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# Nitrogen sources on agronomic traits in *carioca* common bean lines developed under mineral nitrogen fertilization

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**ABSTRACT:** Despite the importance of symbiotic nitrogen fixation (SNF) as a nitrogen source for leguminous plants, there are few reports of common bean breeding programs aiming at efficiency in SNF. This study aimed to analyze the interaction between elite lines of common bean with *carioca* grains and the nitrogen source (mineral fertilizer or SNF), and to select lines with wide adaptability, stability, high agronomic performance, and superiority in nodulation. Nineteen lines with *carioca* grains were evaluated in 12 environments in Brazil. In each environment, one experiment with mineral nitrogen fertilization and another with Rhizobium inoculation were set up, and agronomic traits were evaluated. Nodulation traits were evaluated in two years at one out of five location. Analyses of variance and of stability were performed, and Spearman's correlations and the coincidence of selection of the best lines in both N sources were estimated. There is an effect of the N source and of genetic variability for all the traits. The lines were higher yielding under fertilization with mineral fertilizer, but they were more resistant to lodging when they were inoculated. The line by N source interaction was significant for 100-seed weight and resistance to lodging, but it did not affect selection of the best lines in the N sources. Eleven out of the 19 lines had no difference in grain yields between the two N sources. The line CNFC 15086 is recommended for growing in both N sources since it presented the highest yield and high adaptability and stability under both sources. The genotypes BRS Sublime, CNFC 15010, and CNFC 15003 had a high relative nodulation index and can be used in crosses with higher yielding lines.

**Key words:** *Phaseolus vulgaris* L., *Rhizobium*, symbiotic nitrogen fixation, nodulation.

## INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a food rich in protein and minerals and part of the diet of millions of people. In Brazil, the most consumed type is carioca, representing 70% of the 2.6 million tons produced annually (Embrapa 2023). Common bean can acquire nitrogen through symbiotic nitrogen fixation (SNF). However, this technology use is still low, likely because of the instability of the response to inoculation. This may be attributed to the common bean's promiscuity in attracting a diverse array of microbial species to colonize its nodules, which could explain the frequently reported low rates of nitrogen fixation contribution with this legume (Moura et al. 2022).

Due to variations in the growing of common bean, occurrence of the genotype by environment interaction (G × E) is expected, and it has been reported especially for grain yield in studies using mineral N fertilization and rhizobia inoculation (Pereira et al. 2015, Farid et al. 2017, Dias et al. 2020, Karavidas et al. 2023). Despite the advantages of SNF and the importance of the G × E interaction for common bean, generally studies are conducted on lines evaluated in one or few environments, and the G × E interaction that may occur and change the lines selection in different edaphoclimatic conditions is not examined (Pereira et al. 2015, Farid et al. 2016, 2017).

Studies checking the effect from inoculation with rhizobia compared to the effect from N fertilizer are common for grain yield and 100-seed weight (Fageria et al. 2014a, 2014b, Farid et al. 2016, Dias et al. 2020). The results of these studies are not in agreement, due to the variability among the lines in their ability for N2 fixation, as well as the strong effect environment has on both common bean plants and nitrogen fixing bacteria (Owaresat et al. 2023). Furthermore, research on the impact of SNF on other agronomic traits of interest in common bean, such as sieve yield, plant architecture and plant diseases, is rare (Dias et al. 2020).

Finding a common bean line with high capacity for $N_2$ fixation is laborious, especially because common bean breeding programs have always used mineral N throughout the selection cycles. The evaluation of elite lines under inoculation may be immediate sources of cultivars responsive to SNF. So, the aims of the study were to analyze the effect of the interaction between elite lines of *carioca* common bean and mineral N fertilization or rhizobia inoculation; and to select lines with wide adaptability, yield stability, and high agronomic performance and nodulation when inoculated with rhizobia.

## MATERIALS AND METHODS

Nineteen lines of common bean with *carioca* seed coat (cream-colored seed coat with brown stripes), consisting of three cultivars (BRS Estilo, Pérola, and BRS Sublime) and 16 elite lines, were evaluated in two steps. In the first experiment, agronomic evaluation was conducted in multiple environments, and in the next experiment traits related to nodulation in two environments were evaluated.

Each environment was the combination of location/crop and season/year. In each one, two experiments were set up, both with fertilization of $P_2O_5$ and $K_2O$ at planting in accordance with soil analyses. In the first experiment, nitrogen fertilizer at sowing (20 $kg{\cdot}ha^{-1}$ of N) and in topdressing (60 $kg{\cdot}ha^{-1}$ of N) was applied in the form of urea. In the second one, there was no nitrogen fertilization, and inoculation was made on the seeds with peat inoculant composed of a mixture of 1:1:1 of the strains *Rhizobium freirei* (SEMIA 4080) and *R. tropici* (SEMIA 4077 and 4088), registered for common bean in the Brazilian Ministry of Agriculture. The density of the inoculant was $10^9$ $cells{\cdot}g^{-1}$ of peat, and the inoculant was applied at the rate of 500 g per 50 kg of seeds. The design used in each experiment was randomized blocks, with three replications, in plots consisting of four 4-m-length rows, spaced at 0.45 m, sowing 15 seeds per meter. All the crop treatments were performed as recommended, except for disease control.

Experiments for agronomic evaluation were conducted in five locations in the states of Goiás (Anápolis and Santo Antônio de Goiás), Mato Grosso (Tangará da Serra), and Paraná (Ponta Grossa), and in the Distrito Federal (Brasília), in three different crop seasons (rainy, dry and winter) and two years (2011 and 2012). Grain yield was evaluated in 12 environments; sieve yield, 100-seed weight, and plant architecture in 10 environments; and resistance to lodging in nine environments.

