INTRODUCTION

The common bean is considered a staple food for more than 300 million people in Latin America and Africa, being an important source of protein, fibre, carbohydrates, and minerals (Celmeli et al., 2018; Mukankusi et al., 2019). Although it is one of the most widely produced leguminous crops in the world, the common bean is predominantly cultivated by small farmers, usually in marginal regions, where various biotic and abiotic factors often affect crop productivity (Androcioli et al., 2020; Beebe et al., 2009; Miklas et al., 2006). Stress caused by soil nitrogen (N) deficiency is considered as the main limiting abiotic factor responsible for low productivity of common bean worldwide (Leal et al., 2019).

N is the most important element required by common bean crops (Barros et al., 2018; Chekanai et al., 2018). Although common bean plants can fix atmospheric N (N₂) due to symbiotic interactions with bacteria of the genus Rhizobium (Hungria et al., 2003; Korir et al., 2017), fixation efficiency is generally low compared with other legumes, and N fertilization is recommended to meet the nutritional requirements of the plant (Appelbaum, 2018). Thus, several studies have highlighted the positive effects of N in increasing common bean plant productivity (Chekanai et al., 2018;
Different genetic improvement strategies for low-N tolerance and/or N-use efficiency have been adopted (Kamfwa et al., 2017; Leal et al., 2019). However, selection for both traits is considered a complex process, because genotypes with high tolerance to low-N generally had low N-use efficiency, as previously reported in other crops (Fageria & Baligar, 2005; Maia et al., 2011; Mendonça et al., 2017; Wu et al., 2011). Tolerance to low N can be agronomically defined as the reduction in seed yield under stress conditions compared with ideal crop conditions, whereas N-use efficiency is defined as the ratio of seed yield per unit N available to the plant (Fritsche-Neto & DoVale, 2012). The relationship between low-N tolerance and N-use efficiency has not yet been studied in the common bean.

N-response indices have been used to select superior genotypes under contrasting N conditions. Fageria (2003) proposed using the N-agronomic efficiency (NAE) index to select genotypes with more efficient N use, that is those that are highly productive under minimal available resources. Wu et al., (2011) developed the low-N agronomic efficiency (LNAE) index, in which genotypes with high seed yield are selected under optimal growing conditions and maintain this yield under N-limiting conditions. On the other hand, Miti et al., (2010) aimed to select genotypes with greater behaviour predictability under contrasting N conditions and proposed the low-N tolerance index (LNTI). The performance under contrasting N-(PCN) index was used as a N-response index by Mendonça et al., (2017), which aimed to select genotypes with high seed yield under normal and N-limiting conditions.

The existence of high genetic variability in common bean allows the recombination and selection of genotypes adapted to different environmental conditions (Blair et al., 2010; Kwak & Gepts, 2009). Thus, the joint use of agronomic information and molecular markers is important in allowing breeding programmes to define parents with a higher probability of obtaining transgressive segregants for characteristics of interest (Assefa et al., 2019; Pereira et al., 2019). In this sense, the objectives of the present study were the following: (a) to compare different N-responses indices of common bean cultivars in contrasting N environments, (b) to identify low-N tolerant and high N-use efficiency common bean cultivars, (c) to verify the existing genetic variability among cultivars using single-nucleotide polymorphism (SNP) and amplified fragment length polymorphism (AFLP) markers, and (d) to recommend future crossings to develop cultivars less dependent on N fertilizers.

## MATERIAL AND METHODS

### 2.1 Plant materials

This study evaluated 39 Brazilian carioca common bean (Phaseolus vulgaris L.) cultivars registered in the National Registry of Cultivars of the Ministry of Agriculture, Livestock and Supply (RNC – MAPA) by different public or private research institutions (Table 1). They represent a large part of the genetic variability that exists among carioca common bean cultivars that are currently being cultivated or have been grown during ~50 years of carioca common bean breeding history in Brazil (Zeffa et al., 2020).

