

RESEARCH ARTICLE

# Genetic correlation and path analysis of common bean inoculated with *Rhizobium tropici* and *Azospirillum brasilense*

Lucymara Merquides Contardi<sup>1</sup>, Bruno Ettore Pavan<sup>1</sup>, Patricia de Almeida<sup>1</sup>, Igor Hiroda Kamano<sup>1</sup>, Maria Eduarda Facioli Otoboni<sup>1</sup>, Sebastião Ferreira de Lima<sup>2\*</sup>, Vitória Carolina Dantas Alves<sup>2</sup>, and Rita de Cássia Barros Nunes<sup>3</sup>

<sup>1</sup>Universidade Estadual de São Paulo, Tecnologia de Alimentos e Sócio-Economia, Ilha Solteira, 15385-000, São Paulo, Brasil.

<sup>2</sup>Universidade Federal de Mato Grosso do Sul, Campus de Chapadão do Sul, Chapadão do Sul, 79560-000, Mato Grosso do Sul, Brasil.

<sup>3</sup>Universidade Estadual de Mato Grosso do Sul, Cassilândia, 79540-000, Mato Grosso do Sul, Brasil.

\*Corresponding author (sebastiao.lima@ufms.br).

Received: 29 March 2024; Accepted: 7 July 2024, doi:10.4067/S0718-58392024000500699

## ABSTRACT

The interaction between bean (*Phaseolus vulgaris* L.) genotypes and N-fixing microorganisms has the potential to be utilized in sustainable agricultural production. Thus, the aim of this study was to verify the effects of the interaction for growth, production and nodulation characteristics between the bacteria *Rhizobium tropici* and *Azospirillum brasilense* among different bean genotypes. The experiments were conducted in the Selvíria, Mato Grosso do Sul, Brazil, in 2018 and 2019, in randomized block design with three replicates in a 20 × 3 factorial scheme; 20 common bean genotypes were tested under three forms of inoculation (no inoculation, inoculation with *R. tropici*, co-inoculation of *R. tropici* + *A. brasilense*). The number of grains per pod and nodule mass had the most significant direct positive effect on grain yield. For nodulation, a positive correlation of strong magnitude was observed between mass of the aerial part and number of pods per plant (0.68), stem diameter and mass of nodules (0.92) and first pod insertion height and number of nodules (0.64). In the path analysis, the number of grains per pod had the most significant direct effect (0.57) on the grain productivity variable. Regarding nodulation characteristics, selection for nodule mass was preferred due to its direct effect on grain yield as opposed to the number of nodules, which has a negative effect. This suggests an advantage of larger nodules with greater individual mass than a large number of small nodules for both improved yield and N fixing by common bean plants.

**Key words:** Biological nitrogen fixation, co-inoculation, growth-promoting bacteria, *Phaseolus vulgaris*.

## INTRODUCTION

The common bean (*Phaseolus vulgaris* L.), a noduliferous plant belonging to the family Fabaceae, establishes symbiotic relationships with certain species of bacteria in the family *Rhizobiaceae*, thereby benefiting from atmospheric N fixed through this biological process (Efsthadiadou et al., 2021). In this sense, research must prioritize the search for cultivation methods that reduce costs and environmental impact and make the system more sustainable. One way to achieve this goal is through the use of growth-promoting and/or N-fixing microorganisms, which are bioinputs with high potential for agriculture (Gonçalves et al., 2023; Cabanzo-Atilano et al., 2024).

Biological N fixation can be an alternative for reducing the use of N fertilizers, provided that symbiosis with rhizobia supplies the amount of N necessary for the growth and development of the bean plant. However, the benefits of such inoculation are not well understood owing to the application of high doses of mineral N to recipient crops (Abd-Alla et al., 2023).

The nodulation process is controlled by interactions between the legume (host plant), symbiont bacteria, and environmental factors (Agudelo et al., 2023). The domestication and selection of cultivars with commercially desirable characteristics and the use of N fertilization beyond the capacity of beans to associate with the bacteria *Rhizobium* has influenced the selection of plants that respond better to chemical fertilization (Abd-Alla et al., 2023). As a result, the symbiotic balance between bacteria (*Rhizobium*) and common bean has changed; therefore, it is necessary to reevaluate the biological N fixation capacity of modern bean cultivars (Sousa et al., 2022).

Considering the low availability of bean cultivars with high symbiotic potential, the technique of co-inoculation or mixed inoculation has been used to achieve improved symbiotic performance (Souza and Ferreira, 2017; Steiner et al., 2020).

Among the bacterial groups evaluated, studies have demonstrated the potential of associative bacteria to promote plant growth through various processes, such as the production of growth hormones and the ability to perform biological N fixation, such as bacteria from the genus *Azospirillum* (Timofeeva et al., 2023). Thus, the co-inoculation of *Azospirillum* spp. and *Rhizobium* has yielded positive results in terms of variables analyzed in common bean due to increased root system growth and a high number of atmospheric N-fixing nodules, which may result in increased grain yield (Horácio et al., 2020).