Grain yield was obtained from weighing the grain from the two center rows of the plot and adjusted to 13% moisture. To obtain sieve yield, the grain from a 300 g sample that was retained in a 4.5-mm mesh (no. 12 sieve) was considered. The grain retained in the sieve was weighed, and the percent of grain retained in the sieve was obtained. To obtain the 100-seed weight, a random sample of 100 seeds retained in the sieve was collected, and this sample was weighed.

For evaluations of plant architecture and resistance to lodging, scoring scales from 1 to 9 were used. Score 1 refers to the line ideal for mechanical harvest, with short guides/runners (< 20 cm), high pods (tip of the pod > 15 cm from the ground), and tightly closed branches (angle of main branches with stem less than 10°). Score 9 refers to lines not suitable for mechanized harvest (climbing plant). For the scoring scale for resistance to lodging, score 1 refers to 0% lodged plants, score 2 from 1 to 10% lodged plants and so on successively, up to score 9, with 91 to 100% lodged plants in the plot (Melo 2009).

To evaluate the nodulation traits, the common bean lines were evaluated in one location (Santo Antônio de Goiás) in the 2013 rainy and 2014 winter crop seasons, with rhizobia inoculation and with nitrogen fertilization, as described before. The areas used in these experiments had not received other inoculation experiments previously. For evaluation of nodulation traits, three plants from one of the rows of each plot were collected in the anthesis (R6 phenological stage). The aboveground part was cut at 1 cm from the ground. The root system was removed from the soil carefully to avoid losses of nodules and roots. Then, they were washed over a sieve and dried for 24 hours in the shade. After, the nodules were removed and counted, determining the trait number of nodules (NN). Each sample of nodules were placed in paper bags and dried in a forced-air-circulation oven for 36 h at 72°C. They were then weighed obtaining the nodule dry matter (NDM). To determine the specific weight of nodules (SWN), which represents the size of the nodule, we divided NDM by NN.

The NN, NDM, and SWN were transformed to a scale from 0 to 10, in which the score 10 was attributed to the highest value within the dataset, and the other ones were obtained by cross multiplication. The scalar values were used to obtain the relative nodulation index (RNI) for each line, according to the Eq. 1, adapted from Ferreira et al. (2010):

$$RNI = \frac{(NN*0.4) + (NDM*0.8) + (SWN*1.8)}{3} \quad (1)$$

Individual and combined analyses of variance considering the environment (location/crop season/year) by N source were performed. The combined analysis of variance was carried out taking into account all the experiments, which combines the locations, crop seasons, years, and N source. The effects of environments, lines and N sources were considered fixed. Adjustments in the degrees of freedom of the mean error and of the line by environment interaction were made when the ratio between the highest and the lowest residual mean square was more than 7. When significant, Scott-Knott's test ($\alpha = 0.10$) was used to cluster the means.

We calculated the coincidence between the lines that were classified with the highest yield in the two N sources in each environment, considering the best six lines (selection of the 30%). The RYIN (in percentage) corresponds to the relations between the yield of each line under inoculation and under nitrogen fertilization and was established through the Eq. 2:

$$RYIN_i = \left(\frac{YLD_{inoc}}{YLD_{nitro}}\right) \times 100 \quad (2)$$

where: $YLD_{inoc}$ : yield of line i with rhizobia inoculation; $YLD_{nitro}$ : yield of line i with mineral nitrogen fertilization.

Spearman's correlations for all the traits were also obtained based on the means with inoculation and with N mineral fertilization in each environment to identify if there were line by N source interaction and if there were predominance of the simple or complex interaction. Significant Spearman's correlations from 0–0.40 were considered predominantly complex; from 0.41–0.60 considered intermediate; and from 0.61–1 predominantly of the simple type. For all the traits, the Spearman's correlations were estimated between the overall mean values with inoculation and with mineral nitrogen fertilization. The coincidence between the 30% best lines based on the overall classification was also estimated.

For grain yield, adaptability and stability of the lines were estimated according to Nunes et al. (2005). One analysis considered the experiments with N fertilizer and with rhizobia inoculation together. Other two analyses considered separately the data from the experiments with mineral N fertilization and the data from inoculated experiments. Initially, $z_{ij}$ was obtained, which corresponds to the standardized mean values of the lines for each environment. The constant $k = 4$ was added to all $z_{ij}$, turning all values into positive values. The adaptability was determined by the mean of the $z_{ij}$ values; and the stability of the line corresponds to the coefficient of variation of the $z_{ij}$ ($CV_{zi}$). The lines were considered very stable when they exhibited $CV_{Zi} \leq 20\%$, stable when $20\% < CV_{Zi} \leq 25\%$, and unstable when $CV_{Zi} > 25\%$.

## RESULTS AND DISCUSSION

All sources of variation were significant for grain yield (Table 1). The variability among the lines indicates that it is possible to select genotypes with the best perfomance. Differences in N uptake from the soil and atmosphere contribute to variability among genotypes (Pacheco et al. 2020), so that the N demand is met for some, but not for others. Significance for environments was expected, due to the environmental variations related to the different sowing times, years, locations, and physical and chemical variations of the soils.

**Table 1.** Summary of combined analyses of variance, with decomposition of the line by environment interaction for grain yield (kg·ha$^{-1}$), sieve yield (%), 100-seed weight (g), plant architecture, and resistance to lodging (scores from 1 to 9) of elite lines and cultivars of *carioca* common bean evaluated in experiments in the states of Goiás, Paraná, Mato Grosso, and in the Distrito Federal, Brazil, in three different crop seasons in 2011 and 2012.

