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Genetic parameters and indirect selection of elite lines for efficiency in symbiotic nitrogen fixation in black common bean

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Abstract

This study aimed to evaluate whether managing populations and progenies of black common bean (*Phaseolus vulgaris* L.) in exclusive symbiotic nitrogen fixation (SNF) systems, which refer to environments where the primary nitrogen source in the soil is the SNF, is efficient for selecting lines with high symbiotic performance, using grain yield as a selection criterion, and to verify the relationship between agronomic- and SNF-related traits. A total of 76 black common bean lines from a single population and five checks were evaluated during the winter seasons of 2021 and 2022 under SNF systems. The experimental design was a 9 × 9 triple lattice. Evaluated traits included yield, 100-seed weight, and grain appearance. The top 10 and bottom 10 lines were selected based on grain yield in the field and evaluated with rhizobia inoculation in two greenhouse trials with five checks, using a randomized block design with four replications. Traits assessed included chlorophyll content, shoot and root dry weight, nodule number, nodule dry weight, and specific nodule weight. Genetic variability among the lines allowed for the selection of genotypes with high symbiotic performance. Significant differences were found between the groups of top and bottom lines for specific nodule weight, indicating its potential as a selection indicator. Significant correlations were found among the traits. Specific nodule weight, chlorophyll content, and shoot dry weight were identified as key factors for the indirect selection of genotypes with better symbiotic performance. Eight lines were selected for advanced trials to develop black common bean cultivars for SNF.

Plain Language Summary

Nitrogen deficiency in the soil is a common issue for common bean, prompting many farmers to use chemical fertilizers, of which less than 50% is utilized by plants,

Abbreviations: 100SW, 100-seed weight; CC, chlorophyll content; GY, grain yield; NDW, nodule dry weight; NN, nodule number; RDW, root dry weight; SDW, shoot dry weight; SNF, symbiotic nitrogen fixation; SWN, specific nodule weight; VAG, visual aspect of grains.

leading to waste and pollution. A more sustainable solution is symbiotic nitrogen fixation, where bacteria on plant roots convert atmospheric nitrogen into a usable nutrient. However, breeding programs often overlook this capability. Our study evaluated 76 common bean lines grown with *Rhizobium* bacteria as the sole nitrogen source. We selected the highest and lowest performing lines based on grain yield and assessed their nitrogen-fixing ability. Results indicated that it is feasible to select common bean plants that efficiently utilize nitrogen from bacteria, achieving yields comparable to those with chemical fertilizers. The study identified traits like chlorophyll content and specific nodule weight as key indicators for selecting efficient plants and highlighted eight promising genotypes for sustainable farming.

1 | INTRODUCTION

Symbiotic nitrogen fixation (SNF) is common in the Fabaceae. In this symbiotic process, bacteria, commonly known as rhizobia, inhabit the root nodules and fix atmospheric nitrogen (N_2), which is used by plants in exchange for carbohydrates (Wilker et al., 2019). The main advantages of SNF include the reduction of production costs for farmers since inoculation with rhizobia is more economical compared to nitrogen (N) mineral fertilization, which can increase grain yield (GY) in marginal environments, and the reduction of groundwater contamination through the leaching of applied mineral N (Beaver & Osorno, 2009).

However, symbiotic efficiency varies among legumes, and common bean (*Phaseolus vulgaris* L.) is often characterized as a poor nitrogen fixer (Kamfwa et al., 2019; Reinprecht et al., 2020). The low SNF of common bean can be partly attributed to its short lifecycle (about 90 days) compared to other legumes like soybean (approximately 120 days) (Beaver & Osorno, 2009), promiscuous nodulation with native rhizobia (Moura et al., 2022), and the sensitivity of this biological process to abiotic stresses (Hungria & Vargas, 2000). Furthermore, the emerging efforts of breeding programs to improve SNF in common bean and the complex genetic architecture of this biological symbiosis have also contributed to the limited progress made for this crop (Kamfwa et al., 2019).

Evaluating the nitrogen fixation of common bean genotypes has not been a priority within most common bean breeding programs. Despite the existence of genetic variability for SNF in both wild and traditional genotypes (Knupp et al., 2017; Wilker et al., 2019, 2020) as well as in cultivars and elite lines (Dias et al., 2020, 2024; Farid & Navabi, 2015; Heilig, Wright, et al., 2017; Pereira et al., 2015), incorporating this variability into cultivars has achieved limited progress.

Developing a phenotyping method for SNF capacity that can be easily integrated into the routine of breeding programs is essential to accelerate the progress in selecting common bean cultivars with higher symbiotic efficiency (Heilig, Wright, et al., 2017). For this purpose, yield-related traits can be useful indicators of SNF when plants are grown in environments with minimal or absent levels of N (Bliss, 1993). Additionally, one of the main factors to consider is evaluating segregating populations and lines, from the initial phases of breeding programs, in exclusive SNF systems, which refer to environments where the primary N source in the soil comes from the symbiosis between plants and microorganisms, since mineral N fertilization has been proven to reduce symbiotic fixation capacity in legumes (Jiang et al., 2020; Reinprecht et al., 2020; Sousa et al., 2022). However, recent studies on the development of lines in SNF-dependent systems are not found for common bean.

To promote the expression of SNF, starting in 2014, the common bean breeding program at Embrapa Rice and Beans (*Embrapa Arroz e Feijão*) began making crosses between elite genotypes that were symbiotically promising, identified in several studies (Dias et al., 2020, 2024; Pereira et al., 2015). The populations formed were cultivated in exclusive SNF systems, using GY as an indicator of symbiotic efficiency (Bliss, 1993). Evaluating the genetic variability of these populations by estimating genetic parameters is an important tool to assess the gains obtained by selecting symbiotically efficient genotypes (Farid et al., 2017; Ramalho et al., 2012).

Another crucial factor for breeders is the selection of appropriate traits to monitor the genetic variability associated with SNF (Rodiño et al., 2011). The measurement of SNF capacity can be done directly, by obtaining the amount of nitrogen derived from the atmosphere (Ndfa), or indirectly, by counting the nodule number (NN) and determining the plant biomass, nodule biomass, and chlorophyll content (CC) (Jiang et al., 2020). There are various methods for direct measurement of SNF (^{15}N natural abundance technique, ^{15}N isotopic dilution, acetylene reduction, and others), which, despite being the most accurate, are costly due to the need for specific equipment for analyses (Fonseca-López et al., 2020).

Genetic variability associated with the development of roots and nodules has already been reported for common bean (Farid & Navabi, 2015; Heilig, Beaver, et al., 2017; Jiang et al., 2020; Ramaekers et al., 2013; Rodiño et al., 2011). Thus, the traits used for indirect measurement of SNF may be advantageous as they do not require complex equipment for evaluations, have lower cost, and yield results more quickly (Fonseca-López et al., 2020). However, more studies are needed to detect the relationship that these traits establish among themselves and with GY.