Bean breeding programs in Brazil have aimed to increase economic yield potential, among numerous other factors (Lemos et al., 2020). The selection process aims to improve one primary trait while maintaining or improving the expression of others simultaneously (Reinprecht et al., 2023). Thus, knowledge about the existing relationships between key traits through genetic correlations is of utmost importance for supporting bean selection approaches (Gonçalves et al., 2017; Mesera et al., 2022). However, simplistic correlations can provide distorted information about trait relationships because they are necessarily influenced by other factors (Carloni et al., 2022).

In this context, path analysis can help breeders clarify the influencing factors affecting associations between traits and utilize known correlations into direct and indirect effects (Faysal et al., 2022; Shubha et al., 2024).

It was therefore hypothesized that bean genotypes may respond in their growth and production characteristics to association with N-fixing and/or growth-promoting bacteria. Based on this, in this study, we aimed to estimate the interrelationships of the effects of symbiotic interactions on the growth characteristics, nodulation, production components, and grain yield of common bean.

## MATERIALS AND METHODS

### Experimental area

The experiments were conducted in the field during winter in 2018 and 2019. The study was carried out in the experimental area of the Faculty of Engineering, São Paulo State University, Ilha Solteira Campus in Selvíria (51°22' W, 20°22' S; 335 m a.s.l.), Mato Grosso do Sul, Brazil. The region's climate is Aw according to the Köppen classification (humid tropical with a rainy season in summer and dry winter).

The soil in this area is classified as typic Dystrophic Red Latosol of clayey texture. Before the experimental setup was completed, soil samples were collected and analyzed chemically. Soil analysis was carried out in both sowing years because the areas were fallow. The results obtained were: P resin 23 mg dm<sup>-3</sup>, organic matter 20 g dm<sup>-3</sup>, pH in CaCl<sub>2</sub> = 5.0, K, Ca, Mg, H+Al and cation exchange capacity (all in mmolc dm<sup>-3</sup>) 2.3, 20, 17, 34 and 73.3, respectively, and base saturation 54%.

### Genotypes used

The 20 genotypes evaluated in the experiments comprised four inbred lines and 16 bean cultivars belonging to five national common bean breeding programs (Table 1). Among the materials analyzed, recent and old cultivars were evaluated to represent the genetic variability of the nation's primary improvement programs for common bean.

**Table 1.** Relationship of bean genotypes evaluated in the experiment. Ilha Solteira, São Paulo, 2018/2019. <sup>1</sup>Universidade Estadual Paulista, Faculty of Engineering of Ilha Solteira. <sup>2</sup>Universidade Federal de Lavras. <sup>3</sup>Empresa Brasileira de Pesquisa Agropecuária. <sup>4</sup>Instituto Agronômico do Paraná. <sup>5</sup>Instituto Agronômico de Campinas. Source: Prepared by the author.

Genotypes	Improvement program	Genotypes	Improvement program
1. Ling 03	UNESP <sup>1</sup>	11. BRS Notável	EMBRAPA
2. Ling 06	UNESP	12. Pérola	EMBRAPA
3. Ling 08	UNESP	13. IPR 81	IAPAR <sup>4</sup>
4. Ling 13	UNESP	14. IPR Campos Gerais	IAPAR
5. Carioca MG	UFLA <sup>2</sup>	15. IPR Sabiá	IAPAR
6. Esal 1	UFLA	16. IPR Tangará	IAPAR
7. BRSMG Majestoso	UFLA	17. Imperador	IAC <sup>5</sup>
8. BRSMG Uai	UFLA	18. Sintonia	IAC
9. BRS Cometa	EMBRAPA <sup>3</sup>	19. Milênio	IAC
10. BRS Estilo	EMBRAPA	20. Alvorada	IAC

### Experimental design, treatments and plots

The experiment was conducted using a randomized block design with three replicates in a 20×3 factorial scheme, consisting of a combination of the 20 selected common bean genotypes (Table 1) with three inoculation treatments (no inoculation, inoculation with *Rhizobium tropici*, co-inoculation of *R. tropici* + *Azospirillum brasilense*).

The plots consisted of four 4.0 m long rows with 0.5 m spacing between them. The two central rows were considered valid, and a width of 0.5 m at their edges were disregarded.

### Application of inoculants and experimental conduction

For normal inoculation of bean seeds, an inoculant was applied with a minimum concentration of  $1 \times 10^9$  CFU mL<sup>-1</sup> commercial strains of *R. tropici* SEMIA 4088. For co-inoculation, an inoculant based on *A. brasilense* strains Ab-V5 and Ab-V6 with a concentration of  $2 \times 10^8$  CFU mL<sup>-1</sup> was applied and added to the seeds.

Sowing fertilization was performed in both years of the experiment, according to the soil analysis results, 10 kg N ha<sup>-1</sup>, 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, and 30 kg K<sub>2</sub>O ha<sup>-1</sup> were applied. In addition, covering N fertilization was performed in all treatments at the minimum recommendation for a culture of 20 kg N ha<sup>-1</sup> in both experimental years.