<table>
<thead>
<tr>
<th>Code</th>
<th>Cultivar</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carioca</td>
<td>IAC</td>
</tr>
<tr>
<td>2</td>
<td>IAPAR 14</td>
<td>IDR-Paraná</td>
</tr>
<tr>
<td>3</td>
<td>IAPAR 57</td>
<td>IDR-Paraná</td>
</tr>
<tr>
<td>4</td>
<td>IAPAR 72</td>
<td>IDR-Paraná</td>
</tr>
<tr>
<td>5</td>
<td>Pérola</td>
<td>Embrapa</td>
</tr>
<tr>
<td>6</td>
<td>IAPAR 80</td>
<td>IDR-Paraná</td>
</tr>
<tr>
<td>7</td>
<td>IAPAR 81</td>
<td>IDR-Paraná</td>
</tr>
<tr>
<td>8</td>
<td>ANFc 9</td>
<td>Agro Norte</td>
</tr>
<tr>
<td>9</td>
<td>Princesa</td>
<td>Embrapa</td>
</tr>
<tr>
<td>10</td>
<td>IPR Juriti</td>
<td>IDR-Paraná</td>
</tr>
<tr>
<td>11</td>
<td>BRS Talismã</td>
<td>Embrapa</td>
</tr>
<tr>
<td>12</td>
<td>BRS Pontal</td>
<td>Embrapa</td>
</tr>
<tr>
<td>13</td>
<td>BRS Requinte</td>
<td>Embrapa</td>
</tr>
<tr>
<td>14</td>
<td>IPR Saracura</td>
<td>IDR-Paraná</td>
</tr>
<tr>
<td>15</td>
<td>IPR Colibri</td>
<td>IDR-Paraná</td>
</tr>
<tr>
<td>16</td>
<td>BRS Horizonte</td>
<td>Embrapa</td>
</tr>
<tr>
<td>17</td>
<td>BRS MG Pioneiro</td>
<td>Embrapa</td>
</tr>
<tr>
<td>18</td>
<td>IPR Eldorado</td>
<td>IDR-Paraná</td>
</tr>
<tr>
<td>19</td>
<td>IPR 139</td>
<td>IDR-Paraná</td>
</tr>
<tr>
<td>20</td>
<td>IAC Alvorada</td>
<td>IAC</td>
</tr>
<tr>
<td>21</td>
<td>IPR Tangará</td>
<td>IDR-Paraná</td>
</tr>
<tr>
<td>22</td>
<td>FTS 65</td>
<td>FT Sementes</td>
</tr>
<tr>
<td>23</td>
<td>BRS Estilo</td>
<td>Embrapa</td>
</tr>
<tr>
<td>24</td>
<td>TAA Bola Cheia</td>
<td>Terra Alta</td>
</tr>
<tr>
<td>25</td>
<td>IPR Campos Gerais</td>
<td>IDR-Paraná</td>
</tr>
<tr>
<td>26</td>
<td>IPR Andorinha</td>
<td>IDR-Paraná</td>
</tr>
<tr>
<td>27</td>
<td>BRS MG Madrepérola</td>
<td>Embrapa</td>
</tr>
<tr>
<td>28</td>
<td>BRS Ametista</td>
<td>Embrapa</td>
</tr>
<tr>
<td>29</td>
<td>IPR Curió</td>
<td>IDR-Paraná</td>
</tr>
<tr>
<td>30</td>
<td>BRS Notável</td>
<td>Embrapa</td>
</tr>
<tr>
<td>31</td>
<td>IAC Imperador</td>
<td>IAC</td>
</tr>
<tr>
<td>32</td>
<td>TAA Gol</td>
<td>Terra Alta</td>
</tr>
<tr>
<td>33</td>
<td>IPR Maracanã</td>
<td>IDR-Paraná</td>
</tr>
<tr>
<td>34</td>
<td>TAA Dama</td>
<td>Terra Alta</td>
</tr>
<tr>
<td>35</td>
<td>IPR Quero-quero</td>
<td>IDR-Paraná</td>
</tr>
<tr>
<td>36</td>
<td>IPR Bem-te-vi</td>
<td>IDR-Paraná</td>
</tr>
<tr>
<td>37</td>
<td>IPR Celeiro</td>
<td>IDR-Paraná</td>
</tr>
<tr>
<td>38</td>
<td>IAC Sintonia</td>
<td>IAC</td>
</tr>
<tr>
<td>39</td>
<td>IPR Sabiá</td>
<td>IDR-Paraná</td>
</tr>
</tbody>
</table>
2.2 | Experimental design