From this perspective, the objectives of this study are (i) to evaluate whether the development of lines in exclusive SNF systems is efficient for selecting lines with high symbiotic performance, using GY as a selection criterion, and (ii) to verify the relationship between agronomic- and SNF-related traits, aiming to identify an efficient trait for indirect selection for SNF in common bean.

2 | MATERIAL AND METHODS

2.1 | Field trials

The black common bean population BRS FP403/BRS Esplendor was used in this study to obtain lines. The population was selected from 28 populations obtained in a complete diallel design among eight parents, in 2014, based on productivity and 100-seed weight (100SW), high specific combining abilities, in addition to high general combining abilities of the parents in field evaluations, in soils with low N content and with rhizobia inoculation (SNF systems), in the F_2, F_3, and F_4 generations (N. V. Costa et al., 2025). In each generation, the bulk method was used to compose the subsequent generation. Both parents were selected for high yields in both nitrogen mineral fertilization systems and *Rhizobium* inoculation. The BRS FP403 cultivar also showed a high number and specific nodule weight (SWN) (Dias et al., 2020).

The F_5 generation was planted in Santo Antônio de Goiás, GO, in the winter season of 2019, and 76 random individual plants (lines) were harvested to ensure a representative sample of the population. The plants were multiplied in the greenhouse to obtain a larger number of seeds in the $F_{5:6}$ generation. This study starts from the evaluation of the 76 lines in the $F_{5:7}$ generation, in the winter season of 2021 (sown in May), and in the $F_{5:8}$ generation, in the winter season of 2022 (sown in May), in Santo Antônio de Goiás, GO. Five controls were used: the non-nodulating line NORH 54, the parents BRS FP403 and BRS Esplendor, and the elite lines CNFP 10793 and CNFP 7994. The experiments were arranged in a triple 9 × 9 lattice design, with plots consisting of three rows of 3 m in length, spaced 0.5 m apart.

The climate of the regions where the experiments were conducted is classified as *Aw*, that is, a tropical savanna, according to the Köppen classification, and the predominant soil type is Dark Red Latosol, with clayey texture and flat relief (Embrapa, 2018). The experimental area, being designated for research, is characterized by intensive soil use and low levels of organic matter. The chemical analyses performed prior to the setup of the experiment are shown in Table S1. Limestone was applied to correct soil acidity, and fertilization was carried out only with non-nitrogen fertilizers (P_2O_5 and K_2O) at planting, based on soil analysis.

Core Ideas

- There is genetic variability among the lines for agronomic- and symbiotic nitrogen fixation (SNF)-related traits.
- The specific nodule weight distinguishes more productive lines from less productive ones in SNF systems.
- Chlorophyll content and shoot dry weight are useful for indirectly selecting genotypes with high SNF rate.

Inoculation was performed with liquid inoculant prepared by the Soil Biology Laboratory of Embrapa Rice and Beans, composed of a 1:1:1 mixture of *R. tropici* strains (SEMIA 4077 and 4088) and *R. freirei* (SEMIA 4080), registered with MAPA for common bean. The inoculant has a density of 10^9 cells mL^{-1}. The application was carried out in the sowing furrow at planting, using a directed jet sprayer coupled to the plot planter, at a dosage of 200 mL ha^{-1}.

The yield, 100SW, and visual appearance of the grains were evaluated. GY, adjusted to 13% moisture, was obtained in grams per plot by weighing the grains harvested from all plants in the three rows, with subsequent conversion to kilograms per hectare. The 100SW was obtained by randomly collecting 100 seeds from each plot, for subsequent weighing and determination of the weight in grams. The evaluation of the visual appearance of grains was conducted after harvest using a rating scale from 1 to 3. A grain rated as 1 is considered a typical black bean, exhibiting a uniform dark black color without shape defects. In the case of rating 2, the grains display only a single defect, which may be either in their shape or color uniformity, and do not contain any purple beans. A rating of 3 indicates grains that exhibit defects in both evaluated characteristics, meaning they show nonuniform color and shape defects or contain purple beans.

Individual analyses of variance were performed on the phenotypic data for each year, and after checking homoscedasticity for the residual variances by the Hartley test at 5%, joint analysis was performed. This analysis considered the treatments effects as random and environments as fixed. Selective accuracy was also estimated to assess the informative nature of the experiments (Resende & Duarte, 2007), along with the

TABLE 1 Mean squares of combined variance analyses for the traits grain yield (GY, kg ha^{-1}), 100-seed weight (100SW, g), and visual aspect of grains (VAG, scale of scores from 1 to 3) evaluated in 76 lines and five checks, in Santo Antônio de Goiás, Goiás, in the winter crop seasons of 2021 and 2022, in environments where the main nitrogen source was symbiotic fixation.

Source of variation	*df*	GY	100SW	VAG
Genotypes (G)	80	771,406**	9.79**	0.35**
Lines (L)	75	697,845**	7.96**	0.07**
Checks (C)	4	2,280,668**	46.05**	4.80**
L vs. C	1	251,405	2.43*	3.53**
Years (Y)	1	20,272,323**	633.41**	0.02
G × Y	80	240,411**	0.67*	0.03
L × Y	75	244,435**	0.66*	0.03
C × Y	4	149,893	0.44	0.00
L vs. C × Y	1	300,654	1.89*	0.00
Average residual	320	134,625	0.47	0.04
Overall Mean	–	2264	22.8	1.05
Mean 2021	–	2475	21.7	1.04
Mean 2022	–	2054	23.9	1.05
Coefficient of variation (%)	–	16.24	2.99	18.02
Selective accuracy	–	0.91	0.98	0.95

**and * are significant at 1% and 5% probability, respectively.

coefficient of experimental variation. From the analysis of variance, genetic (σ_g^2), environmental (σ_e^2), and genotype × year interaction (σ_{gy}^2) variances were estimated. The variance components were used to calculate broad sense heritability (H^2) based on the mean input. For combined analysis the following formula was used:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{gy}^2}{e} + \frac{\sigma_e^2}{re}}$$

where *e* is the number of environments and *r* is the number of replications.

Direct selection of the top 10 and bottom 10 lines for GY was performed, and the expected gain with direct selection (GS%) was estimated, considering a selection intensity of 13% (Cruz et al., 2012).

2.2 | Greenhouse trials

Controlled environment experiments were conducted to determine the performance of the top 10 and bottom 10 lines for traits related to SNF and to verify the relationship with GY results obtained in the field. For this, the 20 selected lines (top 10 and bottom 10) were evaluated with five controls: non-nodulating line NORH 54, parent cultivars BRS FP403 and BRS Esplendor, elite line CNFP 7994, and carioca bean cultivar BRS Sublime, which showed high nodulation in another study (Dias et al., 2024).