Seeds were treated the day before the experiment began, using pyraclostrobin, thiophanate-methyl, and fipronil at a dose of 200 mL ai 100 kg<sup>-1</sup> seeds. The bacteria were inoculated using liquid inoculant applied directly to the seeds on the day of sowing. For *R. tropici*, the commercial product Biomax (Vittia Ribeirão Preto, São Paulo, Brazil), was used at a dose of 150 mL 50 kg<sup>-1</sup> seeds and for *A. brasilense*, the commercial product Masterfix grasses (Stoller, Houston, Texas, USA) was used at a dose of 100 mL 50 kg<sup>-1</sup> seeds. The same dosage was used in co-inoculation.

A manual sowing machine with seed distribution was used to obtain 10-12 plants m<sup>-2</sup>. All non-inoculated seeds were sown first to prevent contamination, then seeds treated with *R. tropici* were sown, and finally, seeds co-inoculated with *R. tropici* + *A. brasilense* were sown. Irrigation during the experimental period was provided on demand by a central pivot system, following recommendations for the crop.

Post-emergence weed control was performed with the herbicides fomesafen (250 g ai ha<sup>-1</sup>) and fenoxaprop-P-ethyl (77 g ai ha<sup>-1</sup>) for broad-leaved and narrow-leaved plants, respectively. No chemical control for diseases and pests was applied during the experiment.

### Evaluations

The inoculation response obtained in three plants per plot at the R5 stage was evaluated by measuring the following variables: Dry mass of the aerial part (DMP), number of nodules (NN), and mass of nodules (MN). For DMP, three plants per plot were collected and placed in a forced ventilation oven at an average temperature of 60-70 °C until constant mass was reached. For NN and MN, the root systems of three plants in sequence per

plot were collected using a straight shovel. After counting, the nodules were placed in an oven with forced ventilation at 60 °C until constant mass was reached.

For the production components and plant architecture variables, six plants were collected from each plot and the following were measured. Insertion height (IH): The plants of each plot were measured from the cotyledonary scar to the first pod insertion. Stem diameter (DC): Using a pachymeter, plants were measured at the point of the cotyledonary scar. Total plant height (PH): Using a millimetric tape measure, plant length was measured from the base of the plant to the end of the guide. Number of pods per plant (NPP): The number of pods per plant were counted. Number of grains per plant (NG): The number of grains per plant were counted and used to calculate the number of grains per pod. Number of grains per pod (NGP): The amount of grains/number of pods ratio was calculated. The 100-grain mass (M100G): A sample of 100 grains per plot was evaluated, and its mass was corrected to the wet basis of 13%. Grain yield (GY): All plants in the usable area of the plot were evaluated, and the data were adjusted to 13% moisture.

### Statistical analysis

Initially, a simple statistical analysis by year of cultivation was used; after the homogeneity of residual variances was verified, a joint analysis of experiments was performed using the following model:  $Y_{ijk} = \mu + B_{j(k)} + G_i + A_k + G_iA_k + \varepsilon_{ijk}$ , where  $Y_{ijk}$  is the effect of the plot of the  $i$ -th genotype in the  $j$ -th block in the  $k$ -th year;  $\mu$  is the effect of the general average;  $B_{j(k)}$  is the effect of the  $j$ -th block in the  $k$ -th year;  $G_i$  is the effect of the  $i$ -th genotype;  $A_k$  is the effect of the  $k$ -th year;  $G_iA_k$  is the effect of the interaction between the  $i$ -th genotype and the  $k$ -th year; and  $\varepsilon_{ijk}$  is the effect of experimental error associated with the  $i$ -th genotype of the  $j$ -th block in the  $k$ -th year. From the joint analysis, the matrix of variance and covariance of phenotypic and genotypic variables was obtained due to genetic correlations between the studied traits using the statistical program GENES (Cruz, 2016). To assess the magnitude of the correlation coefficient, we used the classification according to the criteria proposed by Carvalho et al. (2002): Null = 0; weak = 0.1 to 0.30; medium = 0.31 to 0.60; strong = 0.61 to 0.90; very strong = 0.91 to 1.0.

In the path analysis, the total production traits were considered as primary and other secondary; analyses were carried out using the statistical genetic program GENES. However, before the analysis, a multicollinearity diagnosis was made according to the criteria described by Montgomery et al. (2006) to eliminate traits that could have resulted in biased coefficients. Due to moderate-to-strong multicollinearity, path analysis with collinearity was performed at  $k = 0.05$ .

## RESULTS

### Genetic correlation

The values obtained for genetic correlation (Table 2) indicated that there was variation in correlation magnitude and direction. A positive genetic correlation estimate was obtained for the PH variable, in addition to a strong magnitude between PH and IH (0.71), which indicated that plants with greater heights also have higher first pod insertions. Medium magnitude correlations were obtained between PH and NN (0.52) and PH and M100G (0.58), indicating that taller plants have increased 100-grain mass and nodule number in addition to a greater height of first pod insertion. Positive correlations of strong magnitude were detected between DMP and NPP (0.68). Positive correlations of medium magnitudes were identified between DMP and M100G (0.54) and DMP and NN (0.37). This indicates that plants with a high aerial part dry mass also exhibit increased 100-grain mass and nodule number.