Cultivars were evaluated at the Research Stations of Instituto de Desenvolvimento Rural do Paraná – IAPAR – EMATER (IDR- Paraná), Brazil, in Londrina and Ponta Grossa during the rainy season of 2017, and in Santa Tereza do Oeste and Ponta Grossa in the dry season of 2018. Soil physical-chemical analyses and other characteristics related to the assessment sites are presented in Table S1. The experiments were arranged in a randomized complete block design with four replications. Sowing was carried out mechanically and the experimental plots consisted of four 4-m rows, spaced 0.5 m apart, with 15 seeds per linear meter. Two levels of N top-dressing were used in each environment, totaling eight independent experiments. Experiments under high N were fertilized with 40 kg N/ha (urea) at the V₄ development stage (Fernández et al., 1985), whereas the experiments under low N did not receive N top-dressing. All experiments were fertilized at sowing with 300 kg/ha of NPK (04–30–10).

2.3 | N-response indices

Seed yield (SY, kg/ha with 13% corrected humidity) was obtained after manual harvesting and mechanical threshing of plants from the two central rows of each plot. Then, the N-response indices were calculated using the following equations:

i) N-agronomic efficiency (NAE, kg/kg) (Fageria, 2003):

\[
\text{NAE}_{ij} = \frac{\text{SY}_{i(HN)} - \text{SY}_{i(LN)}}{\text{N}}
\]

ii) Low-N tolerance index (LNTI, %) (Miti et al., 2010):

\[
\text{LNTI}_{ij} = \left(1 - \frac{\text{SY}_{i(LN)}}{\text{SY}_{i(HN)}}\right) \times 100.
\]

iii) Low-N agronomic efficiency index (LNAE, kg/ha) (Wu et al., 2011):

\[
\text{LNAE}_{ij} = \left(\frac{\text{SY}_{i(LN)}}{\text{SY}_{i(HN)}}\right) \times \text{SY}_{i(LN)}
\]

iv) Performance under contrasting N (PCN) (Mendonça et al., 2017):

\[
\text{PCN}_{ij} = \frac{2}{\left(\frac{\text{SY}_{i(HN)}}{x_{HN}}\right)^{-1} + \left(\frac{\text{SY}_{i(LN)}}{x_{LN}}\right)^{-1}}
\]

where SYᵢ(ᵢ=₁⁰₀) is the seed yield of cultivar i on repetition j under high N, SYᵢ(LN) is the seed yield of cultivar i on repetition j under low N, xᵢ(HN) is the general mean of seed yield under high-N conditions, xᵢ(LN) is the general mean of seed yield under low-N conditions, and N is the amount of N (kg/ha) applied in the high-N experiment.

2.4 | Genotyping

2.4.1 | DNA extraction

DNA extraction was performed as described by Ariani et al., (2016). After extraction, DNA was purified using genomic DNA Clean and Concentrator Kits (Zymo Research). The quality of the DNA was verified by a NanoDrop™ Lite Spectrophotometer (Thermo Fisher Scientific) and by 1% agarose gel electrophoresis. The genomic DNA was quantified using Quant-iT™ PicoGreen® dsDNA Assay Kits (Thermo Fisher Scientific).

2.4.2 | Molecular markers

The SNP markers were identified using the genotyping-by-sequencing (GBS) method, as described by Ariani et al., (2016). Genomic libraries were sequenced by the Illumina HiSeq 4000 platform (Illumina) via the 100-bp-single-end protocol at the DNA Technologies & Expression Analysis Core Laboratory at the University of California, Davis, USA. Briefly, the CviAll restriction enzyme was used for DNA digestion and, after the polymerase chain reaction (PCR), the assembled products were purified and quantified for sequencing according to the manufacturer’s instructions. The sequencing data were analyzed using the Tassel 5.0 GBS v2 software (Glaubitz et al., 2014) using the standard definitions, except for the minimum quality score (–mnQs 20) and minimum count (–c 10) parameters. The sequences were aligned with the reference genome of Phaseolus vulgaris L. v2.0 bean (Schmutz et al., 2014) acquired from Phytozome (https://phytozome.jgi.doe.gov) using the Burrows-Wheeler Alignment (BWA) software version 0.7.10 (Li & Durbin, 2009). SNP quality control was performed using VCFtools software version 0.1.15 (Danecek et al., 2011), which removed any SNP fulfilling the following criteria: (a) presence of indels; (b) non-biallelic; (c) minor allele frequency (MAF) <0.05; (d) inbreeding coefficient <0.9; (e) linkage disequilibrium (LD) >0.2. Heterozygous SNPs were treated as missing data because common bean plants are predominantly autogamous and, thus, these SNPs may have come from sequencing errors (or residual heterozygosity). The missing data were imputed by the Hidden Markov Model (HMM) using the BEAGLE software version 5.0 ( Browning et al., 2018). After the filtering process, only the high-quality SNPs were used in the subsequent analyses.

