DÂMIANY PÁDUA OLIVEIRA

SELECTION OF ELITE RHIZOBIA STRAINS BY BIOMETRIC TECHNIQUES FOR INOCULATION IN COWPEA AND COMMON-BEAN

LAVRAS - MG

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Thesis presented to the Federal University of Lavras, as part of the requirements of the Postgraduate Program in Soil Science, area of concentration in Soil Biology, Microbiology and Biological Processes, to obtain the title of Doctor.

Profa. Dra. Fatima Maria de Souza Moreira

Advisor

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Approved on August 20th.

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À Deus,
pelas bênçãos e proteção,

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OFEREÇO

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‘‘ ‘UM DIA’ NÃO É TARDE... ”

Sebastião Soares Oliveira – Vovô Tião Quito

GENERAL ABSTRACT

Plant breeding programs seek to obtain genotypes with high yield, yield stability, and wide adaptability to growing environments. The selection of genotypes of microorganisms for adoption as agricultural inoculants has a similar objective. In both cases, the genotype-environment interaction makes it difficult to select materials adapted to highly diverse edaphic and climatic conditions. However, with suitable biometric techniques, variation in the response of each genotype in the environments can be analyzed, allowing selection of those with the desired standard and type of response. For that purpose, Toler and AMMI (additive main effect and multiplicative interaction) modeling and the Annicchiarico methodology were used together with genotypes of nitrogen fixing bacteria in symbiosis with cowpea (*Vigna unguiculata*) and common bean (*Phaseolus vulgaris*) in seven and eight environments, respectively, to determine their adaptabilities and phenotypic stabilities. The response patterns of the symbiont strains for common bean were furthermore determined in four plant cultivars, which composed the 16 macroenvironmental components of the genotype(G)- cultivar(C)-edaphoclimatic environment(E) interaction (GCE interaction). This research showed that the Toler and AMMI models and the Annicchiarico method accurately estimated the effects of adaptability and phenotypic stability of nitrogen fixing bacteria symbiont strains for inoculation in cowpea and common-bean. High adaptability and the potentials of phenotypic stabilities of the genotypes INPA03-11B (of *Bradyrhizobium elkanii*) and UFLA03-164 (*B. uaiense*) for cowpea and UFLA02-127 (of *Rhizobium* sp.) for common bean were confirmed. They were the ones that least contributed to the interaction, with yield capacity equivalent to that from fertilization with high doses of mineral N. They have a foreseeable response and ability to benefit from positive environmental stimuli, but are little to not at all affected by environments of unfavorable quality. They exhibit characteristics idealized by soil microbiologists, by inoculant producers, and by farmers that desire satisfactory yields, with minimal oscillation (under high stability), even with low inputs. INPA03-11B has already been approved as an inoculant by the Brazilian Ministry of Agriculture (MAPA); UFLA03-164 and UFLA02-127 have potential for inclusion as inoculants approved for commercial use for cowpea and common bean, respectively. In addition to UFLA02-127, whose good performance does not depend on the environmental condition and the host cultivar, other strains of *Rhizobium* under the UFLA code tested have yield capacity and adaptabilities higher than those of the CIAT899 strain (of *Rhizobium tropici*) currently approved as an inoculant for common bean in Brazil. The results from CIAT899, however, are enhanced under more stressful conditions and when the host is the Madrepérola cultivar. This was the first time that these biometric techniques were applied for rhizobia selection in field studies. It is recommended that these biometric techniques be used simultaneously for approval of new strains.

Keywords: *Rhizobium. Bradyrhizobium.* Adaptability. Phenotypic stability. Toler and AMMI models. Annicchiarico method. Genotype- cultivar- edaphoclimatic environment interaction. biological N_2 fixation. *Phaseolus vulgaris. Vigna unguiculata*

RESUMO GERAL

Os programas de melhoramento de plantas procuram obter genótipos com alto rendimento, estabilidade de rendimento e ampla adaptabilidade a ambientes de cultivo. A seleção de genótipos de microrganismos para adoção como inoculante agrícola tem objetivo semelhante. Em ambos os casos, a interação genótipo-ambiente torna difícil selecionar materiais adaptados a condições edáficas e climáticas altamente diversas. Porém, com técnicas biométricas adequadas, a variação na resposta de cada genótipo nos ambientes pode ser analisada, permitindo a seleção daqueles com o padrão e tipo de resposta desejados. Para tanto, foram utilizados os modelos de Toler e AMMI (efeitos principais aditivos e análise de interação multiplicativa) e a metodologia Annicchiarico em conjunto com genótipos de bactérias fixadoras de nitrogênio em simbiose com feijão-caupi (*Vigna unguiculata*) e feijão *(Phaseolus vulgaris*) em sete e oito. ambientes, respectivamente, para determinar suas adaptabilidades e estabilidades fenotípicas. Além disso, os padrões de resposta das linhagens simbiontes para o feijoeiro foram determinados em quatro cultivares de plantas, que compunham os 16 componentes macroambientais da interação genótipo (G) - cultivar (C) - ambiente edafoclimático (E) (interação GCE). Esta pesquisa mostrou que os modelos Toler e AMMI e o método Annicchiarico estimaram com precisão os efeitos da adaptabilidade e estabilidade fenotípica de estirpes simbiontes de bactérias fixadoras de nitrogênio para inoculação em feijão-caupi e feijão. A alta adaptabilidade e os potenciais de estabilidades fenotípicas dos genótipos INPA03-11B (de *Bradyrhizobium elkanii*) e UFLA03-164 (*B. uaiense*) para caupi e UFLA02-127 (de *Rhizobium* sp.) para feijão-comum foram confirmados. Eles foram os que menos contribuíram para a interação, com capacidade produtiva equivalente à da fertilização com altas doses do mineral N. Eles têm uma resposta previsível e capacidade de se beneficiar de estímulos ambientais positivos, mas são pouco ou nada afetados pelos ambientes de qualidade desfavorável. Exibem características idealizadas por microbiologistas de solo, por produtores de inoculantes e por agricultores que desejam rendimentos satisfatórios, com oscilação mínima (em alta estabilidade), mesmo com baixos insumos. O INPA03-11B já foi aprovado como inoculante pelo Ministério da Agricultura do Brasil (MAPA); UFLA03-164 e UFLA02-127 têm potencial para inclusão como inoculantes aprovados para uso comercial para caupi e feijão-comum, respectivamente. Além da UFLA02-127, cujo bom desempenho independe das condições ambientais e da cultivar hospedeira, outras estirpes de *Rhizobium* sob o código UFLA testadas apresentam capacidade produtiva e adaptabilidades superiores às da CIAT899 (de *Rhizobium tropici*) atualmente aprovada como inoculante para feijão-comum no Brasil. Os resultados do CIAT899, entretanto, são potencializados em condições mais estressantes e quando o hospedeiro é a cultivar Madrepérola. Esta foi a primeira vez que essas técnicas biométricas foram aplicadas para a seleção de rizóbios em estudos de campo. Recomenda-se que essas técnicas biométricas sejam utilizadas simultaneamente para aprovação de novas estirpes.

Palavras-chave: *Rhizobium*. *Bradyrhizobium*. Adaptabilidade. Estabilidade fenotípica. Modelos Toler e AMMI. Método Annicchiarico. Interação genótipo-cultivar-ambiente edafoclimático. Fixação biológica de N_2. *Phaseolus vulgaris. Vigna unguiculata*

SUMÁRIO

PART ONE - General Introduction

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GENERAL INTRODUCTION

1 INTRODUCTION

Biological nitrogen fixation in leguminous plants in symbiosis with rhizobia is recognized as an important tool for sustainable agriculture. Soybean is grown in some countries through replacement of mineral nitrogen fertilizers by inoculants with selected strains of rhizobia. This biotechnology is spreading to other crops, such as cowpea and common bean. Selection of the rhizobia strains should follow well-established methods under axenic and non-axenic conditions. The latter must include tests under field conditions to verify the suitability of the strain to adapt to edaphic factors and compete with native populations (which are also evaluated) in comparison with nitrogen fertilizers.

Normally, experiments are recommended in different locations or crop seasons for approval of microorganism strains for use as crop inoculants [cf., normative ruling of the Brazilian Ministry of Agriculture: Ministério da Agricultura e Pecuária do Brasil – MAPA, Instrução Normativa - IN 13 of March 24, 2011 (BRASIL, 2011)]. Nevertheless, the genotype by environment interaction (GE interaction) in these experiments makes it difficult to select materials adapted to widely diverse edaphic and climatic conditions. Yet, with the use of appropriate biometric techniques, the variation that occurs among the environments of each genetic material can be analyzed, allowing selection of materials with the type of response and pattern desired, and that respond positively to environmental variations, whether under specific or wide-ranging conditions.

The biometric techniques related to the study of adaptability and phenotypic stability of genotypes [Toler (TOLER and BURROWS, 1998), AMMI (ZOBEL et al., 1988), and Annicchiarico (ANNICCHIARICO, 1992)] are broadly applied in plant breeding. Adaptability refers to the potential ability of genotypes to respond in an advantageous way to environmental stimuli from a yield perspective (MARIOTTI et al., 1976), whereas phenotypic stability is considered to be the ability of genotypes to exhibit consistent performance in the face of variations in environmental quality, or otherwise, the ability of genotypes to exhibit a highly predictable response in accordance with environmental stimuli (CRUZ and REGAZZI, 1994). Among these tools, the additive main effect and multiplicative interaction (AMMI) analysis has become prominent and has been widely applied (ZOBEL et al., 1988). It encompasses, in a single model, the analysis of variance (ANOVA) technique for main effects, and principal component analysis for multiplicative effects, to which the interaction

matrix is applied. Also interesting is the Toler method (TOLER and BURROWS, 1998), based on non-linear regression analysis, which provides adaptive understanding of the genotypes, since it establishes a convex response pattern (when the genotype has a consistent response in unfavorable environments) or concave response pattern (when the genotype has low performance in poor environments and does not respond in favorable environments). The Annicchiarico methodology, for its part, adopts a reliability index of the risk of adoption of each genotype, such that the higher this index, the lower the risk of adoption of the genotype, that is, the lower the probability of lack of success (ANNICCHIARICO, 1992). The three complement each other, adding more consistent information to the study of the GE interaction.

These same methods could be applied in selection of microorganisms of agricultural importance, since they are based on the effects on plant production in edaphic and climatic environments and on plant genotypes, which was the aim of our study. In microbiological studies, however, there are only two reports with this focus for common bean and pea in pots with soil and nutrient solution (GUNNABO et al. 2019; 2020). There are no reports under field conditions, and studies are required in this matter. Thus, this thesis was developed with the following aims: *i)* to test the reliability of use of biometric techniques that estimate the adaptability and phenotypic stability of genotypes and propose applications of them in selection of rhizobia strains for inoculant purposes; *ii)* to aggregate information regarding field performance of elite strains from MAPA and from others in pre-approval for cowpea and common bean; and *iii)* to select rhizobia responsive for inoculation in common bean and cowpea through the study of interactions with the environment and with the genotype of the host symbiont.

2 THEORETICAL REFERENCES

Plant breeding programs seek to obtain genotypes with high yield, yield stability, and wide adaptability to environments. The selection of strains (genotypes) for adoption as agricultural inoculants has a similar objective. However, in both cases, the genotype-environment interaction makes it difficult to select materials adapted to highly diverse edaphic and climatic conditions (MORAIS et al., 2008) and adapted to the genotype of the host symbiont. However, with appropriate biometric techniques, variation in the response of each strain in the environments can be analyzed, allowing selection of those with the desired type of response and pattern.

2.1 The effect of environment on genotypic response

The term environment can be defined as the set of external factors that affect development of a genotype (BORÉM and MIRANDA, 2005; HAVEMAKI, 2014). Combining more than one more factor, as for example, edaphic and climatic conditions and plant cultivars, the more correct designation is then the macroenvironment. In general, environments with characteristics quite different from where the genotype was developed or isolated can affect the performance of the genotypes (SALTZ et al., 2018). Some genotypes may response in an unpredictable manner in environments for which there is no information, that is, in new environments (STAMPS and FRANKENHUIS, 2016). Thus, these genotypes may maintain standard results when grown in known environments, but may differ drastically in their responses to new environments, intensifying the genotype by environment interaction (GE interaction). Therefore, genotypes that have a positive interaction with the environments can constitute the difference between a good genotype and an excellent one (DUARTE and VENCOVSKY, 1999; CHAVES et al., 2001; FERREIRA et al., 2006). The response pattern of strains of N_2 fixing bacteria can guide their introduction into environments of compatible quality, favoring increase in crop yield.

2.2 Symbiotic performance of atmospheric N_2 fixing bacteria (NFB) within the environments

Specifically, for approval of atmospheric N_2 fixing microorganisms for common bean and cowpea, Brazilian legislation considers that genotypes whose viability and agronomic efficiency ensure yields greater than 500 kg ha^{-1} are of interest (BRASIL, 2011). Although results confirm benefits of native rhizobia communities in biological nitrogen fixation (BNF) for cowpea and common bean, the rhizobia community alone established in the soil is not always sufficient to ensure a symbiosis that expressively contributes to increasing the yield of these leguminous plants, and the use of inoculants containing genotypes more efficient in the BNF process is necessary (LACERDA et al., 2004; SOARES et al., 2006; 2014; COSTA et al., 2011; 2014; FARIAS et al., 2016a, b; FERREIRA, L. et al., 2013; FERREIRA, P. , 2019). The adoption of inoculants allows low investment in inputs and can partially or totally replace fertilization with mineral nitrogen (MOREIRA, 2006).

Four genotypes are currently approved by MAPA as inoculants for cowpea, namely, UFLA 03-84, BR3267, and INPA 03-11B, BR 3262 of *Bradyrhizobium viridifuturi* (COSTA et al., 2019), *B. yuanmingense* (LEITE et al., 2018), and *B. elkanii* (GUIMARÃES et al., 2015) and *B. pachyrhizi* (LEITE et al., 2018), respectively. The strains CIAT899 of *R. tropici*

(MARTINEZ-ROMERO et al., 1991; GRAHAM et al., 1994), PRF81 of R. freirei (DALL'AGNOL et al., 2013), and H12 are those approved for common bean. Other strains, such as UFLA03-153 and UFLA03-164 [both of *B. uaiense,* Michel et al., (2020)] and UFLA02-100, UFLA04-195, UFLA02-68, and UFLA02-127 (all four of the genus *Rhizobium*), are in the selection phase and have shown good results in studies preliminary to those of approval. All of them come from the SBMPBS/UFLA (Biology, Microbiology, and Biological Processes Sector of the Universidade Federal de Lavras). The first two strains were isolated from a bauxite mining area of Poços de Caldas, MG, that has slightly alkaline acidity (pH 6.7), with promising results for cowpea. The last four have symbiosis with common bean; they were isolated from acid soils with high Al content from the Brazilian Amazon, and the stresses of that region predominate in tropical soils. In addition to tolerance to acidity, they have high competitive ability with the strains already established and high N_2 fixation ability (SOARES et al., 2006; NOGUEIRA et al., 2017; OLIVEIRA et al., 2017; OLIVEIRA et al.2017, 2018a;b; OLIVEIRA et al., 2017).

2.3 The NFB genotype × environment interaction (GE interaction)

The occurrence of the GE interaction can represent both challenges and opportunities for development of effective inoculants. If the result of the interaction is unpredictable, the GE interaction can represent problems for development of an inoculant with stable performance. If, in contrast, the interaction is predictable, it could allow targeting specific strains to certain environments. In a certain way, this is analogous to directing plant varieties to environments, as is common in plant breeding (GUNNABO et al., 2019).

Biometric studies are recommended so as to refine the selection of NFB in the environmental context, detailing the effect of the GE interaction. This information would optimize processes of approval of genotypes that already show promising results, stratify the environments, characterizing them regarding the effects on each microorganism, and, furthermore, allow the macroenvironmental effect (of the environment and genotype of the host symbiont combination) to be related to the genotypic response of the rhizobia.

2.4 Biometric techniques applied to the study of interaction

The interaction can be statistically detected by a combined analysis of variance of experiments repeated in more than one environment. The most common procedures of the interaction study use regression; the mean of the genotype is the dependent variable and the environmental index is the independent variable (KANG, 1998). When regression is adopted,

the first question is if only one line segment is sufficient to explain the results or if there are two line segments, each one specific for favorable or unfavorable environments, where a basic idea consists of regressing the performance of the genotypes in the environmental means by a linear or non-linear model in the parameters.

The first simple linear regression model projected to explain the GE interaction was that proposed by Finlay and Wilkson (1963), which revealed the scientific community (BECKER and LEON, 1998; TOLER and BURROWS, 1998) due to: *i)* the environmental index (I_j) not being independent from the response variable (Y_{ij})*; ii)* the use of biased estimators of the regression coefficients [because the independent variable is measured with error (STORCK and VENCOVSKY 1994)]; and *iii)* the violation of the presupposition of homogeneity of the residual environmental variations; among others.

Many studies concentrated on improving these methods. In that context, the Toler (TOLER and BURROWS, 1998) and AMMI (additive main effect and multiplicative interaction; ZOBEL *et al.*, 1988) models allow knowledge of the response of the genotype to be obtained and thus, like the Annicchiarico method (ANNICCHIARICO, 1992), they aggregate information on the genotypic response. Nevertheless, it is recommended that the regression models and methods be used simultaneously to estimate the effects of phenotypic stability (FERREIRA et al., 2006). The use of these techniques is already widespread in studies on plant species and many other crops for commercialization (RIBEIRO et al., 2000; BORGES et al., 2010; CARBONELL et al., 2007; BRESEGHELLO et al., 2011; FREIRIA et al., 2018). In microbiological studies, however, there are almost no reports with this focus. Nevertheless, these same methods can be applied in selection of agriculturally important microorganisms, since they are based on the effects on plant production in edaphic and climatic environments.

2.4.1 Biometric techniques applied to the study of the adaptability and phenotypic stability of genotypes in environments

As important as estimating the GE interaction is attributing it to the respective genotypes that were responsible for this component. For that purpose, breeders use statistical-genetic methods able to perform the analysis of adaptability and stability of a group of genotypes in a group of (macro)environments, and in that way, determine those most recommended and most suitable to each condition, which would also provide information regarding the genotypic response of microorganisms.

2.4.1.1 Toler model

The two-segment statistical model proposed by ***Toler*** (TOLER and BURROWS, 1998) to describe adaptability and phenotypic stability is $Y_{ij} = \alpha_i + [Z_j\beta_{1i} + (1\text{-}Z_j)\ \beta_{2i}]\ \mu_j + \delta_{ij} + e_{ij}$, where Y_{ij} is the mean of genotype *i* in environment *j*; α_i is the parameter that reflects the value of the response of genotype *i* in the mean environment ($\mu_j = 0$, where μ_j is the regressor variable); β_{1i} and β_{2i} are the non-linear regression coefficients that measure the response of genotype i to the variations in the environments of inferior and superior quality, respectively; Z_j is an indicator variable that assumes the values 1 (if $\mu_j \leq 0$) and 0 (if $\mu_j > 0$); μ_j is the parameter that measures environmental quality; δ_{ij} is the deviation of this regression; and e_{ij} is the mean experimental error.

The first hypothesis tested is H_0: $\beta_{1i} = \beta_{2i}$; if the test is significant, this hypothesis is rejected and H_1: $\beta_{1i} \neq \beta_{2i}$, is accepted, and thus two straight-line segments occur, which determines a two-segment model. In this case, the genotype will be classified as Group ***A*** (if the response pattern is $\beta_{1i} < 1 < \beta_{2i}$) or as Group ***E*** (if $\beta_{1i} > 1 > \beta_{2i}$). When the hypothesis H_0: $\beta_{1i} = \beta_{2i}$ is accepted, a single straight-line segment is recognized to explain the response of the genotypes (β_i common to the two straight-line segments). This model is given by $Y_{ij} = \alpha_i + \beta_i\ \mu_j + \delta_{ij} + e_{ij}$. The genotypes in this case are classified as Group ***B*** (when the common β_i is significantly different from 1 and $\beta_i > 1$), ***C*** (when $\beta_i = 1$), or ***D*** (when the common β_i is significantly different from 1 and $\beta_i < 1$).

These five groups have the following practical meanings:

A – is the convex and doubly desirable response;

B – is the simple linear response and desirable only in high-quality environments;

C – is the simple linear response that does not deviate from the mean response;

D – is the simple linear response and desirable only in low-quality environments;

E – is the concave and doubly undesirable response.

A convex response or doubly desirable response pattern is therefore characterized when the genotype shows low responsiveness in unfavorable environments ($\mu_j < 0$) and comes to respond satisfactorily when these conditions become favorable ($\mu_j > 0$). A concave response or doubly undesirable response pattern, according to the classification of Toler and Burrows (1998), is characterized when the genotype is highly responsive to low-quality environments and not very responsive in environments with quality above these conditions. The environmental quality measurement μ_j is estimated at the same time as the other

regression parameters, and it is considered favorable (the environment with $\mu_j > 0$) or unfavorable (that with $\mu_j < 0$).

2.4.1.2 AMMI model

The descriptive model of the mean response of a genotype *i* in environment *j* in the ***AMMI*** analysis (ZOBEL; WRIGHT; GAUCH, 1988) is $Y_{ij} = \mu + g_i + e_j + \sum_{k=1}^{n} \lambda_k \gamma_{ik} \alpha_{jk} + \rho_{ij} + \varepsilon_{ij}$, which emerges by taking a traditional model of combined analysis as a basis (μ is the overall mean; g_i is the genotypic effect $_i$; e_j is the effect of environment *j*; ε_{ij} is the effect of the mean experimental error of genotype *i* in environment *j*), for which the classic term of the interaction of genotype i with environment *j*, normally denoted as ge_{ij}, is modeled as $ge_{ij} = \sum_{k=1}^{n} \lambda_k \gamma_{ik} \alpha_{jk} + \rho_{ij}$, where $\rho_{ij} = \sum_{k=n+1}^{p} \lambda_k$; and, furthermore, λ_k are the singular values relative, respectively, to the first n components retained by the method and the last components, from n+1 to p, which are not retained by the method. The method produces scores of principal components of interaction for each genotype, which reflect their contribution to the GE interaction. The genotype with the lowest scores in absolute value or nearer the axis of greater explanation is the most stable. Vectors pointing in common and near directions are indications of specificity/synergy of the genotype to the environment, and, in contrast, vectors pointing in opposite directions are indications of negative interaction.

2.4.1.3 Annicchiarico method

The methodology adopts a reliability index of the risk of adoption of each genotype (ANNICCHIARICO, 1992). The procedures for the calculations initially involve a transformation of the mean values of each cultivar in each environment, in percentage of the mean of the environment. After that, the mean $\bar{Y}_i$ and the standard deviation S_i of the percentages of each cultivar across the environments are estimated. From these estimates, the reliability index I_i is obtained by means of the following estimator: $I_i = \bar{Y}_i - Z_{1-\alpha} S_i$, where I_i is the reliability index (%); $\bar{Y}_i$ is the mean of genotype i in percentage; $Z_{1-\alpha}$ is the quantile of the normal standard distribution, in which the function of accumulated distribution reaches the

percentile value 1-α; and S_i is the standard deviation of the percentage values. The reliability coefficient is adopted at 75%; that is, $\alpha = 0.25$. The greater the reliability index of the genotype, the lower the risk in adoption of the genotype, that is, the lower will be its probability of lack of success.

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PART TWO – Articles

ARTICLE I

Selection of elite *Bradyrhizobium* strains by biometric techniques for inoculation in cowpea

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Abstract

Plant breeding programs seek to obtain genotypes with high yield, yield stability, and wide adaptability to environments. Selection of strains (genotypes) for adoption as crop inoculants has a similar aim. However, in both cases, the genotype × environment interaction hinders selection of materials adapted to the most diverse edaphic and climatic conditions. Nevertheless, with adequate biometric techniques, the variation in the response of each strain in the environments can be analyzed, allowing selection of those with the type of response and standard desired. In the present pioneering field study, the Toler and additive main effects and multiplicative interaction analysis models and the Annicchiarico method were used in a complementary manner with strains of nitrogen-fixing bacteria that are symbionts with cowpea to determine their adaptabilities and phenotypic stabilities. The adaptability and phenotypic stabilities of strains INPA 03-11B and UFLA 03-164 were confirmed by these techniques. These strains have the most stable behavior regardless of the environment. The reliability of adopting these strains exceeded 85%. The good response of strain UFLA03-164,

still in the selection phase, is surprising, with results equivalent to application of mineral N, allowing it to be included among the strains approved as inoculants for cowpea.

Abbreviations: AMMI, Additive Main Effects and Multiplicative Interaction Analysis; BNF, biological nitrogen fixation; EQI, environmental quality index; G×E, genotype × environment; MAPA, Ministério da Agricultura, Pecuária e Abastecimento do Brasil; PSV, principal singular vector; SSGE, sum of squares of the genotype × environment interaction.

Core Ideas

- Five previously selected rhizobia strains were evaluated in seven environments.
- Strains INPA03-11B and UFLA03-164 were adapted to all environments evaluated.
- Strains INPA03-11B and UFLA03-164 had high adaptability and phenotypic stability.
- *Bradyrhizobium uaiense* UFLA03-164 can be used to achieve high cowpea yields.

1 | INTRODUCTION

The aim of plant breeding programs is to obtain genotypes with high yield, yield stability, and wide adaptability to the most varied crop environments. Selection of strains (genotypes) of microorganisms for adoption as crop inoculants has similar aims. According to the Normative Instruction (IN 13 of 24 Mar. 2011) from the Brazilian Ministry of Agriculture Livestock and Food Supply (Ministerio da Agricultura, Pecuaria e Abastecimento [MAPA]) (Republica Federativa do Brasil, 2011), four experiments in different locations or crop seasons are necessary for approval of microorganisms for use as crop inoculants. Nevertheless, for plants and microorganisms, the genotype × environment (G×E) interaction hinders the selection of materials adapted to the most diverse edaphic and climatic conditions (Morais, Moura, Vencovsky, & Pinheiro, 2008). Environments with characteristics quite different from where the genotype was selected may interfere in the performance of the genotypes (Saltz et al., 2018). Some genotypes may respond in an unpredictable manner in new environments for which there is no information (Stamps & Frankenhuis, 2016). Thus, these genotypes can maintain standard results when grown in known environments but may differ drastically in their responses to new environments, intensifying the G×E interaction. Therefore, genotypes that have a positive interaction with diverse environments can constitute the difference between a good and an excellent genotype (Duarte & Vencovsky, 1999; Ferreira, Demetrio, Manly, & Machado, 2006). Interaction can be detected statistically by a combined ANOVA of experiments repeated in more than one environment. The study of adaptability and phenotypic stability of genetic materials is recommended, with the aim of detailing the effect of the G×E interaction. With the use of adequate biometric techniques, the variation occurring among environments of each genetic material can be analyzed, allowing selection of genotypes with the type of response and standard desired and that respond positively to environmental variations, whether under specific or more generalized conditions (Colombari Filho et al., 2013; Ferreira et al., 2006).