The variable DC showed a strong positive correlation with MN (0.92), indicating that the increase in diameter of the stem increased the dry nodule mass. The DC also showed a positive correlation with the NPP (0.37) (Table 2). The IH variable exhibited a strong positive correlation with PH (0.71) and NN (0.64) and medium positive correlation with M100G (0.55). The variables PH and IH correlated positively with each other and with the variables NN and M100G, indicating that the selection of taller plants results in plants with greater first pod insertion heights, higher number of nodules, and greater dry mass of grains. The IH variable exhibited a medium negative correlation with both MN and NPP, at -0.31 and -0.35, respectively (Table 2).

The NPP also correlated negatively with NGP (-0.39), indicating that plants with a higher number of pods formed fewer grains. In general, the NN variable was positively correlated with a medium-to-strong magnitude with four of the analyzed variables (PH, DMP, IH, and M100G). This association indicates that plants with higher number of nodules tend to have increased plant height and higher first pod insertion, higher aerial part, and greater 100-grain mass. This result is critical for improving selection in terms of capacity of nodulation of beans because selecting plants with a higher number of nodules does not exclude other desirable characteristics. The variable MN had a strong positive correlation with DC (0.92) and weak correlations with all other variables. It exhibited a medium negative correlation with IH (-0.35) (Table 2). The variable M100G positively correlated with all variables with magnitudes ranging from weak to medium, indicating that its selection may increase all other related characteristics.

**Table 2.** Genotypic correlation coefficients of total plant height (PH), dry mass of the aerial part (DMP), stem diameter (DC), first pod insertion height (IH), number of pods per plant (NPP), number of nodules (NN), mass of nodules (MN), number of grains per pod (NGP), and 100-grain mass (M100G) of 20 common bean genotypes. Ilha Solteira, São Paulo, 2018/2019.

Variables	DMP	DC	IH	NPP	NN	MN	NGP	M100G
PH	0.11	0.08	0.71	-0.14	0.52	-0.02	-0.07	0.58
DMP		0.19	0.10	0.68	0.37	0.25	0.07	0.54
DC			-0.25	0.36	0.00	0.92	0.12	0.20
IH				-0.31	0.64	-0.35	0.04	0.55
NPP					0.18	0.28	-0.39	0.13
NN						-0.18	-0.15	0.44
MN							0.13	0.18
NGP								0.07

### Path analysis

In the path analysis (Table 3), the direct and indirect effects of the primary explanatory variables on grain yield are presented. The coefficient of determination ( $R^2$ ) of the analysis was equivalent to 73%, indicating that bean grain yield can be explained by the effects of the variables analyzed.

Analysis of the direct effects of the primary components on the principal variable (GY) revealed that the NGP had the most significant direct effect (0.57) on the GY based on the nine primary variables (PH, DMP, DC, IH, NPP, NN, MN, NGP, and M100G) (Table 3). The same effect was observed for the total correlation coefficient, indicating the NGP was a determinant of bean grain yield. The MN was the second variable that most influenced the GY and had a direct effect (0.40) on the GY, and the total correlation was found to be positive (Table 3).

The NPP variable (0.17) had a minimal positive direct effect with GY; however, the indirect and negative effect with NGP (-0.22) made the total correlation negative (-0.12). Therefore, the NPP variable should be included in the selection for increasing grain yield. The variable M100G has a minimal positive direct effect (0.05) on GY but a total negative correlation (-0.27) due to the more significant negative indirect effect on the variables DMP (-0.14) and IH (-0.18).

The variables IH (-0.34), DMP (-0.27), NN (-0.14), and AP (-0.03) had a negative direct effect on GY and negative total correlation coefficients, indicating the minimal contribution of these variables to grain yield. Plants with greater total and first pod insertion heights, greater aerial part volume, and number of nodules failed to significantly impact GY. Although the variable DC showed a negative direct effect (-0.27) with GY, the total correlation (0.26) was positive due to the positive indirect effect of MN (0.37).

**Table 3.** Determining direct and indirect effect components of genotypic correlations of independent explanatory variables: Total plant height (PH), aerial part dry mass (DMP), stem diameter (DC), first pod insertion height (IH), number of pods per plant (NPP), number of nodules (NN), mass of nodules (MN), number of grains per pod (NGP), and 100-grain mass (M100G) on the main dependent variable grain yield (GY). Ilha Solteira, São Paulo, 2018/2019.