| Source of variation | Grain yield | | | Sieve yield | | | 100-seed weight | | | Plant architecture | | | Resistance to lodging | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DF | MS | *p*-value | DF | MS | *p*-value | DF | MS | *p*-value | DF | MS | *p*-value | DF | MS | *p*-value |
| Blocks/ Experiment[!] | 48 | 610,064 | 0.001 | 40 | 77.9 | 0.011 | 40 | 3.0 | 0.018 | 20 | 1.03 | 0.001 | 18 | 2.49 | 0.001 |
| Lines (L) | 18 | 865,509 | 0.001 | 18 | 3,621.5 | 0.001 | 18 | 77.0 | 0.001 | 18 | 7.13 | 0.001 | 18 | 28.53 | 0.001 |
| Environments (E) | 11 | 138,481,351 | 0.001 | 9 | 71,513.2 | 0.001 | 9 | 1,397.4 | 0.001 | 9 | 6.17 | 0.001 | 8 | 63.56 | 0.001 |
| N sources (S) | 1 | 9,921,746 | 0.001 | 1 | 1,284.1 | 0.001 | 1 | 16.1 | 0.004 | 1 | 14.51 | 0.001 | 1 | 56.16 | 0.001 |
| L × E | 198 | 438,324 | 0.001 | 162 | 374.7 | 0.001 | 162 | 5.8 | 0.001 | 162 | 0.42 | 0.006 | 144 | 1.46 | 0.001 |
| L × S | 18 | 303,264 | 0.107 | 18 | 73.2 | 0.080 | 18 | 3.1 | 0.048 | 18 | 0.40 | 0.168 | 18 | 1.68 | 0.027 |
| E × S | 11 | 2,999,437 | 0.001 | 9 | 1,260.2 | 0.001 | 9 | 60.0 | 0.001 | 9 | 1.84 | 0.001 | 8 | 4.73 | 0.001 |
| L × E × S | 198 | 280,466 | 0.004 | 162 | 83.3 | 0.001 | 162 | 2.7 | 0.002 | 162 | 0.35 | 0.155 | 144 | 0.98 | 0.388 |
| Residue | 864 | 211,268 | - | 720 | 48.5 | - | 720 | 1.9 | - | 360 | 0.30 | - | 324 | 0.95 | - |
| Total | 1,367 | | | 1,139 | | | 1,139 | | | 759 | | | 683 | | |
| Overall mean | 2,307 | | | 61.5 | | | 25.6 | | | 4.7 | | | 3.8 | | |
| C (%) | 50 | | | 100 | | | 83 | | | 67 | | | 50 | | |
| Coefficient of variation (%) | 19.9 | | | 11.3 | | | 5.4 | | | 11.7 | | | 25.7 | | |
| $r_s$[6] | 0.45* | | | 0.90** | | | 0.89** | | | 0.81** | | | 0.88** | | |

DF: degrees of freedom; MS: mean square; C: coincidence (%) of selection of the 30% (six) best lines; rs: Spearman's correlation: [ns]not significant (p > 0.05), *significant (p ≤ 0.05), **highly significant (p ≤ 0.01) by the Student's t test; [!]Experiment: combination of location/crop and season/year/N source.

The mean yield values in the two systems confirmed the difference between the N sources (Table 2). The difference of grain yield overall mean between N mineral fertilization and inoculation was 170 kg·ha$^{-1}$. This difference can be considered small when the financial and environmental costs are taken into account. Even with a lower yield, it should be noted that inoculation represents lower cost in relation to mineral fertilizer, and there is lower emission of greenhouse gases and reduction in pollution of ground water (Hungria and Mendes 2015). While inoculating with rhizobia may not consistently result in higher yields, it offers a more favorable cost-to-benefit ratio compared to N mineral, especially when considering the significantly greater environmental and financial expenses associated with nitrogen fertilizer (Sousa et al. 2022).

Several studies show difference in yield under different N sources (Fageria et al. 2014a, 2014b, Pereira et al. 2015). However, another study did not find a difference in yield between the two N sources (Brito et al. 2010). This variation in the results can be attributed to the effect of high temperatures, water deficit, and other abiotic and biotic factors to which both common bean plants and nitrogen fixing bacteria are sensitive (Owaresat et al. 2023).

The positive effects of inoculation on grain yield are reported for Brazilian conditions. The inoculation of cultivar Pérola seeds with *R. tropici* alone or in co-inoculation promoted better nodulation and increased yield in the dry season (Steiner et al. 2019). Furthermore, in the winter season, yield averages close to or above 3,500 kg·ha$^{-1}$ were reported for the cultivars Pérola, BRS Sublime, and BRS Estilo exclusively under inoculation (Andraus et al. 2016).

The line by N source interaction was not significant for yield (Table 1), indicating a similar response of the lines under the two N sources. However, 92% of the Spearman's correlation estimates ($r_s$) between the two N sources by environment were not significant, indicating predominance of a complex interaction, with considerable change in classification of the lines. The estimate of overall rs was intermediate ($r_s$ = 0.45*), indicating that simple and complex interactions are close, and change in the ranking of lines is moderate.

The coincidence in the selection of the 30% best lines was high (C = 50%) (Table 1). The three lines selected in both N sources would be CNFC 15086, CNFC 15082, and CNFC 15097. In seven out of the 12 environments evaluated, C was considered high (≥ 50%), especially with selection intensity of 30%. Thus, the complex interaction detected in the correlation estimates must be due to the change in ranking of the lines with intermediate or low yield. This indicates that the highest yielding lines developed in a system with nitrogen fertilizer can be recommended for systems with use of SNF.