The most common procedures use regression; the mean of the genotype is the dependent variable, and the environmental index is the independent variable (Kang, 1998). When regression is adopted, the first question is whether only one single line segment is sufficient to explain the results or whether there are two straight line segments, each one specific for favorable or unfavorable environments. The basic idea of this consists of

regressing the development of the genotypes in the mean environmental yields by a linear or nonlinearmodel in the parameters. Although there is no unanimous concept for phenotypic stability, various methods and models have been used to explain the G×E interaction (Cruz, Torres, & Vencovsky, 1989; Eberhart & Russell, 1966; Finlay & Wilkinson, 1963; Freeman & Perkins, 1971; Perkins & Jinks, 1968a, 1968b; Shukla, 1972; Silva, 1995a, 1995b; Toler & Burrows, 1998; Verma, Chahal, & Murty, 1978).

The first simple linear regression model applied to explain the G×E interaction was that proposed by Finlay and Wilkson (1963), which was criticized by the scientific community (Becker & Leon, 1988; Toler & Burrows, 1998) due to (a) the environmental index (*Ij*) not being independente of the response variable (*Yij*), (b) the use of biased estimators of the regression coefficients (because the independente variable is measured with error; Storck & Ven-covsky [1994]), and (c) violation of the presupposition of homogeneity of the residual environmental variations. The lack of independence of the environmental index in relation to the response variables extends to all the regression models that were developed after the Finlay and Wilkson model.

A great deal of research has focused on searching for improvements in these methods. In this context, the Toler (Toler & Burrows, 1998) and the Additive Main Effects and Multiplicative Interaction Analysis (AMMI) (Zobel, Wright, & Gauch, 1988) models allow the response of the genotype to be known; thus, like the method of Annicchiarico (1992), they aggregate information regarding genotypic response. The Tolermodels correct the problem of lack of independence of the environmental index with the response variable through use of a model in which the environmental index is a parameter of the model and no longer computed from the response variable, as in all the other regression models. However, it is recommended that the regression models and other methods be used simultaneously to complement each other in estimating adaptability and phenotypic stability effects (Ferreira et al., 2006; Toler & Burrows, 1998). The use of these techniques is widespread in studies on plant species and many other commercial crops (Borges et al., 2010; Breseghello et al., 2011; Carbonell et al., 2007; Freiria et al., 2018). In microbiological studies, almost no reports have this focus (Gunnabo et al., 2019). However, these same methods could be applied in the selection of microorganisms of agricultural importance because they are based on the effects on plant production in edaphic and climatic environments.

Specifically, for approval of atmospheric nitrogen (N_2)-fixing bacteria for cowpea, Brazilian legislation considers strains (genotypes) of interest to be those whose viability and agronomic efficiency ensure yields higher than 500 kg ha^{-1} (Republica Federativa do Brasil, 2011). Although results confirm the benefits of Native rhizobia communities in biological N fixation (BNF) for cowpea, the rhizobia community by itself established in the soil is not always sufficient to ensure a symbiosis that expressively contributes to increase the yield of this legume, and it is necessary to use inoculants containing strains that are more efficient in the BNF process (Costa, Nobrega, Martins, Amaral, & Moreira, 2011, 2014; Farias,Trochmann, Soares, & Moreira, 2016a, 2016b; Ferreira et al., 2019; Lacerda, Moreira, Andrade, & Soares, 2004; Martins et al., 2003; Moreira, 2006; Soares et al., 2006, 2014; Zilli, Marson, Marson, Rumjanek, & Xavier, 2009).

Four strains are currently approved by MAPA as inoculants for cowpea: UFLA 03-84, INPA 03-11B, BR3267, and BR3267. Information regarding adaptability and phenotypic stability of these and of new strains to the most diverse environments should be considered essential due to the high heterogeneity of soils and climates in Brazil. This information would also optimize processes of approval of strains in the selection phase, such as UFLA03-153 and UFLA 03-164, which have already shown promising results (Costa et al., 2011, 2014; Farias et al., 2016a, 2016b; Ferreira et al., 2013, 2019). Furthermore, it would stratify environments, characterizing them regarding the effects of each strain. Consequently, to

gather this information, the present study was proposed applying Toler models on the data of cowpea grain yield obtained after inoculation with five previously selected strains of N_2-fixing bacteria and native rhizobia in seven environments. The researchers further apply AMMI modeling and the Annicchiarico method to the data obtained in the field, thus following the suggestions of Toler and Burrows (1998) in using them as a complement to the nonlinear protocol they developed.

2 | MATERIALS AND METHODS

2.1 | Genotypes evaluated (previously selected strains of nitrogen-fixing bactéria and control treatments with native strains)

The S1 (UFLA 03-84), S2 (BR3267), and S3 (INPA 03-11B) strains, classified as *Bradyrhizobium viridifuturi* (Costa et al., 2019), *Bradyrhizobium yuanmingense* (Leite et al., 2017), and *Bradyrhizobium elkanii* (Guimaraes et al., 2015), respectively, are N_2-fixing bacteria currently approved by MAPA as inoculants for cowpea. The S4 (UFLA 03-153) and S5 (UFLA 03-164) strains, recently classified as *Bradyrhizobium uaiense* (Michel et al., 2020), are in the selection phase and are from the Biology, Microbiology, and Biological Processes Sector of the Universidade Federal de Lavras. The last two strains were isolated from a bauxite-mining area of Pocos de Caldas, MG, which has a slightly alcaline acidity level (pH 6.7), and were included in this study for their potential in fixing N_2 in cowpea (Costa et al., 2011, 2014; Farias et al., 2016a, 2016b; Ferreira et al., 2013; Oliveira-Longatti et al., 2014; Soares et al., 2014). The genotype C6 (native rhizobia) was considered a control because it consisted of native nodule-forming strains from the environments tested. Experiments were conducted to estimate the most probable number of native N_2-fixing nodulating bacteria cells in the 0- to 20-cm soil depth within each experimental field area (Soares et al., 2006, 2014). Numbers around 10^3 cells g^{-1} of soil were obtained for all areas. A control fertilized with N through 70 kg N-urea ha^{-1} on native rhizobia (called C7) was inserted among the treatment mentioned above.

The inoculants containing the strains S1, S2, S3, S4, and S5 were prepared from turf sterilized in an autoclave at a ratio of 3:2 (w/v) of turf and 79 semisolid culture medium (Fred & Waksman, 1928) in the log phase (after 5 d of growth). The resulting material was applied at the rate of 10 g kg^{-1} of cowpea seeds. The quality of the inoculants was monitored through counting of colony-forming units, meeting the minimum legal number of viable cells of around 10^9 colony-forming units per gram of inoculant at sowing (Republica Federativa do Brasil, 2011).

2.2 | Evaluated environments

Seven field experiments were conducted at different locations and years/crop seasons, which are referred to as “environment” (E) (Table 1). In the 2010 dry season (seeds were sown in February), Experiments E1 (in Lavras, MG), E2 (in Presidente Olegario, MG), and E3 (in Bambui, MG) were set up. In April, in the winter crop season of this same year, E4 (in Patos de Minas, MG) was set up. The last three experiments—E5 (in Lavras, MG), E6 (in Luminarias, MG), and E7 (in Presidente Olegario, MG)—were set up in November and December 2010 in the 2010/2011 rainy crop season. The results of chemical and physical analyses of combined samples of the soils of the environments, the geographic coordinates, the climate of the areas, and the specifications of fertilization and crop history in these

environments are shown in Table 1. In Figure 1, a summary of the main climate occurrences over the time of conducting the experiments is shown.

2.3 | Experimental design and crop management practices

The experimental design was always randomized blocks, with four replications and the seven treatments previously described. In each one of the seven environments, the experimental unit (24m2) consisted of six 4-m rows spaced at 1.0 m. A schema for the experimental strategy is provided in Supplemental Figure 1.

Soil tillage was through plowings; two harrow passes were also made in E5 and E6. In the seven environments, all the plots received phosphate and potassium fertilization, defined according to soil analysis (Table 1). In all the experiments, the cowpea cultivar used was BR-17 Gurgueia, and seeds were sown manually immediately after inoculation at a density of 15 seeds per linear meter, which was maintained throughout the cycle.

The crops in E1, E2, E5, E6, and E7 were grown under conditions of low use of inputs and technology, without irrigation or application of pesticides. The soil was not amended in any of the seven environments. Nonvegetative pest control was not necessary in any of the environments. Manual weeding was performed in all the experiments whenever necessary. In E3 and E4, manual weeding complemented application of a mixture of 0.5 L of fomesafen (Flex) + 1.0 L of bentazon + imazamox (Amplo, BASF) + 1.0 L of fluazifop-butyl (Fusilade, Syngenta) per hectare. In analogy with common bean crops, the treatments provided to E3 and E4 would be equivalent to technology levels 3 and 4 (Chagas et al., 1999), respectively, and E4 varied only through use of a higher rate of potassium fertilizationand by adoption of sprinkler irrigation throughout the crop cycle due to the requirements of the crop season.

2.4 | Statistical analyses

At grain maturity, the yield in each environment was obtained from the total weight of grain produced in the part of the plot used for data collection (Rows 4 and 5), adjusting moisture to 130 g kg^{-1}. Individual ANOVA was conducted on all the data with Sisvar 5.7 software (Ferreira, 2019) after the normality test (Shapiro–Wilk test) and homoscedasticity test (Bartlett's test) had been used on the residues of the models using R software (R Development Core Team, 2019). After that, combined ANOVA wasconducted on the field experiments, observing the homogeneity of the residual mean squares. The mean value for each G×E interaction was calculated from the four replicates in that experiment/environment for all further analyses. From this information, parameters of adaptability and phenotypic stability were estimated through use of the Toler (Toler & Burrows, 1998) and AMMI (Zobel et al., 1988) models and the Annicchiaricomethod (Annicchiarico, 1992) in the Estabilidade statistical software (Ferreira & Zambalde, 1997). To confirm the specific response of the inoculated strains (S1, S2, S3, S4, and S5), the same biometric analyses were applied after controls C6 and C7 were excluded (Supplemental Tables 1–3; Supplemental Figures 2 and 3). For analysis of the Toler models, the nonlinear least squares technique was used by iterative processes, applying the modified Gauss–Newton method for estimation of these parameters (Rosse & Vencovsky, 2000; Rosse, Vencovsky, & Ferreira, 2002).

TABLE 1 Chemical characteristics, geographic coordinates, and altitude of the environments studied, with the respective information regarding crop season/year, preceding crop, and fertilization adopted

Characteristic	Environment [Location]							
	Unit	E1 [Lavras]	E2 [Presidente Olegário]	E3 [Bambuí]	E4 [Patos de Minas]	E5 [Lavras]	E6 [Luminárias]	E7 [Presidente Olegário]
pH	-	5.7 G	6.5 H	6.0 G	6.0 G	6.0 G	4.8 L	4.8 L
P	*mg dm^{-3}*	5.2 M	1.8 VL	260.0 VG	35.7 VG	3.1 VL	1.2 VL	1.7 VL
K	*mg dm^{-3}*	81.0 G	253.0 VG	374.0 VG	75.0 G	51.0 M	62.0 M	41.0 M
Ca	*cmol$_c$ dm^3*	2.9 G	4.7 VG	10.4 VG	1.8 M	1.9 M	0.2 VL	0.8 L
Mg	*cmol$_c$ dm^3*	1.3 G	1.2 G	1.1 G	0.8 M	0.5 M	0.2 L	0.4 L
Al	*cmol$_c$ dm^3*	0.0 VL	0.0 VL	0.1 VL	0.1 VL	0 VL	0.7 M	0.8 M
H + Al	*cmol$_c$ dm^3*	3.6 M	2.3 L	2.9 M	5 M	2.1 L	6.3 G	13.7 VG
SB	*cmol$_c$ dm^3*	4.4 G	6.5 VG	12.5 VG	2.8 M	2.5 M	0.6 VL	2.1 M
T	*cmol$_c$ dm^3*	8.0 G	8.9 G	15.4 VG	7.8 G	4.6 M	6.9 G	15.0 G
t	*cmol$_c$ dm^3*	4.4M	6.5 G	12.6 VG	2.9 M	2.5 M	1.3 L	2.1 L
m	*%*	0.0 VL	0.0 VL	0.8 VL	3.4 VL	0.0 VL	55.6 H	38 M
V	*%*	55.1 M	73.8 G	81.1 VG	35.6 L	55.0 M	8.2 VL	8.7 VL
OM	*dag kg$^-$*	2.6 M	3.6 M	0.8 VL	4.4 G	2.9 M	4.0 M	4.6 G
Zn	*mg dm^{-3}*	0.8 L	2.8 H	15.1 H	6.9 H	1.5 M	0.3 VL	1 M
Fe	*mg dm^{-3}*	71.9 H	74.8 H	28.3 M	32.7 G	75.2 H	36.9 G	1.0 VL
Mn	*mg dm^{-3}*	7.1 M	11.8 G	144.8 H	96.3 H	12.0 H	3.2 L	1 VL
Cu	*mg dm^{-3}*	0.8 L	1.4 G	5.8 H	10.9 H	1.4 H	1.7 G	1 M
B	*mg dm^{-3}*	0.2 L	0.0 VL	0.3 L	0.3 VL	0.1 VL	0.2 L	0.1 VL
S	*mg dm^{-3}*	25.5 H	16.6 H	8.4 M	7.7 M	11.8 H	8.9 M	13.8 H
Geographic coordinates	-	21°14’ S 45°00’ W	20°46’ S 43°11’ W	20°00’ S 45°58’ W	18°34 S 46° 31’ W	21°14’ S 45°00’ W	21°30’ S 44°54’ W	20°46’ S 43°11’ W
Altitude	*m*	920	591	706	833	920	957	591
Crop season/Year	-	2010 Dry	2010 Dry	2010 Dry	2010 Autumn/Winter	2010/11 Rainy	2010/11 Rainy	2010/11 Rainy
Preceding crop	-	Maize	Brachiaria	Deactivated garden crops	Various crops	Maize	Clean field	Maize
BF[†]: P_2O_5	*kg ha^{-1}*	70	80	70	70	70	70	70
BF[‡]: K_2O	*kg ha^{-1}*	20	20	20	40	20	20	20

[a]BF, base fertilization; m, exchangeable aluminum saturation; OM, organic matter; SB, sum of exchangeable bases; T, cation exchange capacity at pH 7; t, cation exchange capacity; V, base saturation. [b]G, good; H, high; L, low; M, medium; VG, very good; VL, very low (according to Ribeiro, Guimaraes, & Alvarez. 1999).

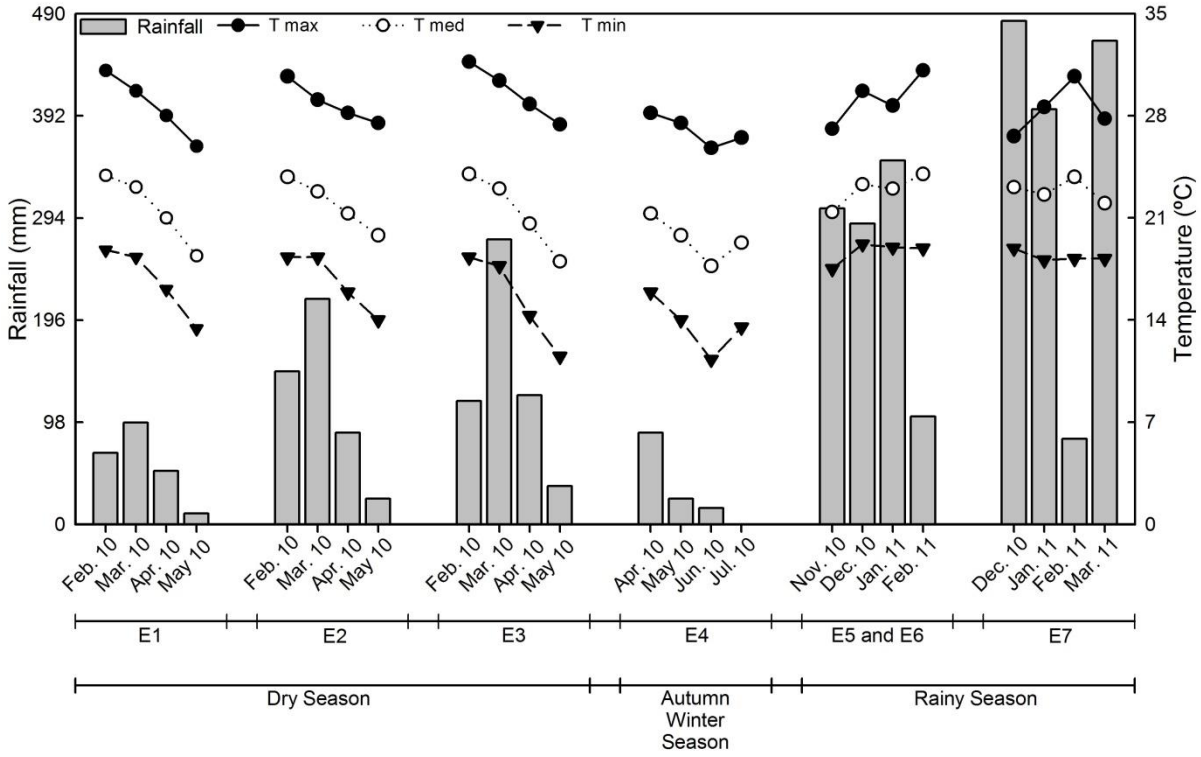

FIGURE 1 Monthly variation of the maximum, mean, and minimum temperatures and of rainfall in environments E1 (Lavras), E2 (Presidente Olegario), E3 (Bambui), E4 (Patos de Minas), E5 (Lavras), E6 (Luminarias), and E7 (Presidente Olegario) during the experimental period.

After that, the genotypic means were clustered by the Scott–Knott test ($P < .05$) using Sisvar 5.7 software. The AMMI model was adjusted using decomposition of the singular value of the interaction matrix, and the F tests and degrees of freedom were determined according to Gollob (1968). Biplots of the AMMI model were developed using the 'agricolae' package in R.

2.5 | Detailing of themodels and method

2.5.1 | Toler models

The bi-segmented statistical model proposed by Toler & Burrows (1998) to describe adaptability and phenotypic stability is: $Y_{ij} = \alpha i + [Z_j\beta_{1i} + (1 - Z_j)\ \beta_{2i}]\ \mu_j + \delta_{ij} + e_{ij}$, where Y_{ij} is the mean of genotype i in environment j; α_i is the parameter that reflects the value of the response of genotype i in the mean environment ($\mu_j = 0$, where μj is the regressor variable, which is a parameter of the model); β_{1i} and β_{2i} are nonlinear regression coefficients that measure the response of genotype i to the variations in the environments of lower and higher quality, respectively; Z_j is an indicator variable that assumes the value $Z_j = 1$ if $\mu_j \leq 0$ and $Z_j = 0$ if $\mu_j > 0$; μ_j is the parameter that measures environmental quality; δ_{ij} is the deviation of this regression; and eij is the mean experimental error.

The first hypothesis tested is H0: $\beta_{1i} = \beta_{2i}$; if the test is significant, this hypothesis is rejected, accepting H1: $\beta_{1i} \neq \beta_{2i}$, thus occurring as two straight line segments, which determines the model as bi-segmented. In this case, the genotype will be classified as Group

A if the response pattern is $\beta_{1i} < 1 < \beta_{2i}$, obtaining a convex and doubly desirable response, or the genotype will be classified as Group E if $\beta_{1i} > 1 > \beta_{2i}$, obtaining a concave and doubly undesirable response. When the hypothesis H0: $\beta_{1i} = \beta_{2i}$ is accepted, a single line segment is recognized to explain the response of the genotypes (βi common to the two straight lines segments). This model is given by $Y_{ij} = \alpha_i + \beta_i\ \mu_j + \delta_{ij} + e_{ij}$. The genotypes in this case are classified as Group B, C, or D. Groups are classified as B when common β_i is significantly different from 1 and $\beta_i > 1$; as C when $\beta_i = 1$; and as D when common β_i is significantly different from 1 and $\beta_i < 1$. The measurement of environmental quality μ_j is estimated simultaneously with the other regression parameters. The environment with $\mu_j > 0$ is considered favorable, and that with $\mu_j < 0$ is considered unfavorable.

Toler and Burrows (1998) describe the practical meanings of these five groups in the following manner: A is the convex and doubly desirable response, B is the simple linear response that is desirable only in high quality environments, C is the simple linear response that does not deviate from the mean response, D is the simple linear response that is desirable only in low-quality environments, and E is the concave and doubly undesirable response. A convex response or doubly desirable response pattern is therefore characterized when the genotype has low responsiveness in the unfavorable environments ($\mu_j < 0$) and comes to respond satisfactorily when these conditions become favorable ($\mu_j > 0$). A concave response or doubly undesirable response pattern, according to the classification of Toler and Burrows (1998), is characterized when the genotype is highly responsive to the environments of low quality and exhibits minimal response in environments with quality above these conditions.

2.5.2 | AMMI models

The descriptive model of the mean response of a genotype i, in an environment j, in the ***AMMI*** analysis (Zobel et al., 1988) is $Y_{ij} = \mu + g_i + e_j + \sum_{k=1}^{n} \lambda_k \gamma_{ik} \alpha_{jk} + \rho_{ij} + \varepsilon_{ij}$, which arises based on a traditional combined analysis model (μ is the overall mean; g_i is the genotype effect *i*; e_j is the effect of environment j; ε_{ij} is the effect of the mean experimental error of genotype *i* in environment *j*), for which the classic term of interaction of genotype i with environment *j*, normally denoted as ge_{ij}, is modeled as: $ge_{ij} = \sum_{k=1}^{n} \lambda_k \gamma_{ik} \alpha_{jk} + \rho_{ij}$, where $\rho_{ij} = \sum_{k=n+1}^{p} \lambda_k \gamma_{ik} \alpha_{jk}$, and λ_k's are the singular values relative, respectively, to the first n components retained by the method and the last components, from n+1 to p, which are not retained by the method. The new terms of this model result from what is called singular value decomposition (SVD) from the matrix of estimated interactions: $GE_{(ge)} = [ge_{ij}]$, with: $ge_{ij} = Y_{ij} - Y_i - Y_j + \bar{Y}$. From the SVD, the sum of squares of the interaction is $SSGE = \sum_{k=1}^{p} r\lambda_k^2$, k = 1, 2, ..., p, where p is the GE matrix rank, λ_k is obtained, which is the k-th singular value of GE (scalar); γ_{ik} is the element corresponding to the *i*-th genotype of the k-th singular vector relative to genotypes (γ_k); α_{jk} is the element corresponding to the *j*-th environment, in the *k*-th singular vector relative to environments (α_k); and r is the number of replications of the genotypes means in each environment. The method produces principal component scores of interactions for each genotype that reflect their contribution to the GE interaction. Thus, the genotype with the lowest score(s) in absolute value is the most stable.

2.5.3 | Annicchiarico Method

The method proposed by Annicchiarico (1992) adopts a reliability index of the risk of adoption of each genotype. The procedures for the calculations initially involve a transformation of the means of each cultivar in each environment in percentage of the mean of the environment. After that, the mean $\bar{Y}_i$ and the standard deviation S_i of the percentages of each cultivar across the environments are estimated. From these estimates, the reliability index I_i is obtained by means of the following estimator: $I_i = \bar{Y}_i - Z_{1-\alpha} S_i$, where I_i is the reliability index (%); $\bar{Y}_i$ is the mean of genotype i in percentage; $Z_{1-\alpha}$ is the quantile of standard normal distribution, in which the function of accumulated distribution reaches the percentile value 1-α; and S_i is the standard deviation of the percentage values. The reliability coefficient adopted was 75%, i.e., $\alpha = 0.25$. The greater this index, the lower the risk in adoption of the genotype. The greater the reliability index of the genotype, the lower the probability of lack of success.

3 | RESULTS

Cowpea yield was significantly affected by the genotype (strains of N_2-fixing bacteria and control treatments with native rhizobia) and environment and by the G×E interaction (Table 2; $P<.01$). This shows that the genotypes exhibited different responses in the environments analyzed (Table 3). This justifies the study of genotypic response to identify the magnitude of the G×E interaction in its adaptability and phenotypic stability.

The potential of adaptability and phenotypic stability of some strains was confirmed by the biometric techniques in the study: Toler, AMMI, and Annicchiarico. Detailed results as extracted fromanalyses of each one of the models and method are presented below.

TABLE 2 Summary of combined analysis of variance of the data on cowpea grain yield in accordance with genotypes (strains of nitrogen-fixing bacteria and control treatments with native rhizobia) and crop environments

Source of Variation	Degrees of Freedom	Yield (mean square)
Block (Environment)	21	179,182.9686**
Genotype (G)[a]	6	562,250.7518**
Environment (E)	6	7,375,475.8191**
G×E interaction	36	199,356.417**
Residual	126	61,161.9447
Coefficient of variation (%)	20.37	-

[a]Includes previously selected strains of nitrogen-fixing bacteria and control treatments with native rhizobia.
**Significant by the F test ($P < 0.01$).

TABLE 3 Cowpea grain yield obtained from genotypes (strains of nitrogen-fixing bacteria and control treatments with native strains) in each environment studied

Genotype[a]	**Environment [site]**						
	***E1* [*Lavras*]**	***E2* [*Presidente Olegário*]**	***E3* [*Bambuí*]**	***E4* [*Patos de Minas*]**	***E5* [*Lavras*]**	***E6* [*Luminárias*]**	***E7* [*Presidente Olegário*]**
	kg ha^{-1}						
S1 [UFLA 03-84]	1,407.55	843.99	1,735.19	1,815.63	1,193.19	571.16	719.46
S2 [BR3267]	1,339.28	1,305.71	1,332.25	1,632.81	813.11	281.37	781.93
S3 [INPA 03-11B]	1,656.49	1,483.82	2,252.39	1796,88	1,147.25	345.70	732.27
S4 [UFLA 03-153]	1,434.56	1,360.06	2,058.17	1445,32	523.39	233.20	826.95
S5 [UFLA 03-164]	1,451.69	1,416.21	2,126.17	1628,13	1,250.96	438.70	1,272.30
C6 [native rhizobia]	1,621.61	978.09	1,162.50	1,712.50	736.50	241.77	859.76
C7 [native rhizobia fertilized with mineral N]	1,581.91	1,266.65	2,238.15	1,660.94	1,403.58	339.90	1,021.98
Overall mean	1,499.01	1,236.36	1,843.54	1,670.31	1,009.71	350.26	887.51

[a]C6, native rhizobia; C7, native rhizobia fertilized with mineral N (urea); S1, S2, S3, S4, and S5, selected strains of nitrogen-fixing bacteria

3.1 | Toler models

The E1 (Lavras), E2 (Presidente Olegario), E3 (Bambui), and E4 (Patos de Minas) environments exhibited a positive environmental quality index (EQI) (Table 4), indicating a strong contribution to an increase in mean yield from the environment factor. For their part, E5 (Lavras), E6 (Luminarias), and E7 (Presidente Olegario) contributed to a decrease in the environmental mean; they were considered the most unfavorable environments (EQI of greatest negative values; Table 4).