The experimental units consisted of 5-L plastic pots filled with a 2:1 mixture of sand and soil. Fertilization with non-nitrogen fertilizers (P_2O_5 and K_2O) was carried out according to the soil chemical analysis, which can be seen in Table S1. Before planting, seeds were disinfected as described by Kipe-Nolt et al. (1992). Five seeds were sown per pot, and thinning was performed 7 days after plant emergence, leaving two plants per pot. Plant inoculation was also performed 7 days after emergence, using a pipette to apply 1 mL plant^{-1} of the liquid inoculant produced by the Soil Biology Laboratory of Embrapa Rice and Beans, composed of a 1:1:1 mixture of *Rhizobium tropici* strains (SEMIA 4077 and 4088) and *R. freirei* (SEMIA 4080), registered with MAPA for common bean. The inoculant has a concentration of 10^9 cells mL^{-1}.

Two experiments were conducted, one planted in April and another in July 2023. During the development period, the plants were kept in a greenhouse located in the experimental area of the School of Agronomy at the Federal University of Goiás, in Goiânia, GO. Irrigation was performed using a drip system. The experimental design used was a randomized block design with four replications. Evaluations for all traits were performed when a minimum of one plant in the pot had at least one open flower (R6 stage).

The CC (Falker chlorophyll index [FCI]) of the plants in each plot was measured using a portable digital chlorophyll meter (ClorofiLog—CFL 1030; Falker). Three readings were taken per plant, using the third completely expanded trifoliate leaf from the apex, as recommended by Maia et al. (2017). The shoot dry weight (SDW, g plant^{-1}) was obtained by removing the shoot of the two plants in each plot using pruning shears at 1 cm above the soil and placing them in labeled paper bags. The paper bags were placed in a forced-air circulation oven for 72 h at 65°C. After this period, the samples were weighed to determine the mass.

After separating the shoots, the roots were carefully removed from the pots, separated from the substrate, and washed over a sieve under running water to eliminate maximum impurities. Then, the nodules present on the roots were detached and counted to determine the NN (units per plant). The roots and nodules were placed in paper bags and dried in a forced-air circulation oven for 36 h at 72°C. After this period, they were weighed to determine the root dry weight (RDW, g plant^{-1}) and nodule dry weight (NDW, mg plant^{-1}). The SWN (mg nodule^{-1}) was obtained by dividing the NDW by the NN.

Individual and combined analyses of variance were performed for all traits. The effects of genotypes and environments were considered fixed. For the combined analysis, homogeneity of variances was verified and selective accuracy was estimated. The Wilcoxon nonparametric test was used to

TABLE 2 Estimates of genetic variance ($\hat{\sigma}^2_g$), variance of line × year interaction ($\hat{\sigma}^2_{gy}$), and heritability at the mean level ($\hat{H}^2$) for the traits grain yield (GY, kg ha^{-1}), 100-seed weight (100SW, g), and visual aspect of grains (VAG, scale of scores from 1 to 3) of 76 lines of black common bean evaluated in the winter crop seasons of 2021 and 2022 and in the combined analysis, in environments where the main nitrogen source was symbiotic fixation.

	GY			100SW			VAG		
Year	$\hat{\sigma}^2_g$	$\hat{\sigma}^2_{gy}$	$\hat{H}^2$(%)	$\hat{\sigma}^2_g$	$\hat{\sigma}^2_{gy}$	$\hat{H}^2$(%)	$\hat{\sigma}^2_g$	$\hat{\sigma}^2_{gy}$	$\hat{H}^2$(%)
2021	149,156	–	77.2	1.03	–	89.4	0.004	–	25.5
2022	75,187	–	62.2	1.53	–	89.0	0.007	–	34.3
Combined analysis	93,870	18,301	80.7	1.25	0.03	94.1	0.006	0.001	49.8

compare the means of the top and bottom lines for each trait obtained in the greenhouse. The means were grouped by the Scott–Knott test at 5% probability.

For the top 10 and bottom 10 lines and two controls (BRS FP403 and BRS Esplendor) common in field and greenhouse experiments, adjusted means (Best Linear Unbiased Estimators [BLUEs]) were obtained using the "lm" function and the "emmeans" package (Lenth, 2021) of R software (R Core Team, 2022). Genetic correlation coefficients between traits measured in different experiments and phenotypic correlation between traits measured in the same experiment were calculated using the "metan" package (Olivoto & Lúcio, 2020).

The multivariate principal component analysis was performed using the "FactoMineR" (Lê et al., 2008) and "factoextra" (Kassambara & Mundt, 2022) packages of R software. Correlation and principal component analyses were carried out using data from the 20 lines (top 10 and bottom 10) and two controls (BRS Esplendor and BRS FP403), which were common to field and greenhouse trials.

3 | RESULTS

3.1 | Field trials

Significant differences among lines were observed for all traits, except for visual grain appearance, during the 2021 winter season. The environmental coefficient of variation was 14.7% and 18.0% for GY, 2.8% and 3.1% for 100SW, and 17.1% and 18.9% for visual grain appearance for the years 2021 and 2022, respectively. Selective accuracy estimates were high for GY (>0.80) and very high for other traits (>0.90) (Resende & Duarte, 2007).

In the combined analysis, significant differences among genotypes were observed for all traits (Table 1). The effect of years significantly influenced GY and 100SW, with differences of 421 kg ha^{-1} and 2.2 g among the years, respectively. However, no variation was observed for the effect of years on visual grain appearance, which is confirmed by the similarity between the means of the 2 years (1.04 and 1.05 in 2020 and 2021, respectively). The lines × years interaction was significant for GY and 100SW.

The estimates of genotype × year interaction variance ($\hat{\sigma}^2_{gy}$) were always lower than genetic variance estimates ($\hat{\sigma}^2_g$) for all traits evaluated (Table 2). Heritability estimates at the mean level ($\hat{H}^2$) ranged from low to moderate (25.5%–49.8%) for visual grain appearance. For GY, heritability was high, with specific values of 77.2% in 2021, 62.2% in 2022, and 80.7% in the joint analysis. Similarly, 100SW also exhibited high heritability estimates, reaching 89.4% in 2021, 89.0% in 2022, and 94.1% in the combined analysis.

The overall mean GY of the lines was 2264 kg ha^{-1}. Of the 76 lines evaluated, 19 (20%) had yields higher than the parent BRS FP403 (2554 kg ha^{-1}) and 32 (42%) were superior to the parent BRS Esplendor (2313 kg ha^{-1}). Excluding the non-nodulating line NORH 54, which presented the lowest GY (1088 kg ha^{-1}), the mean yield of the control lines was 2441 kg ha^{-1}, representing a superiority of only 7.25% compared to the mean of the 76 lines.