Direct effect	PH	DMP	DC	IH	NPP	NN	MN	NGP	M100G
Direct GY	-0.03	-0.27	-0.27	-0.34	0.17	-0.14	0.40	0.57	0.05
Indirect via PH		0.00	0.00	-0.02	0.00	-0.01	0.00	0.00	-0.01
Indirect via DMP	-0.03		-0.05	-0.03	-0.18	-0.10	-0.07	0.02	-0.14
Indirect via DC	-0.02	-0.05		0.07	-0.10	0.00	-0.25	-0.03	-0.05
Indirect via IH	-0.24	-0.03	0.08		0.10	-0.21	0.12	-0.01	-0.18
Indirect via NPP	-0.02	0.12	0.06	-0.05		0.03	0.05	-0.07	0.02
Indirect via NN	-0.07	-0.05	0.00	-0.09	-0.03		0.03	0.02	-0.06
Indirect via MN	-0.01	0.10	0.37	-0.14	0.11	-0.07		0.05	0.07
Indirect via NGP	-0.04	-0.04	0.07	0.02	-0.22	-0.09	0.08		0.04
Indirect via M100G	0.03	0.03	0.01	0.03	0.01	0.02	0.01	0.00	
Total (correlation)	-0.43	-0.22	0.26	-0.56	-0.12	-0.59	0.39	0.59	-0.27
Coefficient of determination (R <sup>2</sup> ): 0.73									

## DISCUSSION

Knowledge of the existing relationships among the characteristics through genetic correlations is fundamental for plant breeding (Gonçalves et al., 2017) (Table 2). Thereby, it is necessary to interpret them considering three factors: Magnitude, direction, and significance (Santos et al., 2022). However, for genetic correlations, the significance is not essential because their existence enables us to determine whether there is a genetic relationship between the traits, which may be due to pleiotropy or genetic linkage (Chebib and Guillaume, 2021).

A strong genotypic correlation between plant height and first pod insertion (0.63) was reported by Arevalo et al. (2020), who described the correlations of 22 bean genotypes including BRS Estilo, BRSMG Uai, IPR Campos Gerais, and Pérola. Our result (Table 2) differed from the magnitude of correlation reported by Gonçalves et al. (2017), whose study evaluated the genetic correlations of 40 accessions of common bean cultivated by Empaer of Cáceres, Mato Grosso, and showed a weak genetic correlation between plant height and first pod insertion (0.17).

The relationship between plant height and first pod insertion is essential for genotype selection and thus for improving mechanized harvesting (Kuzbakova et al., 2022). Different magnitudes in genetic correlations found in varying sets of genotypes suggest that such correlations are due to gene linkage rather than pleiotropism (Chebib and Guillaume, 2021). This aids the selection of genotypes in any direction off of large segregating populations, thus enabling a greater probability of gene exchange from crossing-over in gamete formation (Silva et al., 2018). Therefore, a higher genetic correlation in selected genotypes is expected considering the mechanized harvest.

The strong positive correlation between stem diameter (DC) and mass of nodules (MN) and DC and number of pods per plant (NPP) (Table 2) may have occurred because the increase in diameter of the stem influenced the amount of photoassimilates transported, leading the increase in weight of the dry nodule mass. Similar results were obtained by Rocha et al. (2009), where 16 genotypes were evaluated to assess the components of the common bean aerial part, and the correlation found between stem diameter and the number of pods per plant was 0.37. Therefore, these variables must have a positive correlation because an increase in the number of pods cannot be supported by a plant that does not show a parallel increase in stem diameter (Velho et al., 2017).

The positive correlations between total plant height (PH), insertion height (IH), MN and 100-grain mass (M100G) (Table 2) indicate the possibility of selecting plants that facilitate the management of the crop in the field, favoring the development of improved cultivation technologies for increasing crop yields and facilitating

treatment of large crop areas. Thus, mechanized harvesting operations that lead to improved bean quality require improved breeding to obtain plants that are more upright with less lodging and greater pod insertion heights (Klasener et al., 2022; Silva et al., 2023).

The negative correlations between number of pods per plant (NPP) and number of grains per plant (NGP) (Table 2) show the compensation effect that the bean promotes among production components (Silva et al., 2023), such as the increase or reduction in seed size in response to total pod number. A strong negative correlation among these variables has been reported by Arevalo et al. (2020). When a correlation coefficient equals zero occurs as verified between the variables number of nodules (NN) and DC (Table 2), which does not imply a lack of relationship but only expresses the absence of a linear relationship between them.

For a breeding program, it is important to seek a balance between plant characteristics to obtain a productive genotype considering other traits associated with yield. Therefore, breeders must aim to achieve a balance between the target trait and other associated characteristics (Sampaio Filho et al., 2023).

Correlation measures association, so a high correlation does not imply a cause-and-effect relationship between the variables. Hence, it is not possible to infer cause and effect relationships from correlation data (Zuffo et al., 2018). Thus, pathway analysis can help breeders understand the causes and effects involved in associations between variables by breaking down known correlation coefficients into direct and indirect effects for a basic variable (Diniz and Oliveira, 2019).

The importance of the NPP to determine of bean grain yield has also been verified by another author (Ribeiro et al., 2018), who found more significant positive direct effects for the variable number of grains per pod in the path analysis. For breeding purposes, it is crucial to identify the characteristics that are most highly correlated with the main trait and have a more significant direct effect for selection so that the correlated response through indirect selection is satisfactory (Silva et al., 2023). In light of this, selecting bean cultivars with higher NPP and MN will result in genotypes with higher grain yields. Therefore, characteristics with medium-to-strong direct correlations that consistently present in the different groups of genotypes tested indicate that their links are due in part to pleiotropic genes and that indirect selection can be performed in any population, segregated or not. This approach will yield satisfactory results provided that the correlation is in the direction of the target improvement (Chebib and Guillaume, 2021).