**Table 2.** Means of grain yield (GY – kg·ha$^{-1}$) of elite lines and *carioca* common bean cultivars under mineral nitrogen fertilization and inoculation with rhizobia, estimate of Zi, coefficient of variation (CVi), and relationship between yield with inoculation and yield with mineral nitrogen fertilization (RYIN, %), evaluated in 24 experiments (overall) and 12 environments per N source (nitrogen fertilizer and inoculated with rhizobia), in 2011 and 2012.

| Line | Overall | | | Nitrogen Fertilization | | | Inoculated with Rhizobia | | | RYIN |
|---|---|---|---|---|---|---|---|---|---|---|
| | GY | Zi | CVi | GY | Zi | CVi | GY | Zi | CVi | |
| CNFC 15086 | 2,559 A | 4.7 | 19.1 | 2,690 Aa | 4.9 | 14.6 | 2,428 Ab | 4.6 | 25.2 | 90 |
| CNFC 15097 | 2,470 A | 4.5 | 29.8 | 2,653 Aa | 4.7 | 30.7 | 2,287 Ab | 4.3 | 35.9 | 86 |
| CNFC 15082 | 2,422 B | 4.4 | 17.4 | 2,514 Aa | 4.3 | 23.5 | 2,329 Aa | 4.4 | 17.3 | 93 |
| CNFC 15049 | 2,378 B | 4.3 | 22.0 | 2,540 Aa | 4.4 | 26.9 | 2,217 Bb | 4.1 | 23.3 | 87 |
| CNFC 15038 | 2,374 B | 4.2 | 16.3 | 2,483 Aa | 4.3 | 20.5 | 2,266Ab | 4.0 | 16.7 | 91 |
| CNFC 15070 | 2,371 B | 4.1 | 21.9 | 2,443 Aa | 4.1 | 25.5 | 2,300 Aa | 4.1 | 25.1 | 94 |
| CNFC 15018 | 2,357 B | 4.2 | 21.7 | 2,513 Aa | 4.5 | 21.8 | 2,202 Bb | 3.8 | 24.9 | 88 |
| CNFC 10762 | 2,342 B | 4.2 | 25.4 | 2,304 Ba | 4.0 | 40.1 | 2,380 Aa | 4.4 | 20.8 | 103 |
| CNFC 15025 | 2,326 B | 4.1 | 17.3 | 2,485 Aa | 4.3 | 18.1 | 2,166 Bb | 3.9 | 20.1 | 87 |
| CNFC 15003 | 2,285 C | 3.8 | 27.4 | 2,345 Ba | 3.8 | 36.0 | 2,224 Ba | 3.9 | 29.3 | 95 |
| CNFC 15033 | 2,281 C | 3.9 | 18.3 | 2,355 Ba | 3.9 | 27.1 | 2,207 Ba | 4.0 | 17.2 | 94 |
| CNFC 15035 | 2,266 C | 3.9 | 28.5 | 2,392 Ba | 4.0 | 46.4 | 2,139 Bb | 3.8 | 20.6 | 89 |
| CNFC 15023 | 2,258 C | 3.8 | 20.1 | 2,235 Ba | 3.5 | 30.3 | 2,281 Aa | 4.1 | 15.4 | 102 |
| CNFC 15010 | 2,234 C | 3.8 | 18.5 | 2,274 Ba | 3.6 | 18.1 | 2,194 Ba | 3.9 | 22.1 | 96 |
| CNFC 15001 | 2,227 C | 3.7 | 25.6 | 2,200 Ba | 3.5 | 47.0 | 2,254 Aa | 4.0 | 16.1 | 102 |
| Pérola | 2,220 C | 3.9 | 37.4 | 2,310 Ba | 3.8 | 42.7 | 2,131 Ba | 3.9 | 43.4 | 92 |
| CNFC 15044 | 2,198 C | 3.7 | 23.9 | 2,317 Ba | 3.7 | 35.5 | 2,080 Bb | 3.6 | 22.9 | 90 |
| BRS Estilo | 2,140 C | 3.5 | 20.3 | 2,183 Ba | 3.4 | 30.0 | 2,098 Ba | 3.6 | 19.6 | 96 |
| BRS Sublime | 2,130 C | 3.4 | 25.1 | 2,222 Ba | 3.4 | 34.7 | 2,039 Ba | 3.4 | 26.6 | 92 |
| Mean | 2,307 | - | - | 2,392 a | - | - | 2,222 b | - | - | 93 |

Mean values followed by the same lowercase letters in the row and uppercase letters in the column (Scott-Knott, $\alpha = 10\%$) do not differ statistically.

Comparing the RYIN of the highest yielding lines (CNFC 15086, and CNFC 15097), the line that most stood out was CNFC 15086 (RYIN = 90%) (Table 2). The lines CNFC 10762, CNFC 15023, and CNFC 15001 had RYIN higher than 100%, indicating better performance in the SNF system. These lines do not show potential for recommendation as cultivars for use in both systems, but they may be directed specifically for growing under SNF or for crosses as a source of alleles for SNF in combination with higher yielding parents. Similar results were found by Farid et al. (2017), who showed that the absence of correlation between SNF and yield indicates that selection of the best lines for SNF does not necessarily lead to the highest yields.

In general, the lines had higher yield when fertilized with nitrogen fertilizer (Table 2), suggesting that yield is directly affected by the N source used in the selection phase. Since the beginning of the Brazilian Agricultural Research Company (Embrapa) common bean breeding program in 1984, segregating populations have been grown, and selection of lines has been carried out along with the use of nitrogen fertilizer. Thus, naturally, the lines have a superior response under these conditions (Pereira et al. 2015). Even so, lines and even cultivars could be identified with a similar response under the two N sources (Table 2). Furthermore, among them, the lines CNFC 15082, CNFC 15070, CNFC 10762, CNFC 15023, and CNFC 15001 had high yield under inoculation and, for that reason, merit attention, as they show potential for introduction in the breeding program as a source of favorable alleles for SNF.

About stability and adaptability, the highest yielding lines in both N sources were generally also the most adapted. The line CNFC 15086 was not only very high yielding, but also very stable (CVi ≤ 20%) in the general classification and under nitrogen fertilizer; yet, it was unstable under inoculation (CVi > 25%). The lines CNFC 15023, CNFC 15001, CNFC 15038, and CNFC 15082 were very stable under inoculation. Studies of adaptability and stability under the SNF condition for common bean are not common. However, Farid et al. (2016) were able to identify eight recombinant inbred lines (RILs) that showed broad adaptation, regardless of the N source.