The AFLP protocol was adapted from Vos et al., (1995). Approximately 700 ng of DNA was digested with 5 U of EcoRI and 1 U of Msel at 37°C for 4 hr, after which the adapters were bound using 2 U T4 DNA ligase at 22°C for 1 hr. Incubation at 70°C for 10 min was performed to thermally inactivate the restriction enzymes. The pre-selective amplification reaction was performed using EcoRI + A and Msel + C primers in a volume of 10 µL containing 3 µL 1x diluted template. The PCR conditions were as follows: 72°C for 2 min, followed by 20 cycles at 94°C for 1 s, 56°C for 30 s, and 72°C for 2 min, with a final extension for 30 min at 60°C. The selective reaction was
conducted with the Eco + ACA/Mse + CAC, Eco + ACG/Mse + CAA, Eco + ACT/Mse + CAA, and Eco + ACG/Mse + GAC primer pairs, with 2.5 μL six times diluted template and in a final volume of 10 μL. The amplification conditions followed an initial cycle of 94°C for 2 min, followed by 65°C for 30 s, 72°C for 2 min, eight cycles at 94°C for 1 s, 64°C for 30 s (with a decrease of 1°C per cycle) and 72°C for 2 min, 23 cycles at 94°C for 1 s, 56°C for 30 s and 72°C for 2 min, with a final extension 30 min at 60°C. The PCR products were separated by capillary electrophoresis using an ABI 3500 XL system (Applied Biosystems), with GS-600 LIZ (Applied Biosystems) used as a molecular weight marker. GENEMAPPER® v.4.1 software (Applied Biosystems) was used to detect electropherogram peaks generated by the AFLP technique and for fragment determination.

2.5 | Phenotypic data analysis

Data analysis was performed using the best linear unbiased prediction (BLUP) and restricted maximum likelihood (REML) methods through the R software version 3.6.0 (https://www.r-project.org/) using ‘lme4’ package (Bates et al., 2007). Analyses of deviance (ANODEV) were performed considering the following statistical model:

\[
Y_{ijkm} = \mu + g_i + b_{i/k/m} + e_k + n_m + ge_i + gn_m + en_{km} + gen_{km}\]

where \(\mu\) is the overall mean, \(g_i\) is the random effect of the \(i\)-th genotype, \(b_{i/k/m}\) is the random effect of the \(j\)-th block within the \(k\)-th location and within the \(m\)-th N fertilization, \(l_j\) is the fixed effect of the \(k\)-th local, \(n_m\) is the fixed effect of the \(m\)-th N fertilization, \(g_{i/k/m}\) is the random effect of the genotype \(\times\) location interaction, \(ln_{km}\) is the random effect of the genotype \(\times\) N fertilization interaction, \(ln_{km}\) is the fixed effect of the local \(\times\) N fertilization interaction, \(gn_{km}\) is the random effect of the genotype \(\times\) local \(\times\) N fertilization interaction, and \(f_{ijkm} \sim N(0, \sigma^2)\) is the random effect of the error associated with each experimental unit. Random and fixed effects were evaluated using the likelihood ratio test (LRT) and Wald tests, respectively, at 5% probability.