As expected, selecting the bottom 10 lines resulted in a high negative gain for GY (−18.6%) (Table 3), with a reduction of 520 kg ha^{-1} from the original mean. Conversely, selecting the top 10 lines for GY provided a selection gain of 20.4% for the trait, an increase of 571 kg ha^{-1} from the original mean. The mean GY of the top 10 lines was 2836 kg ha^{-1}, while the mean of the bottom 10 lines was 1739 kg ha^{-1}, a difference of nearly 40%. Furthermore, the mean of the top 10 lines was significantly higher than that of the best control lines, with a difference of 425 kg ha^{-1}.

Direct selection for GY resulted in losses for 100SW and visual grain appearance. For 100SW, selecting the top 10 lines resulted in a gain of −2.51% (Table 3), a decrease of 0.6 g from the original mean (22.8 g). For visual grain appearance, there was a gain of 1.75%, with the selection of the top 10 lines, representing an increase of 0.04 on the rating scale, raising the mean from 1.04 to 1.08.

3.2 | Greenhouse trials

In the greenhouse, the coefficients of variation were adequate for CC (17%) and RDW (14%) but very high (> 50%) for the other traits (Table 4). Conversely, selective accuracy estimates ranged from high (>0.70) to very high (>0.90)

TABLE 3 Selection gains and means of the top 10 lines (10+), bottom 10 lines (10−), and checks evaluated in environments where the main nitrogen source was symbiotic fixation, for the traits grain yield (GY, kg ha^{-1}), 100-seed weight (100SW, g), and visual aspect of grains (VAG, scale of scores from 1 to 3).

Genotypes	GY	100SW	VAG
Top 10 (10+)			
CNFP 21622	3312	23.3	1.50
CNFP 21630	2914	23.0	1.00
CNFP 21681	2905	21.6	1.00
CNFP 21629	2853	22.4	1.00
CNFP 21649	2806	22.3	1.00
CNFP 21619	2739	22.6	1.00
CNFP 21661	2726	22.1	1.00
CNFP 21662	2725	20.9	1.00
CNFP 21651	2723	23.3	1.00
CNFP 21632	2659	20.8	1.33
Mean	2836	22.2	1.08
SG_{10+} (%)[a]	20.36	−2.5	1.75
Bottom 10 (10−)			
CNFP 21654	1881	20.6	1.17
CNFP 21637	1839	23.4	1.00
CNFP 21687	1831	23.9	1.33
CNFP 21677	1819	22.3	1.00
CNFP 21645	1815	21.4	1.00
CNFP 21670	1792	22.5	1.00
CNFP 21656	1758	23.1	1.50
CNFP 21667	1665	25.3	1.00
CNFP 21646	1649	21.9	1.00
CNFP 21682	1339	21.2	1.17
Mean	1739	22.6	1.12
SG_{10-} (%)[b]	−18.74	−1.12	3.36
Checks			
NORH 54	1088	21.6	3.00
BRS FP403	2554	25.4	1.00
CNFP 10793	2566	25.5	1.00
BRS Esplendor	2313	20.0	1.00
CNFP 7994	2331	20.0	1.00
Mean[c]	2441	22.7	1.00

[a] Genetic gain from direct selection of the top 10 lines for grain yield.

[b] Genetic gain from direct selection of the bottom 10 lines for grain yield.

[c] Values do not include the check NORH 54.

(Resende & Duarte, 2007). Significant differences between lines were observed for all traits except for RDW. The effect of environments was significant for all traits. Differential behavior of lines between environments was observed only for NN.

Significant differences between the top 10 and bottom 10 lines were found only for SWN (Figure 1). The average of the top lines was 0.48 mg nodule^{-1} and of the bottom lines was 0.38 mg nodule^{-1}, a difference of 0.10 mg nodule^{-1}. For the other traits, it is not possible to assert that the top and bottom genotypes for GY are also the best and worst for other traits measured under controlled conditions.

For CC, the means of the lines ranged from 20.3 to 33.4 FCI, with the highest value observed in line CNFP 21651 (Table 5). The overall means for SDW and RDW were 2.11 g plant^{-1} and 0.51 g plant^{-1}, respectively, with line CNFP 21682 presenting the highest mean for SDW, while NORH 54 stood out for RDW. The means for NN demonstrated a wide variation, ranging from 161 to 665 nodules plant^{-1}, with CNFP 21687 reaching the highest count. The overall mean for NDW was 129 mg plant^{-1}, and for SWN, there was a range from 0.25 to 0.78 mg nodule^{-1}, with CNFP 21651 again achieving the highest value.

CC showed a positive and significant phenotypic correlation with SWN (0.66) (Figure 2). NDW was significantly correlated with multiple traits, including NN (0.52), SWN (0.46), and root dry weight (0.66). In parallel, SDW had significant correlations with RDW (0.80), NDW (0.82), NN (0.49), and 100SW (0.49). Furthermore, 100SW was significantly correlated with both NDW (0.54) and NN (0.45). In contrast, GY did not show significant correlations with any of the traits assessed.

The first two principal components explained 67% (42% PC1 and 25% PC2) of the total variation (Figure 3). The GY vector aligned with the CC and SWN vectors, which overlapped in the graph. This suggests that yield has a consistent relationship with the other two traits in terms of their contribution to overall data variability, and this relationship is certainly nonlinear, as it was not captured by genetic correlations (Figure 2). Principal component analysis also showed an association between SDW, RDW, NDW and 100SW, reinforcing the correlation analysis results (Figure 2).

4 | DISCUSSION

4.1 | Genetic parameters and agronomic performance of lines in exclusive SNF systems

The presence of genetic variability among lines and controls evaluated in inoculated environments indicates the possibility of selecting superior genotypes. Differences in atmospheric nitrogen absorption may have contributed to the variability among genotypes so that the nitrogen demand is met for some but not for others (Dias et al., 2024; Pacheco et al., 2020).

Environmental factors, particularly climatic conditions present during the 2 years of evaluation, significantly impacted the expression of GY and 100SW. SNF is highly sensitive to environmental variations such as temperature, water

TABLE 4 Mean squares of combined variance analyses for traits related to symbiotic nitrogen fixation (SNF), in two greenhouse trials, conducted to evaluate 20 lines, the top 10 and bottom 10 for grain yield, and five checks.