In addition to the yield performance of a line, traits linked to grain quality, such as sieve yield and 100-seed weight, are essential for a new cultivar to gain acceptance. There are differences among the lines and among the environments for both traits (Table 1). The effect of environment, including management of nitrogen fertilization and inoculation, on 100-seed weight is widely reported in the literature (Carbonell et al. 2010, Pereira et al. 2012, Fageria et al. 2014a, 2014b). Greater sieve yield was generally obtained when there was inoculation with rhizobia (62.6%, Table 3). However, for 100-seed weight, a positive effect of mineral nitrogen fertilization was observed (25.7 g). Though the effects of the N source were significant, probably due to the number of replications and to the high experimental accuracy, the differences in the mean values do not represent a practical difference.

**Table 3.** Mean values of sieve yield (%), 100-seed weight (g), plant architecture, and resistance to lodging (scores from 1 to 9) of elite lines and cultivars of *carioca* common bean under mineral nitrogen fertilization and inoculation with rhizobia, evaluated in multiple environments in 2011 and 2012.

| Line | Sieve yield | | | 100-seed weight | | | Architecture | | | Lodging | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | Nitro | Inoc | Mean | Nitro | Inoc | Mean | Nitro | Inoc | Mean | Nitro | Inoc |
| CNFC 15086 | 64 D | 63 Da | 64 Ca | 28.5 A | 28.6 Aa | 28.4 Aa | 5.2 D | 5.4 Db | 5.0 Ca | 4.5 D | 4.7 Da | 4.3 Ca |
| CNFC 15097 | 80 A | 79 Aa | 81 Aa | 27.6 B | 27.7 Ba | 27.4 Ba | 4.6 C | 4.8 Cb | 4.4 Ba | 4.4 D | 4.9 Eb | 3.8 Ca |
| CNFC 15082 | 77 B | 75 Bb | 80 Aa | 26.5 C | 26.4 Ca | 26.5 Ca | 5.0 D | 5.2 Db | 4.8 Ca | 4.9 D | 5.2 Eb | 4.6 Da |
| CNFC 15049 | 67 C | 65 Db | 70 Ba | 24.3 G | 23.7 Gb | 25.0 Ea | 4.4 B | 4.6 Bb | 4.3 Aa | 3.1 B | 3.4 Ca | 2.8 Aa |
| CNFC 15038 | 56 F | 54 Gb | 59 Da | 26.0 D | 26.1 Ca | 25.9 Da | 4.7 C | 5.0 Cb | 4.5 Ba | 3.3 B | 3.6 Ba | 3.0 Ba |
| CNFC 15070 | 55 F | 55 Ga | 56 Ea | 25.5 E | 25.5 Da | 25.5 Ea | 4.5 C | 4.5 Ba | 4.6 Ba | 3.2 B | 3.5 Cb | 2.9 Aa |
| CNFC 15018 | 55 F | 56 Ga | 55 Ea | 26.9 C | 27.1 Ba | 26.7 Ca | 5.0 D | 5.2 Db | 4.8 Ca | 4.6 D | 5.2 Eb | 4.0 Ca |
| CNFC 10762 | 68 C | 68 Ca | 68 Ba | 24.4 G | 24.4 Fa | 24.4 Fa | 5.4 E | 5.6 Eb | 5.2 Ca | 5.4 E | 6.0 Fb | 4.9 Da |
| CNFC 15025 | 55 F | 54 Ga | 57 Ea | 25.9 D | 26.2 Ca | 25.7 Da | 4.3 B | 4.4 Aa | 4.3 Aa | 3.0 B | 3.5 Cb | 2.6 Aa |
| CNFC 15003 | 53 G | 51 Hb | 55 Ea | 24.8 F | 24.9 Ea | 24.6 Fa | 4.5 C | 4.5 Ba | 4.5 Ba | 3.2 B | 3.1 Aa | 3.2 Ba |
| CNFC 15033 | 59 E | 57 Fb | 61 Da | 26.1 D | 26.2 Ca | 25.9 Da | 4.2 A | 4.3 Aa | 4.2 Aa | 2.8 A | 3.1 Aa | 2.6 Aa |
| CNFC 15035 | 53 G | 50 Hb | 56 Ea | 24.7 F | 25.2 Ea | 24.3 Fb | 4.7 C | 4.9 Cb | 4.6 Ba | 3.6 C | 3.7 Ba | 3.4 Ba |
| CNFC 15023 | 57 F | 57 Fa | 57 Ea | 25.0 F | 25.4 Da | 24.7 Fb | 4.6 C | 4.6 Ba | 4.5 Ba | 3.3 B | 3.4 Ba | 3.2 Ba |
| CNFC 15010 | 60 E | 60 Ea | 61 Da | 25.3 E | 25.6 Da | 25.0 Ea | 4.1 A | 4.2 Aa | 4.1 Aa | 2.6 A | 2.8 Aa | 2.3 Aa |
| CNFC 15001 | 55 F | 55 Ga | 56 Ea | 25.4 E | 25.5 Da | 25.3 Ea | 4.6 C | 4.6 Ba | 4.6 Ba | 3.0 B | 2.8 Aa | 3.2 Ba |
| Pérola | 61 E | 61 Ea | 60 Da | 25.2 E | 25.1 Ea | 25.3 Ea | 5.8 F | 6.0 Fb | 5.6 Da | 5.6 E | 5.9 Fb | 5.2 Da |
| CNFC 15044 | 58 E | 58 Fa | 59 Da | 25.3 E | 25.6 Da | 25.0 Ea | 5.0 D | 5.1 Da | 4.9 Ca | 3.7 C | 3.9 Ba | 3.5 Ba |
| BRS Estilo | 66 C | 64 Db | 69 Ba | 24.6 F | 24.9 Ea | 24.3 Fb | 4.4 B | 4.7 Cb | 4.1 Aa | 3.8 C | 4.4 Db | 3.2 Ba |
| BRS Sublime | 67 C | 66 Da | 67 Ba | 24.3 G | 24.4 Fa | 24.1 Fa | 4.7 C | 4.9 Cb | 4.5 Ba | 4.0 C | 4.2 Ba | 3.8 Ca |
| Mean | 61 | 60 b | 63 A | 25.6 | 25.7 a | 25.5 b | 4.7 | 4.9 b | 4.6 a | 3.8 | 4.1 b | 3.5 a |

Mean values followed by the same lowercase letters in the row and uppercase letter in the column (Scott-Knott, α = 10%) do not differ statistically.*Mean values followed by the same lowercase letters in the row and uppercase letter in the column (Scott-Knott, α= 10%) do not differ statistically.