The ANODEV for the N-response indices were performed using the model presented above excluding the effect of N level and its interaction with the other effects. Broad-sense heritability \((h^2)\) was estimated using the following formula: \(h^2 = \sigma_g^2/\sigma_y^2 + 1 + \sigma_e^2/\sigma_y^2\), where \(\sigma_g^2\) is the genotypic variance, \(\sigma_e^2\) is the genotype \(\times\) environment interaction variance, \(\sigma_r^2\) is the residual variance, \(r\) is the number of environments, and \(n\) is the number of repetitions per environment. Selective accuracy \((\bar{r})\) and genotypic correlation \((\rho)\) were obtained, respectively, as follows: \(\bar{r} = \sqrt{1 - PEV}/\sigma_y\) and \(\rho = \sigma_g^2/\sigma_y^2 + \sigma_e^2/\sigma_y^2\), in which \(PEV\) is the variance of the error in predicting genotypic values, \(\sigma_g^2\) is the genotypic variance, and \(\sigma_e^2\) is the genotype \(\times\) environment interaction variance.

Analysis of the adaptability and stability of the N-response indices was also performed using the mixed model approach with the harmonic mean relative performance of genetic values (HMRPGV) method using Selegen – REML/BLUP software (Resende, 2016). The HMRPGV method was used to simultaneously capitalize on the effects of adaptability and stability through the following equation:

\[
HM\text{RPGV}_j = n/ \sum_{i=1}^{n} Vg_i/Vg_j \]

where \(n\) is the number of environments in which the \(i\)-th cultivar was evaluated, \(Vg_i\) is the genotypic value of the \(i\)-th cultivar in environment \(j\), and \(Vg_j\) is the general genotypic mean in environment \(j\). HMRPGV were expressed in the unit of each N-response indices multiplying the values obtained by the general mean of each index.

The eight best cultivars (selection intensity 20%) were selected for each index. Analysis of the correlation between N-response indices was performed by Pearson’s linear correlation coefficient \((\rho)\) using the following formula: \(\rho = \text{cov}(x, y)/\sqrt{\text{var}(x) \times \text{var}(y)}\), where \(\text{cov}(x, y)\) is the covariance between \(x\) and \(y\), whereas \(\text{var}(x)\) and \(\text{var}(y)\) are the variances of \(x\) and \(y\), respectively. The significance of the correlation estimates was checked by \(t\)-tests at 5% significance level. The selection coincidence index \((\text{SC, in } \%\)) between N-response indices was evaluated using the following equation: \(\text{SC} = (CC/n) \times 100\), where CC is the number of cultivars coincidentally selected between the indices, and \(n\) is the total number of cultivars selected by the selection intensity \((n = 8)\). These analyses were performed in R software version 3.6.0 through the ‘ggstatsplot’ (Patil & Powell, 2018) and ‘ggplot2’ packages (Wickham, 2011).

2.6 | Genotypic data analysis

Cluster analysis was carried out aiming to identify groups of cultivars that are genetically distinct. Thus, genetic dissimilarity matrices were calculated for both the SNP and AFLP markers using the mean Euclidean distance. The cultivars were grouped using Ward’s hierarchical method (Ward, 1963), as this method has greater ability to maximize homogeneity within groups and, consequently, to maximize differences between groups. Analysis of the correlation between the SNP- and AFLP-based distance matrices was performed using Mantel’s test (Mantel, 1967) with 1,000 permutations.

The combined distance between the two marker types was calculated as follows: \(d_{ij} = d_{ij}(\text{SNP}) + d_{ij}(\text{AFLP})/2\), where \(d_{ij}(\text{SNP})\) is the mean Euclidean distance between cultivars \(i\) and \(j\) based on SNP markers, and \(d_{ij}(\text{AFLP})\) is the mean Euclidean distance between cultivars \(i\) and \(j\) based on AFLP markers. Then, the cultivars were grouped using Ward’s method (Ward, 1963), whereas the optimal number of groups formed in the dendrogram was defined by the Mojena criterion (Mojena, 1977). This criterion is based on computing the highest amplitude between clusters through the following formula: \(a_j > a + \omega S\), where \(j = 1, 2, ..., n\) is the number of clusters; \(a_j\) is the correspondence joint point to \(n - j + 1\) clusters; \(a\) and \(S\) are the mean and the standard deviation of \(a\); \(\omega\) is the constant equal to 1.25, as suggest by Milligan and Cooper (1985). These analyses were performed with R software version 3.6.0 using the ‘factoextra’ (Kassambara & Mundt, 2017), ‘dendextend’ (Gallili, 2015), and ‘ade4’ (Dray & Dufour, 2007) packages.
3 | RESULTS