TABLE 4 Cowpea grain yield, environmental quality index estimated by the Toler model (in accordance with crop environments), and mean percentage of yield in relation to the environmental mean and reliability index estimated by the Annicchiarico method in accordance with genotypes (strains of nitrogen-fixing bacteria and control treatments with native strains)

Environment	**Yield[a] kg ha^{-1}**	**Toler Model**	
		Environmental Quality Index[a] (Standard Error)	
E1 [Lavras]	1,499.01 C	285.1556 (40.8223)	
E2 [Presidente Olegário]	1,236.36 D	22.5045 (41.6917)	
E3 [Bambuí]	1,843.53 A	629.6795 (42.0872)	
E4 [Patos de Minas]	1,670.31 B	456.4556 (40.8567)	
E5 [Lavras]	1,009.71 E	-204.1422 (42.5948)	
E6 [Luminárias]	350.25 F	-863.6012 (42.9693)	
E7 [Presidente Olegário]	887.80 E	-326.0519 (42.6917)	
Genotype[d]		Annicchiarico Method	
		Reliability Index[b] (i)	**Mean percentage in relation to the environmental mean**
S1 [UFLA 03-84]	1,183.73 B	53.1264	103.89
S2 [BR3267]	1,069.49 B	69.0851	87.70
S3 [INPA 03-11B]	1,344.97 A	85.3843	107.87
S4 [UFLA 03-153]	1,125.94 B	51.7724	87.92
S5 [UFLA 03-164]	1,369.16 A	89.7759	116.66
C6 [native rhizobia]	1,044.67 B	55.2573	84.53
C7 [native rhizobia fertilized with mineral N]	1,359.01 A	86.8468	111.43
Overall mean	1,213.86	-	100.00

[a]Main effects of the "Genotype" factor are indicative of adaptability ($P < .01$).
[b]Level of significance adopted = .05.
[c]Mean values followed by the same letter in the column belong to the same group ($P < .01$).
[d]C6, native rhizobia; C7, native rhizobia fertilized with mineral N (urea); S1, S2, S3, S4, and S5, selected strains of nitrogen-fixing bacteria.

Fitting the uni- and bi-segmented models of Toler represented 99.71 and 97.55% of the G×E interaction, respectively. Thus, high-quality classification of the genotypes by the nonlinear protocol adopted is expected. Initially, the need or not for adoption of a bi-segmented model in detriment to the single segmented model (uni-segmented) was checked. Therefore, because $\beta_{1i} \neq \beta_{2i}$ (Table 5), the hypothesis that the response of S1 and S2 could be represented by a regression line was discarded. The two straight lines segment (bi-segmented)

model was most suitable for those genotypes; for the others, it was the uni-segmented model because the hypothesis mentioned was not rejected for them.

The responsiveness of the genotypes of unisegmented response decreased in the following order: S3 > S4 > C7 > S5 > C6 (see β, Table 6). According to the response patterns suggested by Toler and Burrows (1998), the genotypes were classified by the significance of the β estimates (Table 6) into Group A: S1 (of $\beta_{1i} < 1 < \beta_{2i}$); Group B: S3 and S4 ($\beta > 1$); Group C: S5, C6, and C7 ($\beta = 1$), and Group E: S2 ($\beta_{1i} > 1 > \beta_{2i}$).

TABLE 5 Estimates and standard errors (in parentheses) of $\beta_{2i} - \beta_{1i}$ of the bi-segmented model of Toler and test of hypothesis H0: $\beta_{1i} = \beta_{2i}$

Genotype[†]	**(β_{2i} - β_{1i}) Estimate**
S1 [UFLA 03-84]	0.71* (0.3291)
S2 [BR3267]	-0.89* (0.3358)
S3 [INPA 03-11B]	0.05 (0.3268)
S4 [UFLA 03-153]	0.28 (0.3268)
S5 [UFLA 03-164]	-0.14 (0.3849)
C6 [native rhizobia]	-0.63 (0.3488)
C7 [native rhizobia fertilized with mineral N]	0.09 (0.3448)

[a]C6, native rhizobia; C7, native rhizobia fertilized with mineral N (urea); S1, S2, S3, S4, and S5, selected strains of nitrogen-fixing bacteria
*Significant at the .05 probability level.

TABLE 6 Estimates and standard errors (in parentheses) of the regression coefficients β_{1i} and β_{2i} of the bi-segmented model and of the β of the uni-segmented model, both models of Toler, along with the tests of hypotheses for the equality of these parameters at 0 and 1, as well as classification of the Toler response pattern according to the models of the genotypes and tests performed

Genotype[a]	**Model**		**Response pattern**
	β_{1i} Estimate	β_{2i} Estimate	
Bi-segmented			
S1 [UFLA 03-84]	0.50[b,c] (0.1730)	1.30[b,c] (0.2152)	A
S2 [BR3267]	1.16[b,c] (0.1782)	0.42[b,c] (0.2175)	E
Uni-segmented	(β_i) Estimate		
S3 [INPA 03-11B]	1.25[b,c] (0.0916)		B
S4 [UFLA 03-153]	1.15[b,c] (0.0913)		B
S5 [UFLA 03-164]	0.94[c] (0.0911)		C
C6 [native rhizobia]	0.84[c] (0.0913)		C
C7 [native rhizobia fertilized with mineral N]	1.10[c] (0.0912)		C

[a]C6, native rhizobia; C7, native rhizobia fertilized with mineral N (urea); S1, S2, S3, S4, and S5, selected strains of nitrogen-fixing bacteria.
[b]Significantly different from 0 by the F test ($P < .05$).
[c]Significantly different from 1 by the F test ($P < .05$).

3.2 | AMMI model

Biplots resulting from analysis by the AMMI1 and AMMI2 models are presented in Figures 2 and 3, respectively. The first two singular axes related to the effects of the interaction captured 50.30 and 27.50% of the sum of squares of the genotype × environment interaction (SS_{GE}). Therefore, the model AMMI2 explains 77.80% of the SS_{GE}.The AMMI1 biplot contains the variation of the principal additive effect of the genotypes and environments and is represented on the horizontal axis. The variation of the multiplicative effects of the interaction is shown on the vertical axis. On the AMMI2 biplot, the multiplicative effects of the interaction containing the first two singular axes (principal singular vector [PSV]) are represented. Genotypes with PSV values near zero show stability to test environments.

Combinations of genotypes and environments with PSV scores of the same sign have specific positive interactions; combinations of opposite sign have specific negative interactions. In the AMMI2 biplot, stable genotypes and environments (with a small contribution to the SSGE) are those whose points are near the origin (i.e., with scores practically zero for the two interaction axes).

In general, the strains S3, S5, and C7 and the environments E1 (Lavras), E5 (Lavras), E6 (Luminarias), and E7 (Presidente Olegario) contributed little to the G×E interaction because they exhibited low score magnitudes on the interaction axis (Figure 2), which was confirmed in the AMMI2 biplot (Figure 3). This means that they are the most stable among those tested. An opposite situation was shown by the C6 genotype and by the environment E3 (Bambui) (Figures 2 and 3). There was possible adaptive synergy between the pairs (genotype-environment) S1-E5 (Lavras), S2-E1 (Lavras), and S4-E2 (Presidente Olegario) (Figure 3) and negative interaction of the S2 strain with the E3 environment (Bambui) (vectors pointing in opposite directions; Figure 3). The E6 (Luminarias) and E7 (Presidente Olegario) environments proved to be very discrepant from E3 (Bambui) (Figures 2 and 3).

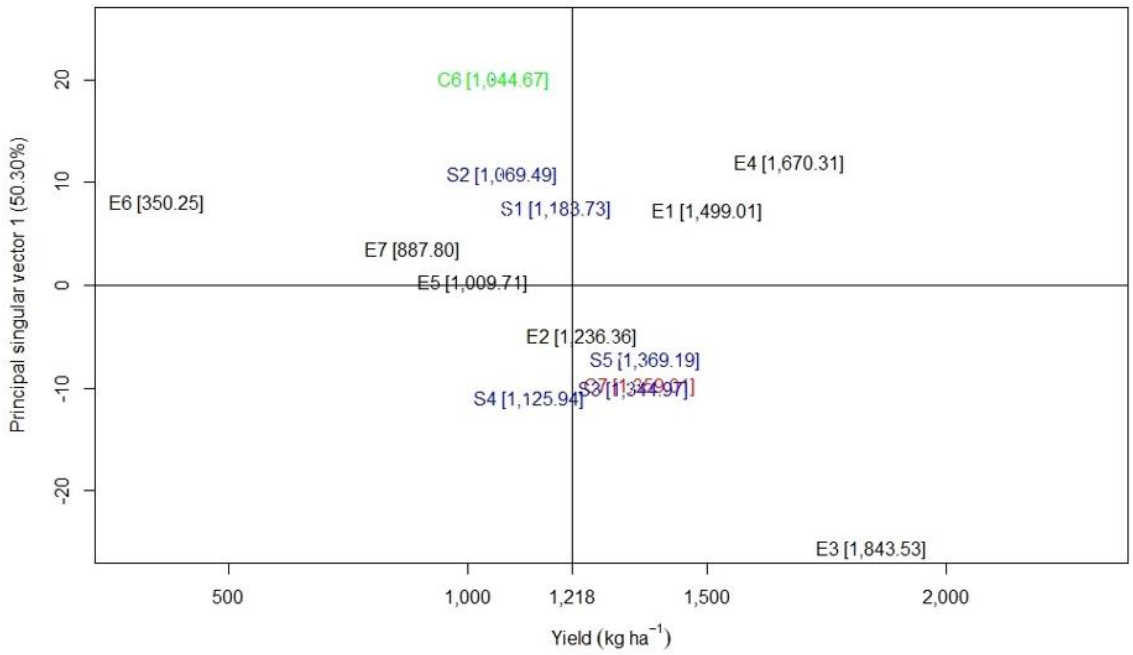

FIGURE 2 Additive Main Effects and Multiplicative Interaction (AMMI) 1 biplot for the data on cowpea obtained by genotypes (strains ofN_2-fixing bacteria: S1-UFLA 03-84, S2-BR3267, S3-INPA03- 11B, S4-UFLA 03-153, S5-UFLA 03-164, and C6-native rhizobia, and

a control fertilized with mineral N on native rhizobia, designated C7), and seven environments (E1, Lavras; E2, Presidente Olegario; E3, Bambui; E4, Patos deMinas; E5, Lavras; E6, Luminarias; and E7, Presidente Olegario), with their grain yield (in kg ha^{-1}) in brackets

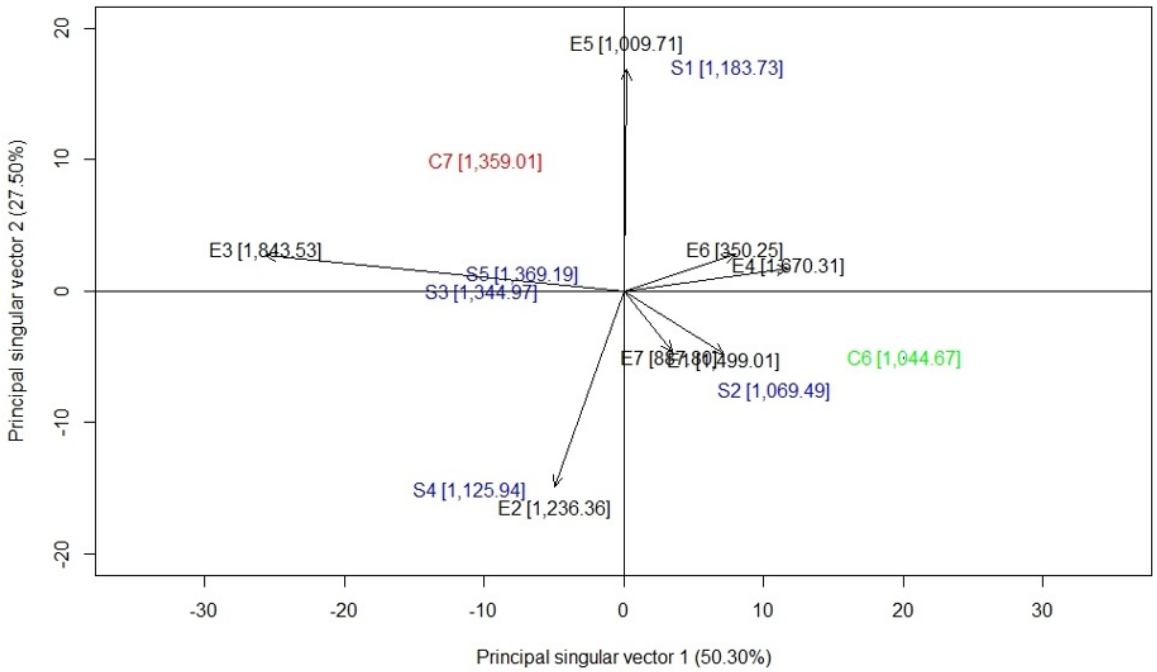

FIGURE 3 Additive Main Effects and Multiplicative Interaction (AMMI) 2 biplot by genotypes (strains of nitrogen-fixing bacteria: S1-UFLA 03-84, S2-BR3267, S3-INPA 03-11B, S4-UFLA 03-153, S5- UFLA 03-164, and C6-native rhizobia, and a control fertilized with mineral N on native rhizobia, designated C7) and seven environments (E1, Lavras; E2, Presidente Olegario; E3, Bambui; E4, Patos de Minas; E5, Lavras; E6, Luminarias; and E7, PresidenteOlegario), with their grain yield (in kg ha^{-1}) in brackets

3.3 | Annicchiarico method

The genotypes S3 (INPA 03-11B), S5 (UFLA 03-164), and C7 (native rhizobia fertilized with mineral N) were more adapted to the environments evaluated, achieving 7.87, 16.66, and 11.43% greater yield estimates, respectively, than the mean of 1,213.86 kg ha^{-1} of the study (Table 4). The reliability of adoption of these three genotypes exceeds 85%, nearing 90% if the S5 strain is used (Table 4). A high probability of lack of successwas registered if the native C6 genotype (reliability index = 55.25%) is adopted, which is confirmed by means of its yield, which was lower than that of the other genotype.

The same behavior of strains (genotypes) S1, S2, S3, S4, and S5 was found when all these biometric analyses were applied, excluding the controls (C6 and C7) (Supplemental Tables 1–3; Supplemental Figures 2 and 3).

4 | DISCUSSION

Biometric techniques are widely applied to plant Breeding for analysis and interpretation of genotype response under environmental interactions, without restrictions for use in microbiological studies. In spite of this possibility, only application of the AMMI modeling is found in the literature associated with study of the interaction of N_2-fixing bacterial strains in pots with a sterile solution (Gunnabo et al., 2019). It is encouraging to study the G×E interaction with grain yield results in the field, above all in Brazil, where there is high heterogeneity of soils and climate conditions. An important characteristic of the AMMI is the possibility of obtaining graphs of the principal components maintained in analysis of the model and graphs in relation to mean yield. However, the pattern of environmental response cannot be estimated directly from this model. Such information must be identified through other models, such as the Toler models.

In the present study, the Toler, AMMI, and Annicchiarico biometric techniques allowed identification of more adapted, responsive, and stable genotypes in relation to the most diverse environments. In agreement with Toler and Burrows (1998) and Ferreira et al. (2006), simultaneous use of these techniques aggregated information concerning the genotypes.

Significant contributions of the genotypes S3 (INPA 03-11B), S5 (UFLA 03-164), and C7 (native rhizobia fertilized with mineral N) were found. With different degrees of responsiveness, the S1 (UFLA 03-84), S2 (BR3267), S4 (UFLA 03-153), and C6 (native rhizobia) exhibited yield capacity below the overall mean, which translates to low adaptability to the environments.

Given the particular aspects of each of the methods of analysis used in the study, complementary information could be detected. By the Tolermodels, for example, a possible effect of the crop seasons was observed: The rainy crop season was the one with the greatest negative impact (negative EQI). This is due to excessive moisture during the crop cycle. When water availability exceeds 450 mm per crop cycle and with poor distribution in water supply at crucial stages, cowpea yield may be compromised (Oliveira et al., 2017). This occurred in E5 (Lavras), E6 (Luminarias), and E7 (Presidente Olegario) (Figure 1). Furthermore, in these last two environments, another aggravating factor was related to the high acidity of H+ and Al3+ of their soils (Table 1) because pH and aluminum saturation (m%) act both in plant development and in rhizobia– cowpea symbiosis as they interfere with nutrient availability for the symbionts (Ferreira, Bomfeti, Soares, &Moreira, 2012;Moreira, 2006). Specifically in these last two environments, under the conditions of the study, yield advantages of genotypes of Group A (such as S1 [UFLA 03-84 strain], with $\beta_{1i} < 1 < \beta_{2i}$) or Group B (such as strains S3 [INPA 03-11B] and S4 [UFLA 03-153], with $\beta_i > 1$) would not be expected; but rather, negative impacts under genotypes of Group E (such as S2 [BR3267] strain, with $\beta_{1i} > 1 > \beta_{2i}$). Further experiments in the same areas could confirm the relationships between edaphic and climatic conditions and strains.

According to the response patterns suggested by Toler and Burrows (1998), genotypes of Group A are those with a doubly desirable response pattern, characterized when the genotype has low responsiveness in the unfavorable environments and comes to respond satisfactorily when conditions become favorable. In other words, they are genotypes that are demanding in levels of environmental quality to express all their genetic yield potential. Rosse and Vencovsky (2000) argue that for genotypes of Group A to achieve this potential, the use of technology in the environment is necessary because under adverse conditions (e.g., without soil amendment in high-acidity soils, such as those found in E6 and E7), these materials show low responsiveness. Pattern B genotypes are like those of Group A in their ability to efficiently exploit favorable environments, although they experience the effects of

unfavorable environments at low intensity (Morais et al., 2008). The responses of Group E are concave, following a doubly undesirable pattern (Toler & Burrows, 1998). That means that the genotype belonging to this group is highly responsive to the environments of low quality and is not very responsive in environments with quality above these conditions.

The responses of S2 (BR3267) corresponded to the response expected from Group E. The yield of S1 (UFLA 03-84) from Group A (Toler & Burrows, 1998) was not diminished by the conditions of E6 and E7, but any possible yield increase in the favorable environments contributed less to its final mean value than in that of the S3 (INPA 03-11B) or S4 (UFLA 03-153) of Group B. Strains S3 and S4 expressed high yield even in lower-quality environments, achieving even more satisfactory levels in those with favorable characteristics; however, S4 stands out for adaptability due to possible intrinsic characteristics (Table 4).

The yield limitation of S1 (UFLA 03-84) does not exclude its use as an inoculant. Lacerda et al. (2004), Rufini et al. (2014), Soares et al. (2006), and Ferreira et al. (2019) observed yields of S1 in the order of 1,200 kg ha^{-1} in other environments in the state of Minas Gerais. Mean values representative of this strain ranged from 690 kg ha^{-1} in thesemi-arid regions of Piaui (Costa et al., 2011, 2014; Ferreira et al., 2013) to 797 kg ha^{-1} in Maranhao (Farias et al., 2016a, 2016b); these values were always above the mean yield of the crop in Brazil in 2018/2019 (mean, 504 kg ha^{-1}) (Conab, 2019)] even though they were not as expressive as those of MinasGerais. Possibly because of this capacity of providing above-average yields at costs lower than production costs with mineral fertilizer, S1 (UFLA 03-84) is among the elite strains approved by MAPA for cowpea.

The lower performance of the C6 (native rhizobia) was foreseen. This result is consistent with other reports from the literature (Farias et al., 2016a, 2016b; Ferreira et al., 2013, 2019; Lacerda et al., 2004; Soares et al., 2006). Although it could have an advantage over the other genotypes because of inhabiting environments to which the others were introduced, C6 did not stand out. It responded in an unstable way to environmental variations, it proved to be active in root infection but benefitted little from environmental stimuli, and it was the genotype with lowest responsiveness among those of Group C, with lowest β (Table 6). The native rhizobia communities of the soil alone are not always sufficient for establishing the symbiosis that contributes significantly to an increase in cowpea yield; the use of inoculants containing strains that are more efficient in the BNF process is necessary (Moreira, 2006). The strains INPA 03-11B (S3) and UFLA 03-164 (S5) exhibited this capacity.

Some strains seem to exhibit adaptation to specific environments (interpretation by the AMMI model). The pH of E2 (Presidente Olegario), which is slightly alkaline (pH 6.5), is similar to the conditions of origin of the S4 strain (UFLA 03-153) that was isolated from soil with pH 6.7 (Soares et al., 2014). This explanation applies to adaptation of the S1 strain (UFLA 03-84) to the E5 environment (Lavras), with pH and Al^{3+} saturation equivalent to those of the environment from which this strain was isolated (pH 6.1; m% = 0) (Soares et al., 2014). Coming from the semi-arid Northeast region (Leite et al., 2017; Martins, Rumjanek, & Neves, 1997, 2003), where the main limitation is water supply, S2 (BR3267) found better conditions for establishing itself and for BNF in the E1 environment (Lavras), which had greatest rainfall restriction (Figure 1) and was not irrigated.

The potential negative interaction of S2 (BR3267) in E3 (Bambui) detected by the AMMI model confirms that the predictions of the Toler model, which associate a bisegmented response to S2, are not very responsive in environments with favorable qualities. Thus, S2 was not greatly benefitted, for example, by the fertility of E3 (Table 1). There is also the hypothesis that substances such as antibiotics produced by populations of microorganisms of this environment may have interfered in the cowpea–S2 strain symbiosis. Oliveira-Longatti et al. (2014) reported that BR3267/S2 has low resistance to erythromycin, ampicillin, rifamycin, and amoxicillin. Consequently, S2 would have to overcome this chemical stress to

colonize plant roots even more. Tolerance to antibiotics is of particular importance in all soil conditions because it is one of the mechanisms that rhizobia can use to overcome antagonism exercised by other organisms in the soil (Barret, Morrissey, & O'Gara, 2011; Florentino, Sousa, Silva, Silva, & Moreira, 2010).

The good performance of S3 (INPA 03-11B) corroborates its approval by MAPA as an inoculant strain for cowpea. The good response of S5 (UFLA 03-164), a strain still in the selection phase, is surprising. Even exposed to some stressful environmental conditions (responses strongly shown in E6 [Luminarias] and E7 [Presidente Olegario]), these strains maintained high and constant yields, always above the mean of the environment. Therefore, it is not surprising that the reliability of success in adoption of these strains came close to 90% in the Annicchiarico method. Together, these strains obtained mean yields near 1,360 kg ha^{-1}. This represents 12% more than the overall mean of grain yield in the study (1,213.86 kg ha^{-1}); 30% more than the mean of the C6 (1,044.67 kg ha^{-1}), which was considered the control genotype; and 270% more than the Brazilian mean of 504 kg ha^{-1} achieved by the crop in 2018/2019 (Conab, 2019). These values are equivalent to obtaining 2.45, 5.25, and 14.27 60-kg bags of grain more per hectare, respectively. These increases are the aim of any grower, above all because they are comparable to results with the application of mineral N (C7), which would increase cowpea production costs. All these reasons therefore indicate that the strain UFLA 03-164 (S5) has potential for approval from MAPA and for use by Brazilian producers and consumers of inoculants.

5 | CONCLUSIONS

Native rhizobia respond in an unstable way to environmental variations, responding little to increases in environmental quality.

By the Toler model, the strains UFLA 03-84 and BR3267 follow the bi-segmented response pattern of Groups A and E, respectively. The INPA 03-11B and UFLA 03-153 strains follows the Group B pattern, and UFLA 03-164 follows the Group C pattern.

The strains INPA 03-11B and UFLA 03-164 are the most adapted to the environments evaluated, resulting in yields equivalent to those of the control fertilizedwithmineral N.

The strain UFLA 03-164 of *B. uaiense* has potential for approval as a commercial inoculant for cowpea.

The Toler and AMMI models and the Annicchiarico method estimate the effects of adaptability and phenotypic stability of strains of N_2-fixing bacteria symbionts for inoculation on cowpea. It is recommended that these biometric techniques be used simultaneously for approval of new strains.

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AUTHOR CONTRIBUTIONS.

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Bruno Lima Soares: Conceptualization; Formal analysis; Investigation; Methodology; Resources; Validation; Writing-review & editing.
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CONFLICT OF INTEREST. The authors declare no conflict of interest.