The line by N source interaction was significant only for 100-seed weight (Table 1). There are few studies in the literature concerning the genotype by environment interaction (G × E) for grain quality traits (Pereira et al. 2012, 2017, Fageria et al. 2014a). In general, the G × E interaction is detected, but without representing much importance. Most of the estimates of Spearman's correlation (85%) by environment for the two traits were intermediate or high ($r_s > 0.40^*$). The overall Spearman's correlations were high (0.90** and 0.89** for sieve yield and 100-seed weight, respectively) (Table 1), indicating the predominance of the simple type of interaction and small variation in classification of the lines. Confirming these results, coincidently, the six best lines (C = 100%) would be selected for sieve yield and five out of the six best (C = 83%) for 100-seed weight. Thus, the line by N source interaction significant for 100-seed weight represented little practical effect for purposes of selection of the best lines.

For these two traits, the controls evaluated are considered to be high performance; therefore, lines that exceed these cultivars are considered excellent. The elite lines that stood out for sieve yield were CNFC 15097 and CNFC 15082 (Table 3). These lines were also among those of best performance under both N sources and had sieve yield greater than 70%, a value considered suitable for meeting industry demands (Carbonell et al. 2010). The CNFC 15086 line was prominent for high yield, and though it had a sieve yield lower than 70%, it was still superior to the Pérola cultivar, considered a standard for grain quality.

For a cultivar to be successful in the market, it must have not only high yield potential and commercial quality of its grain, but also upright plant architecture and resistance to lodging, which reduces losses in mechanized harvest and contact of the pods with the ground, limiting deterioration of the commercial quality of the grain (Pereira et al. 2012). The effects of lines, environments, and N sources were significant for both traits (Table 1). In general, the lines had better architecture and better resistance to lodging under SNF (Table 3).

The line by N source interaction was significant only for resistance to lodging (Table 1). The Spearman's correlation estimates per environment for plant architecture ranged from low to intermediate (Table 1), confirming the importance of the interaction. However, for resistance to lodging, most of estimates per environment (88%) were intermediate or high, suggesting moderate or low change in the ranking of the lines. The overall Spearman's correlation estimates for both traits were high, suggesting that the best lines regarding plant architecture and resistance to lodging are the same under both N sources. Corroborating what was indicated by the overall Spearman's correlation estimate, the coincidences in selection for plant architecture and resistance to lodging were high (C = 67% and C = 50%, respectively). Likewise in soybean, Serunjogi et al. (2002) evaluated the effect of inoculation and of mineral fertilization and did not observe an effect of the line by N source interaction on resistance to lodging, indicating that there was no influence of the source of N on selection of soybean lines for resistance to lodging.

The same two lines stood out for plant architecture and resistance to lodging (CNFC 15010 and CNFC 15033) (Table 3) in mean value and also under both N sources. This confirms the observation that the effect of the line by N source interaction, when present, occurs due to the change in the classification from intermediate to inferior lines, not affecting selection of lines with better plant architecture and resistance to lodging. Generally, lines with better plant architecture are also among the best lines regarding resistance to lodging (Table 3), confirming that cultivars with upright architecture are less susceptible to lodging (Pereira et al. 2012). The Pérola cultivar had relatively high mean scores for plant architecture and resistance to lodging, which is consistent with its semi-prostrate architecture. However, there was a significant improvement in its architecture and resistance to lodging upon being inoculated with rhizobia (Table 3). Qu et al. (2016) also observed that soybean lines significantly increased their resistance to lodging when inoculated with *Sinorhizobium meliloti*.

Differences were observed among lines, environments and N sources for all the traits related to nodulation (Table 4). Regarding the N source, higher mean values were observed when inoculation with rhizobia was performed (Table 5), which was expected, since nodulation and efficient nitrogen fixation are observed when there are inoculation with rhizobia and absence of or low application rate of mineral N (Kontopoulou et al. 2017).

The line by N source interaction was highly significant for all the nodulation traits (Table 4). The estimates of coincidence and the Spearman's correlation reinforce the importance of interaction between lines and N sources, and the N source used during the selection process for all the nodulation traits is important. All the Spearman's correlation estimates by environment and overall were not significant, indicating predominance of the complex type of interaction. The selection coincidences were low, 33% for number of nodules and for relative nodulation index; and 17% for nodule dry matter and specific weight of nodules.