3.1 | Analyses of deviance and seed yield genetic parameters

The ANADEV for SY revealed significant effects ($p < .01$) for all sources of variation and their interactions, excluding cultivar × N × location (Table 2). Broad-sense heritability ($h^2$) and selective accuracy ($\hat{r}$) estimates were 0.62 and 0.78, respectively, and correlation values were 0.57 (across locations), 0.93 (across N levels), and 0.31 (across N × location interactions). Under low-N conditions, the means between locations ranged from 1,709.40 kg/ha (STO18) to 2,512.39 kg/ha (PG17), whereas under high N the means ranged from 1,936.59 kg/ha (STO18) to 3,098.05 kg/ha (PG17) (Figure 1).

3.2 | Analyses of deviance and genetic parameters among N-response indices

The ANADEV revealed significant effects of cultivar ($p < .01$) and location ($p < .05$) for all N-response indices (Table 3). However, the cultivar × location interaction only significantly affected $SY_{LNTI}$, $SY_{LNAE}$, and PCN ($p < .01$). The $h^2$ estimates ranged from 0.21 (LNTI) to 0.69 (PCN), whereas the $\hat{r}$ values ranged from 0.45 (LNTI) to 0.83 (PCN). Genetic correlation estimates ranged from 0.24 (LNTI) to 0.79 (NAE).

### TABLE 2 | Analysis of deviance (ANADEV) and genetic parameters for seed yield of 39 carioca bean cultivars evaluated under contrasting N environments

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Seed yield (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed effects</td>
<td></td>
</tr>
<tr>
<td>Nitrogen (N)</td>
<td>430.96**</td>
</tr>
<tr>
<td>Location (L)</td>
<td>40.13**</td>
</tr>
<tr>
<td>N × L</td>
<td>28.75**</td>
</tr>
<tr>
<td>Random effects</td>
<td></td>
</tr>
<tr>
<td>Cultivar (C)</td>
<td>28.65**</td>
</tr>
<tr>
<td>C × N</td>
<td>18.87**</td>
</tr>
<tr>
<td>C × L</td>
<td>460.12**</td>
</tr>
<tr>
<td>C × N × L</td>
<td>0.55 ns</td>
</tr>
<tr>
<td>Genetic parameters</td>
<td></td>
</tr>
<tr>
<td>Heritability ($h^2$)</td>
<td>0.62</td>
</tr>
<tr>
<td>Selective accuracy ($\hat{r}$)</td>
<td>0.78</td>
</tr>
<tr>
<td>Genetic correlation through location (L)</td>
<td>0.57</td>
</tr>
<tr>
<td>Genetic correlation through nitrogen (N)</td>
<td>0.93</td>
</tr>
<tr>
<td>Genetic correlation through L × N</td>
<td>0.31</td>
</tr>
<tr>
<td>Mean</td>
<td>2,150.70</td>
</tr>
</tbody>
</table>

Note: ns and ** not significant at the 5% probability level and significant at the 1% probability level by Wald (fixed effects) or likelihood ratio test (random effects), respectively.

3.3 | N-response indices, adaptability, and stability

The adaptability and stability values of the N-response indices are presented in Figure 2. Seed yield (SY) ranged from 1,708.01 (Carioca) to 2,497.55 kg/ha (IAC Sintonia). When analyzed separately under contrasting N conditions, SY ranged from 1,618.31 (Carioca) to 2,241.43 kg/ha (IAC Sintonia) under low-N conditions, and from 1,877.27 (Carioca) to 2,789.06 kg/ha (IPR Sábiá) under high-N conditions. Based on a selection intensity of ~ 20%, the BRS Notável, IPR Quero-quero, IPR Bem-te-vi, IAC Sintonia, and IPR Sábiá cultivars were selected based on SY, $SY_{LNTI}$, and $SY_{LNAE}$. These same cultivars were also selected using the PCN index.