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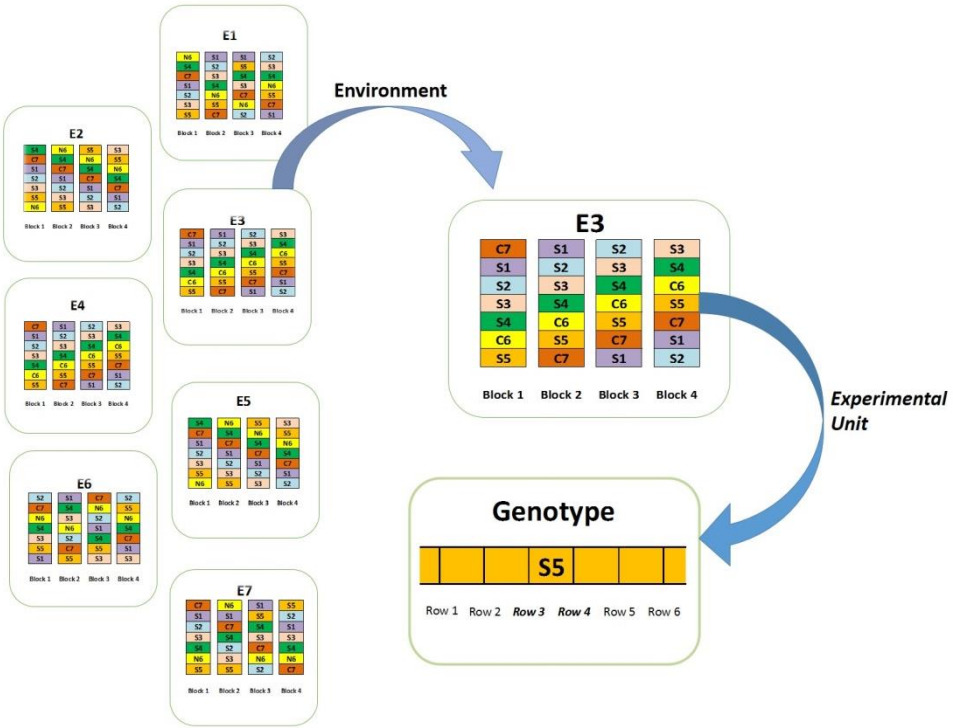
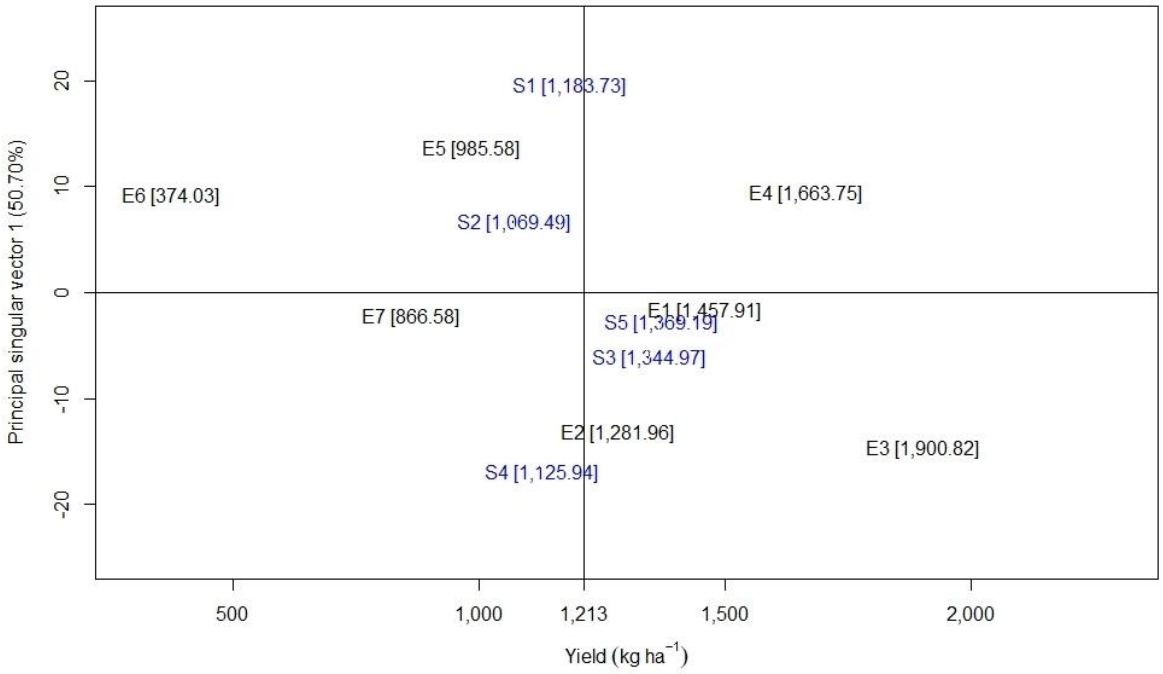
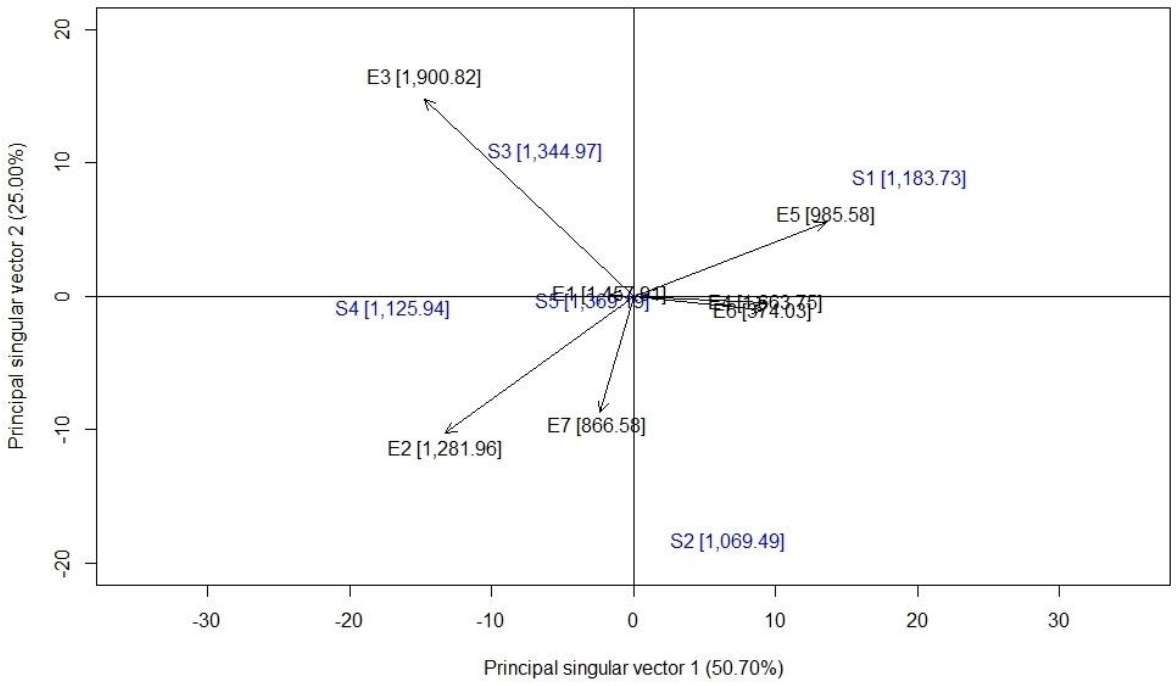
SUPPLEMENTAL MATERIAL



SUPPLEMENTAL FIGURE 1. Schema of the experimental design (randomized blocks, with four replications and the seven treatments) in the seven field experiments that were conducted at different locations (environments). Treatments were: S1 [UFLA 03-84], S2 [BR3267], S3 [INPA 03-11B], S4 [UFLA 03-153] and S5 [UFLA 03-164] strains, C6 [native rhizobia populations] and C7 [native rhizobia populations + 70 kg N-urea ha^{-1}].



SUPPLEMENTAL FIGURE 2. AMMI1 biplot for the data on cowpea obtained by five strains of nitrogen-fixing bacteria (S1-UFLA 03-84, S2-BR3267, S3-INPA 03-11B, S4-UFLA 03-153, and S5-UFLA 03-164) and seven environments (E1-Lavras, E2-Presidente Olegário, E3-Bambuí, E4-Patos de Minas, E5-Lavras, E6-Luminárias, and E7-Presidente Olegário), with their grain yield (in kg ha^{-1}) in brackets in the legend.



SUPPLEMENTAL FIGURE 3. AMMI2 biplot of the additive main effects and multiplicative interaction (AMMI) for strains of nitrogen-fixing bacteria (S1-UFLA 03-84,

S2-BR3267, S3-INPA 03-11B, S4-UFLA 03-153, and S5-UFLA 03-164) and seven environments (E1-Lavras, E2-Presidente Olegário, E3-Bambuí, E4-Patos de Minas, E5-Lavras, E6-Luminárias, and E7-Presidente Olegário), with their grain yield (in kg ha^{-1}) in brackets in the legend.

SUPPLEMENTAL TABLE 1. Cowpea grain yield, environmental quality index estimated by the Toler model (in accordance with crop environments) and mean percentage of yield in relation to the environmental mean and Reliability Index estimated by the Annicchiarico method (in accordance with five genotypes of nitrogen-fixing bacteria).

Environment	**Yield[a] kg ha^{-1}**	**Toler Model**	
		Environmental Quality Index[a] (Standard Error)	
E1 [Lavras]	1,457.91 C	239.2542 (48.4677)	
E2 [Presidente Olegário]	1,281.96 D	63.2963 (49.3349)	
E3 [Bambuí]	1,900.82 A	682.1603 (49.9817)	
E4 [Patos de Minas]	1,663.75 B	445.0903 (48.4289)	
E5 [Lavras]	985.58 E	-233.0817 (50.0149)	
E6 [Luminárias]	374.03 F	-844.6000 (50.6546)	
E7 [Presidente Olegário]	866.58 E	-352.0837 (49.9046)	
Genotype[d]		Annicchiarico Method	
		Reliability Index[b] (i)	**Mean percentage in relation to the environmental mean**
S1 [UFLA 03-84]	1,183.73 B	56.3068	102.70
S2 [BR3267]	1,069.49 B	67.8786	87.13
S3 [INPA 03-11B]	1,344.97 A	85.1603	107.03
S4 [UFLA 03-153]	1,125.94 B	51.8470	87.22
S5 [UFLA 03-164]	1,369.16 A	88.0004	115.83
Overall mean	1,218.13	-	100.00

[a]Main effects of the "Genotype" factor are indicative of adaptability ($P < .01$).
[b]Level of significance adopted = .05.
[c]Mean values followed by the same letter in the column belong to the same group ($P < .01$).
[d]S1, S2, S3, S4, and S5, selected strains of nitrogen-fixing bacteria.
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SUPPLEMENTAL TABLE 2. Estimates and standard errors (in parentheses) of $\beta_{2i} - \beta_{1i}$ of the bi-segmented model of Toler and test of hypothesis H0: $\beta_{1i} = \beta_{2i}$

Genotype[†]	**(β_{2i} - β_{1i}) Estimate**
S1 [UFLA 03-84]	0.71* (0.3466)
S2 [BR3267]	-0.89* (0.3537)
S3 [INPA 03-11B]	0.05 (0.3442)
S4 [UFLA 03-153]	0.28 (0.3442)
S5 [UFLA 03-164]	-0.14 (0.3421)
S5 [UFLA 03-164]	-0.14 (0.3421)

[a]S1, S2, S3, S4, and S5, selected strains of nitrogen-fixing bacteria
*Significant at the .05 probability level.

SUPPLEMENTAL TABLE 3. Estimates and standard errors of the regression coefficients β_{1i} and β_{2i} of the bi-segmented model and of the β of the uni-segmented model, both Toler models, along with the tests of hypotheses for the equality of these parameters at 0 and 1, as well as classification of the Toler response pattern according to the models of the strains of nitrogen-fixing bacteria and tests performed.

Genotype[a]	**Model**		**Response pattern**
	β_{1i} Estimate	β_{2i} Estimate	
Bi-segmented			
S1 [UFLA 03-84]	0.51[b,c] (0.1809)	1.22[b,c] (0.1973)	A
S2 [BR3267]	1.23[b,c] (0.1810)	0.33[b,c] (0.2034)	E
Uni-segmented	(β_i) Estimate		
S3 [INPA 03-11B]	1.25[b,c] (0.0873)		B
S4 [UFLA 03-153]	1.16[b,c] (0.0874)		B
S5 [UFLA 03-164]	0.93[c] (0.0827)		C

[a]S1, S2, S3, S4, and S5, selected strains of nitrogen-fixing bacteria.
[b]Significantly different from 0 by the F test ($P < .05$).
[c]Significantly different from 1 by the F test ($P < .05$).

ARTICLE II

Selection of elite *Rhizobium* strains by biometric techniques for inoculation in common bean

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Abstract

Variation in plant responses to inoculation with rhizobia strains in different environments can be analyzed with the aid of biometric techniques. The Annicchiarico methodology and the Additive Main Effects and Multiplicative Interaction Analysis (AMMI) and Toler models are here applied to the genotype × environment (GE) interaction to determine the adaptability and phenotypic stability of N_2-fixing bacteria in symbiosis with common bean (*Phaseolus vulgaris* L.) in environments with high, moderate, and low acidity and with deficiencies or excessive concentrations of nutrients. A randomized block design was used in eight environments, with four replications and seven bacterial genotypes, including two controls with native soil bacterial communities (with or addition without mineral-N fertilization). Individual and combined analyses of variance and biometric analyses were conducted on the yield values. CIAT899, UFLA02-127, and native rhizobia were the most stable genotypes in relation to environments (AMMI models). CIAT899 and native rhizobia are the least adapted (Toler models), whereas the genotype UFLA02-127 has the best response to the environments (Toler models). The Annicchiarico method showed that the reliability of adoption of UFLA02-127 and native rhizobia fertilized with mineral N exceeds 85%, indicating high

probability of increase in common bean yield if adopted in the field. Conversely, native rhizobia have <65% reliability.

Abbreviations: AMMI. Additive Main Effects and Multiplicative Interaction Analysis; *C6*. native rhizobia; *C7*. native rhizobia fertilized with mineral nitrogen (urea); CFU. colony-forming units; E. environment; EQI. Environmental Quality Index; G. genotype; GE interaction. genotype × environment interaction; MAPA. National Supply Company Brazilian Ministry of Agriculture (Ministério da Agricultura, Pecuária e Abastecimento); S. strain; SS_{GE}. sum of squares of the interaction; SVD. singular value decomposition.

Core Ideas

- This is a pioneering field study with three biometric techniques for *Rhizobium* selection.
- Five previously selected *Rhizobium* sp. strains were evaluated in eight environments.
- *Rhizobium* strain UFLA02-127 had the highest adaptability and phenotypic stability.
- Yields from all UFLA strains were equivalent to yields from mineral-N fertilizer.

1 | INTRODUCTION

Studies in the Amazon have indicated a wide diversity of rhizobia with the ability to tolerate stresses predominant in tropical soils (Ferreira et al., 2012; Guimarães et al., 2012; Oliveira-Longatti et al., 2013, 2014; Soares et al., 2014). Furthermore, *Rhizobium* strains isolated from the Brazilian Amazon region have shown the capacity for biological N_2 fixation with leguminous species (Costa et al., 2019; Ferreira et al., 2019; Guimarães et al., 2015; Nogueira et al., 2017; Oliveira et al., 2017, 2020; Oliveira, Ferreira, et al., 2018; Oliveira, Soares, et al., 2018; Oliveira-Longatti et al., 2013; Pádua Oliveira et al., 2017; Soares et al., 2006; 2014; Zilli et, al.,2009) and, for that reason, have received special attention in studies directed to the production of inoculants. In addition, attention to inoculant production has increased due to the possible adverse effect of chemical fertilizers on the environment and their production costs.

Nevertheless, various factors can affect the efficiency of symbiotic rhizobia and legume species under field conditions. Some of them are intrinsic to the bacterial strain and plant species, whereas others are extrinsic, that is, adaptability of the rhizobia and legume species to biological, physical, and chemical attributes in the environment. Predominant characteristics of tropical conditions are soil acidity; deficiency in nutrients such as calcium, phosphorus, and magnesium; toxic concentrations of sulfur, manganese, copper, and aluminum; and climate with high temperatures and wide amplitude in rainfall amounts (Ferreira et al., 2012; Graham et al., 1994; Hungria & Vargas, 2000; Moreira et al., 2006; Oliveira et al., 2017; Pádua Oliveira et al., 2017; Raposeiras et al., 2002; Rufini et al., 2011; Ruschel & Reuszer, 1973; Tsai et al., 1993). As these situations can have mainly negative effects on bacterial performance, accurate selection of strains more adapted to the intended environment is necessary. This selection occurs through testing, by introducing these isolates into diverse environmental (soil and climate) conditions, and conclusive interpretation of these tests regarding the response pattern must also consider the interaction of these genotypes with environments (GE interaction) (Ferreira et al., 2006; Morais et al., 2008; Wu, R. & O'Malley, 1998).

Some bacterial genotypes may exhibit similar results in a wide range of environments (soil and climate) and results which are similar to their response in environments of isolation

or in those with properties conducive to their good performance. Their responses may likewise differ drastically in environments to which they are not yet adapted or that have antagonistic characteristics, exhibiting the intensity of the effects of the GE interaction (Ferreira et al., 2006; Saltz et al., 2018; Stamps & Frankenhuis, 2016). Seeking to evaluate the effect of this GE interaction, the study of adaptability and phenotypic stability of bacterial genotypes is recommended.

There are techniques widely used in the literature on plant breeding (Annicchiarico, 1992; Toler & Burrows, 1998; Zobel et al., 1988) that can also be successfully used in the analysis and interpretation of GE interaction for microbiological genotypes (Gunnabo et al., 2019, 2020; Oliveira et al., 2020). Such tools allow selection of plant or microbial genotypes according to the type of response and pattern desired, preferring those genotypes that respond positively to the specific or broad environmental variations; these tools also allow the environments to be stratified regarding the effect obtained from each microorganism (Colombari Filho et al., 2013; Ferreira et al., 2006; Gunnabo et al., 2019, 2020; Oliveira et al., 2020), that is, environments with better response to inoculation can be selected.

If the bacterial genotype has satisfactory adaptability and phenotypic stability, it is qualified as promising for use as an inoculant. By determining responsiveness in a positive interaction with favorable environments, it is even possible to distinguish a good genotype from an excellent one (Ferreira et al., 2006; Gunnabo et al., 2019, 2020; Oliveira et al., 2020). Specifically, in determination of symbiosis with common bean (*Phaseolus vulgaris* L.), the response pattern of strains of N_2-fixing bacteria can direct introduction of the strains into compatible environments, favoring an increase in crop yield. Adoption of inoculants allows low investment in inputs and may partially or totally replace mineral nitrogen fertilization as, for instance, in cowpea [*Vigna unguiculata* (L.) Walp.], with strains selected by biometric techniques (Oliveira et al., 2020). Similar studies can be carried out with *Rhizobium* spp. in symbiosis with other leguminous species, as in this study. With this information, it will be possible to advance the process of selecting strains of symbionts with beneficial effects to common bean. Therefore, biometric techniques were applied to evaluate the adaptability and phenotypic stability of pre-selected strains and of native bacterial communities that nodulate common bean in environments with high, moderate, and low acidity and with deficiencies or excessive concentrations of nutrients in different tropical Oxisols.

2 | MATERIALS AND METHODS

2.1 | Environmental characterization

In the 2010 dry crop season, which consisted of sowing in February and March, experiments EI (in Presidente Olegário, MG), E2 (in Patos de Minas, MG), and E3 (in Bambuí, MG) were set up. In April, in the winter crop season of the same year, experiments E4 (in Patos de Minas, MG) and E5 (in Pitangui, MG) were set up. The last three experiments, E6 (in Lavras, MG), E7 (in Luminárias, MG), and E8 (in Guarda-Mór, MG), were set up in November and December 2010, in the 2010/2011 rainy crop season.

The environments had soils with high (E1 and E7), moderate (E2, E3, E4, E5, and E6), or low (E8) active acidity, some of which were less saturated in exchangeable bases, such as calcium and magnesium, and highly saturated with aluminum (E1and E7) or with high concentrations of sulfur, manganese, and copper (E2). A broader characterization of these environments, with specifications of fertilization and crop history is shown in Table 1. A summary of the main climate occurrences during the time of the experiments is shown in Figure 1.

TABLE 1. Chemical characteristics, geographic coordinates, and altitude of the environments studied, with the respective information regarding crop season/year, preceding crop, and fertilization adopted

Characteristic	Environment (Location)								
	Unit	E1 (Presidente Olegário)	E2(Patos de Minas)	E3 (Bambuí)	E4(Patos de Minas)	E5 (Pitangui)	E6 (Lavras)	E7 (Luminárias)	E8 (Guarda-Mór)
pH	–	**4.8L** [a]	5.5 G	6.0 G	6.0 G	5.5 G	6.0 G	**4.8 L**	6.2 H
P	mg kg^{-1}	1.7 VL	56.4	260.0 VG	35.7 VG	14.9 G	3.1 VL	1.2 VL	2.0
K	mg kg^{-1}	41.0 M	83.0 G	374.0 VG	75.0 G	181 VG	51.0 M	62.0 M	34.0 L
Ca	mg kg^{-1}	**1603 L**	360.7 M	20084.2 VG	360.7 M	340.7 M	380.7 M	**40,1 VL**	681,4 G
Mg	mg kg^{-1}	**50.2 L**	11.5 M	138.1 G	100.4 M	87.9 M	62.8 M	**25.1 L**	414.3 VG
Al	$cmol_c$ dm^{-3}	**0.8 M**	0.1 VL	0.1 VL	0.1 VL	0.1 VL	0.0 VL	**0.7 M**	0.0 VL
H + Al	$cmol_c$ dm^{-3}	**13.1 VH**	0.3 VL	2.9 M	5 M	2.3 L	2.1 L	**6.3 H**	2.9 M
SB	$cmol_c$ dm^{-3}	2.1 M	2.7 M	12.5 VG	2.8 M	2.9 M	2.5 M	**0.6 VL**	6.8 VG
T	$cmol_c$ dm^{-3}	15.0 G	9.0 G	15.4 VG	7.8 G	5.2 M	4.6 M	6.9 G	9.7 G
t	$cmol_c$ dm^{-3}	**2.1 L**	2.8 M	12.6 VG	2.9 M	3.0 M	2.5 M	**1.3 L**	6.8 G
m	%	**38 M**	3.6 VL	0.8 VL	3.4 VL	3.4 VL	0.0 VL	**55.6 H**	0.0 VL

Characteristic	Environment (Location)								
	Unit	**E1 (Presidente Olegário)**	**E2(Patos de Minas)**	**E3 (Bambuí)**	**E4(Patos de Minas)**	**E5 (Pitangui)**	**E6 (Lavras)**	**E7 (Luminárias)**	**E8 (Guarda-Mór)**
V	%	**8.7 VL**	74.6 G	81.1 VG	35.6 L	55.3 M	55.0 M	**8.2 VL**	70.1 G
OM	%	4.6 G	3.6 M	0.8 VL	4.4 G	2.2 M	2.9 M	4.0 M	5.1 G
Zn	mg kg^{-1}	1 M	3.9 H	15.1 H	6.9 H	4.1 H	1.5 M	0.3 VL	0.8 L
Fe	mg kg^{-1}	1.0 VL	21.1 M	28.3 M	32.7 G	80.9 H	75.2 H	36.9 G	45.0 H
Mn	mg kg^{-1}	1 VL	**137.9 H**	**44.8 H**	**96.3 H**	**31.5 H**	12.0 H	3.2 L	5.2 M
Cu	mg kg^{-1}	1 M	**13.4 H**	**5.8 H**	**10.9 H**	1.2 M	**1.4 H**	1.7 G	0.8 M
B	mg kg^{-1}	0.1 VL	0.1 VL	0.3 L	0.3 VL	0.1 VL	0.1 VL	0.2 L	0.2 L
Sulfur	mg kg^{-1}	13.8	80.0	8.4 M	7.7 M	5.0	**11.8 H**	8.9 M	12.3
Geographic coordinates	–	-18.40806; −46.43721	-18.46330; −46.43086	-20.03172; −46.01053	–18.53975; −46.45979	–19.71308; −44.89134	–21.22517; −44.97256	–21.53309, −44.96174	-17.91229; −47.16348
Altitude	m	591	833	706	833	709	920	957	616

Characteristic	Environment (Location)								
	Unit	**E1 (Presidente Olegário)**	**E2(Patos de Minas)**	**E3 (Bambuí)**	**E4(Patos de Minas)**	**E5 (Pitangui)**	**E6 (Lavras)**	**E7 (Luminárias)**	**E8 (Guarda-Mór)**
Crop season/ Year	–	2010 Dry	2010 Dry	2010 Dry	2010 autumn/winter	2010 autumn/winter	2010/11 Rainy	2010/11 Rainy	2010/11 Rainy
Preceding crop	–	Brachiaria	Various crops	Deactivated garden crops	Various crops	Various crops	Maize	Clean field	Maize
BF[b]: P_2O_5	kg ha^{-1}	80	70	70	70	70	70	70	105
BF[c]: K_2O	kg ha^{-1}	20	20	20	40	40	20	20	35

Note. SB: sum of exchangeable bases, T: cation exchange capacity at pH 7, m: exchangeable aluminum saturation, V: base saturation, OM: organic matter, BF: base fertilization, L: low, G: good, H: high, VL: very low, VG: very good, M: medium, VH: very high, according to Ribeiro et al. (1999).

[a] Stress conditions highlighted in bold.

[b] Source: Triple superphosphate.

[c] Source: Potassium chloride.

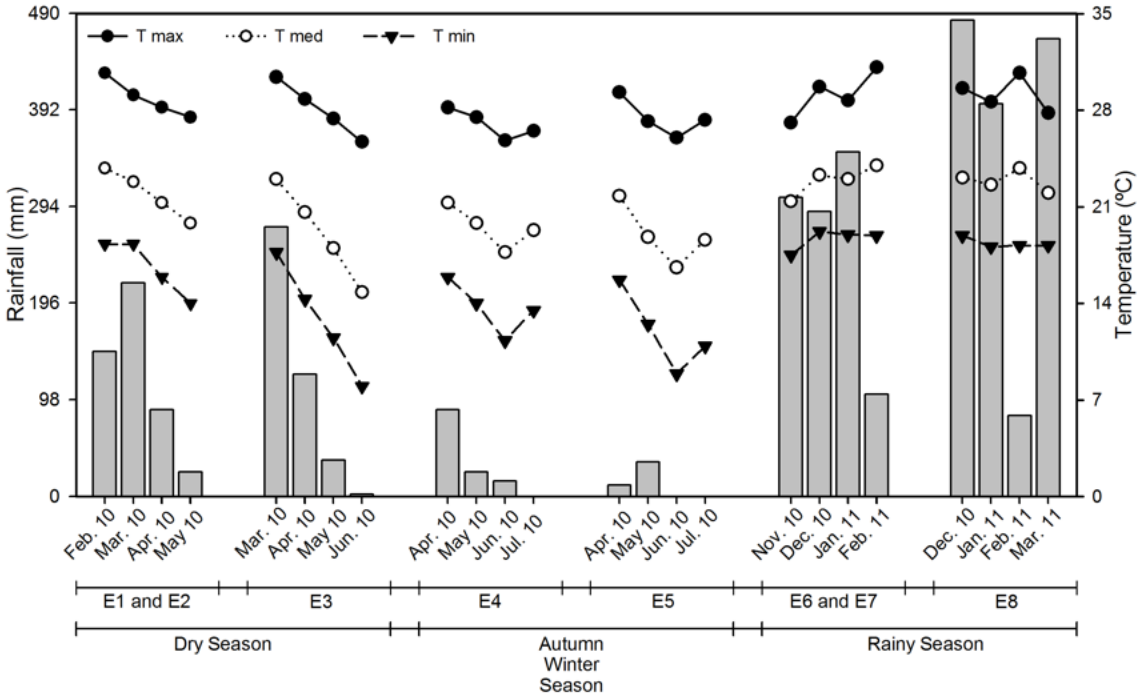

FIGURE 1 Monthly variation of the maximum, mean, and minimum temperatures and of rainfall in environments: E1(Presidente Olegário), E2 (Patos de Minas), E3 (Bambuí), E4 (Patos de Minas), E5 (Pitangui), E6 (Lavras), E7 (Luminárias), and E8 (Guarda-Mór) during the experimental period

2.2 | Genotypic characterization

The S1 genotype consisted of the strain CIAT899 of *Rhizobium tropici* (Graham et al., 1994), approved by the Brazilian Ministry of Agriculture (Ministério da Agricultura, Pecuária e Abastecimento) for manufacture of commercial inoculants for common bean seeds. The other strains inoculated - UFLA02-100 (S2), UFLA04-195 (S3), UFLA02-68 (S4), and UFLA02-127 (S5) – also belong to the *Rhizobium* genus (all *Rhizobium* sp.). The four UFLA strains belong to the SBMPBS/UFLA collection (Biology, Microbiology, and Biological Processes Sector collection of the Federal University of Lavras) and were isolated by those responsible for the lab. CIAT899 was provided by Esperanza Martinez-Romero, who described this species. They were isolated from acid soils with high Al content from the Brazilian Amazon; high acidity and high Al content stresses predominate in tropical soils. In addition to tolerance to acidity, the strains have high competitive ability with the already established strains and high N_2 fixation capacity (Nogueira et al., 2017; Soares et al., 2006, as described in Oliveira, Soares, et al., 2018; Pádua Oliveira et al., 2017). The C6 genotype was considered a control treatment because it consisted of native strains from the environments tested. Experiments were conducted to estimate the most probable number of colony-forming units (CFU) of native nodular N_2-fixing bacteria at the 0-to-20-cm soil depth of each experimental area, according to methods described in Soares et al. (2006). Numbers of around 10^4 CFU per gram of soil were obtained in the environments. Native populations fertilized with 80 kg N-urea ha^{-1} received the denomination C7, this being a second control treatment inserted within the previously mentioned genotypes. The Brazilian Ministry of Agriculture (Ministério da Agricultura, Pecuária e Abastecimento [MAPA]) guidelines, given by Normative Instruction (IN 13) of 24 Mar. 2011, which defines methodologies for approval of inoculants for common bean, recommend that the strains be compared to nitrogen fertilization at the dose of 80 kg N-

urea ha^{-1} (applied in part at sowing and in part as topdressing). We consider this nitrogen content in urea, which has 45% N in its composition. That is, we applied approximately 178 kg ha^{-1} of urea in treatment C7 (divided at sowing and as topdressing as a crop management practice).