**Table 4.** Summary of combined analyses of variance, with decomposition of the line by environment interaction, for number of nodules per plant (NN), node dry matter per plant (NDM, in mg·plant$^{-1}$), specific weight of nodules (SWN, in mg·nodule$^{-1}$), and relative nodulation index (RNI) of four *carioca* common bean experiments conducted in Santo Antônio de Goiás, GO, Brazil, in the 2013 rainy crop season and 2014 winter crop season.

| Source of variation | DF | NN | | NDM | | SWN | | RNI | |
|---|---|---|---|---|---|---|---|---|---|
| | | MS | *p*-value | MS | *p*-value | MS | *p*-value | MS | *p*-value |
| Blocks/Experiment* | 8 | 237 | 0.001 | 107 | 0.005 | 0.1 | 0.448 | 0.09 | 0.774 |
| Lines (L) | 18 | 1,450 | 0.001 | 5,390 | 0.001 | 1.9 | 0.001 | 4.00 | 0.001 |
| Environments (E) | 1 | 39,113 | 0.001 | 26,050 | 0.001 | 1.8 | 0.001 | 2.49 | 0.001 |
| N sources (S) | 1 | 116,197 | 0.001 | 525,223 | 0.001 | 49.5 | 0.001 | 308.20 | 0.001 |
| L × E | 18 | 1,470 | 0.001 | 3,133 | 0.001 | 1.3 | 0.001 | 2.39 | 0.001 |
| L × S | 18 | 1,607 | 0.001 | 6,229 | 0.001 | 1.6 | 0.001 | 4.15 | 0.001 |
| E × S | 1 | 9,004 | 0.001 | 1,757 | 0.001 | 17.0 | 0.001 | 12.24 | 0.001 |
| L × E × S | 18 | 1,148 | 0.001 | 3,245 | 0.001 | 1.8 | 0.001 | 2.96 | 0.001 |
| Residue | 144 | 62 | - | 37 | - | 0.1 | - | 0.14 | - |
| Total | 227 | - | | - | | - | | - | |
| Overall mean | | 37.9 | | 64.2 | | 1.7 | | 2.72 | |
| C (%) | | 33 | | 17 | | 17 | | 33 | |
| Coefficient of variation (%) | | 20.8 | | 9.4 | | 22.4 | | 14.2 | |
| $r_s$ | | $-0.28^{ns}$ | | $-0.27^{ns}$ | | $0.12^{ns}$ | | $-0.04^{ns}$ | |

DF: degrees of freedom; MS: mean square; C: coincidence (%) of selection of the 30% (six) best lines; $r_s$: Spearman's correlation: $^{ns}$not significant ($p > 0.05$) by the Student's t test; *combination of location/crop and season/year/N source.

**Table 5.** Mean values of number of nodules (NN, in unit·plant$^{-1}$), nodule dry matter (NDM, in mg·plant$^{-1}$), specific weight of nodules (SWN, in mg·nodule$^{-1}$), and relative nodulation index (RNI) of elite lines and cultivars of carioca common bean under mineral nitrogen fertilization and inoculation with rhizobia, evaluated in Santo Antônio de Goiás, GO, Brazil, in the 2013 rainy crop season and 2014 winter crop season.

| Line | NN | | NDM | | SWN | | RNI | |
|---|---|---|---|---|---|---|---|---|
| | Nitro | Inoc | Nitro | Inoc | Nitro | Inoc | Nitro | Inoc |
| CNFC 15086 | 4.7 Cb | 54.4 Da | 6.8 Eb | 123.2 Da | 2.28 Aa | 2.47 Ba | 2.66 Ab | 4.38 Da |
| CNFC 15097 | 9.4 Cb | 52.9 Da | 12.7 Db | 80.3 Ga | 1.97 Ba | 1.49 Db | 2.40 Ab | 2.85 Fa |
| CNFC 15082 | 18.5 Bb | 32.5 Fa | 21.2 Cb | 72.7 Ha | 1.22 Cb | 2.43 Ba | 1.71 Bb | 3.68 Ea |
| CNFC 15049 | 24.9 Bb | 73.2 Ba | 17.9 Cb | 92.2 Fa | 0.70 Db | 1.24 Da | 1.15 Cb | 2.85 Fa |
| CNFC 15038 | 12.3 Cb | 57.7 Da | 11.6 Db | 78.7 Ga | 0.89 Db | 1.68 Da | 1.20 Cb | 3.09 Fa |
| CNFC 15070 | 13.1 Cb | 41.9 Ea | 21.7 Cb | 95.6 Fa | 1.51 Cb | 2.31 Ba | 2.01 Bb | 3.84 Ea |
| CNFC 15018 | 9.4 Cb | 66.5 Ca | 18.7 Cb | 117.9 Da | 1.83 Bb | 2.25 Ba | 2.31 Ab | 4.18 Da |
| CNFC 10762 | 18.9 Bb | 30.4 Fa | 14.0 Cb | 89.2 Fa | 0.54 Db | 2.79 Aa | 0.88 Db | 4.24 Da |
| CNFC 15025 | 19.8 Bb | 55.2 Da | 12.1 Db | 72.8 Ha | 0.59 Db | 1.25 Da | 0.94 Db | 2.53 Ga |
| CNFC 15003 | 5.3 Cb | 103.3 Aa | 3.0 Eb | 200.7 Aa | 0.66 Db | 2.06 Ca | 0.81 Db | 5.06 Ba |
| CNFC 15033 | 12.0 Cb | 59.6 Da | 2.7 Eb | 79.6 Ga | 0.35 Db | 1.63 Da | 0.50 Db | 3.06 Fa |
| CNFC 15035 | 11.8 Cb | 76.3 Ba | 13.8 Cb | 108.7 Ea | 0.94 Db | 1.68 Da | 1.28 Cb | 3.52 Ea |
| CNFC 15023 | 15.9 Cb | 43.6 Ea | 15.9 Cb | 73.3 Ha | 1.35 Ca | 1.67 Da | 1.79 Bb | 2.93 Fa |
| CNFC 15010 | 9.3 Cb | 55.3 Da | 8.5 Db | 158.6 Ba | 0.71 Db | 3.11 Aa | 0.95 Db | 5.46 Aa |
| CNFC 15001 | 36.2 Aa | 42.9 Ea | 44.4 Ab | 62.0 Ia | 1.49 Ca | 1.46 Da | 2.38 Aa | 2.57 Ga |
| Pérola | 6.5 Cb | 68.0 Ca | 8.0 Db | 155.2 Ba | 1.24 Cb | 2.35 Ba | 1.52 Cb | 4.67 Ca |
| CNFC 15044 | 11.0 Cb | 80.3 Ba | 10.9 Db | 148.2 Ca | 0.90 Db | 2.03 Ca | 1.20 Cb | 4.33 Da |
| BRS Estilo | 20.5 Bb | 48.3 Da | 30.5 Bb | 122.2 Da | 1.25 Cb | 2.81 Aa | 1.86 Bb | 4.71 Ca |
| BRS Sublime | 32.1 Ab | 107.1 Aa | 33.7 Bb | 201.0 Aa | 1.23 Cb | 2.65 Aa | 1.96 Bb | 5.74 Aa |
| Mean | 15.3 b | 60.5 A | 16.2 b | 112.2 a | 1.14 b | 2.07 a | 1.55 b | 3.88 a |