Source of variation	*df*	CC[1]	SDW[2]	RDW[3]	NN[4]	NDW[5]	SWN[6]
Genotypes (G)	24	43.9**	1.49**	0.12**	131,015**	14,682**	0.17**
Lines (L)	19	31.2**	1.18*	0.04	106,931**	9119*	0.12**
Checks (C)	4	92.1**	2.62**	0.52**	234,127**	32,951**	0.31**
L vs. C	1	91.2*	2.84*	0.06	178,013**	48,355**	0.61**
Environment (A)	1	526.0**	80.79**	7.34**	1,544,218**	20,603*	0.80**
G × A	24	16.4	0.63	0.06**	39,327*	4006	0.06
L × A	19	16.8	0.77	0.03	43,600*	5019	0.07
C × A	4	16.3	0.12	0.16**	18,175	931	0.02
L vs. C × A	1	8.6	0.00	0.18*	34,325	155	0.11
Average residual	144	13.6	0.65	0.03	23,412	4899	0.05
Coefficient of variation (%)		17	53	14	66	66	68
Selective accuracy		0.83	0.75	0.88	0.91	0.82	0.82

Abbreviations: CC, chlorophyll content (Falker chlorophyll index [FCI]); NDW, nodule dry weight (mg plant^{-1}); NN, nodule number (unit plant^{-1}); RDW, root dry weight (g plant^{-1}); SDW, shoot dry weight (g plant^{-1}); SWN, specific nodule weight (mg nodule^{-1}).
** and * are significant at 1% and 5% probability, respectively.

availability, and soil nutrient content (Pacheco et al., 2020), which may have contributed to the differences found.

Differential behavior of the lines over the years has already been reported by other authors for yield and 100SW under rhizobia inoculation (Barbosa et al., 2018; Dias et al., 2020, 2024; Pereira et al., 2015). However, no such difference in behavior was observed for the visual aspect of the grains. For black common bean, the grain color varies with harvest moisture: the lower the moisture, the higher the color uniformity. In the winter season, harvest moisture is low, so it was expected that the lines would show similar color uniformity in both years.

The existence of variability among lines was confirmed by genetic variance estimates ($\hat{\sigma}^2_g$) (Table 2). The high superiority of genetic variance estimates in relation to the genotype × year interaction variance estimates ($\hat{\sigma}^2_{gy}$) for all traits indicates that the lines were minimally affected by year-to-year environmental variation, suggesting good potential for successful selection of superior genotypes for SNF. This stability in performance may have contributed to the high heritability estimates observed.

Farid et al. (2017) found heritability estimates for GY in common beans under SNF of 32% in non-stressed environments and 25% in water-stressed environments. Thus, the variation in heritability estimates can result from the environmental conditions in which they were obtained and the genetic variability of the genotypes. The high heritability values obtained for GY may have reflected the uniform environmental conditions in which the plants were cultivated (Ramalho et al., 2012). This suggests that the SNF systems were favorable for line evaluation, allowing the selection of superior genotypes based on observed phenotype.

The high direct genetic gain obtained for GY through the selection of the top 10 lines can be explained by the high estimates of genetic variance and heritability found for this trait. The losses observed in 100SW and visual aspect of grains (VAG) resulting from direct selection for GY were not significant. The preferred 100SW varies according to the commercial type of bean (Pereira et al., 2012), and for black common beans, it is desirable that the weight exceeds 22 g. Despite the observed decrease, the 100SW for the selected lines was 22.2 g, which is still considered favorable, especially when compared to the average of the parent BRS Esplendor (20.0 g), a black common bean cultivar with small grains (J. G. C. D. Costa et al., 2011). Regarding the VAG, the increase of 0.04 on the rating scale indicates a slight and minimal deterioration in grain color.

The large number of lines with superior GY compared to the controls, especially BRS FP403, a cultivar well accepted by producers and with high yield in SNF systems in previous studies (Dias et al., 2020), demonstrates the high potential of the lines to become recommended cultivars for SNF systems. The mean of the top lines was higher than those found in studies evaluating recombinant lines (Farid et al., 2017; Heilig, Wright, et al., 2017), elite lines, and cultivars under SNF (Dias et al., 2020, 2024; Farid & Navabi, 2015; Fonseca et al., 2013; Pereira et al., 2015). Although cultivars and elite lines have high agronomic potential, allowing their direct use in SNF systems, these genotypes were developed and selected in soils fertilized with mineral N, highlighting that the N source used in the development process influences yield expression under SNF (Pereira et al., 2015).

The lines evaluated in this study originated from a population formed by parents selected for their high performance