2. 3 | Experimental design and crop treatments

A randomized block statistical design was always used, with four replications of each one of the previously described genotypes. In the eight environments, the experimental unit (24 m^2) consisted of six 4-m length rows, spaced at 1.0 m, and the four center rows were used for data collection. Soil tillage was through plowing, as well as two passes with a disk in environments E6 and E7. The doses of phosphorus (source – single superphosphate) and of potassium (source – potassium chloride) applied (Table 1) were defined according to technology levels, as proposed by Ribeiro et al. (1999). In all experiments, the common bean cultivar used was BRSMG Majestoso, which was manually sown immediately after inoculation. A sowing density of 15 seeds per linear meter was adopted.

Soil amendments were not made in any of the eight environments. Crops were produced under the condition of low use of inputs, without use of irrigation or application of agricultural chemicals in E1, E2, E6, E7, and E8. Pest control was not necessary in any of the environments. Manual weeding was performed in the experiments whenever necessary. In E3, E4, and E5, the weeding was complementary to application of a mixture of 0.5 L of fomesafen (Flex) + 1.0 L of bentazon + imazamox (Amplo) + 1.0 L of fluazifop-butyl (Fusilade) per hectare. Only E4 and E5 received constant sprinkler irrigation throughout the crop cycle, due to the requirements of the growing season.

2.4 | Preparation of inoculants

Inoculants containing the strains S1, S2, S3, S4, and S5 were prepared in peat sterilized in an autoclave at the proportion of 3:2 (w:v) of peat and 79 culture medium at 28 °C and constant shaking (110 rpm) for 48 h (log phase of the bacterial growth), as suggested by Soares et al. (2006) and Ferreira et al. (2009). The quality of the inoculant was monitored through CFUs, meeting the requirements of minimum legal number of viable cells, around 10^9 CFU of *Rhizobium* per gram of inoculant at sowing (Brasil, 2011). The resulting material was used at the rate of 100 g per kilogram of seed.

2.5 | Statistical and biometric analyses

At grain maturity, the yield (kg ha^{-1}) of each environment was obtained from the total weight of the grain produced in the area of the plot used for data collection, adjusted to moisture content of 130 g kg^{-1}. Individual analysis of variance was performed with the Sisvar 5.7 software (Ferreira, 2019) after the normality test (Shapiro–Wilk test) and the homoscedasticity test (Bartlett test) on the residues of the models through the R software (R Development Core Team, 2019). After that, combined analysis of variance of the data was performed, observing the homogeneity of the residual mean squares. The mean value for the GE interaction was calculated from the four replications of grain yield of each genotype per environment. From this information, the adaptability and phenotypic stability parameters were estimated according to the methodology and models described below.

2.5.1 | *Annicchiarico method*

The confidence index of Annicchiarico (Annicchiarico, 1992; Delgado et al., 2019), regarding the risk of adoption of each genotype, was computed. The higher this index, the lower the risk of adoption of the genotype and the probability of failure.

2.5.2 | *AMMI model*

After that, the Additive Main Effects and Multiplicative Interaction Analysis (AMMI) (Gauch et al., 2008; Zobel et al., 1988) was applied, which is a technique widely reported in the literature on plant breeding for analysis and interpretation of the genotype by interactions in the environment, and also successfully used in microbiological studies in pots with solution (Gunnabo et al., 2019, 2020) and in the field (Oliveira et al., 2020). Its main aim is to reduce the multidimensional patterns of interaction into a small number of components. This is achieved by fitting a statistical model that first subtracts the main effects of genotype (G) and environment (E) before applying singular value decomposition (SVD) of the effects of the GE interaction. Details of this methodology can be found in Oliveira et al. (2020). The genotype with the lowest scores in absolute value or nearer the axis of greater explanation is the most stable. Vectors pointing to near directions in common are indicative of the specificity/synergy of the genotype to the environment; and, in contrast, vectors pointing in opposing directions are indicative of negative interaction.

2.5.3 | *Toler models*

Finally, the bi-segmented statistical model proposed by Toler (Toler & Burrows, 1998); Delgado et al., 2019) was fitted to describe the adaptability and phenotypic stability, which is given by the following equation: $Y_{ij} = \alpha_i + [Z_j\beta_{1i} + (1\text{-}Z_j)\ \beta_{2i}]\ \mu_j + \delta_{ij} + e_{ij}$, where Y_{ij} is the mean of genotype i in environment *j*; α_i is the parameter that reflects the value of the response of genotype i in the mean environment [$\mu_j = 0$, where μ_j is the non-observable (latent) regression variable, but which is a parameter of the model]; β_{1i} and β_{2i} are non-linear regression coefficients that measure the response of genotype i to the variations in the environments of lower and higher quality, respectively; Z_j is a variable that assumes the value $Z_j = 1$ if $\mu_j < 0$ and $Z_j = 0$ if $\mu_j > 0$; μj is the parameter that measures environmental quality; δ_{ij} is the deviation from this regression; and eij is the mean experimental error.

Initially, the hypothesis H0: $\beta_{1i} = \beta_{2i}$ should be tested; if this hypothesis is rejected, it is an indication of there being two straight line segments that determine the model as bi-segmented. When the hypothesis H0: $\beta_{1i} = \beta_{2i}$ is not rejected, a single line segment is considered to explain the response of the genotypes (with the angular coefficient βi common to the two straight line segments). This single-segment model is given by the following equation: $Y_{ij} = \alpha_i + \beta_i\ \mu_j + \delta_{ij} + e_{ij}$.

With the models fitted, the genotypes are classified in groups A, B, C, D, or E. A summary of Groups: Criteria and Type of Response attributed to them follows:

A. The hypothesis $\beta_{1i} = \beta_{2i}$ is rejected and $\beta_{1i} < 1 < \beta_{2i}$ is accepted – Convex and doubly desirable response;

B. The hypothesis $\beta_{1i} = \beta_{2i}$ is not rejected and H_0: β_i = 1is rejected, and the common angular coefficient is $\beta > 1$ – Simple linear response and desirable only in high quality environments;
C. The hypothesis $\beta_{1i} = \beta_{2i}$ is not rejected and H_0: β_i = 1is not rejected – Simple linear response that does not deviate from the mean response;
D. The hypothesis $\beta_{1i} = \beta_{2i}$ is not rejected and H_0: β_i = 1is rejected, and the common angular coefficient is $\beta < 1$ – Simple linear response and desirable only in low quality environments;
E. The hypothesis $\beta_{1i} = \beta_{2i}$ is rejected and $\beta_{1i} > 1 > \beta_{2i}$ – Concave and doubly undesirable response.

According to the classification of Toler and Burrows (1998), a convex response or doubly desirable response pattern is characterized when the genotype has low responsivity in the unfavorable environments ($\mu_j < 0$) and comes to respond satisfactorily when these conditions become favorable ($\mu_j > 0$). The concave response or doubly undesirable response pattern is characterized when the genotype is highly responsive to the low-quality environments and little responsive in environments with quality greater than these conditions. The measurement of environmental quality μ_j is estimated simultaneously with the other regression parameters. The environment with $\mu_j > 0$ is considered favorable and the environment with $\mu_j < 0$, unfavorable. Main effects of the "Genotype" factor are indicative of adaptability.

2.6 | Auxiliary tools

The adaptability and phenotypic stability parameters were estimated by the *Estabilidade* statistical software (Ferreira & Zambalde, 1997). The AMMI model was fitted by singular value decomposition of the interaction matrix, and the *F* tests and degrees of freedom were determined according to Gollob (1968); biplots were developed using the *agricolae* package in R. For analysis of the Toler models, the nonlinear least squares technique was used by iterative processes, applying the modified Gauss–Newton method for estimation of these parameters (Rosse & Vencovsky, 2000; Rosse et al., 2002). After that, the genotypic means were clustered by the Scott–Knott test ($P < .05$) by the Sisvar 5.7 software (Ferreira, 2019).

3 | RESULTS

Common bean yield was significantly affected by the genotypes - G (N_2-fixing bacterial strains and native controls), environments – E, and the GE interaction (Table 2; $P < .01$; Figure 2). This shows that the genotypes exhibited different responses in the environments analyzed. Thus, the study of the genotypic response to identify the magnitude of the genotype by environment interaction in their adaptability and phenotypic stability is justified.

TABLE 2 Summary of combined analysis of variance of the data on common bean grain yield in accordance with genotypes (strains of nitrogen-fixing bacteria and control treatments with native rhizobia) and crop environments; common bean grain yield and environmental quality index estimated by the Toler model (in accordance with crop environments); and mean percentage of yield in relation to the environmental mean and Reliability Index estimated by the Annicchiarico method [in accordance with genotypes]

		Yield

Source of variation	df	Mean square
Block (Environment)	24	174,369.6149**
Genotype (G)[a,b]	6	513,925.6728**
Environment (E)	7	6,915,375.0511**
GE interaction	42	158,810.1744**
Residual	144	72,774.8474
Coefficient of variation, %	22.41	–
Environment (Location)	**Yield, kg ha^{-1}**	**Toler model** **Environmental Quality Index[c] (Standard Error)**
E1 (Presidente Olegário)	968.34 D[d]	–235.75 (69.3467)
E2 (Patos de Minas)	1,155.49 C	–48.56 (69.3754)
E3 (Bambuí)	1,286.41 B	82.37 (69.3485)
E4 (Patos de Minas)	2,277.19 A	1,073.15 (69.4194)
E5 (Pitangui)	1,355.16 B	151.12 (69.7554)
E6 (Lavras)	985.86 D	–218.18 (69.7453)
E7 (Luminárias)	558.32 E	–645.72 (69.2350)
E8 (Guarda-Mór)	1,045.61 D	–158.43 (69.4396)
Genotype[e]	**Yield, kg ha^{-1}**	**Annicchiarico method** **Reliability Index[c] (i)** / **Mean percentage in relation to the environmental mean**
S1 (CIAT899)	1,119.18[a,d] B	71.79 / 94.75
S2 (UFLA02-100)	1,230.67 A	54.45 / 104.66
S3 (UFLA04-195)	1,219.21 A	69.35 / 100.40

		Yield	
Source of variation	**df**	**Mean square**	
S4 (UFLA02-68)	1,211.64 A	71.04	100.31
S5 (UFLA02-127)	1,291.22 A	85.34	106.27
S6 (native rhizobia)	979.19 C	63.57	79.32
C7 (native rhizobia fertilized with mineral N)	1,377.25 A	92.56	114.29
Overall mean	1,204.05	–	–

[a] Main effects of the "Genotype" factor are indicative of adaptability ($P < .01$).
[b] Includes previously selected strains of nitrogen-fixing bacteria and control treatments with native rhizobia.
[c] Level of significance adopted = .05.
[d] Mean values followed by the same letter in the column belong to the same group ($P < .01$).
[e] S1, S2, S3, S4, and S5: selected strains of nitrogen-fixing bacteria; C6: native rhizobia; and C7: native rhizobia fertilized with mineral N (urea).
** Significant by the F test ($P < .01$).

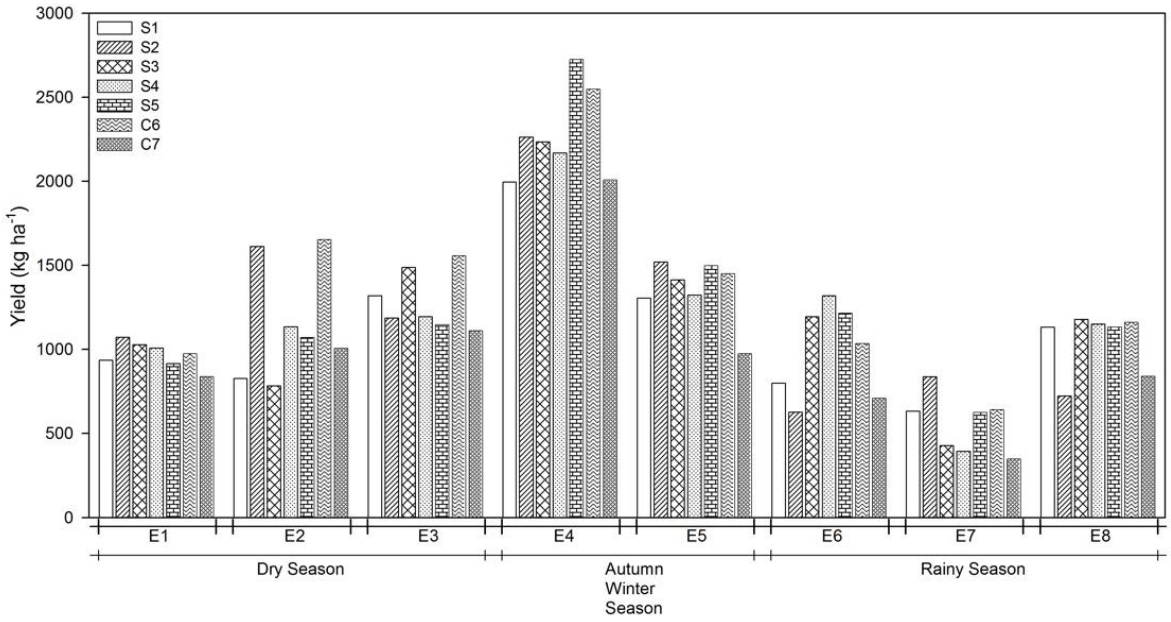

FIGURE 2 Common bean grain yield obtained from genotypes (strains of nitrogen-fixing bacteria and control treatments with native strains) in each environment studied. Strains of nitrogen-fixing bacteria: S1 (CIAT899), S2 (UFLA02-100), S3 (UFLA04-195), S4 (UFLA02-68), S5 (UFLA02-127), C6 (native rhizobia), and C7 (a control fertilized with mineral N on native rhizobia) in eight environments: E1 (Presidente Olegário), E2 (Patos de Minas), E3 (Bambuí), E4 (Patos de Minas), E5 (Pitangui), E6 (Lavras), E7(Luminárias), and E8 (Guarda-Mór)

3.1 | Annicchiarico method

The genotypes S1 (CIAT899), S5 (UFLA02-127), and C7 (native rhizobia fertilized with mineral N) exhibited the expected positive responses, but only the latter two exceeded the mean yield of the study (Table 2). The reliability of adoption of S5 and C7exceeds 85%, indicating high chance of increase in common bean yield if adopted in the field. The rate of success in adoption of the genotypes S2 (UFLA02-100) and C6 (native rhizobia) was <65%; however, only C6 produced below the environmental mean (Table 3), which represented nearly 20% less in this study.

TABLE 3 Estimates and standard errors of $\beta_{2i} - \beta_{1i}$ of the bi-segmented model of Toler and test of the hypothesis Ho: $\beta_{1i} = \beta_{2i}$

Genotype[a]	$(\beta_{2i} - \beta_{1i})$ Estimate[b]	Standard Error
S1 (CIAT899)	0.07	0.3099
S2 (UFLA02-100)	0.28	0.3099
S3 (UFLA04-195)	–0.28	0.3096
S4 (UFLA02-68)	–0.38	0.3098
S5 (UFLA02-127)	0.61	0.3112
C6 (native rhizobia)	–0.01	0.3089
C7 (native rhizobia fertilized with mineral N)	–0.28	0.3104

[a] S1, S2, S3, S4, and S5: selected strains of nitrogen-fixing bacteria; C6: native rhizobia; and C7: native rhizobia fertilized with mineral N (urea).
[b] Significant by the *F* test ($P < .05$).

3.2 | AMMI model

Biplots resulting from analysis by the AMMI1 and AMMI2 models are shown in Figures 3 and 4, respectively. The first two singular axes related to the effects of the interaction captured 57.50 and 20.50% of the sum of squares of the interaction (SS_{GE}), amounting to 78% explanation on SS_{GE}. Interpretation of a biplot regarding the GE interaction is carried out by observing the magnitude and the signal of the genotype and environment scores for the interaction axis. In the AMMI1 biplot, stability is evaluated on the ordinates (first singular axis: Principal singular vector [PSV] 1 – PSV1). The horizontal axis of this biplot considers the variation of the main additive effect of the genotypes and environments. The multiplicative effects of the interaction containing the first two PSV are represented in the AMMI2 biplot (Figure 3). In any of the situations, low scores, nearer the PSV of greater explanation or near zero indicate genotypes and environments that contributed little or nearly nothing to the interaction, characterizing them as stable. Combinations of genotypes and environments with PSV scores of the same sign have specific positive interactions; combinations of opposite sign have specific negative interactions. Thus, S1

(CIAT899), S5 (UFLA02-127), and C6 (native rhizobia) were the genotypes that least contributed to the interaction and were therefore the most stable in this study. In relation to the environments of little interference in the interaction, the following were most prominent: E1 (Presidente Olegário), E4 (Patos de Minas), and E5 (Pitangui) (Figure 3). The environments E7 (Luminárias) and E2 (Patos de Minas) and the genotypes S2 (UFLA02-100) and S3 (UFLA04-195) contributed most directly to the interaction. There was synergy between the following pairs (genotype - environment): S1 and C6-E1, S2 and C6-E7, S2-E2, and S5-E4 (Figures 3 and 4). A negative interaction was registered between S2-E6 and S3-E2 (vectors pointing in opposing directions). The environments E1 and E4 and the genotypes S2 and S4 (UFLA02-68) proved to be very discrepant from each other (Figures 3 and 4).

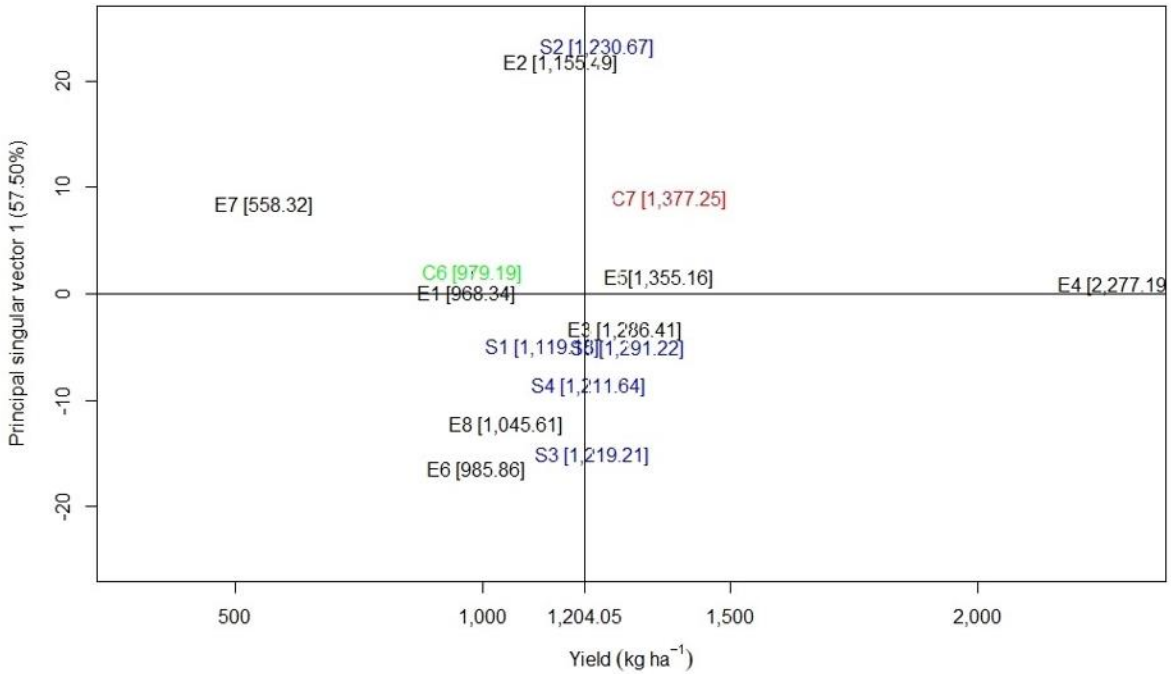

FIGURE 3 AMMI1 biplot of the additive main effects and multiplicative interaction (AMMI) by genotypes, consisting of strains of nitrogen-fixing bacteria: S1(CIAT899), S2(UFLA02-100), S3(UFLA04-195), S4(UFLA02-68), S5(UFLA02-127), C6 (native rhizobia), and C7 (a control fertilized with mineral N on native rhizobia), in eight environments: E1(Presidente Olegário), E2(Patos de Minas), E3(Bambuí), E4 (Patos de Minas), E5 (Pitangui), E6 (Lavras), E7(Luminárias), and E8(Guarda-Mór), with their grain yield (in kg ha^{-1}) in brackets

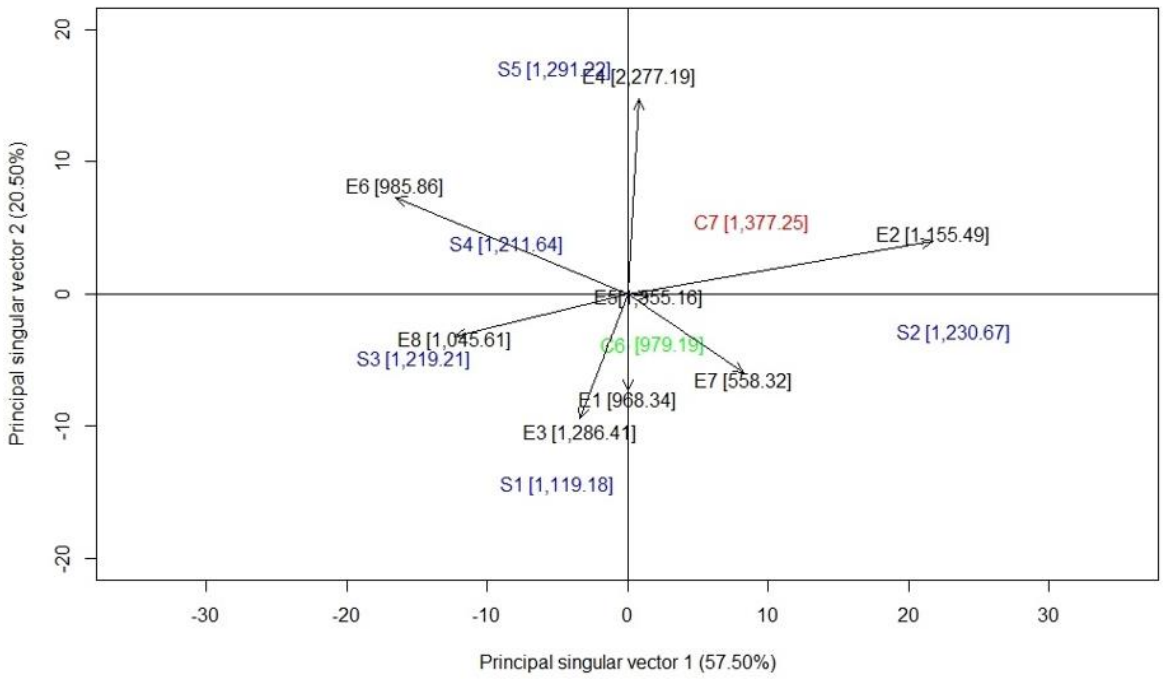

FIGURE 4 AMMI2 biplot of the additive main effects and multiplicative interaction (AMMI) by genotypes, consisting of strains of nitrogen-fixing bacteria: S1(CIAT899), S2(UFLA02-100), S3(UFLA04-195), S4(UFLA02-68), S5(UFLA02-127), C6 (native rhizobia), and C7 (a control fertilized with mineral N on native rhizobia), in eight environments: E1(Presidente Olegário), E2(Patos de Minas), E3(Bambuí), E4 (Patos de Minas), E5 (Pitangui), E6 (Lavras), E7(Luminárias), and E8(Guarda-Mór), with their grain yield (in kg ha^{-1}) in brackets

3.3 | Toler models

The environments E4 (Patos de Minas) and E5 (Pitangui) exhibited a higher positive Environmental Quality Index (EQI) (Table 2), indicating strong contribution to the increase in mean yield of the environmental factor. The EQI of the E2 environment was intermediate (Patos de Minas), near the limit of environmental contribution in yield (Table 2). In contrast, E1 (Presidente Olegário) and E7 (Luminárias) were those that most contributed to reduction in the environmental mean, and were considered the environments most unfavorable to the crops (EQI of higher negative values).

Genotypic adaptability, which is the ability of the genotypes to respond to the environmental stimuli in an advantageous manner (Toler & Burrows, 1998), decreased in the following order: S2 = S3 = S4 = S5 = C7 > S1 > C6 (Table 1).

The performance of the S5 strain (UFLA02-127) followed the bi-segmented model, with a pattern corresponding to cluster A of Toler (Tables 3 and 4). This conclusion was obtained after the difference between β_{1i} and β_{2i} was found, discarding the hypothesis that the S5 response could be represented by a single line of regression. The other genotypes exhibited a single-segment response, which decreased in the following order: C7 > S3 > S2 = C6 > S4 > S1 (cf. β, Table 4). By the significance of $\beta_i = 1$, they were then categorized within the type C response pattern of Toler. The fitting of the single (uni-segmented) and bi-segmented models represented 98.04 and 97.13% of the GE interaction, respectively.

