}	1.5944*	0.3932^{ns}	-1.1680^{ns}	-0.2892^{ns}	0.7994**
EA-10	0.1244^{ns}	0.6798^{ns}	-1.5431*	-0.4050^{ns}	1.1440^{ns}	-1.1391**
EA-11	0.8344^{ns}	1.7564*	1.3210^{ns}	-0.0327^{ns}	-3.879**	-0.2489*
EA-12	-2.1404*	0.7215^{ns}	0.1391^{ns}	0.8742^{ns}	0.4057^{ns}	-0.1388^{ns}
YD-1	-0.4172^{ns}	0.4360^{ns}	0.8501^{ns}	-2.6399*	1.7711*	-0.9365**
YD-2	1.8631*	-0.0468^{ns}	-1.9439*	-0.7736^{ns}	0.9012^{ns}	0.637**
YD-3	1.1631^{ns}	-0.2882^{ns}	-0.8496^{ns}	-0.2235^{ns}	0.1982^{ns}	-1.0756**
YD-4	-0.1513^{ns}	-1.7351*	0.8218^{ns}	1.8906*	-0.8259^{ns}	0.5378**
YD-5	0.3486^{ns}	-0.5593^{ns}	-0.7034^{ns}	0.4709^{ns}	0.4432^{ns}	0.0937^{ns}
YD-6	1.3121^{ns}	-0.4978^{ns}	0.7256^{ns}	-1.0606^{ns}	-0.4793^{ns}	-0.6015**
YD-7	1.6262*	-3.5221**	0.2688^{ns}	1.3328^{ns}	0.2943^{ns}	-1.6729**

YD-8	0.8632^{ns}	-0.1014^{ns}	-0.4473^{ns}	-0.2975^{ns}	-0.0170^{ns}	-0.0314^{ns}
YD-9	1.3066^{ns}	-1.4398^{ns}	0.5819^{ns}	-0.8271^{ns}	0.3784^{ns}	0.1185^{ns}
YD-10	-0.0613^{ns}	1.3734^{ns}	0.2402^{ns}	-0.2702^{ns}	-1.2820^{ns}	-0.8879**
YD-11	0.8350^{ns}	-0.2636^{ns}	0.5578^{ns}	2.1043*	-3.2334**	-0.7687**
YD-12	-0.0796^{ns}	0.2848^{ns}	1.0529^{ns}	-0.8785^{ns}	-0.3795^{ns}	0.3632**
GCC	-2.152**	1.5523**	-0.1411**	-1.3544**	2.0951**	
			Fresh weight of nodules			
EA-1	0.0331^{ns}	-0.0830^{ns}	-0.0683^{ns}	0.0693^{ns}	0.0489^{ns}	0.0049^{ns}
EA-2	-0.0250^{ns}	-0.1551*	0.0146^{ns}	0.0209^{ns}	0.1445*	0.0410**
EA-3	-0.0631^{ns}	-0.0120^{ns}	0.0359^{ns}	-0.0180^{ns}	0.0571^{ns}	0.0911**
EA-4	-0.0635^{ns}	0.0928^{ns}	-0.0880^{ns}	-0.0479^{ns}	0.1065^{ns}	0.0805**
EA-5	-0.0601^{ns}	0.0160^{ns}	0.0195^{ns}	0.0201^{ns}	0.0044^{ns}	0.0648**
EA-6	0.1009^{ns}	-0.0545^{ns}	-0.0036^{ns}	-0.1015^{ns}	0.0586^{ns}	0.0324**
EA-7	-0.0454^{ns}	0.0544^{ns}	-0.0616^{ns}	-0.1005^{ns}	0.1531*	0.1284**

Prog. / Strains	**SCA**					
	SEMIA 5079	**SEMIA 587**	**SEMIA 5019**	**UFLA 06-24**	**INPA 03-11B**	**GCA**
			Fresh weight of nodules			
EA-8	-0.0212^{ns}	0.0562^{ns}	0.0119^{ns}	0.0065^{ns}	-0.0534^{ns}	0.0239*
EA-9	-0.0221^{ns}	0.0927^{ns}	0.0077^{ns}	-0.1225^{ns}	0.0441^{ns}	0.0924**
EA-10	0.0633^{ns}	0.0397^{ns}	-0.0986^{ns}	-0.0378^{ns}	0.0333^{ns}	0.0537**
EA-11	-0.0811^{ns}	0.1090^{ns}	-0.0018^{ns}	0.1113^{ns}	-0.1374*	0.1304**
EA-12	-0.2571**	0.0858^{ns}	-0.0425^{ns}	0.1178^{ns}	0.0959^{ns}	0.0261*
YD-1	-0.0093^{ns}	0.0780^{ns}	0.1455*	-0.2127*	-0.0016^{ns}	-0.1112**
YD-2	0.1317^{ns}	-0.0310^{ns}	-0.0965^{ns}	0.0646^{ns}	-0.0688^{ns}	0.0526**
YD-3	0.0201^{ns}	-0.1278^{ns}	0.1939*	0.0022^{ns}	-0.0884^{ns}	-0.1349**
YD-4	-0.1541*	-0.0560^{ns}	0.1927*	0.1523*	-0.1351*	-0.0132^{ns}
YD-5	0.0995^{ns}	-0.0679^{ns}	-0.0107^{ns}	-0.0061^{ns}	-0.0150^{ns}	-0.0038^{ns}
YD-6	0.0327^{ns}	0.0413^{ns}	0.0310^{ns}	-0.1041^{ns}	-0.0010^{ns}	-0.0680**