The research indicates that NPP variable should be included in the selection for increasing grain yield (GY). Additionally, visual selection for the higher number of pods per plant is more readily implemented in bean breeding programs than selecting for a higher number of grains per pod (Ribeiro et al., 2018). However, selection should be carried out carefully due to the growth stages of the plant wherein pod formation occurs before grain formation. Furthermore, in the case of common beans, there may be a compensatory effect when the plant undergoes stress; hence, investing in a higher number of pods per plant may lead to smaller grains (Klasener et al., 2022). Caution should be exercised when selecting only for NPP, as this may not increase grain production.

Due to the positive indirect effect of MN, the selecting for nodule mass is likely to promote GY and increase stem diameter. Table 2 shows that the variables DC and MN had a strong positive correlation. Therefore, direct selection of DC to increase GY without considering the capacity of biological N fixation (in this case, MN) can lead to plants with larger stem diameters and lower productivity. Conversely, DC accompanied by intense N assimilation by biological N fixation, represented by MN, can guarantee an adequate supply of photoassimilates for both plant architecture and production.

Regarding the effects of nodulation variables (number of nodules [NN] and MN), selecting for MN appeared more impactful due to its direct positive effect on grain yield, whereas NN had a negative effect. Consequently, it is more important to have larger nodules than a higher number of small nodules, and the size of the nodule, although unusual as a criterion for nodulation, is essential. Furthermore, there is a correlation between nodule mass and N content accumulated in legumes (Zoffoli et al., 2021).

Small nodules indicate a low efficiency of biological N fixation and contribute little or nothing to N nutrition. In addition, this trait leads to poor plant health as it transfers photosynthates for maintenance and functioning without large amounts of N fixed by the nodules. Nodule mass is also an indicator of biological N fixation efficiency. The increased MN could be due to increased nodule size, i.e., as a characteristic of the physiology of the plant and not necessarily due to a larger number of nodules (Iqbal et al., 2022).

The negative genetic correlation between MN and NN variables (Table 2) indicates that plants with a higher NN had lower MN or plants with an increase in nodule dry mass exhibited a decrease in NN. In this sense, co-inoculation with *A. brasilense*, a growth promoter, may have contributed to the increase in MN, consequently increasing N fixation, which may result in higher bean GY. Therefore, it would be ideal for bean breeders to maintain desirable commercial characteristics while maintaining a high symbiotic capacity, with a large MN. This combination is possible because the correlation of NN was positive with plant architecture traits and weak and negative between NN and MN, indicating that their relationship is characterized by few pleiotropic genes and high genetic distance. Furthermore, such a correlation indicates that their weak genetic linkage is close to independent, facilitating simultaneous selection of both characteristics for the same side as the size of segregating populations is adjusted to this end.

## CONCLUSIONS

The number of nodules in the common bean has positive correlations of medium-to-high magnitudes with key plant architecture traits, whereas nodule mass has positive correlations of medium-to-high magnitudes with production components. With regard to selecting common bean genotypes for increased grain yield, the nodule mass trait is more promising than the nodule number.

### Author contribution

Conceptualization: L.M.C., B.E.P. Methodology: L.M.C., B.E.P. Software: L.M.C., S.F.L. Validation: P.A. Formal analysis: L.M.C., S.F.L. Investigation: L.M.C., M.E.F.O. Writing-original draft: L.M.C. Writing-review & editing: L.M.C., P.A., H.H.K., S.F.L., M.E.F.O., V.C.D.A., R.C.B.N. Visualization: P.A. V.C.D.A., R.C.B.N. Supervision: B.E.P., H.H.K. All co-authors reviewed the final version and approved the manuscript before submission.

### Acknowledgements

The author would like to thank Embrapa Arroz e Feijão, IAPAR, and Universidade Federal de Lavras for donating seeds for this study, and support from the Federal University of Mato Grosso do Sul and the State University of Mato Grosso do Sul. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001.