TABLE 4 Estimates and standard errors of the regression coefficients β_{1i} and β_{2i} of the bi-segmented model and of the β of the single-segment model, both Toler models, along with the tests of hypotheses for the equality of these parameters at 0 and 1, and classification of the Toler response pattern according to the models of the genotypes and tests performed

Genotype[a]	**Model**				**Response pattern**[b]
	Bi-segmented				
	β_{1i} Estimate	Standard Error	β_{2i} Estimate	Standard Error	
S5 (UFLA02-127)	0.82[c,d]	0.2321	1.44[c,d]	0.1382	A
	Uni-segmented				
	β_i Estimate		Standard Error		
S1 (CIAT899)	0.82[c]		0.0952		C
S2 (UFLA02-100)	0.94[c]		0.0950		C
S3 (UFLA04-195)	0.99[c]		0.0949		C
S4 (UFLA02-68)	0.93[c]		0.0954		C
C6 (native rhizobia)	0.94[c]		0.0950		C
C7 (native rhizobia fertilized with mineral N)	1.13[c]		0.0951		C

[a] S1, S2, S3, S4, and S5: selected strains of nitrogen-fixing bacteria; C6: native rhizobia; and C7: native rhizobia fertilized with mineral N (urea).
[b] Response pattern A: The hypothesis $\beta_1 = \beta_2$ is rejected and $\beta_1 \leq 1 \leq \beta_2$ is accepted – Convex and doubly desirable response; response pattern C: The hypothesis $\beta_1 = \beta_2$ is not rejected and H_0: β = 1 is not rejected – Simple linear response that does not deviate from the mean response.
[c] Significantly different from 0 by the *F* test ($P < .05$).
[d] Significantly different from 1 by the *F* test ($P < .05$).

4 | DISCUSSION

The soil characteristics of the environments that were inoculated with the pre-selected strains and the native communities (with fertilization and without fertilization with mineral N) were characterized by acidity levels that were high (E1and E7), moderate (E2, E3, E4, E5, and E6), and low (E8), very high aluminum saturation (E1 and E7), high concentrations of sulfur, manganese, and copper (E2), and reduced or minimum concentrations

of calcium and magnesium (these are essential bases, but they decreased in the V% saturation component in E1and E7) (Table 1).

Soils in the E3, E4, E5, E6, and E8 environments had lower chemical restrictions. However, these characteristics were not advantageous in the soils of E3, E6, and E8, possibly as a result of their inadequate moisture (Figure 1). The low residual moisture in the soil in part of the crop cycle is common in the dry crop season of these regions, which may need to be complemented by rescue irrigation. This low moisture may have been responsible for the impact in E3 on adaptability (Table 2). Certainly, this was the condition that impeded the yields in E3 from reaching the levels of the irrigated crops (E4 and E5). This environment (E3) had good fertility and low organic matter content (Table 1), suitable for stimulating nodulation and promoting biological nitrogen fixation (BNF) if the water limitation were resolved (Figure 1). In E6 and E8, high water concentration, typical of the rainy crop season, may have interfered in the genotypic responses, decreasing production. However, even so, production remained high, indicating good performance of the pre-selected strains (Figures 1 and 2).

The low effect of the E1, E4, and E5 environments on the interaction was indicative of the stability of the contributions of these environments in the genotypes – the E1 environment negatively affected the yields of all genotypes (low in this environment), whereas the opposite, that is, positive effect on the yields of all genotypes, prevailed in E4 and E5 (with high yields in these environments). In contrast, E7 and E2 had the greatest effect on the interaction and on the response of the genotypes (Figures 3 and 4). E7 had more stressful characteristics than those of E2, with the EQI of the latter at the limit between favorable quality and unfavorable quality (Table 2).

Environments such as E7 accommodate rhizobia that developed tolerance mechanisms to survive the stresses that characterize the environment. These rhizobia have stable responses in the face of environmental changes. That may be the reason for better performance (highest yield) of C6 and UFLA02-100 (S2) in E7 (Figure 4). Such mechanisms range from maintaining the intracellular levels at near neutral levels to releasing peripheral cellular structures that are in direct contact with soil acidity (Kaur, Selvakumar & Ganeshamurthy, 2019).

Some condition of E2 certainly contributed to its effect on the GE interaction and to the low performance of the UFLA04-195 (S3) strain, whose E2-S3 vectors pointed in opposite directions in the AMMI and AMM2 biplots (Figures 3 and 4). The high concentrations of sulfur, manganese, and copper of the soil of the E2 environment (80.0, 137.9, and 13.4 mg kg^{-1}, respectively) may have been toxic to the strain (Matsuda et al., 2002) or were even worsened by possible antagonism with the native rhizobia communities. In spite of being tolerant to 8 out of 12 antibiotics tested, the UFLA04-195 (S3) strain is sensitive to rifamycin, kanamycin, gentamicin, and vancomycin (Oliveira-Longatti et al., 2013) released by other soil microorganisms, which may have favored the native strains adapted to this condition. In contrast, E2 contributed to the high performance of UFLA02-100 (S2) and C7. The strain UFLA02-100 (S2) was successful in both E7and E2 (Figures 2, 3, and 4), suggesting that it has greater tolerance to stressful conditions.

The growth capacity of the UFLA02-100 (S2) strain under high (pH 4.9–5.0: Soares et al., 2006; Rufini et al., 2011), moderate (pH 5.6–6.0: Rufini et al., 2011; Nogueira et al., 2017; Oliveira, Ferreira, et al., 2018; Oliveira, Soares, et al., 2018; Pádua Oliveira et al., 2017), and low (pH 6.4: Pádua Oliveira et al., 2017) active acidity provides conditions for adaptation to different environments, even though conditions for its development appear to be optimal since it had variable performance in this study, above all in E2 and E7, which affected the GE interaction. Even so, its yield remained at around 1,200 kg grain ha^{-1} in the varied environments (Table 1). Through its specificity in E7 (of high active acidity, pH 4.8)

and negative interaction in E6 (of low active acidity, pH 6.0), it appears to develop better under high acidity (Figure 4). This would be a possible condition of divergence between the strains UFLA02-100 (S2) and UFLA02-68 (S4); the latter is moderately stable in our study and indifferent to this acidity range in the studies of Rufini et al. (2011). These variants assist in understanding the opposing positions of S2 and S4 in the AMMI biplots (Figures 3 and 4). Nevertheless, in spite of this divergent response, both strains (S1 and S4) show resistance to the antibiotics azithromycin, streptomycin, erythromycin, ampicillin, chloramphenicol, rifamycin, nalidixic acid, clarithromycin, amoxicillin, and vancomycin, as well as high capacity for degradation of the phenol pollutant as a carbon source, constituting additional advantages to adaptation to stresses such as acidity (Oliveira-Longatti et al., 2013).

The genotypes C6 and C7 had responses that were most expected, negative for C6 and positive for C7. Conversely, CIAT899 (S1) and UFLA02-127 (S5) were the most stable genotypes in all the environments by all the biometric techniques (Tables 2, 3, and 4; Figures 3 and 4).

Even numerous and competitive native soil bacterial populations are not always able to establish efficient symbiosis that significantly contributes to common bean growth. Thus, it may be necessary to employ inoculants containing strains that are more efficient in N_2 fixation (Ferreira et., 2009; Moreira, Siqueira & Brussaard, 2006; Oliveira et al., 2019; Oliveira, Ferreira, et al., 2018; Oliveira, Soares, et al., 2018; Pádua Oliveira et al., 2017; Soares et al., 2006), such as those found in the present study (Tables 2 and 4; Figures 2, 3, and 4). The contribution of these populations rises, in turn, when complemented with 80 kg N-urea ha^{-1} (C7), that is, the increase in yield was due to the nitrogen fertilizer; however, C7 showed less stability than C6. As C7 is dependent on mineral-N fertilization, it is likely to have impaired efficiency under adverse conditions, such as nutritional limitation to the plant and water restriction in incorporation of nitrogen fertilizer (which would volatilize the urea) or excessive water supply (which would leach the mineral N). Efficiency may also be impaired in soils rich in organic or mineral N. For that reason, the yield from the C7 treatment does not deviate from the environmental mean even with additional N supply, with definition of yield increases becoming unstable. In a practical sense, the use of nitrogen fertilizer (C7) in an environment with limitations or with abundant fertility would be a wasted investment.

CIAT899 (S1) of *Rhizobium tropici* is one of the strains approved as an inoculant for common bean in Brazil, where acidic conditions are predominant in soils. It is more competitive at pH 5.0 (Frey & Blum, 1994), a value quite similar to the 4.8 of E1(Presidente Olegário), where the strain showed most synergy in this study (Figures 3 and 4). CIAT899 (S1) is also not very sensitive to pH variations from 5.0 to 6.9 (Rufini et al., 2011; Ferreira et al., 2012). This wide tolerance to acidity favors phenotypic stability.

The strain UFLA02-127 (S5), just as the others of the UFLA code studied here, was isolated from acid soils of the Brazilian Amazon and also belongs to the *Rhizobium* genus. It is tolerant to acidity, has high ability in competition with already established strains, and has high N_2 fixation capacity (Nogueira et al., 2017; Oliveira, Ferreira, et al., 2018; Oliveira, Soares, et al., 2018; Pádua Oliveira et al., 2017; Soares et al., 2006). Furthermore, It has performance comparable to that of CIAT899 in peat (Nogueira et al., 2017; Pádua Oliveira et al., 2017) and liquid (Oliveira, Ferreira, et al., 2018) vehicles, which increases its chance of being adopted by farmers.

With the exception of UFLA02-127 (S5), all of the strains had a simple linear response, corresponding to that of group C of Toler ($\beta_i = 1$; Toler & Burrows, 1998); these strains respond to improvement in environmental quality, but have little deviation in generating yields other than those expected in the environments (Rosse, Vencovsky & Duarte, 2002). The native rhizobia (C6 and C7controls) fit in the same group C; but without

complementation with mineral N, the adaptability of C6 is quite inferior to C7, a condition that is certainly related to its low BNF efficiency with common bean (Moreira, Siqueira & Brussaard, 2006). Inoculation with the UFLA02-127 (S5) strain, however, would be a positive aggregation to the crops because S5 acts as strains of Toler group A, with a doubly desirable response pattern ($\beta 1i < 1 < \beta 2i$; Toler & Burrows, 1998).

In agronomic interpretation, convex and doubly desirable response mean null or minimal losses in environments of low quality and substantial gains in those with superior quality, that is, the idealized response in a promising genotype. Having confirmed the existence of this attribute, lower losses would occur in introduction of the strain under critical conditions (such as those of E1, E2, and E7), and its inoculation would be more advantageous in favorable environments, such as soils that are fertile or highly technified (e.g., E3, E4, and E5), where they would express all their genetic potential for increasing yield (Oliveira et al., 2020; Rosse & Vencovsky, 2000; Toler & Burrows, 1998). Such was the performance of the UFLA02-127 (S5) strain in this study, with evident synergy in E4, an environment of better quality and where this strain was the one that led to highest yield.

It is not by chance that the good performance of the UFLA02-127 (S5) strain occurred in so many situations in this study, or that it often appears in Brazilian studies. Its efficiency in snap bean, also *P. vulgaris*, was greater than that of the native population and equivalent to that of the native control with mineral N in an experiment in pots with soil at pH 5.8 (Oliveira, Ferreira, et al., 2018). With common bean in the field, the strain led to a mean of 1,700 kg ha^{-1} in four rainy season crops in acid or moderately acid soils without soil amendment and with low use of inputs such as micronutrients or agricultural chemicals (Ferreira et al., 2009; Nogueira et al., 2017; Pádua Oliveira et al., 2017; Soares et al., 2006), reaching levels near 3.0 t ha^{-1} in one of them (Nogueira et al., 2017). This mean value is 64.57% greater than the estimate for the crop in the 2020 crop year in Brazil by National Food Supply Agency (Companhia Nacional de Abastecimento - Conab). In the mean of the three crop seasons in our study, under different conditions, this strain led to yield 25% greater than the 1,031 kg ha^{-1} mean yield produced in Brazil in 2019, confirming its good performance and adaptability, regardless of the crop season. Thus, as in the reports cited above, it achieved yield equivalent to that obtained with mineral N (genotype C7). Therefore, inoculation with the UFLA02-127 (S5) strain is viable and can replace nitrogen fertilization without hurting the crop, which means not only savings on N fertilizers but, above all, a significant ecological contribution.

The fact of being successful in low quality environments and having positive interaction in favorable environments is what differentiates a good from an excellent genotype (Ferreira et al., 2006; Gauch, Piepho & Annicchiarico, 2008; Morais et al., 2008). In this respect, the UFLA02-127 (S5) strain most aggregates value as an inoculant for common bean among the bacterial genotypes studied here. UFLA02-127 (S5) exhibits characteristics idealized by soil microbiologists, by inoculant producers, and by farmers that desire satisfactory yields, maintaining oscillation to a minimum (under high stability) even with low inputs. For all these reasons, the UFLA02-127 strain has potential for approval by MAPA.

5 | CONCLUSIONS

CIAT899, UFLA02-127, and native rhizobia were the most stable genotypes in relation to the environments (AMMI models).

CIAT899 and native rhizobia are the least adaptable (Toler models), whereas the genotype UFLA02-127 has the best response to the environments (Group A of Toler).

The Annicchiarico method showed that the reliability of adoption of UFLA02-127 and native rhizobia fertilized with mineral N exceeds 85%, indicating high probability of increase in common bean yield if adopted in the field. Conversely, native rhizobia have <65% reliability.

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AUTHOR CONTRIBUTIONS

Dâmiany Pádua Oliveira: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Resources; Validation; Visualization; Writing-original draft.
Bruno Lima Soares: Conceptualization; Formal analysis; Investigation; Methodology; Resources; Validation; Writing-review & editing.
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Thiago Ribeiro Passos: Investigation.
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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ARTICLE III

Adaptabilidade e estabilidade fenotípica de rizóbios em 16 macroambientes brasileiros compostos por genótipos de feijão-comum e ambientes edafoclimáticos

Artigo redigido em formato preliminar de submissão ao periódico Crop Science

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ABSTRACT. A existência de interação genótipo de rizóbio × cultivar hospedeira × ambiente (interação *GCA*) tem implicações no desenvolvimento de inoculantes para leguminosas e pode representar desafios e oportunidades para a melhoria da fixação biológica de nitrogênio (FBN) no feijão-comum. Com auxílio de ferramentas biométricas apropriadas é possível explorar essa interação, definindo-se a contribuição de cada fator sobre o desempenho relativo dos simbiontes. Rizóbios que apresentem ampla adaptação ao ambiente serão capazes de superar fatores limitantes e manter uma maior capacidade de FBN. Outra característica importante é que possuam estabilidade nas respostas desejadas, sobretudo na produtividade, que é o principal atributo de interesse dos agricultores, que farão uso dessa biotecnologia. Por meio dos modelos de Toler e AMMI (efeito principal aditivo e interação multiplicativa) e do método de Annicchiarico, a resposta diferencial de cada rizóbio ao longo dos ambientes pode ser analisada, permitindo a seleção daqueles com padrão e tipo de resposta ideal. No presente estudo, essas técnicas biométricas foram utilizadas na determinação da adaptabilidade e estabilidade fenotípicas de rizóbios em simbiose com cultivares brasileiras de feijão-comum, em campo. Da combinação entre cultivares hospedeiras e ambientes edafoclimáticos foram constituídos os 16 macroambientes nos quais os rizóbios foram avaliados. Conclui-se que: apesar do desempenho estável, rizóbios nativos não asseguram sucesso em cultivos que deles dependam como fonte nitrogenada. A dose 20 kg N-ureia ha^{-1} eleva a estabilidade do rizóbio nativo e amplia sua capacidade produtiva, que, ainda assim, é inferior à da estirpe UFLA02-127 (*Rhizobium* sp.). A inoculação com CIAT899 (*R. tropici*) e fertilização com 80 kg N-ureia ha^{-1} têm grande interferência na interação *GCA*. Há sinergia na simbiose entre a estirpe CIAT899 e cultivar BRSMG-Madrepérola. A estirpe UFLA02-127 tem alta adaptabilidade e estabilidade fenotípicas. Apresenta padrão de resposta previsível e capacidade de se beneficiar de estímulos ambientais positivos, sendo pouco ou nada afetada em ambientes de qualidade reduzida. Incrementa a produtividade do feijoeiro-comum independente da cultivar hospedeira ou do ambiente. Ainda, suas médias se assemelham às da

aplicação de N-ureia. Pelas vantagens listadas, a estirpe UFLA02-127 tem potencial para aprovação como inoculante para o feijoeiro-comum.

PALAVRAS-CHAVE. Fixação biológica de N_2, *Rhizobium*, *Phaseolus vulgaris*, Produtividade de grãos, Interação genótipo-cultivar-ambiente, Múltiplas combinações, Técnicas biométricas

1 | INTRODUÇÃO

Os fertilizantes nitrogenados são amplamente utilizados na cultura do feijão-comum (*Phaseolus vulgaris* L.) no Brasil, onerando o custo de produção e podendo afetar negativamente o meio ambiente (Oliveira et al., 2018; Pelegrin et al., 2009). A fixação biológica de nitrogênio (FBN) pode reduzir os impactos negativos relacionados ao uso excessivo de fertilizantes nitrogenados (Dias et al., 2020; Moreira and Siqueira, 2006; Pelegrin et al., 2009; Soares et al., 2016). Entretanto alguns fatores podem interferir negativamente no desempenho das estirpes de rizóbio e eficiência da FBN em condições de campo (Hungria and Vargas, 2000; Oliveira et al., 2018; Ruschel and Reuszer, 1973).

Como essas situações em maioria podem interferir negativamente sob o desempenho das estirpes, torna-se imprescindível uma seleção criteriosa de estirpes adaptadas ao ambiente pretendido, o que se faz por meio de testagem de introdução desses isolados em diversas condições ambientais ("IN SDA 13 de 24-03-2011 Inoculantes.pdf — Português (Brasil)," n.d.). Entretanto a interpretação conclusiva quanto ao padrão comportamental é dificultada pela interação desses rizóbios, principalmente com o genótipo leguminosa hospedeira e com o ambiente edafoclimático (Gunnabo et al., 2019; Oliveira et al., 2021a; Sánchez-Rodríguez et al., 2020).

A existência da interação genótipo de rizóbio × cultivar hospedeira × ambiente (interação *GCA*) tem grandes implicações no desenvolvimento de inoculantes e pode representar desafios e oportunidades para a melhoria da FBN no feijão-comum. Isso porque pode existir variabilidade no padrão de respostas de fixação de N_2 dos rizóbios (Moreira and Siqueira, 2006; Oliveira et al., 2021a) e de genótipos de feijão-comum em relação à eficiência de absorção de nitrogênio (N) (Dias et al., 2020; Fageria et al., 2013; Fageria et al., 2014), o que tanto pode estar relacionado com a genética dos materiais como com a influência do fator ambiental (Saltz et al., 2018). Buscam-se, portanto, genótipos de rizóbios e de feijoeiros que mantenham boas performances, independentemente da combinação *GCA*.

Estirpes que apresentem adaptação a uma gama de hospedeiros e ambientes são capazes de superar fatores limitantes e manter uma maior capacidade de fixação do N_2. Outra característica importante que deve ser considerada ao selecionar um rizóbio nativo para uso como inoculante é a capacidade de produzir constantemente as respostas desejadas no aumento da produtividade, apresentando comportamento estável e previsível de acordo com variações hospedeiro-ambientais (Gunnabo et al., 2019; Oliveira et al., 2021a, 2020; Soares et al., 2006).

Recentemente técnicas biométricas amplamente utilizadas no melhoramento de plantas para a análise e interpretação do genótipo por interações no ambiente, foram utilizadas pela primeira vez para avaliar o desempenho de estirpes simbiontes com a cultivar Majestoso de feijão-comum na interação envolvendo oito ambientes brasileiros (Oliveira et al., 2021a). Dentre as estirpes avaliadas encontravam-se a CIAT899 [*Rhizobium tropici* (Martinez-Romero et al., 1991)], atualmente aprovada pelo Ministério da Agricultura Pecuária e Abastecimento brasileiro, e a UFLA02-127 (*Rhizobium* sp.), ainda em fase de seleção, mas com grande potencial às fases de aprovação, pelo bom desempenho em condições axênicas e em campo. Ambas destacaram-se pela tolerância a estresses predominantes em solos tropicais e pela capacidade de incrementar as produtividades do feijão-comum (Ferreira et al., 2012, 2009; Longatti et al., 2013; Nogueira et al., 2017; Oliveira et al., 2021b, 2017; Oliveira et al., 2018a; Rufini et al., 2011; Soares et al., 2006), mas apenas a UFLA 02-127 aliou competência de adaptabilidade e de estabilidade fenotípica (Oliveira et al., 2021a) nos ambientes testados.

A manutenção do bom desempenho simbiótico também em outras cultivares de boa aceitação no país pode incentivar ao uso de bioinoculantes, e, ainda, pode nortear programas de melhoramento vegetal que visam à seleção de cultivares responsivas à FBN. Nesse intuito, portanto, foram determinadas a adaptabilidade e a estabilidade fenotípica de rizóbios em simbiose com quatro cultivares brasileiras de feijão-comum, em campo, aos quais após combinados (cultivares e ambientes) constituíram os 16 macroambientes em estudo. No estudo da interação *GCA* contou-se com o auxílio dos modelos de Toler e AMMI e do método de Annicchiarico, os quais permitem ainda estratificar os macroambientes conforme suas contribuições na interação *GCA.*

2 | MATERIAIS E MÉTODOS

Caracterização dos fatores da interação GCA

Ambientes edafoclimáticos. Na safra das águas de 2016, que consistiu em semeaduras em outubro e novembro, foram instalados experimentos no Estado de Minas Gerais: em Lavras, em Lambari (Lambari1) e em Patos de Minas. O quarto experimento foi instalado na safra da seca 2016 (fevereiro/2016), também no município de Lambari (Lambari2), mas em área de maior acidez. Uma caracterização mais ampla desses ambientes, com dados climáticos (temperatura e precipitação média), além de especificações de adubação e histórico dos cultivos, encontram-se na Tabela 1.

Genótipos de rizóbios. Os genótipos de rizóbios inoculados consistiram na estirpe CIAT899 de *Rhizobium tropici* (Martinez-Romero et al., 1991) e UFLA02-127 de *Rhizobium* sp.. A primeira já é aprovada pelo Ministério da Agricultura, Pecuária e Abastecimento Brasileiro para a fabricação de inoculantes comerciais para a cultura do feijão-comum. A estirpe UFLA 02-127 foi isolada de solo ácido e com alto teor de Al da Amazônia brasileira, estresses esses predominantes em solos tropicais. Além da tolerância a acidez, possui alta habilidade de competição com as estirpes já estabelecidas e elevada capacidade de fixação do N_2 (Ferreira et al., 2009; Nogueira et al., 2017; Oliveira et al., 2018a; Oliveira et al., 2021b, 2017; Rufini et al., 2011; Soares et al., 2006). Os rizóbios nativos de cada ambiente edafoclimático foram considerados um tratamento controle. A dose 20 kg N-ureia ha^{-1} foi considerada na literatura (Oliveira et al., 2018b; Oliveira et al., 2019, 2016) como potencialmente estimuladora aos rizóbios nativos, e em razão dessa afirmativa, foi inserida no estudo. A dose 80 kg N-ureia ha^{-1} é padrão em ensaios com rizóbios [Ministério da Agricultura, Pecuária e Abastecimento - Instrução Normativa 13, de 24 de março de 2011]. Experimentos foram conduzidos para estimar o número mais provável de unidades formadoras de colônias (UFC) de rizóbios nativos na profundidade de 0 a 20 cm do solo de cada área experimental (Soares et al., 2006). Números em torno de 10^3 UFC por grama de solo foram obtidos nos ambientes edáficos.

Tabela 1 Características químicas e físicas das amostras de solo coletadas na camada de 0-20 cm em Lavras, Patos de Minas, Lambari 1 e Lambari2.

Characteristicas[a]	**Unidades**	**Ambientes**			
		Lavras	**Lambari 1**	**Patos de Minas**	**Lambari2**
pH	-	5,5 MA	5,8 MA	5,7 MA	6,1 LA
P disponível	(mg dm^{-3})	46,82 MB	80,0 MB	38,78 MB	12,50 M
K	($cmol_c$ dm^{-3})	0,48 MBa	0,1082	0,076 MBa	0,25
Ca	($cmol_c$ dm^{-3})	3,20 B	3,75 B	0,86 Ba	1,09 Ba
Mg	($cmol_c$ dm^{-3})	0,70 M	0,54 M	0,24 Ba	0,35 Ba
Al	($cmol_c$ dm^{-3})	0,06 MBa	0,11 MBa	0,12 Ba	0,54 M
H + Al	($cmol_c$ dm^{-3})	4,00 M	2,10 Ba	6,24 A	7,37 A

SB	($cmol_c\ dm^{-3}$)	4,43 B	4,40 B	1,18 Ba	1,69 Ba
t	($cmol_c\ dm^{-3}$)	4,50 M	6,50 M	1,30 MBa	2,23 Ba
T	($cmol_c\ dm^{-3}$)	8,43 M	4,51 M	7,42 M	9,06 B
m	(%)	1,30 MBa	2,44 MBa	9,23 Ba	24,22 M
V	(%)	52,58 M	67,67 B	35,86 Ba	18,67 Ba
MO	($dag\ kg^{-1}$)	3,01 M	2,25 M	2,84 M	2,82 M
Zn	($mg\ dm^{-3}$)	17,48 A	2,02 B	2,18 A	4,72 A
Fe	($mg\ dm^{-3}$)	79,79 A	94,00 A	54,46 A	41,35 A
Mn	($mg\ dm^{-3}$)	19,24 A	79,52 A	80,57 A	14,92 A
Cu	($mg\ dm^{-3}$)	2,60 A	4,43 A	13,74 A	1,03 M
B	($mg\ dm^{-3}$)	0,04 MBa	0,02 MBa	0,08 MBa	0,02 MBa
S	($mg\ dm^{-3}$)	7,15 M	34,65 MB	85,09 MB	40,32 MB
Análises físicas					
Argila	($g\ kg^{-1}$)	560	460	340	460
Silte	($g\ kg^{-1}$)	110	230	410	140
Areia	($g\ kg^{-1}$)	330	310	250	400
Altitude	-	920	986	940	986
Coordenadas GPS	-	-21.229270, -44.977572	-21.962340. -45.351902	-18.587258, -46.514675	21° 56' S 45° 18' W
Classificação climática de Köppen	-	Cwa		Aw	Cwa
Safra/Ano	-	Águas 2015/16			Seca 2016
AB[1]: P_2O_5	$kg\ ha^{-1}$	90	70	70	90
AB[2]: K_2O	$kg\ ha^{-1}$	20	20	20	50
Cultivo anterior	-	Milho			Brachiaria

[a]pH em água; SB: Soma de bases trocáveis; t: Capacidade de troca de cátions; T: Capacidade de troca de cátions a ph 7; m: Alumínio trocável; V: Saturação por bases; MO: Matéria organica; AB: Adubação de base. [LA (Low acid), MA (Medium Acid), Ba (Baixo), M (Médio), B (Bom), A (Alto), MB (Muito Bom), MBa (Muito baixo) (63)

Cultivares hospedeiras. As cultivares de feijão-comum utilizadas (IAC Alvorada, BRSMG Madrepérola, BRS Notável e BRS Estilo) são recomendadas para o estado de Minas Gerais. As cultivares IAC Alvorada (Morais Carbonell et al., 2008) e BRS Estilo (Melo et al., 2009) têm ciclo normal variável de 85 a 95 dias. A cultivar BRS Notável tem ciclo precoce de 75-85 dias (Santos Pereira et al., 2012). A cultivar BRSMG Madrepérola possui ciclo semi-precoce de 80 dias (Carneiro et al., 2012). Todas pertencem ao grupo carioca e apresentam hábitos de crescimento indeterminado dos tipos II (Estilo, arquitetura ereta e Notável, arquitetura semi-ereta), III (Madrepérola, prostrada) e II / III (Alvorada, semi-ereta) e produtividade média da ordem de 2500 $kg\ ha^{-1}$, com potencial produtivo de até 4472 $kg\ ha^{-1}$ (Notável).