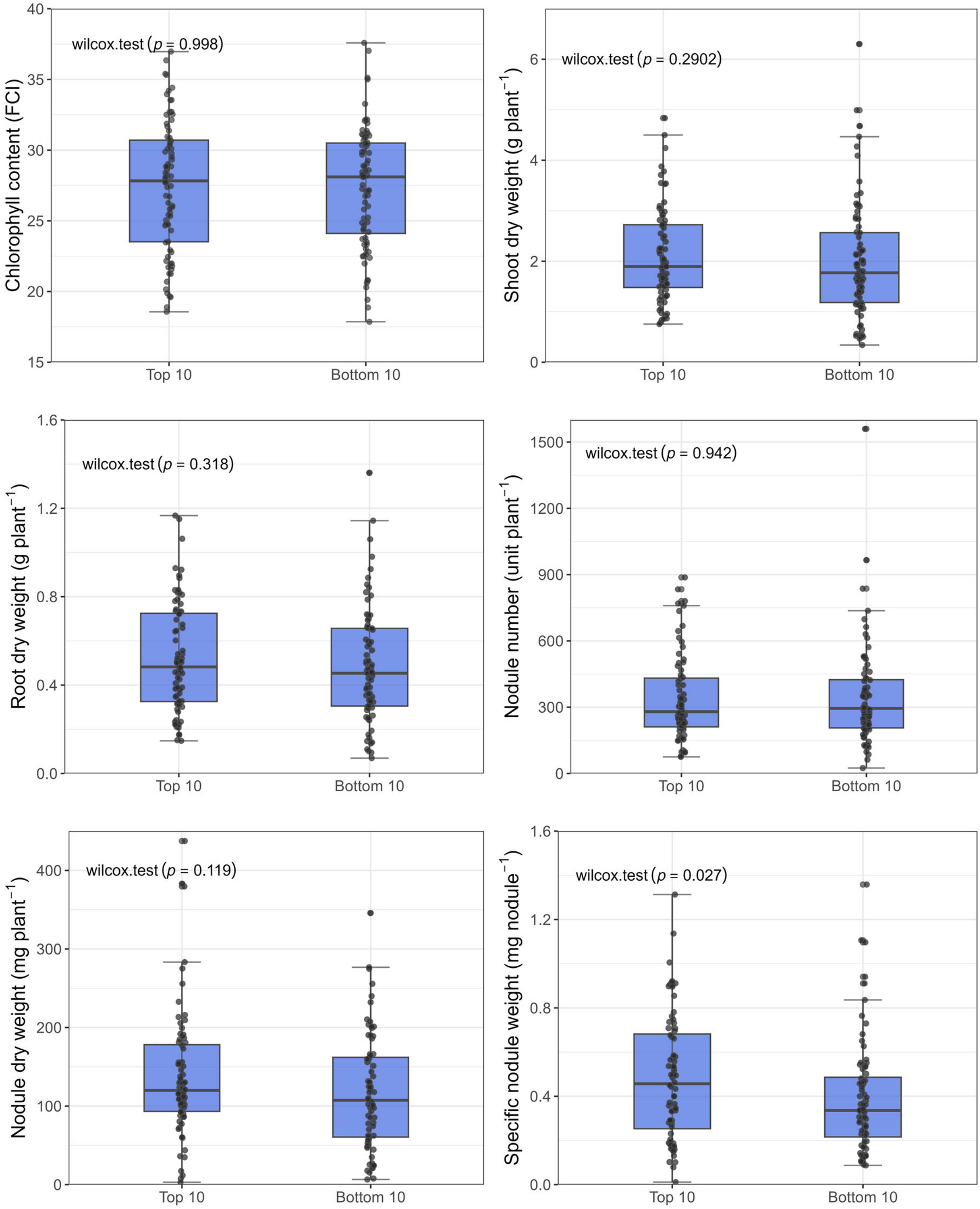

FIGURE 1 Comparison between the means of the top 10 and bottom 10 line groups for grain yield evaluated in the field, considering the following traits: chlorophyll content (Falker chlorophyll index [FCI]), shoot dry weight (g plant^{-1}), root dry weight (g plant^{-1}), nodule number (unit plant^{-1}), nodule dry weight (mg plant^{-1}), and specific nodule weight (mg nodule^{-1}) evaluated in a greenhouse.

in BNF systems for yield and nodulation. The population was advanced through the bulk method in replicated experiments, using rhizobia inoculation as the sole N source. Thus, natural selection may have acted to select the most productive and competitive genotypes under SNF, contributing to the high means found. However, it is important to emphasize that the evaluated genotypes may also have efficiency in N absorption, which may have influenced the observed yield results. The capacity to absorb available N in the soil is a relevant factor to consider, especially in soils with low levels of the nutrient.

TABLE 5 Means of the top 10 (10+) and bottom 10 (10−) lines for grain yield and five checks, considering the traits chlorophyll content (CC, Falker chlorophyll index [FCI]), shoot dry weight (SDW, g plant^{-1}), root dry weight (RDW, g plant^{-1}), nodule number (NN, unit plant^{-1}), nodule dry weight (NDW, mg plant^{-1}), and specific nodule weight (SWN, mg nodule^{-1}) measured in two plant nursery trials.

Genotypes	CC	SDW	RDW	NN	NDW	SWN
Top 10 (10+)						
CNFP 21619	27.2b	1.91ab	0.46bc	351bcd	122a	0.44ab
CNFP 21622	24.8bc	2.43ab	0.58bc	570ab	194a	0.43ab
CNFP 21629	29.5b	1.93ab	0.52bc	288cd	112a	0.39ab
CNFP 21630	27.9b	2.29ab	0.60bc	228cd	126a	0.63ab
CNFP 21632	26.1bc	2.09ab	0.55bc	419abcd	132a	0.38ab
CNFP 21649	26.2bc	2.05ab	0.49bc	323bcd	155a	0.48ab
CNFP 21651	33.4a	2.64ab	0.59bc	265cd	204a	0.78a
CNFP 21661	27.6b	2.10ab	0.47bc	383abcd	150a	0.40ab
CNFP 21662	24.5bc	2.00ab	0.47bc	338bcd	93a	0.29b
CNFP 21681	25.6bc	1.97ab	0.53bc	287cd	113a	0.55ab
Mean	27.3	2.14	0.53	345	140	0.48
Bottom 10 (10−)						
CNFP 21637	26.8b	2.16ab	0.55bc	390abcd	106a	0.29b
CNFP 21645	26.1bc	1.35b	0.46bc	224cd	69a	0.36ab
CNFP 21646	29.4b	1.55ab	0.45bc	161d	97a	0.57ab
CNFP 21654	25.8bc	1.98ab	0.42bc	323bcd	93a	0.35ab
CNFP 21656	27.1b	2.36ab	0.48bc	400abcd	159a	0.46ab
CNFP 21667	27.3b	2.67ab	0.50bc	536abc	149a	0.26b
CNFP 21670	27.6b	1.84ab	0.44bc	269cd	95a	0.33ab
CNFP 21677	27.0b	1.74ab	0.41bc	254cd	98a	0.43ab
CNFP 21682	28.8b	3.00a	0.71b	397abcd	166a	0.43ab
CNFP 21687	26.3bc	2.19ab	0.53bc	665a	155a	0.34ab
Mean	27.2	2.08	0.50	362	119	0.38
Overall mean	27.3	2.11	0.51	354	129	0.43
Checks						
BRS ESPLENDOR	25.1bc	1.38b	0.36c	328bcd	68a	0.25b
BRS FP403	29.6b	2.58ab	0.66b	317bcd	171a	0.55ab
CNFP 7994	26.1bc	1.52ab	0.30c	256cd	80a	0.32ab
BRS SUBLIME	26.8b	2.19ab	0.48bc	468abc	123a	0.33ab
NORH 54	20.3c	1.33b	0.94a	0	0	0
Mean[a]	27.2	2.10	0.50	371	125	0.40

Note: Values followed by equal letters, in the columns, do not differ statistically from each other using the Scott–Knott means test at 5% probability.

[a] Values do not include the check NORH 54.

4.2 | Indirect selection and performance of lines for SNF-related traits

The lines showed genetic variability for traits related to SNF. Environmental factors, such as temperature and humidity, influenced the expression of these traits, even in greenhouse conditions. However, the lines maintained consistent responses for most SNF-related traits, regardless of variations in cultivation environments.

The selection of contrasting genotypes (top and bottom) for evaluation in the greenhouse was based on the premise that the top and bottom lines for GY would also be the top and bottom for SNF-related traits since nitrogen is the nutrient required in the largest amounts by common beans, and the most productive lines could be those with the best symbiotic performance. As observed, the superiority of the top lines for SNF traits occurred only for SWN (Figure 1).