### References

- Abd-Alla, M.H., Al-Amri, S.M., El-Enany, A-W.E. 2023. Enhancing *Rhizobium*-legume symbiosis and reducing nitrogen fertilizer use are potential options for mitigating climate change. *Agriculture* 13:2092. doi:10.3390/agriculture13112092.
- Agudelo, M.G., Ruiz, B., Capela, D., Remigi, P. 2023. The role of microbial interactions on rhizobial fitness. *Frontiers in Plant Science* 14:1277262. doi:10.3389/fpls.2023.1277262.
- Arevalo, A.C.M., Caardoso, D.L., Kraeski, M.J., Santana, D.C., Arguelho, S.B.D. 2020. Genetic parameters, correlations and principal components for agronomic characters in common bean genotypes of the carioca group. *Research, Society and Development* 9:1-21. doi:10.33448/rsd-v9i11.9831.
- Cabanzo-Atilano, I., Sandoval-Villa, M., Almaraz-Suárez, J.J., García-Cué, J.L., Pedraza-Santos, M.E., Peralta-Sánchez, M.G. 2024. Efficiency of plant growth promoting rhizobacteria (PGPR) in the vegetative development of blackberries (*Rubus* spp.) in greenhouse. *Chilean Journal of Agricultural Research* 84:70-83. doi:10.4067/S0718-58392024000100070.
- Carloni, P.R., Melo, P.G.S., Melo, L.C., Faria, L.C., Souza, T.L.P.O., Almeida, V.M., et al. 2022. Genotype by environment interaction in common bean cultivars for iron and zinc concentration in grains. *Semina: Ciências Agrárias* 43:1787-1804. doi:10.5433/1679-0359.2022v43n4p1787.
- Carvalho, C.G.P., Arias, C.A.A., Toledo, J.F.F., Almeida, L.A., Kiihl, K.A.S., Oliveira., M.F. 2002. Genotype × environment interaction on soybean yielding performance in Paraná State. *Pesquisa Agropecuária Brasileira* 37:989-1000. doi:10.1590/S0100-204X2002000700013.
- Chebib, J., Guillaume, F. 2021. Pleiotropy or linkage? Their relative contributions to the genetic correlation of quantitative traits and detection by multitrait GWA studies. *Genetics* 10:219. doi:10.1093/genetics/iyab159.
- Cruz, C.D. 2016. Genes Software - extended and integrated with the R, Matlab and Selegen. *Acta Scientiarum* 38:547-552. doi:10.4025/actasciagron.v38i4.32629.
- Diniz, R.P., Oliveira, E.J. 2019. Genetic parameters, path analysis and indirect selection of agronomic traits of cassava germplasm. *Anais da Academia Brasileira de Ciências* 91:e20180387. doi:10.1590/10.1590/0001-3765201920180387.