YD-7	0.1812*	-0.1774*	-0.0625^{ns}	0.1551*	-0.0965^{ns}	-0.1353**
YD-8	-0.0309^{ns}	0.0185^{ns}	0.0032^{ns}	0.0140^{ns}	-0.0049^{ns}	0.0626**
YD-9	0.0724^{ns}	-0.0355^{ns}	-0.0683^{ns}	0.0388^{ns}	-0.0076^{ns}	-0.2085**
YD-10	0.0381^{ns}	0.1895*	-0.0773^{ns}	-0.0520^{ns}	-0.0984^{ns}	-0.1314**
YD-11	0.0567^{ns}	-0.0612^{ns}	0.0265^{ns}	0.0456^{ns}	-0.0676^{ns}	-0.0740**
YD-12	0.0028^{ns}	-0.0129^{ns}	-0.0029^{ns}	-0.0158^{ns}	0.0288^{ns}	-0.0043^{ns}
GCA	-0.1467**	0.1219**	0.0460**	-0.1214**	0.1003**	
Dry weight of nodules						
EA-1	0.0589^{ns}	-0.0366^{ns}	-0.0584^{ns}	0.0511^{ns}	-0.0149^{ns}	-0.017**
EA-2	0.0420^{ns}	-0.0429^{ns}	0.0380^{ns}	0.0547^{ns}	-0.0918*	-0.0304**
EA-3	-0.0145^{ns}	-0.0427^{ns}	0.0330^{ns}	0.0005^{ns}	0.0237^{ns}	0.0233**
EA-4	-0.0058^{ns}	0.0060^{ns}	-0.0313^{ns}	-0.0131^{ns}	0.0442^{ns}	-0.0046^{ns}
EA-5	-0.0198^{ns}	0.0332^{ns}	-0.0326^{ns}	0.0491^{ns}	-0.0299^{ns}	0.0012^{ns}
EA-6	0.0140^{ns}	-0.0425^{ns}	-0.0056^{ns}	0.0074^{ns}	0.0267^{ns}	-0.0131*
EA-7	-0.0359^{ns}	0.0414^{ns}	-0.0289^{ns}	-0.0237^{ns}	0.0471^{ns}	0.0742**
EA-8	0.0435^{ns}	0.0090^{ns}	-0.0066^{ns}	0.0054^{ns}	-0.0513^{ns}	0.0079^{ns}
EA-9	0.0018^{ns}	-0.0064^{ns}	0.0553^{ns}	-0.0430^{ns}	-0.0077^{ns}	0.0383**
EA-10	0.0229^{ns}	0.0047^{ns}	-0.0536^{ns}	-0.0059^{ns}	0.0319^{ns}	0.0419**
EA-11	-0.0120^{ns}	0.0343^{ns}	0.0090^{ns}	0.0465^{ns}	-0.0778*	0.0468**
EA-12	-0.1444*	0.0479^{ns}	-0.0480^{ns}	0.0623^{ns}	0.0823*	0.0243**
YD-1	-0.0059^{ns}	0.0474^{ns}	0.0908*	-0.1302*	-0.0020^{ns}	-0.0397**
YD-2	0.0797*	-0.0207^{ns}	-0.0506^{ns}	0.0187^{ns}	-0.0271^{ns}	0.0316**
YD-3	-0.0704^{ns}	-0.0208^{ns}	0.0786*	-0.0024^{ns}	0.0151^{ns}	-0.0685**
YD-4	-0.0909*	-0.0148^{ns}	0.0771*	0.0271^{ns}	0.0016^{ns}	0.0422**
YD-5	0.0552^{ns}	-0.0058^{ns}	-0.0361^{ns}	-0.0256^{ns}	0.0124^{ns}	-0.0071^{ns}
YD-6	0.0400^{ns}	0.0190^{ns}	0.0589^{ns}	-0.0748*	-0.0431^{ns}	-0.0211**
YD-7	0.0915*	-0.1014*	-0.0298^{ns}	0.0775*	-0.0378^{ns}	-0.0667**
YD-8	-0.0461^{ns}	0.0507^{ns}	-0.0124^{ns}	-0.0284^{ns}	0.0363^{ns}	0.0522**