The results indicate that the symbiotic performance of the top and bottom lines did not differ statistically for most traits. This suggests that managing the segregating population by bulk in the field over successive generations, in environments with rhizobia inoculation, was efficient. Only those

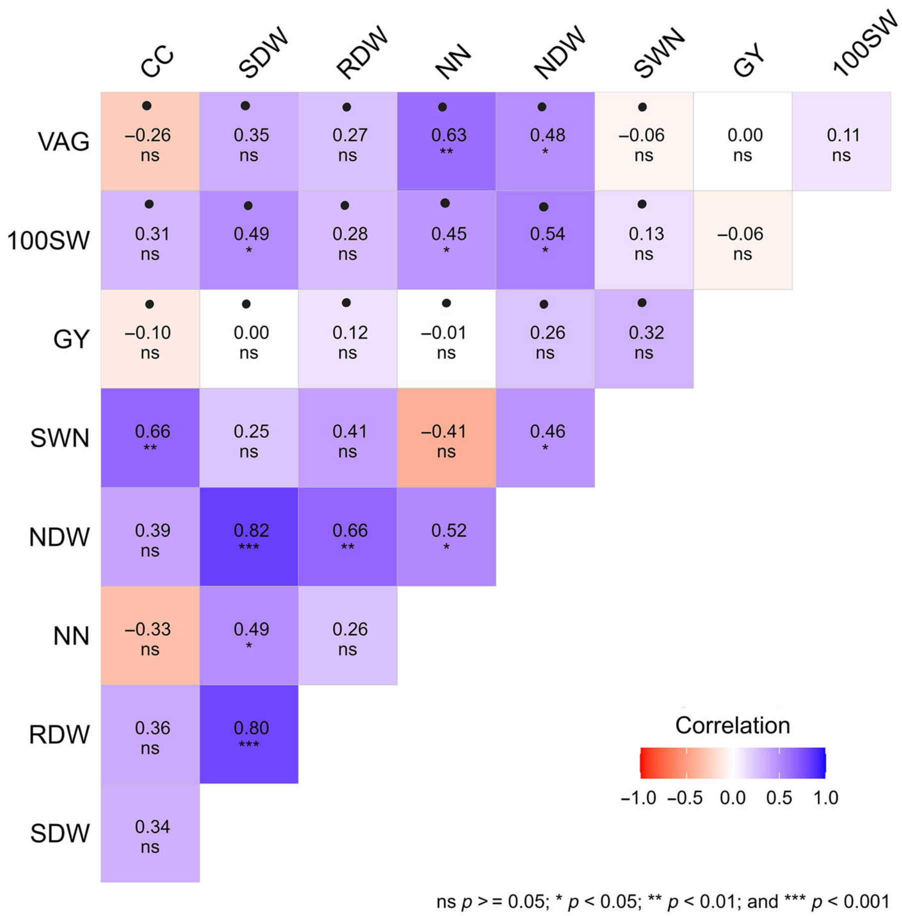

FIGURE 2 Genetic correlation coefficients (●) among traits measured in different experiments and phenotypic correlation (without symbol) among traits measured in the same experiments, in evaluations for symbiotic nitrogen fixation of 20 lines and two black common bean checks. In greenhouse experiments, chlorophyll content (CC, Falker chlorophyll index [FCI]), shoot dry weight (SDW, g plant^{-1}), root dry weight (RDW, g plant^{-1}), nodule number (NN, unit plant^{-1}), nodule dry weight (NDW, mg plant^{-1}), and specific nodule weight (SWN, mg nodule^{-1}) were measured, while in field experiments, grain yield (GY, kg ha^{-1}), 100-seed weight (100SW, g), and visual aspect of grains (VAG, scale of grades from 1 to 3) were assessed. *, ** and *** significant at 5%, 1%, and 0.1% probability, respectively. ns, nonsignificant.

genotypes that were best associated with the bacteria to obtain the necessary nitrogen for their development remained in the population. Therefore, the differences observed in GY between the top and bottom lines are minimally related to SNF and more to other factors such as the genetic constitution of the genotypes, plant architecture, lifecycle, disease resistance, among others.

Since SNF-related traits are difficult to measure (Nagpal et al., 2023; Oladzad et al., 2020), making their routine incorporation in breeding programs unfeasible, an association between greenhouse and field data could enable the use of greenhouse trials only in the final stages of SNF breeding programs, where the number of genotypes to be evaluated is smaller.

Thus, in the initial stages of the programs, genotypes would be evaluated for their field performance under rhizobia inoculation for traits commonly included in breeding programs, such as GY (Bliss, 1993). In the more advanced stages, experiments in the greenhouse would be conducted to evaluate SNF-related traits, as these can be more precise and require less time and space than a full field evaluation, besides being possible to conduct at any time of the year (Heilig, Beaver, et al., 2017; Heilig, Wright, et al., 2017; Rodiño et al., 2011).

The high variations between the means of the lines, particularly for CC and NN, allow for the identification of superior-performing genotypes under SNF systems. Nitrogen is an important component of chlorophyll molecules and plays a fundamental role in maintaining plant photosynthetic rates. Chlorophyll pigment concentration is a reliable indicator of plant physiological condition, and studies have reported significant increases due to rhizobia inoculation (Farid et al., 2017; Ramaekers et al., 2013; Reinprecht et al., 2020).

For the NN, the means found were higher than those reported by Fonseca et al. (2013), Farid and Navabi (2015), and Dias et al. (2020), whose highest means for the trait were 392, 170, and 98 nodules plant^{-1}, respectively, in