- Efstathiadou, E., Ntatsi, G., Savvas, D., Tampakaki, A.P. 2021. Genetic characterization at the species and symbiovar level of indigenous rhizobial isolates nodulating *Phaseolus vulgaris* in Greece. *Scientific Reports* 11(1):8674. doi:10.1038/s41598-021-88051-8.
- Faysal, A.S.M., Ali, L., Azam, M.G., Sarker, U., Ercisli, S., Golokhvast, K.S., et al. 2022. Genetic variability, character association, and path coefficient analysis in transplant Aman rice genotypes. *Plants* 11(21):2952. doi:10.3390/plants11212952.
- Gonçalves, D.L., Barelli, M.A.A., Oliveira, T.C., Santos, P.R.J., Silva, C.R., Poletine, J.P., et al. 2017. Genetic correlation and path analysis of common bean collected from Caceres Mato Grosso State, Brazil. *Ciência Rural* 47:1-7. doi:10.1590/0103-8478cr20160815.
- Gonçalves, G.C., Ferbonink, G.F., Hemkemeier, C., Caione, G., Yamashita, O.M., Luiz, S.A.R., et al. 2023. Vegetative and productive characteristics of soybean under doses of boron and inoculation of *Trichoderma atroviride*. *Chilean Journal of Agricultural Research* 83:159-167. doi:10.4067/S0718-58392023000200159.
- Horácio, E.H., Zucareli, C., Gavilanes, F.Z., Yunes, J.S., Sanzovo, A.W.S., Andrade, D.S. 2020. Co-inoculation of rhizobia, azospirilla and cyanobacteria for increasing common bean production. *Semina: Ciências Agrárias* 41(5supl1):2015-2028. doi:10.5433/1679-0359.2020v41n5supl1p2015.
- Iqbal, N., Sadras, V.O., Denison, R.F., Zhou, Y., Denton, M.D. 2022. Clade-dependent effects of drought on nitrogen fixation and its components—Number, size, and activity of nodules in legumes. *Field Crops Research* 284:108586. doi:10.1016/j.fcr.2022.108586.
- Klasener, G.R., Ribeiro, N.D., Santos, G.G.D. 2022. Genetic divergence and selection of common bean cultivars based on plant architecture and grain yield. *Revista Caatinga* 35:818-828. doi:10.1590/1983-21252022v35n409rc.
- Kuzbakova, M., Khassanova, G., Oshergina I., Tem, E., Jatayev, S., Yezhebeyeva, R., et al. 2022. Height to first pod: A review of genetic and breeding approaches to improve combine harvesting in legume crops. *Frontiers in Plant Science* 16:948099. doi:10.3389/fpls.2022.948099.
- Lemos, R.C., Abreu, A.F.B., Souza, E.A., Santos, J.B., Ramalho, M.A.P.A. 2020. A half century of a bean breeding program in the South and Alto Paranaíba regions of Minas Gerais. *Crop Breeding and Applied Biotechnology* 20:e295420211. doi:10.1590/1984-70332020v20n2c27.
- Mesera, E., Shifaraw, G., Alamerew, S., Amsalu, B. 2022. Genetic variability analysis and association of traits in common bean (*Phaseolus vulgaris* L.) landraces collected from Ethiopia at Jimma. *Advances in Agriculture* 2022:4400711. doi:10.1155/2022/4400711.
- Montgomery, D.C., Peck, E.A., Vining, G.G. (eds.) 2006. Introduction to linear regression analysis. 4<sup>th</sup> ed. Wiley Series in Probability and Statistics. Wiley-Interscience, Hoboken, New Jersey, USA.
- Reinprecht, Y., Schram, L., Perry, G.E., Morneau, E., Smith, T.H., Pauls, K.P. 2023. Mapping yield and yield-related traits using diverse common bean germplasm. *Frontiers in Genetics* 14:1246904. doi:10.3389/fgene.2023.1246904.
- Ribeiro, N.D., Santos, G.G.D., Maziero, S., Steckling, S.D. 2018. Phenological, plant architecture, and grain yield traits on common bean lines selection. *Revista Caatinga* 31:657-666. doi:10.1590/1983-21252018v31n314rc.
- Rocha, F., Berili, L.D., Garcia, S.H., Modena, R., Coimbra, J.L.M., Guidolin, A.F., et al. 2009. Selection in bean (*Phaseolus vulgaris* L.) mutant populations for adaptive characters. *Biotemas* 22:19-27.
- Sampaio Filho, J.S., Olivoto, T., Oliveira, E.J.D. 2023. Multi-trait selection in multi-environments for performance and stability in cassava genotypes. *Frontiers in Plant Science* 14:1282221. doi:10.3389/fpls.2023.1282221.
- Santos, G.G.D., Ribeiro, N.D., Maziero, S.M., Santos, G.G.D. 2022. Genetic diversity among bean landraces and cultivars for agronomy traits and selection of superior parents. *Revista Ciência Agronômica* 53:e20217873. doi:10.5935/1806-6690.20220031.
- Shubha, K., Choudhary, A.K., Dubey, A.K., Tripathi, K., Kumar, R., Kumar, S., et al. 2024. Evaluation of lablab bean [*Lablab purpureus* (L.) sweet] genotypes: Unveiling superior pod yield, nutritional quality, and collar rot resistance. *Frontiers in Nutrition* 10:1243923. doi:10.3389/fnut.2023.1243923.
- Silva, J.G., Ferreira, E.P.B., Nascente, A.S., Sarmiento, P.H.L., Messias, M. 2023. Pod positioning and grain yield of common bean as affected by sowing density, nitrogen fertilization and fertilization depth. *Bioscience Journal* 39:e39084. doi:10.14393/BJ-v39n0a2023-67282.
- Silva, F.D.C., Pereira, H.S., Melo, P.G.S., Melo, L.C. 2018. Selection of parents and segregating populations of common bean with high agronomic potential and slow seed-coat darkening. *Pesquisa Agropecuária Tropical* 48:75-82. doi:10.1590/1983-40632018v4849519.
- Sousa, W.S., Soratto, R.P., Peixoto, D.S., Campos, T.S., Silva, M.B., Souza, A.G.V., et al. 2022. Effects of *Rhizobium* inoculum compared with mineral nitrogen fertilizer on nodulation and seed yield of common bean. A meta-analysis. *Agronomy for Sustainable Development* 42:52. doi:10.1007/s13593-022-00784-6.
- Souza, J.E.B., Ferreira, E.P.B. 2017. Improving sustainability of common bean production systems by co-inoculating rhizobia and azospirilla. *Agriculture, Ecosystems & Environment* 237:250-257. doi:10.1016/j.agee.2016.12.040.
- Steiner, F., da Silva Oliveira, C.E., Zoz, T., Zuffo, A.M., de Freitas, R.S. 2020. Co-Inoculation of common bean with *Rhizobium* and *Azospirillum* enhance the drought tolerance. *Russian Journal of Plant Physiology* 67:923-932. doi:10.1134/S1021443720050167.

- Timofeeva, A.M., Galyamova, M.R., Sedykh, S.E. 2023. Plant growth-promoting soil bacteria: Nitrogen fixation, phosphate solubilization, siderophore production, and other biological activities. *Plants* 12:4074. doi:10.3390/plants12244074.
- Velho, L.P.S., Gemeli, M.S., Trevisani, N., Pereira, T.C.V., Cerutti, P.H., Melo, R.C., et al. 2017. Phenotypic correlation and direct and indirect effects of aerial part components with root distribution of common bean. *Pesquisa Agropecuária Brasileira* 52:328-334. doi:10.1590/S0100-204X2017000500006.
- Zoffoli, B.C., Brito, L.F., Straliozzo, R., Araújo, A.P.D. 2021. Early nitrogen supplementation stimulates the nodulation and growth of common bean plants inoculated with rhizobium. *Acta Scientiarum Agronomy* 43:e55105. doi:10.4025/actasciagron.v43i1.55105.
- Zuffo, A.M., Ribeiro, A.B.M, Brizi, A.T., Zambiazzi, E.V., Fonseca, W.L. 2018. Correlations and path analysis in soybean cultivars grown at different plant densities. *Revista Cultura Agronômica* 27:78-90. doi:10.32929/2446-8355.2018v27n1p78-90.