YD-9	0.0015^{ns}	-0.0029^{ns}	-0.0295^{ns}	-0.0003^{ns}	0.0312^{ns}	-0.0517**
YD-10	0.0254^{ns}	0.0664^{ns}	-0.0422^{ns}	-0.0232^{ns}	-0.0264^{ns}	-0.0538**
YD-11	-0.0143^{ns}	-0.0052^{ns}	0.0367^{ns}	-0.0128^{ns}	-0.0043^{ns}	-0.0316**
YD-12	-0.0165^{ns}	-0.0170^{ns}	-0.0113^{ns}	-0.0168^{ns}	0.0617^{ns}	0.0219**
GCA	-0.0498**	0.0299**	0.0473**	-0.044**	0.0165**	

Prog. / Strains	**SCA**					
	SEMIA 5079	**SEMIA 587**	**SEMIA 5019**	**UFLA 06-24**	**INPA 03-11B**	**GCA**
			Z index			
EA-1	0.3834^{ns}	-0.4467^{ns}	-0.8478^{ns}	2.1709*	-1.2599^{ns}	-0.3078*
EA-2	0.4442^{ns}	-0.6234^{ns}	0.7661^{ns}	0.5986^{ns}	-1.1856^{ns}	0.1794^{ns}
EA-3	-0.0546^{ns}	-1.4939^{ns}	1.1071^{ns}	0.4641^{ns}	-0.0226^{ns}	0.5658**
EA-4	1.0171^{ns}	-0.4114^{ns}	-0.4014^{ns}	-0.4155^{ns}	0.2112^{ns}	0.1196^{ns}
EA-5	0.0057^{ns}	1.0111^{ns}	-1.3254^{ns}	1.1065^{ns}	-0.7979^{ns}	-0.3705**
EA-6	-0.5111^{ns}	-1.0415^{ns}	1.3103^{ns}	0.6195^{ns}	-0.3772^{ns}	-0.374**
EA-7	-0.8165^{ns}	-0.4601^{ns}	0.7026^{ns}	-0.5359^{ns}	1.1099^{ns}	0.9445**
EA-8	-0.4354^{ns}	-0.8005^{ns}	0.3202^{ns}	0.5219^{ns}	0.3938^{ns}	-0.4244**
EA-9	-0.0880^{ns}	0.6754^{ns}	1.9655*	-2.0293*	-0.5235^{ns}	0.3514**
EA-10	-0.5912^{ns}	0.1779^{ns}	-0.6842^{ns}	-0.6308^{ns}	1.7284*	-0.0117^{ns}
EA-11	-1.5415^{ns}	1.2753^{ns}	0.5231^{ns}	0.8807^{ns}	-1.1377^{ns}	0.5684**
EA-12	-1.5217^{ns}	1.7351*	-1.6901*	0.2081^{ns}	1.2686^{ns}	0.1847^{ns}
YD-1	0.0128^{ns}	0.1866^{ns}	1.5965*	-1.6954*	-0.1005^{ns}	-1.069**
YD-2	1.9488*	-0.0111^{ns}	-2.0406*	0.5094^{ns}	-0.4065^{ns}	1.2056**
YD-3	0.1155^{ns}	-1.1076^{ns}	1.1292^{ns}	-0.3024^{ns}	0.1654^{ns}	-0.9866**
YD-4	-2.0255*	0.7756^{ns}	0.9467^{ns}	0.5125^{ns}	-0.2093^{ns}	1.0473**
YD-5	0.7363^{ns}	-0.5402^{ns}	-0.7004^{ns}	0.2619^{ns}	0.2425^{ns}	-0.108^{ns}
YD-6	0.9994^{ns}	0.4278^{ns}	0.9355^{ns}	-1.7039^{ns}	-0.6588^{ns}	-0.1363^{ns}
YD-7	2.6099*	-2.9703*	-0.6220^{ns}	1.5443*	-0.5619^{ns}	-1.7542**
YD-8	-1.8215*	1.8243*	-0.3677^{ns}	-0.4751^{ns}	0.8401^{ns}	2.1444**

YD-9	0.2705^{ns}	0.5836^{ns}	-0.8658^{ns}	-0.3661^{ns}	0.3779^{ns}	-0.714**
YD-10	1.1994^{ns}	1.0856^{ns}	-1.9085*	-0.0423^{ns}	-0.3343^{ns}	-0.9503**
YD-11	0.2542^{ns}	0.6084^{ns}	0.3197^{ns}	-1.3425^{ns}	0.1602^{ns}	0.1099^{ns}
YD-12	-0.5902^{ns}	-0.4598^{ns}	-0.1686^{ns}	0.1409^{ns}	1.0777^{ns}	-0.2141^{ns}
GCA	-0.5489**	0.2405**	0.4357**	-0.324**	0.1966**	

^{ns}: not significant, and *, **: differ significantly from zero to 1 and 5% probability, respectively, by student's t-test.

Source: The author (2020).