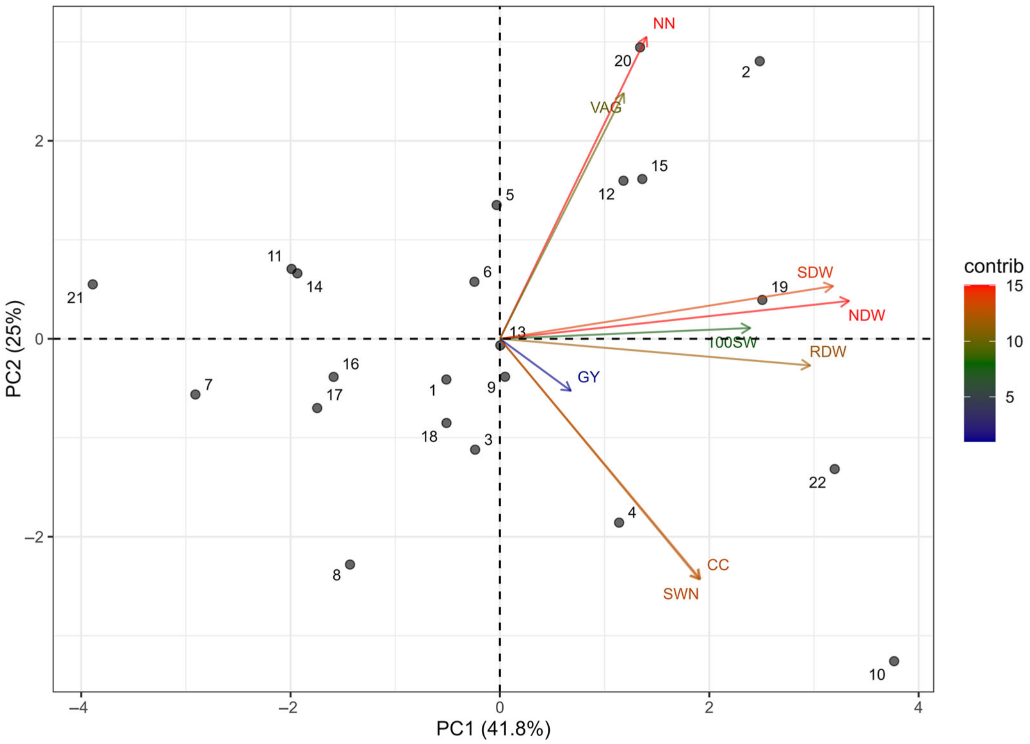

FIGURE 3 Principal component analysis of 22 common bean genotypes, comprising the top 10 (1–10) and bottom 10 (11–20) for grain yield, and two checks (21 and 22), for the traits evaluated in the greenhouse: chlorophyll content (CC, Falker chlorophyll index [FCI]), shoot dry weight (SDW, g plant^{-1}), root dry weight (RDW, g plant^{-1}), nodule number (NN, unit plant^{-1}), nodule dry weight (NDW, mg plant^{-1}), and specific nodule weight (SWN, mg nodule^{-1}); and the traits evaluated in the field: grain yield (GY, kg ha^{-1}), 100-seed weight (100SW, g), and visual aspect of grains (VAG, scale of scores from 1 to 3).

evaluations of commercial cultivars, and similar to those reported by Knupp et al. (2017), who found a range of 170–663 nodules plant^{-1} in germplasm bank accessions. The fact that the lines were developed in exclusive SNF systems may have contributed to their greater association with rhizobia, ensuring intense nodule formation. However, it is worth noting that common beans typically produce a high number of small nodules with low fixing activity (Isoi & Yoshida, 1991), meaning that a higher nodule number does not necessarily imply more effective SNF.

The relationship between nodule size and nitrogen-fixing ability in common beans needs to be clearly established (Knupp et al., 2017). Matoso (2014) studied the minimum nodule size that effectively contributes to nitrogen accumulation in the shoot and growth of common beans. Although the results were inconclusive regarding symbiotic capacity, medium-sized nodules (>2 mm) favored plant growth. In the study by Rodiño et al. (2011), it was evident that genotypes with large nodules (>2 mg nodule^{-1}), despite having fewer nodules, had greater nodule, root, and shoot biomass compared to genotypes with smaller nodules (<1.5 mg nodule^{-1}).

Understanding the relationships among various traits is crucial for enhancing SNF efficiency. An important association between CC and SWN was detected. Some studies have demonstrated correlations between CC and the percentage of ndfa (Farid et al., 2017; Reinprecht et al., 2020) and GY (Ramaekers et al., 2013), recommending its use in indirect selection for SNF. However, this recommendation is only valid when there are no other nitrogen sources available, as these directly increase the trait (Jiang et al., 2020; Reinprecht et al., 2020).

The correlations involving NDW indicated that nodule biomass increases with greater quantity and size of the nodules, as well as with root development. NDW is considered one of the most reliable traits related to SNF, as established by classic studies on common bean and soybean (Döbereiner, 1966; Hungria & Bohrer, 2000), since it shows a good association with the total nitrogen in plants, unlike nodule number.

GY did not show significant correlation with the evaluated traits (Figure 2). Selecting high-yielding genotypes with high SNF efficiency has been slow in common beans (Reinprecht et al., 2020). While some studies report an association between GY and SNF (Barbosa et al., 2018; Farid & Navabi, 2015; Heilig, Wright, et al., 2017), others emphasize that selection for both traits should be performed simultaneously due to low or absent correlations (Farid et al., 2017;

Reinprecht et al., 2020). These results highlight the complexity of the interaction between yield and SNF in common beans, underscoring the need for more studies to ensure consistent advances for both traits.

The association found between SWN, CC, and GY in principal component analysis enables recommending the first two traits for indirect selection in breeding common beans for yield and SNF capacity. It should be noted that SWN was the only trait that allowed discrimination between the top and bottom genotypes for GY. Additionally, phenotyping CC with a chlorophyll meter at the beginning of flowering offers a faster, easier, low-cost, and nondestructive alternative for the plants, as it does not involve the root system (Ramaekers et al., 2013).

Principal component analysis also indicated an important association between SDW, RDW, NDWs and 100SW (Figure 3). Both traits contribute to plant biomass, which is directly related to good nitrogen supply and total nitrogen content in plant tissues (Hungria & Bohrer, 2000). Some studies recommend evaluating SDW for indirect SNF selection in soybean and common bean genotypes (Heilig, Wright, et al., 2017; Hungria & Bohrer, 2000; Nicolás et al., 2002). Therefore, the results found reinforce the relevance of the trait as a reliable indicator of SNF, especially in soils with low nitrogen levels.

The genotypes selected in this study will be evaluated in the advanced trials of the program, where they will be tested in a greater number of environments where the primary nitrogen source is SNF. For this, the selection of the top 10 lines for GY evaluated in this study is recommended, except for CNFP 21632 and CNFP 21662, which showed low means for 100SW (Table 4), not meeting current market expectations. The remaining eight lines showed good means for agronomic traits and similar and satisfactory performance for SNF-related traits. In the next evaluations, it is also important to consider N uptake efficiency, as this can significantly influence the yield and overall performance of the selected lines.

5 | CONCLUSION

The development of lines exclusively in environments where SNF is the main nitrogen source is efficient for selecting lines with high symbiotic efficiency (top 10 lines), allowing for significant genetic gains in GY (20.36%). CC, SDW, and SWN can be recommended for indirect selection of genotypes with better SNF performance. It is possible to identify superior lines for continued evaluations aiming at the release of cultivars adapted to production systems based on SNF as the main nitrogen source.

AUTHOR CONTRIBUTIONS

Nayana Valéria Costa: Data curation; formal analysis; investigation; methodology; software; supervision; validation; visualization; writing—original draft; writing—review and editing. **Patrícia Guimarães Santos Melo**: Conceptualization; formal analysis; funding acquisition; investigation; methodology; supervision; validation; writing—original draft; writing—review and editing. **Helton Santos Pereira**: Conceptualization; data curation; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; writing—review and editing. **Renato Gomide de Sousa**: Data curation; formal analysis; investigation; methodology; software; visualization. **Enderson Petronio de Brito Ferreira**: Conceptualization; funding acquisition; investigation; project administration; supervision; validation; visualization; writing—review and editing. **Adriano Moreira Knupp**: Conceptualization; data curation; investigation; methodology; supervision; validation; writing—review and editing. **Leonardo Cunha Melo**: Conceptualization; data curation; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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