Composição dos Macroambientes: Os macroambientes foram denominados como sendo: LaAlv, LaEst, LaMad, LaNot, PMAlv, PMEst, PMMad, PMNot, Lb1Alv, Lb1Est, Lb1Mad, Lb1Not, Lb2Alv, Lb2Est, Lb2Mad e Lb2Not. Essa denominação foi dada pela junção das iniciais dos nomes dos ambientes edafoclimáticos [Lavras (La), Patos de Minas (PM) e Lambari (Lb) e das cultivares hospedeiras [BRS Estilo (Est), IAC Alvorada (Alv), BRSMG

Madrepérola (Mad), BRS Notável (Not)]. Considerando que dois experimentos foram conduzidos em Lambari, utilizou-se as codificações 1 e 2 para relacioná-los à safra de implantação (1: safra das águas 2015/16; 2 safra da seca 2016).

Desenho experimental e Tratos culturais. O delineamento estatístico foi sempre blocos ao acaso, com três repetições. Em cada ambiente edafoclimático a unidade experimental (14.4 m^2) foi constituída de seis linhas de 4 m de comprimento, espaçadas de 0.6 m e a área útil correspondeu às quatro linhas centrais. O preparo do solo foi feito através de uma aração. As fontes de fósforo e de potássio foram sempre superfosfato simples e cloreto de potássio, respectivamente, e suas doses foram definidas de acordo com os níveis de tecnologia propostos pela Comissão de Fertilidade do Solo do Estado de Minas Gerais (Gerais, 1999) (Tabela 1). Em todos os experimentos a semeadura manual foi feita imediatamente após a inoculação, adotando-se a densidade de 15 sementes por metro linear.

Sob os rizóbios nativos foram aplicadas as doses 0, 20 e 80 kg N-ureia ha^{-1}. A dose 20 foi aplicada integralmente em semeadura enquanto a dose 80 foi fracionada entre semeadura (40 kg ha^{-1}) e cobertura (40 kg ha^{-1}). As doses da semeadura foram aplicadas juntamente das fontes de P e K. A cobertura com N-ureia ocorreu também manualmente, tendo a ureia sido distribuída lateralmente às plantas, em um fluxo contínuo ao longo das linhas de cultivo, entre os estádios V3 e V4.

Em nenhum dos ambientes foi feita correção do solo. Os cultivos se deram em condição de baixa utilização de insumos, não tendo sido aplicados defensivos fitossanitários. Houve irrigação de salvamento em Lambari2, na safra da seca, de modo a atender a demanda hídrica em etapas imprescindíveis ao desenvolvimento da simbiose e da cultura. Não foi necessário controle de pragas em nenhum ambiente. Em todos os experimentos foi realizada capina manual sempre que necessário. As capinas foram complementares à aplicação de uma mistura de 0,5 L de fomesafem (Flex) + 1,0 L de bentazona + imazamox (Amplo) + 1,0 L de fluazifop-butil (Fusilade) por hectare.

Preparo dos inoculantes. Os inoculantes contendo as estirpes CIAT899 e UFLA02-127 foram preparados com turfa esterilizada em autoclave, na proporção 3:2 (m:v) de turfa e cultura em meio 79. A qualidade do inoculante foi monitorada por meio de UFC, atendendo ao número mínimo legal de células viáveis, em torno de 10^9 UFC de *Rhizobium* por grama de inoculante na semeadura (IN SDA 13 de 24-03-2011 Brasil2011). O material resultante foi empregado na base de 100 g por kg de semente.

Análises estatísticas. Na maturidade dos grãos, o rendimento em cada macroambiente foi obtido a partir do peso total dos grãos produzidos na área útil da parcela, ajustando a umidade para 130 g kg^{-1}. A análise individual de variância foi realizada em todos os dados com o software Sisvar 5.7 (Ferreira, 2019) após o teste de normalidade (teste de Shapiro-Wilk) e o teste de homocedasticidade (teste de Bartlett) nos resíduos dos modelos por meio do software R (R Development Core Team). Em seguida, foi realizada análise combinada de variância nos experimentos de campo, observando a homogeneidade dos quadrados médios residuais. O modelo estatístico nessa análise combinada é:

$$Y_{ijkm} = \mu + b_{j(m)} + g_i + c_k + gc_{ik} + e_m + ge_{im} + ce_{km} + gce_{ikm} + \varepsilon_{ijkm},$$

em que Y_{ijkm} é a observação do i-ésimo genótipo, no j-ésimo bloco do m-ésimo experimento na k-ésima cultivar, μ é o efeito da constante geral do modelo; $b_{j(m)}$ é o efeito do j-ésimo bloco no m-ésimo experimento, g_i é o efeito do i-ésimo genótipo, c_k é o efeito da k-ésima cultivar, gc_{ik} é o efeito da interação do i-ésimo genótipo com a k-ésima cultivar, e_m é o efeito do m-ésimo ambiente, ge_{im} é o efeito da interação do i-ésimo genótipo com o m-ésimo ambiente, ce_{km} é o efeito da interação da k-ésima cultivar com o m-ésimo ambiente, gce_{ikm} é o efeito da interação entre o i-ésimo genótipo, a k-ésima cultivar e o m-ésimo ambiente e ε_{ijkm} é o efeito do erro experimental associado a observação Y_{ijkm} com distribuição normal, independente e homocedástico, ou seja, $\varepsilon_{ijkm} \sim N(0, \sigma^2)$.

Uma vez detectada a interação tripla, o valor médio para cada interação *GCA* foi calculado a partir das três repetições dos genótipos de rizóbio por ambiente para todas as análises posteriores. Os fatores cultivares e ambientes edafoclimáticos foram combinados em um único fator denotado ambiente, sendo que equivaleria na verdade a um macroambiente. Isso foi realizado para permitir que os métodos clássicos de análise de estabilidade pudessem ser aplicados, uma vez que eles pressupõem um único fator ambiental (Novaes Rosse e Vencovsky, 2000; Rosse et al., 2002). A partir desses resultados e procedimentos, os parâmetros de adaptabilidade e estabilidade fenotípica foram estimados de acordo com a metodologia e modelos descritos a seguir:

Análises Biométricas.

Método Annicchiarico (Annicchiarico, 1992)**.** Esse método adota um índice de confiança do risco de adoção de cada genótipo de rizóbio. Os procedimentos para os cálculos envolvem transformação das médias de cada rizóbio em cada macroambiente e estimação das médias. De posse dessas estimativas, o índice de confiança $I\%_i$ é obtido por meio de um estimador

apropriado (Annicchiarico, 1992; Oliveira et al., 2021b, 2020). Quanto maior esse índice, menor o risco na adoção do genótipo. Quanto maior o índice de confiança do genótipo, menor a probabilidade de insucesso.

Modelos de Toler (Toler and Burrows, 1998)**.** A primeira hipótese testada é H0: $\beta_{1i} = \beta_{2i}$; se o teste for significativo, essa hipótese é rejeitada, aceitando H1: $\beta_{1i} \neq \beta_{2i}$. Ocorrem assim dois segmentos de retas, os quais determinam o modelo bissegmentado $Y_{ij} = \alpha_i + [Z_j\beta_{1i} + (1\text{-}Z_j)\ \beta_{2i}]$ $\mu_j + \delta_{ij} + e_{ij,;}$ [Y_{ij} como sendo a média dos genótipos rizobianos i no ambiente j; α_i como o parâmetro que reflete o valor da resposta desse rizóbio i, no ambiente médio ($\mu_j = 0$, em que μ_j é a variável regressora, que é um parâmetro do modelo); β_{1i} e β_{2i} são coeficientes de regressão não lineares que medem a resposta do genótipo i às variações nos ambientes de qualidade inferior e superior, respectivamente; Z_j como sendo uma variável indicadora, que assume o valor $Z_j = 1$ se $\mu_j \leq 0$ e $Z_j = 0$ se $\mu_j > 0$; μ_j representa o parâmetro que mede a qualidade ambiental; δ_{ij} é o desvio dessa regressão; e e_{ij} é o erro experimental médio. O genótipo de rizóbio será então classificado como do grupo A (resposta $\beta_{1i} < 1 < \beta_{2i}$) ou E (resposta $\beta_{1i} > 1 > \beta_{2i}$)].

Quando a hipótese H0: $\beta_{1i} = \beta_{2i}$ é aceita, um segmento de linha única é reconhecido para explicar a resposta genotípica (β_i comum aos dois segmentos de linha reta). Neste caso, o modelo é dado por $Y_{ij} = \alpha_i + \beta_i\ \mu_j + \delta_{ij} + e_{ij}$. Os genótipos nesse caso serão classificados como grupo *B, C* ou *D* - no grupo B, quando β_i comum é significativamente diferente de 1 e $\beta_i > 1$; como C, quando $\beta_i = 1$; e como *D* quando β_i comum é significativamente diferente de 1 e $\beta_i < 1$. A medição da qualidade ambiental μ_j é estimada simultaneamente com os outros parâmetros de regressão. O ambiente com $\mu_j > 0$ é considerado favorável e o ambiente com $\mu_j < 0$, desfavorável.

Os significados práticos desses grupos são: ***A***- é a resposta convexa e duplamente desejável [caracterizada quando o genótipo tem baixa responsividade nos ambientes desfavoráveis ($\mu_j < 0$) e chega a responder satisfatoriamente quando essas condições se tornam favoráveis ($\mu_j > 0$)]; ***B***- é a resposta linear simples que é desejável apenas em ambientes de alta qualidade; ***C***- é a resposta linear simples que não se desvia da resposta média; ***D***- é a resposta linear simples que é desejável apenas em ambientes de baixa qualidade; e ***E***- é a resposta côncava e duplamente indesejável (caracterizada quando o genótipo do rizóbio for altamente responsivo aos ambientes de baixa qualidade e pouco responsivo em ambientes com qualidade acima dessas condições).

Modelos AMMI (Zobel et al., 1988)**.** Após estabelecer a existência da interação *GCA*, foi utilizado um modelo aditivo de efeito principal e interação de multiplicação (*AMMI*) para decompor a interação. Seu principal objetivo é reduzir os padrões multidimensionais de interação em um pequeno número de componentes que contêm o máximo de informações possível sobre a interação. Isso é conseguido ajustando-se um modelo estatístico que subtrai primeiro os efeitos principais de cada fator antes de aplicar a decomposição de valor singular (SVD) dos efeitos de interação de *GCA* O modelo final que descreve o genótipo pelos dados do macroambiente torna-se *ij*: $GCA_{(ge)} = [gca_{ij}]$, com: $gca_{ij} = Y_{ij} - Y_i - Y_j + \bar{Y}$. A partir do SVD, a soma dos quadrados da interação é, k = 1, 2, ..., p, onde p é a classificação da matriz *GCA*, λ_k é obtido, que é o k-ésimo valor singular de *GCA* (escalar); γ_{ik} é o elemento correspondente ao i-ésimo genótipo do k-ésimo vetor singular em relação aos genótipos de rizóbios (γk); α_{jk} é o elemento correspondente ao j-ésimo ambiente, no k-ésimo vetor singular em relação aos macroambientes (αk); e r é o número de repetições dos genótipos de rizóbio em cada ambiente. O método produz escores de interações dos componentes principais para cada genótipo que refletem sua contribuição para a interação *GCA*. Assim, o rizóbio com as pontuações mais baixas em valor absoluto ou mais próximo do eixo de maior explicação é o mais estável.

Ferramentas auxiliares. Os parâmetros de adaptabilidade e estabilidade fenotípica foram estimados por meio do no software estatístico *Estabilidade.* O modelo *AMMI* foi ajustado pela decomposição do valor singular da matriz de interação, e os testes F e graus de liberdade foram determinados de acordo com Gollob (Gollob, 1968). Biplots foram desenvolvidos usando o pacote *agricolae* em R. Para análise dos modelos de *Toler*, a técnica de mínimos quadrados não lineares foi utilizada por processos iterativos, aplicando o método de Gauss-Newton modificado para estimação desses parâmetros (Novaes Rosse and Vencovsky, 2000; Rosse et al., 2002). Em seguida, as médias genotípicas foram agrupadas pelo teste de Scott-Knott (P <0,05) por meio do software Sisvar 5.7 (Ferreira, 2019).

3 | RESULTADOS

Estudo da interação *GCA – Adaptabilidade e Estabilidade fenotípica*

A produtividade do feijão-comum foi significativamente influenciada pelos genótipos-G [duas estirpes de bactérias fixadoras de N_2: CIAT899 e UFLA2-127; e controles de estirpes nativas com três doses de N mineral: 0 (NR0N), 20 (NR20N) e 80 kg N-ureia ha^{-1} (NR80N)],

macroambientes-CA (combinação dos componentes: cultivar-C e ambiente edafoclimático-A) e pela interação *GCA* (Tabela Suplementar 1 e 2). Portanto o desempenho dos genótipos de estirpes tem comportamento diferencial em função do macroambiente a que é inserido. Justifica-se, assim, o estudo do comportamento genotípico em sua adaptabilidade e estabilidade fenotípica, de modo identificar a magnitude da interação *GCA* e traçar o perfil dos fatores nela envolvidos.

Método Annicchiarico (Annicchiarico, 1992)**.** Os reliability index (I%), que indicam a probabilidade de sucesso na adoção dos rizóbios, decresceram na seguinte ordem: UFLA02-127 > NR80N > CIAT899 > NR20N > NR0N (Tabela 2). Os maiores I% foram os dos genótipos UFLA02-127 (86.06%) e NR80N (79.91%), de produtividades estimadas em 109% da média macroambiental. Em uma faixa intermediária situaram-se os genótipos CIAT899 e NR20N, com I% de respectivamente 68% e 72%; NR20N, entretanto, com estimativa de 3% de perdas percentuais em relação à produtividade média macroambiental. O menor I% foi o do genótipo nativo (NR0N, 61.42%), cujo insucesso na produtividade reflete em prejuízos da ordem de 18.18% na estimativa macroambiental (Tabela 2).

Tabela 2 Índice de confiabilidade estimado pelo método Annicchiarico [de acordo com os genótipos (estirpes de bactérias fixadoras de nitrogênio e tratamentos de controle com estirpes nativas)]) e porcentagem média em relação à média macroambiental.

Genótipo de rizóbio[a]	Índice de confiabilidade[b] (I%)	Porcentagem media em relação à media ambiental
CIAT899	67,61	102,53
UFLA02-127	86,06	109,86
Rizóbio nativo (NR0N)	61,42	81,83
NR20N	71,64	97,01
NR80N	79,91	108,77

Valores médios seguidos da mesma letra na coluna pertencem ao mesmo grupo (P <0,01). [a]CIAT899 e UFLA02-127: estirpes selecionadas de bactérias fixadoras de nitrogênio; rizóbio nativo (NR) e rizóbio nativo fertilizado com N mineral [0 kg N-ureia ha^{-1} (NR0N), 20 kg N-ureia ha^{-1}(NR20N) and 80 kg N-ureia ha^{-1} (NR80N)]. [b] Nível de significância adotado = 0,05.

Modelos de Toler (Toler and Burrows, 1998)**.** Os índices de qualidade ambiental (EQI) variaram de -696.24 a 727.24 (Tabela 3). O ambiente com EQI > 0 é considerado favorável e o ambiente com EQI < 0, desfavorável. O maior EQI positivo foi o de Lb2Est (727.24), seguido, em ordem decrescente, pelos macroambientes Lb1Alv > PMAlv > Lb1Not > Lb1Est > LaAlv > Lb1Mad > LaEst. Prevaleceram dentre os de EQI positivo os macroambientes compostos pelas cultivares BRS Estilo e IAC Alvorada (sufixos ‘Est’ e ‘Alv’ das denominações macroambientais) e pelo ambiente edafoclimático 'Lambari1' (prefixo ‘Lb1’).

Lb2Alv (-696.24) e Lb2Mad (-569.38) foram os macroambientes mais desfavoráveis ao incremento das produtividades. Macroambientes compostos pela cultivar BRSMG Madrepérola (sufixo 'Mad') e pelos ambientes edafoclimáticos 'Lambari2' e 'Patos de Minas' predominaram dentre os de EQI negativo (prefixos 'Lb2' e 'PM' das denominações).

A adaptabilidade fenotípica, traduzida na capacidade de genótipos responderem vantajosamente à melhoria do ambiente, elevou-se na seguinte ordem: NR < NR20N = CIAT899 < UFLA02-127 = NR80N (Tabela 3).

Tabela 3 Produtividade de grãos do feijão e índice de qualidade ambiental estimado pelo modelo Toler (de acordo com macroambientes).

Macroambientes (cultivares de feijão e ambientes edafoclimáticos)	**Produtividade† kg ha^{-1}**	***Toler Model*** **Índice de qualidade ambiental‡**
LaAlv	1780,45 B	118,03
LaEst	1757,61 B	95,21
LaMad	1478,41 C	-184,00
LaNot	1480,60 C	-181,82
PMAlv	1925,40 B	262,99
PMEst	1552,03 C	-110,39
PMMad	1553,59 C	-108,82
PMNot	1582,92 C	-79,49
Lb1Alv	1983,57 B	321,15
Lb1Est	1806,89 B	144,48
Lb1Mad	1764,11 B	101,70
Lb1Not	1846,68 B	184,27
Lb2Alv	966,17 D	-696,24
Lb2Est	2389,65 A	727,24
Lb2Mad	1093,03 D	-569,38
Lb2Not	1637,48 B	-24,93

Genótipo de rizóbio[a]	**Produtividade † kg ha^{-1}** (indicativo de adaptabilidade)
CIAT899	1681,48 B
UFLA02-127	1841,52 A
Rizóbio nativo (NR0N)	1346,26 C
NR20N	1629,33 B
NR80N	1813,46 A
Média	1662,41

Valores médios seguidos da mesma letra na coluna pertencem ao mesmo grupo (P <0,01). [a]Efeitos principais do fator "*Genótipo*" são indicativos de adaptabilidade (P <0,01). [b]Nível de significância adotado = 0,05. §CIAT899 e UFLA02-127: estirpes selecionadas de bactérias fixadoras de nitrogênio; rizóbio nativo (NR) e rizóbio nativo fertilizado com N mineral [0 kg N-ureia ha^{-1}(NR20N); 20 kg N-ureia ha^{-1} (NR20N) and 80 kg N-ureia ha^{-1} (NR80N)]; Macroambientes compostos pelas cultivares IAC-Alvorada, BRS-Estilo, BRS-Notável e BRSMG-Madrepérola (sufixos 'Alv', 'Est', 'Not' e 'Mad' das denominações macroambientais) e por condições

edafoclimáticas Lavras, Patos de Minas , Lambari1 e Lambari 2 (prefixos 'Lav', 'PM', 'Lb1' e 'Lb2' das denominações macroambientais)

O desempenho dos genótipos UFLA02-127 e NR80N seguiu o modelo bissegmentado, com um padrão correspondente ao do grupo A de Toler (Tabela 4). Essa conclusão foi obtida após constatar uma diferença significativa ($p < 0.05$) entre β_{1i} e β_{2i}, descartando a hipótese de que a resposta de ambos os genótipos poderia ser representada por uma única linha de regressão. Os genótipos CIAT899 e NR20N exibiram resposta de segmento único ($\beta_{1i} = \beta_{2i}$), que diminuiu em função das estimativas β (CIAT899 > NR20N; Tabela 4). Pela significância de $\beta_i = 1$, eles foram então categorizados como de padrão de resposta tipo C, que é definido por médias que não se desviam das produtividades esperadas aos ambientes. Com coeficiente angular $\beta_i < 1$, o genótipo NR enquadrou-se no grupo D, apresentando resposta linear simples e desejável apenas em ambientes de baixa qualidade (Tabela 4).

Tabela 4 Estimativas de $\beta_{2i} - \beta_{1i}$ do modelo bissegmentado de Toler e teste da hipótese Ho: $\beta_{1i} = \beta_{2i}$, e estimativas e erros padrão dos coeficientes de regressão β_{1i} e β_{2i} do modelo bissegmentado e do β do modelo de segmento único, ambos modelos Toler, juntamente com os testes de hipóteses de igualdade desses parâmetros em 0 e 1, e classificação do padrão de resposta do Toler de acordo com os modelos dos genótipos e testes realizados.

Genótipo[a]	**$\beta_{2i} - \beta_{1i}$ Estimativa**
CIAT899	-0,3514
UFLA02-127	-1,3195*
Rizóbio nativo (NR0N)	0,3603
NR20N	-0,5957
NR80N	1,9064*

Genotype[a]	**MODELO**				**Padrão de Resposta**[b]
	Bissegmentado				
	β_{1i} Estimativa	**Erro Padrão**	**β_{2i} Estimativa**	**Erro Padrão**	
UFLA02-127	1,20[c]	0,3461	1,19[c,d]	0.3677	A
NR80N	0,97[c]	0,3558	2,38[c,d]	0.4151	A
	Unissegmentado				
	β_i Estimate		**Error Padrão**		
CIAT899	1,29[c]		0,1893		C
Rizóbio nativo (NR0N)	0,94[c,d]		0,1902		D
NR20N	0,99[c]		0,1884		C

*Significativo pelo teste F (P <0,05) .[a]CIAT899 e UFLA02-127: estirpes selecionadas de bactérias fixadoras de nitrogênio; rizóbio nativo (NR) e rizóbio nativo fertilizado com N mineral [0 kg N-ureia ha^{-1} (NR0N), 20 kg N-ureia ha^{-1} (NR20N) and 80 kg N-ureia ha^{-1} (NR80N)]. [b]Padrão de resposta A: A hipótese $\beta_{1i} = \beta_{2i}$ é rejeitada e $\beta_1 < 1 < \beta_2$ é aceita - Resposta convexa e duplamente desejável; padrão de resposta C: A hipótese $\beta_{1i} = \beta_{2i}$ não é rejeitada e H0: $\beta = 1$ não é rejeitada - Resposta linear simples que não se desvia da resposta média; padrão de resposta D: A hipótese $\beta_{1i} = \beta_{2i}$ não é rejeitada e H0: $\beta = 1$ é rejeitada, e o coeficiente angular comum é $\beta < 1$ - Resposta linear simples e desejável apenas em ambientes de baixa qualidade. Significativamente diferente de [c]0 e de [d]1 pelo teste F (P <0,05).

Modelos AMMI (Zobel et al., 1988)**.** A interpretação do biplot AMMI em relação à interação *GCA* foi realizada observando-se a magnitude e o sinal dos escores do genótipo e macroambiente para o eixo de interação. No biplot AMMI1 (Fig. 1), a estabilidade é avaliada nas ordenadas [primeiro eixo do singular: vetor singular principal (PSV)1]. O eixo horizontal deste biplot considera a variação do principal efeito aditivo dos genótipos e macroambientes. Os efeitos multiplicativos da interação contendo os dois primeiros PSV são representados no biplot AMMI2 (Fig. 2). Em qualquer uma das situações, escores baixos, próximos ao PSV de maior explicação ou próximos a zero indicam genótipos e macroambientes que pouco ou quase nada contribuem para a interação. Neste contexto, a estirpe UFLA02-127, os genótipos nativos NR20N e NR0N e os macroambientes Lb1Est, LaAlv, LaEst, PMAlv, Lb2Alv, LaNot e PMEst foram os que menos contribuíram para a interação *GCA* (Figs. 1 e 2). Apresentam, portanto, alta estabilidade. No sentido oposto, ou seja, como grandes responsáveis pelos efeitos na interação, citam-se a estirpe CIAT899, o genótipo NR80N e os macroambientes Lb1Alv, PMMad e PMNot (Figs. 1 e 2). Foram estes os cinco tratamentos que mais se afastaram dos eixos PSV dos biplots AMMI. Outros macroambientes como Lb1Not, Lb1Mad, Lb2Est, Lb2Not e LaMad também interferiram na interação, mas com efeitos menos consistentes (Figs. 1 e 2). Houve ainda sinergia adaptativa entre rizóbios e macroambientes listados a seguir (Figs. 1 e 2): CIAT899 e PMMad, PMNot, Lb1Mad, Lb2Mad, LaMad; UFLA02-127 e Lb1Alv, LB1Est; NR80N e Lb2Est, Lb1Not, Lb2Not; NR20N e PMAlv, LaEst; e NR0N e Lb2Mad, Lb2Alv, Lb2Not (Fig. 1).