Mean values followed by the same lowercase letters in the row and uppercase letters in the column (Scott-Knott, $\alpha = 10\%$) do not differ statistically; nitro: mineral nitrogen fertilization; inoc: inoculation with rhizobia.

Regarding the mean values of the lines for number of nodules, a wide amplitude was observed among the mean values when there was inoculation (Table 5), showing the influence of genetic variations. Under SNF, the cultivar BRS Sublime and the line CNFC 15003 stood out, both with mean values greater than 100 nodules per plant. Among them, the cultivar BRS Sublime had a high number of nodules also when fertilized with mineral N, and it can be recommended for crosses as a source of alleles for nodulation.

There was nodulation of the lines when grown without inoculation and with fertilization with nitrogen fertilizer, which is common, and this indicates the presence of native strains (Hungria et al. 2013, Otsubo et al. 2013). Although there are observations regarding native strains that are able to nodulate efficiently and in a manner equivalent to selected strains (Figueiredo et al. 2016), in general the NN in the lines not inoculated and fertilized with nitrogen fertilizer is lower than the NN when there is inoculation (Table 5).

For nodule dry matter under SNF, the line CNFC 15003 and the cultivar BRS Sublime stood out, with mean values greater than 200 mg·plant$^{-1}$. The overall mean of the lines under inoculation was approximately seven times greater than that observed under fertilization with nitrogen fertilizer (Table 5). Otsubo et al. (2013) also observed the influence of the nitrogen source on nodule dry matter, with reduction in this trait in lines that were fertilized with nitrogen fertilizer and in those whose nodulation occurred by native strains.

The lines CNFC 15010 and CNFC 10762 and the cultivars BRS Estilo and BRS Sublime had the highest specific weights of nodules, with mean values that ranged from 3.11 to 2.65 mg nodule-1 under SNF (Table 5). The nodules coming from native strains in the non-inoculated lines were not only fewer in number, but also smaller. Although a higher NN does not always mean more effective SNF, nodule size is an indicative of symbiotic efficiency (Li et al. 2015).

The relative nodulation index allows selection based on the most important traits of nodulation and has been used, above all, in selection of wild lines and accessions of the germplasm bank (Ferreira et al. 2010, Knupp et al. 2013). The cultivar BRS Sublime and the lines CNFC 15010 and CNFC 15003 are notable in regard to this index, and they can be recommended as potential parents for making crosses with the aim of increasing capacity for N2 fixation (Table 5). The cultivar BRS Sublime (Wendland et al. 2018) recently released and selected under conditions of application of nitrogen fertilizer, proved to be responsive to inoculation and was consistent in its response under SNF, exhibiting high nodulation and larger nodule size when inoculated.

In general, the genotypes superior for the nodulation traits (BRS Sublime, CNFC 15010, and CNFC 15003) do not have the highest yields (Table 2). In an analogous way, among the lines with highest yield under SNF, only CNFC 10762 stood out with high SWN. There was no association between the traits of nodulation and grain yield. To all these traits, it is required to conduct a breeding program specifically with inoculation with selected rhizobia, without the use of fertilization with mineral N, making crosses between the lines with high nodulation, such as BRS Sublime, CNFC 15510, and CNFP 15003, and the highest yielding lines, such as CNFC 15086.

## CONCLUSION

The elite lines of *carioca* common bean obtained in a system with mineral N fertilization are higher yielding under fertilization with nitrogen fertilizer, but they are more resistant to lodging when inoculated with rhizobia.

There is a small effect of N sources for plant architecture, sieve yield, and 100-seed weight. However, the line by N source interaction does not affect selection of the best lines.

The line CNFC 15086 is recommended for growing both in systems with mineral nitrogen fertilization and in systems with the use of SNF, because it has high yield and wide adaptability and yield stability.

There is genetic variability among elite *carioca* common bean lines for nodulation traits. The cultivar BRS Sublime and the lines CNFC 15010 and CNFC 15003 are selected for presenting the highest relative nodulation index.

## CONFLICT OF INTEREST

Nothing to declare.

## AUTHORS' CONTRIBUTION

**Conceptualization:** Melo, L. C. and Pereira, H. S. **Data curation:** Melo, L. C.; Souza, T. L. P. O.; Faria, L. C.; Pereira, H. S.; Dias, P. A. S. and Almeida, V. M. **Formal analysis:** Dias, P. A.S.; Melo, P. G. S.; Ferreira, E. P. B. and Pereira, H. S. **Funding acquisition:** Melo, P. G. S.; Ferreira, E. P. B.; Melo, L. C. and Pereira, H. S. **Methodology:** Melo, P. G. S.; Ferreira, E. P. B.; Melo, L. C. and Pereira, H. S. **Writing – original draft:** Dias, P. A. S.; Melo, P. G. S.; Ferreira, E. P. B.; Melo, L. C. and Pereira, H. S. **Writing – review and editing:** Dias, P. A. S.; Melo, P. G. S.; Ferreira, E. P. B.; Melo, L. C. and Pereira, H. S.

## DATA AVAILABILITY STATEMENT

Data will be made available on request to the corresponding author.

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