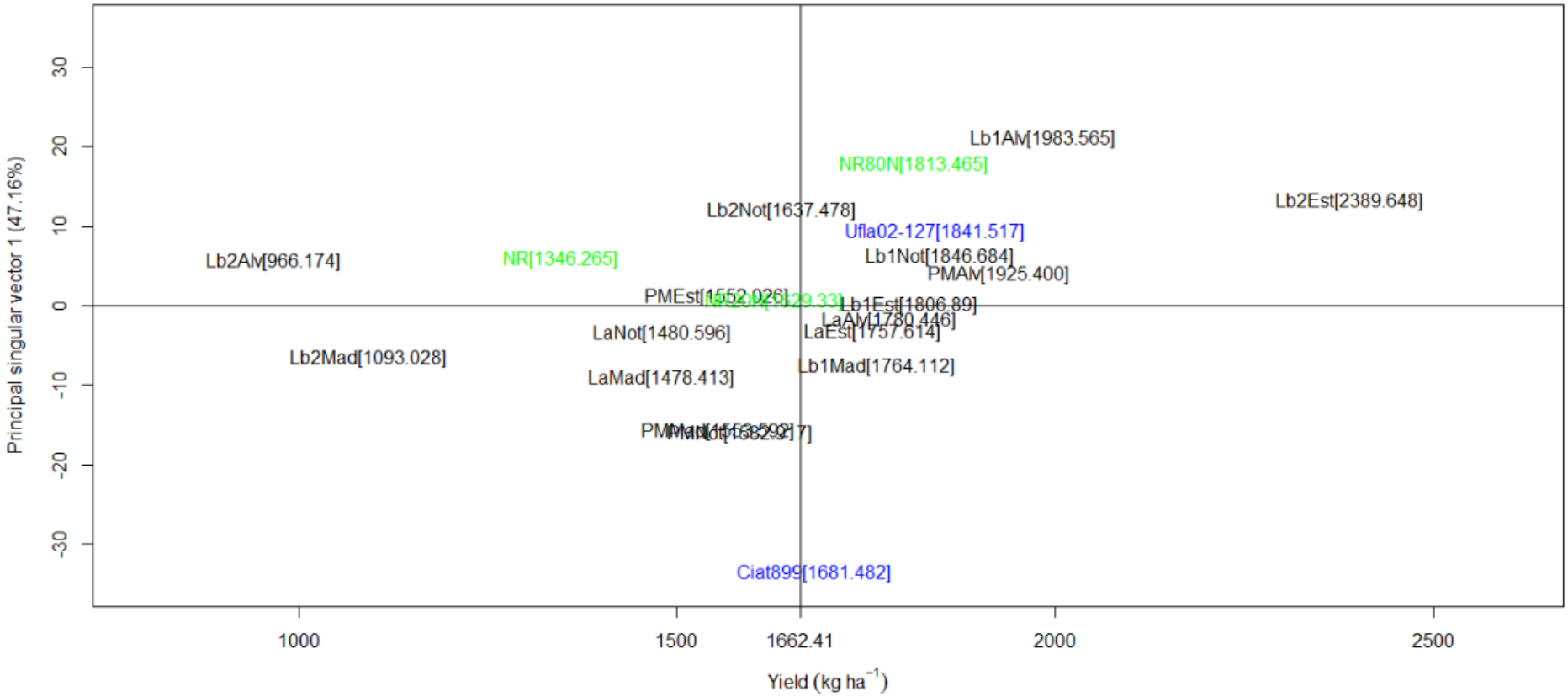
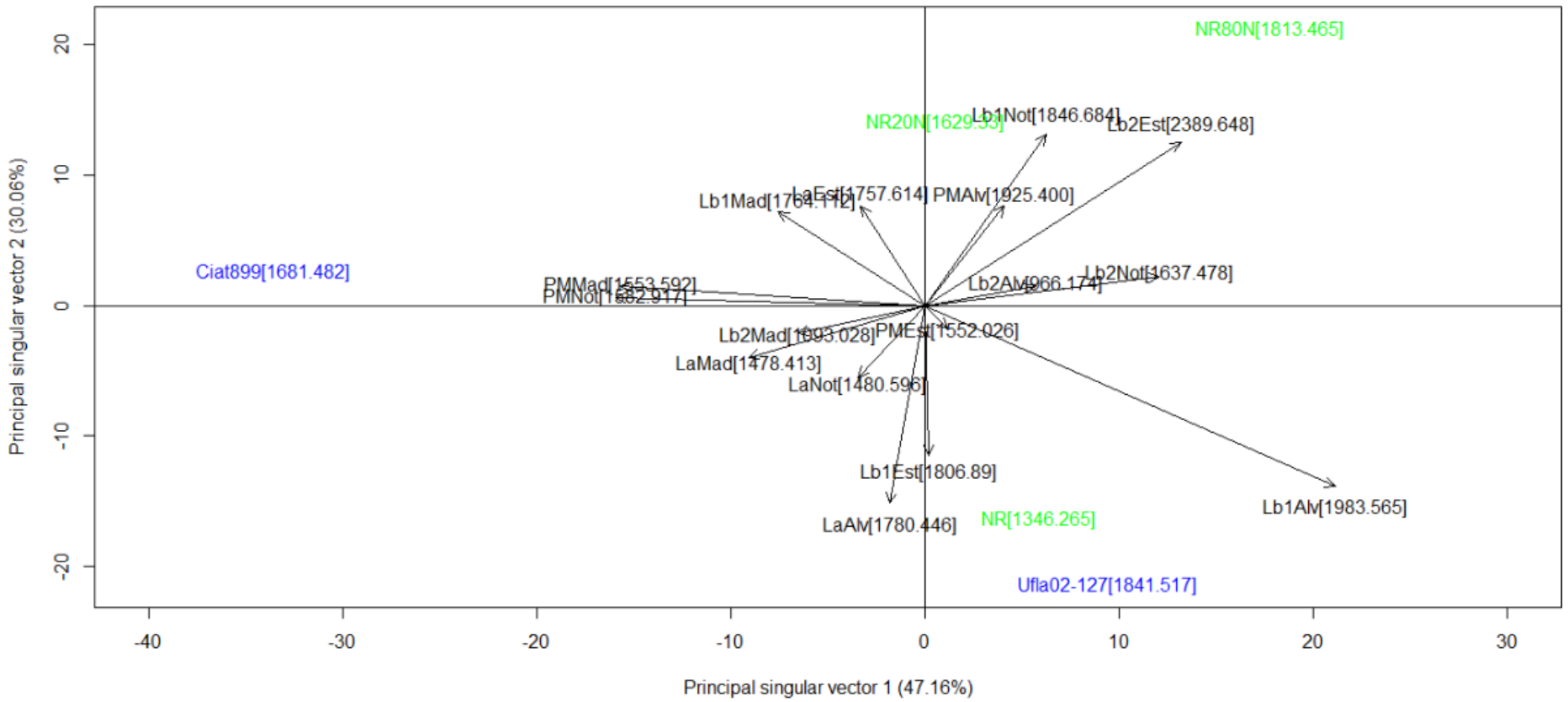


FIGURA 1. Biplot AMMI1 para dados de feijão obtidos por rizóbio nativo (NR0N, NR20N e NR80N) e rizóbio inoculado (CIAT899 de *Rhizobium tropici* e UFLA02-127 de *Rhizobium* sp.) em 16 macroambientes compostos pelos cultivares IAC-Alvorada, BRS-Estilo, BRS-Notável, e BRSMG-Madrepérola (sufixos 'Alv', 'Est', 'Not' e 'Mad' dos nomes macroambientais) e pelas condições edafoclimáticas Lavras, Patos de Minas, Lambari1 e Lambari2 (prefixos 'Lav', 'PM' , 'Lb1' e 'Lb2' dos nomes macroambientais), com sua produtividade de grãos (em kg ha^{-1}) entre colchetes



375

376 **FIGURA 2.** Biplot AMMI2 para dados de feijão obtidos por rizóbio nativo (NR0N, NR20N e NR80N) e rizóbio inoculado (CIAT899 de
377 *Rhizobium tropici* e UFLA02-127 de *Rhizobium* sp.) em 16 macroambientes compostos pelos cultivares IAC-Alvorada, BRS-Estilo, BRS-
378 Notável, e BRSMG-Madrepérola (sufixos 'Alv', 'Est', 'Not' e 'Mad' dos nomes macroambientais) e pelas condições edafoclimáticas Lavras, Patos
379 de Minas, Lambari1 e Lambari2 (prefixos 'Lav', 'PM' , 'Lb1' e 'Lb2' dos nomes macroambientais), com sua produtividade de grãos (em kg ha^{-1})
380 entre colchetes

4 | DISCUSSÃO

As técnicas utilizadas nesse estudo permitiram estratificar os macroambientes conforme o padrão de resposta de seus efeitos na interação *GCA*. Foram ainda reveladas diferenças no desempenho relativo dos genótipos, os quais foram definidos com base em sua adaptabilidade e estabilidades fenotípicas, o que não teria sido possível identificar considerando apenas os rendimentos médios baseados em uma ANOVA simples.

Metade dos macroambientes apresentaram IQE positivos, que são indicativos de possuírem qualidade favorável ao bom desenvolvimento genotípico no incremento das produtividades ambientais. Estes ambientes demonstram possuir essa característica, sobretudo, os macroambientes compostos pelas cultivares Estilo (Melo et al., 2009) e Alvorada (Morais Carbonell et al., 2008) e pelo ambiente edafoclimático 'Lambari1'. Em contrapartida, macroambientes compostos pela cultivar Madrepérola e pelos ambientes edafoclimáticos 'Lambari2' e ‘Patos de Minas’ demonstraram qualidade inferior.

Para acréscimos nas produtividades destes últimos é necessário adoção de insumos e tecnologias específicas, como, por exemplo, a de correção da fertilidade do solo. Lambari2' e ‘Patos de Minas’ possuíam deficiência em nutrientes como cálcio, fósforo (em Lambari2) e magnésio; concentrações tóxicas de enxofre (sobretudo em Patos de Minas) e alumínio; além de alta acidez potencial e baixas CTC efetiva e saturação de bases (Materiais e Métodos - Tabela 1). Condições essas, caso não corrigidas, podem limitar as possibilidades de ganho ambiental.

Fatores podem afetar a eficiência de rizóbios simbióticos e espécies de leguminosas em condições de campo. Alguns deles são intrínsecos à estirpe bacteriana e às espécies vegetais, enquanto outros são extrínsecos, relacionados a atributos biológicos, físicos e químicos do meio ambiente. Por isso a importância da seleção de genótipos de alta adaptabilidade aos mais diversos ambientes, sobretudo em um país de condição tropical como o Brasil, onde predominam condições de acidez do solo; deficiência de nutrientes como cálcio, fósforo e magnésio; concentrações tóxicas de enxofre, manganês, cobre e alumínio; e clima com altas temperaturas e ampla amplitude nas chuvas (Hungria and Vargas, 2000; Moreira et al., 2006; Oliveira et al., 2018; Ruschel and Reuszer, 1973).

Números de cerca de 10^3 unidades formadoras de colônia de rizóbios nativos por grama de solo foram obtidos nos macroambientes estudados. Esses rizóbios têm comportamento estável, mas negativamente previsível. Enquadram-se no grupo D de Toler (Toler and Burrows, 1998), com padrão de resposta desejável apenas nos ambientes de baixa

qualidade. Isso significa que as comunidades nativas conseguem sobreviver e induzir benefícios em condições adversas, as quais poderiam comprometer o desempenho de outros genótipos de rizóbio sem tal aptidão. Entretanto, o incremento que ele proporciona é muito baixo e supera apenas o do grupo E (de Figueiredo et al., 2015; Delgado et al., 2019; Oliveira et al., 2020; Toler and Burrows, 1998). Por certo, o melhor desempenho nativo prevaleceu nos macroambientes de qualidade reduzida, sendo ele mais frequente em 'Lambari2' (macroambiente de prefixo 'Lb2'). Possivelmente NR0N tenha desenvolvido mecanismos de tolerância aos estresses que caracterizaram esse ambiente (Tabela 1). Apesar dessa aptidão, é baixa a probabilidade de sucesso em sua adoção em cultivos que dele dependam para atender a demanda nitrogenada do feijão-comum (baixo I% - Método Annichiaricco).

As comunidades nativas de rizóbio do solo por si só nem sempre são suficientes para estabelecer uma simbiose que contribua significativamente para um aumento na produção de leguminosas. Essa afirmação é recorrente na literatura (Ferreira et al., 2009; Nogueira et al., 2017; Oliveira et al., 2021a, 2020, 2017; Paula Rodiño et al., 2011; Soares et al., 2006). A complementação com baixa dose N-mineral pode aumentar a eficiência de simbiose dessa comunidade nativa (Oliveira et al., 2018b; Oliveira et al., 2019, 2016), mas não garantir que os resultados equivalham aos da inoculação com estirpes selecionadas pela boa eficiência de fixação de N_2.

A dose 20 kg N-ureia ha^{-1} (genótipo NR20N) elevou a estabilidade do rizóbio nativo e ampliou sua capacidade produtiva (em comparação ao NR0N) de D à do grupo C de Toler (Toler and Burrows, 1998), o mesmo grupo da estirpe CIAT899 (Martinez-Romero et al., 1991), a primeira dentre as atualmente aprovadas como inoculante para feijão-comum no Brasil pelo MAPA. No grupo C as respostas em produtividade não se afastam da média macroambiental (Toler and Burrows, 1998). Isso amplia a confiabilidade de adoção do genótipo, pela maior chance de êxito com o rizóbio. Essa mesma dose trouxe benefícios ao genótipo nativo em simbiose com a cultivar Madrepérola (Carneiro et al., 2012) em quatro outros ambientes brasileiros estudados no Brasil (Oliveira, 2013), os quais resultaram em incrementos equivalentes ao da inoculação com a estirpe CIAT899.

A sinergia entre o genótipo CIAT899 e a cultivar Madrepérola no atual estudo é indicativo de especificidade entre este par estirpe-feijoeiro. Esse resultado foi recorrente em todos os macroambientes que continham essa cultivar (PMMad, Lb1Mad, LB2Mad e LaMad). Autores já haviam mencionado a alta responsividade dessa estirpe com a cultivar (da Silveira Cardillo et al., 2019; Figueiredo et al., 2016; Fonseca et al., 2013;Oliveira et al.,

2018b), cuja produtividade de grãos pode se assemelhar ao da fertilização com 80 kg N-ureia ha^{-1} (Oliveira et al., 2018a; Oliveira et al., 2019, 2017). Essa sinergia indica que, mesmo sendo a CIAT899 uma estirpe bastante promíscua [capaz de estabelecer nódulos fixadores de N_2 eficazes com várias espécies de leguminosas, incluindo feijão-comum (*P. vulgaris*), *Leucaena* spp., *Lotus japonicus, Lotus burttii* e *Gliricidia* spp. (Datola Tullio et al., 2019; Del Cerro et al., 2017)], pode ter seu desempenho melhorado na presença de cultivares específicas. Embora os melhores resultados com a estirpe tenham ocorrido com a Madrepérola, produtividades de até 1667 kg ha^{-1} resultaram da simbiose com outras cultivares (Tabela Suplementar 2). Há relatos de resultados semelhantes com a CIAT899 em estudos da mesma natureza com foco em outros genótipos de *P. vulgaris* (Fageria et al., 2014; Grange et al., 2007; Oliveira et al., 2018a).

A FBN tem se intensificado na cultura do feijão-comum nos últimos anos, com resultados positivos e consistentes sendo alcançados para várias cultivares (Andraus et al., 2016; Cardoso et al., 2017; Fageria et al., 2014; Ferreira et al., 2009; Grange et al., 2007; Knupp et al., 2017; Moreira et al., 2017; Nogueira et al., 2017; Oliveira et al., 2018, 2018a; Pereira et al., 2015; Soares et al., 2006). Rizóbios eficientes (nativos ou introduzidos por inoculação) e cultivares responsivas têm proporcionado rendimentos satisfatórios – equivalentes ou até superiores aos obtidos com a fertilização com nitrogênio mineral. No entanto, existem materiais que são mais dependentes de fertilizantes nitrogenados para seu desenvolvimento (Fageria et al., 2014; Grange et al., 2007; Pereira et al., 2015). A elevada oferta de N mineral durante o ciclo de seleção do feijoeiro certamente tem influência nessa demanda nitrogenada. Esse pode ser o caso da cultivar BRS Notável (Santos Pereira et al., 2012), a qual está associada aos principais resultados de sinergia do genótipo fertilizado com 80 kg N-ureia ha^{-1} (NR80N). Pesquisas, contudo, indicam que, ainda que algumas cultivares apresentem rendimento mais alto sob fertilização com N mineral, tornam-se mais resistentes a doenças quando inoculado com rizóbio (Ferreira et al., 2020; Dias et al., 2020).

A capacidade de simbiose da CIAT899 com a cv. Madrepérola, por exemplo, agrega às potencialidades da cultivar. Lançada há menos de uma década, essa cultivar tem tido grande aceitação no Brasil (Carneiro et al., 2012), mas recebido ainda pouca atenção quanto à sua capacidade de simbiose com rizóbios. Por seus bons resultados com a principal estirpe inoculante nacionalmente aprovada para o feijão-comum, assim como com os outros rizóbios, pode ser então incluída como referência em programas de melhoramento voltados para FBN.

CIAT899 foi a estirpe que mais interferiu na interação. Possivelmente seja ela a responsável pelos expressivos resultados de PMMad e PMNot, cujos efeitos, dentre todos os macroambientes, mais se intensificaram sobre a interação *GCA*. Algo interferiu positivamente à sua adaptabilidade em Patos de Minas, especificamente nas cultivares Madrepérola e Notável. Conceitualmente, adaptabilidade designa a capacidade de genótipos responderem vantajosamente à melhoria do ambiente, do ponto de vista da produtividade (Mendonça et al., 2007). A adaptabilidade da CIAT899, que resultou em maior eficiência de FBN da estirpe em Patos de Minas, pode estar relacionada com fatores inerentes à cultivar hospedeira (pela exsudação de metabólitos importantes, por exemplo), os quais podem ter sido potencializados pela inter-relação com a microbiota nativa presente na rizosfera daquele macroambiente (Andraus et al., 2016; de Oliveira-Longatti et al., 2014; Ferreira et al., 2012; Moreira and Siqueira, 2006).

Os genótipos UFLA02-127 e NR80N apresentaram o comportamento padrão do grupo A de Toler, que é o de resposta duplamente desejável [$\beta_{1i} < 1 < \beta_{2i}$; (Toler and Burrows, 1998)]. Isso significa que apresentam perdas mínimas em ambientes de baixa qualidade e ganhos substanciais naqueles com qualidade superior. Por isso as sinergias de UFLA02-127 e do genótipo fertilizado com 80 kg N-ureia ha^{-1} serem evidenciadas em macroambientes de alto EQI (Tabela 3). Apesar de pertencerem ao mesmo agrupamento, apresentaram estabilidades distintas – a alta estabilidade atribuída à estirpe UFLA02-127 (de escores mais próximos do eixo PSV1, o que agrega maior explicação à interação *GCA*) e a baixa associada ao genótipo nativo fertilizado com 80 kg N-ureia ha^{-1}. Neste último caso, seguramente o aumento na produtividade deveu-se à adubação nitrogenada mineral, já que doses elevadas de N inibem a atuação rizobiana (Moreira and Siqueira, 2006; Oliveira et al., 2018a; Silva et al., 2021).

A alta confiabilidade de adoção de NR80N (I% ≡ 80%) não garante que sua eficiência agronômica seja aproveitada ao seu máximo potencial, o que foi indicado pela baixa estabilidade do tratamento neste estudo. Como dependente da adubação nitrogenada mineral é provável que NR80N possa ter eficiência comprometida sob condições adversas (como por exemplo, de limitação nutricional à planta e de ocorrência de umidade em níveis inadequados para incorporação do fertilizante nitrogenado mineral). Sua eficiência pode ser até anulada em solos ricos em N orgânico ou mineral. Torna-se instável, portanto, a definição dos seus incrementos na produtividade (Oliveira et al., 2021b, 2020). Isso é coerente com os resultados AMMI e com outros estudos envolvendo esse tratamento (Oliveira et al., 2021b, 2020). Na

prática, o uso de altas doses do fertilizante nitrogenado em um ambiente com limitações ou com fertilidade abundante, seria um investimento perdido, além de causar impactos negativos no meio ambiente. Por sua vez as vantagens da adoção da UFLA02-127 englobam a previsibilidade de ganhos em ambientes favoráveis, pela incorporação dos estímulos ambientais vantajosos, e expectativa de prejuízos irrelevantes em ambientes de qualidade inferior.

Neste estudo, a estirpe UFLA02-127 foi considerada a de maior estabilidade. Seu bom desempenho não esteve condicionado a determinado ambiente edafoclimático ou a alguma cultivar específica. Isso denota o amplo espectro de simbiose dessa estirpe, cuja produtividade foi consistentemente mais elevada que a dos outros rizóbios e equivalente à da fertilização com 80 kg N-ureia ha^{-1}. Esta estirpe pode, portanto, ser utilizada com sucesso em diversas cultivares e ainda substituir a fertilização com nitrogênio sem prejudicar a cultura, o que significa não apenas economia de fertilizantes em N, mas, acima de tudo, uma significativa contribuição ecológica. Não é por acaso seu bom desempenho apareça com frequência em estudos brasileiros com *P. vulgaris* (Figueiredo et al., 2016; Nogueira et al., 2017; Oliveira et al., 2018a; Oliveira et al., 2021b, 2017; Soares et al., 2006), os quais corroboram seu potencial genético elevado, indicado por elevadas produtividades, sobretudo, quando inserida em ambiente favorável, com boas condições de cultivo. Por razões como essas a estirpe tem potencial para compor a relação de inoculantes brasileiros para a cultura do feijão-comum.

O trabalho enfatiza ainda a importância de se avaliar e selecionar cultivares que foram desenvolvidas em sistema de cultivo com fertilização com N mineral, mas que apresentam desempenho superior em sistema com inoculação de *Rhizobium*. Esse pode ser um gargalo de incentivo à difusão da biotecnologia de inoculação e à adoção de cultivares de feijão-comum em campo. Como as cultivares Alvorada, Madrepérola e Estilo (em ordem de lançamento comercial), e mesmo a Alvorada (de sinergia com a máxima adubação nitrogenada mineral, mas também de resultados consideráveis com as inoculações), já apresentam vários fenótipos agronômicos favoráveis acumulados ao longo dos anos de seleção, com também resultados satisfatórios com as estirpes CIAT899 e UFLA02-127, podem ser utilizadas em cruzamentos nos programas de melhoramento visando a FBN, como fonte de alelos de características agronômicas importantes também na simbiose de fixação do N_2 (Dias et al., 2020; Pereira et al., 2015). Considerando a excelente adaptabilidade e estabilidade fenotípica da estirpe UFLA02-127 seria interessante também a recomendação dessa estirpe, em substituição a

adubação nitrogenada, durante as fases de seleção de novas cultivares nos programas de melhoramento genético.

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555 **Tabela Suplementar 1**Resumo da análise de variância conjunta dos dados de produtividade de grãos de feijão, em função de genótipos (estirpes
556 de bactérias fixadoras de nitrogênio e controles nativos com rizóbio nativo) e macroambientes

Fonte de variação	Graus de liberdade	Produtividade (quadrado médio)
Bloco (Ambiente)	32	496993,2251**
Genótipo (G)[a]	15	1696170,9640**
Macroambiente (CA)[b]	4	1875633,7116**
Interação *GCA*	60	311396,7116*
Resíduo	128	220493,8903
Coeficiente de variação (%)	28,25	-

557 [a]Inclui estirpes previamente selecionadas de bactérias fixadoras de nitrogênio e tratamentos de controle com rizóbio nativo. [b]Inclui 16 macroambientes compostos pelas
558 cultivares IAC-Alvorada, BRS-Estilo, BRS-Notável e BRSMG-Madrepérola e pelas condições edafoclimáticas Lavras, Patos de Minas, Lambari1 e Lambari2. Significativo
559 pelo teste F (** P <0,01 e * P <0,05).

Tabela Suplementar2 Produtividade de grãos de feijão (in kg ha^{-1}) obtida por rizóbio em 16 macroambientes compostos pelas cultivares IAC Alvorada, BRS Estilo, BRS Notável e BRSMG Madrepérola (sufixos 'Alv', 'Est', 'Not' e 'Mad' das denominações macroambientais) e por condições edafoclimáticas Lavras, Patos de Minas, Lambari1 e Lambari2 (prefixos 'Lav', 'PM', 'Lb1' e 'Lb2' das denominações macroambientais).

Ambiente edafoclimático	Cultivar	Macroambiente (Ambiente edafoclimático e Cultivar)	Genótipo de rizóbio[a]				
			CIAT899	UFLA02-127	Rizóbio Nativo (NR0N)	NR20N	NR80N
Lavras	Alvorada	LaAlv	1736,99 b	2549,63 a	1424,27 b	1842,87 b	1348,47 b
Lavras	Estilo	LaEst	1802,72 a	1931,05 a	1025,24 b	2238,37 a	1790,69 a
Lavras	Madrepérola	LaMad	1782,21 a	1556,81 a	1311,00 a	1373,49 a	1368,56 a
Lavras	Notável	LaNot	1593,95 a	1697,50 a	1315,20 a	1360,80 a	1435,53 a
Patos de Minas	Alvorada	PMAlv	1968,67 a	2126,25 a	1309,42 b	1646,67 b	2576,00 a
Patos de Minas	Estilo	PMEst	1466,67 a	1726,38 a	1337,92 a	1658,33 a	1570,83 a
Patos de Minas	Madrepérola	PMMad	2145,67 a	1618,75 a	1041,04 a	1429,17 a	1533,33 a
Patos de Minas	Notável	PMNot	2225,00 a	1522,50 a	1258,75 a	1266,67 a	1641,67 a
Lambari1	Alvorada	Lb1Alv	1361,04 b	2717,14 a	2020,90 a	1429,08 b	2389,66 a
Lambari1	Estilo	Lb1Est	1771,10 a	2313,16 a	1634,96 a	1664,90 a	1650,33 a
Lambari1	Madrepérola	Lb1Mad	2034,71 a	1749,59 a	1212,31 a	1926,77 a	1897,18 a
Lambari1	Notável	Lb1Not	1709,30 a	1744,97 a	1364,09 a	1965,80 a	2449,26 a
Lambari2	Alvorada	Lb2Alv	830,66 a	948,84 a	916,74 a	789,95 a	1344,68 a
Lambari2	Estilo	Lb2Est	1982,14 b	2501,78 a	1796,42 b	2652,99 a	3014,91
Lambari2	Madrepérola	Lb2Mad	1350,55 a	1070,68 a	1005,69 a	886,64 a	1151,58 a
Lambari2	Notável	Lb2Not	1142,33 a	1689,25 a	1566,28 a	1936,77 a	1852,75 a

Na linha, valores médios seguidos dos mesmas letras minúsculas pertencem ao mesmo grupo de acordo com o teste de Scott-Knott ($p < 0,05$). [a]CIAT899 e UFLA02-127: estirpes selecionadas de bactérias fixadoras de nitrogênio; rizóbio nativo (NR0N - 0 kg N-ureia ha^{-1}) e rizóbio nativo fertilizado com N mineral [20 kg N-ureia ha^{-1}(NR20N) e 80 kg N-ureia ha^{-1} (NR80N)].

CONFLITO DE INTERESSES

Os autores declaram não haver conflito de interesses.

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