The cultivars selected using the LNTI index were IAC Sintonia, Princesa, IPR Saracura, IPR Quero-quero, IPR Celeiro, IPR Maracanã, TAA Gol, and IPR Tangará. For the LNAE index, the selected cultivars were IAC Sintonia, Princesa, IPR Saracura, IPR Quero-quero, BRS Notável, TAA Dama, BRS Ametista, and IPR Bem-te-vi. The NAE index was the most discordant in cultivar selection, as it selected IPR Sábiá, IPR 139, IPR Campos Gerais, BRSMG Madrepérola, BRS Pontal, TAA Bola Cheia, FTS 65, and IPR Eldorado. The cultivars selected based on their N-response indices are represented in biplot graphs of the relationship between SY adaptability and stability at low and high N (Figure 3).

3.4 | Correlation and coincidence of selection

The correlation and coincidence estimates in the selection among N-response indices are presented in Figure 4a,b, respectively. Positive and significant correlations ($p < .05$) ranged from 0.44 (NAE × SYLN) to 1.00 (SY × PCN), whereas negative correlations ranged from – 0.69 (LNAE × LNTI) to – 0.35 (PCN × LNTI). Although they were positively correlated ($\rho = 0.86$), the NAE and LNTI indices have a conceptually inverse relationship because the genotypes that are more low-N tolerant have the lowest LNTI values (Figure 4c). Selection coincidence index percentages ranged from 0 (NAE × LNTI and NAE × LNAE) to 100% (SY × PCN). Among the N-response indices, PCN was the most promising index to select genotypes under low and high N conditions, as it exhibited high correlation and concordance values with SY, $SY_{LNTI}$, and $SY_{LNAE}$. On the other hand, the NAE index showed the lowest values.

3.5 | Genetic variability determined by SNP and AFLP markers

A total of 889 polymorphic markers were identified across the 39 carioca common bean cultivars, composed of 351 SNPs and 538 AFLP markers. The mean dissimilarity ($d$) between cultivars was $0.776 \pm 0.063$ based on SNP markers. The IAPAR 72 and IAPAR 57 cultivars ($d = 0.282$) were the closest genetically, whereas IAPAR 80 and TAA Bola Cheia ($d = 0.911$) were the most distant (Figure S1). The mean genetic dissimilarity was $0.540 \pm 0.083$ based on AFLP.
markers, with the IPR Curió and IAPAR 81 cultivars \( (d = 0.350) \) being the most similar, and the IPR Bem-te-vi and IAPAR 72 \( (d = 0.766) \) cultivars being the most distant. The Mantel's test showed no correlation \( (r_m = 0.06; p = .16) \) between the distance matrices, indicating complementarity between the two types of markers (Figure 5a).

The mean dissimilarity using the combination of SNP and AFLP markers was \( 0.658 \pm 0.054 \) for the studied cultivars. The ANFc 9 and BRSMG Madrepérola cultivars were the closest genetically \( (d = 0.406) \), whereas the IPR Sabiá and IAPAR 80 cultivars were the most distant \( (d = 0.798) \). The Mojena method indicated the existence of four distinct groups in the dendrogram (cutoff point = 0.87) obtained by Ward's method (Figure 5b). Group 1 (orange) was composed of 24 cultivars (IPR Juriti, Pérola, IAPAR 81, IPR Bem-te-vi, IPR 139, BRS Pontal, IPR Curió, IAC Imperador, IPR Celeiro, BRSMG Talismã, IAC Alvorada, BRS Requinte, IPR Saracura, BRS Amestista, IPR Maracanaí, BRSMG Pioneiro, BRS Notável, BRS Estilo, IAC Sintonia, Princesa, IPR Eldorado, IAPAR 14, BRSMG Madrepérola, and AnFc 9), and group 2 (yellow) consisted of the IPR Sabiá, IPR Quero-quero, IPR Tangará, IPR Campos Gerais, and FTS 65. On the other hand, four cultivars (IAPAR 80, IAPAR 57 IAPAR 72, and BRS Horizonte) were included in group 3 (light blue), whereas the cultivars TAA Dama, TAA Bola Cheia, TAA Gol, Carica, IPR Colibri, and IPR Andorinha formed group 4 (dark blue). In general, the cultivars that showed the high performance in relation to the N-response indices were grouped in groups 1 and 2, whereas the cultivars with poor performance were grouped in groups 2 and 3.

### 4 DISCUSSION

#### 4.1 Genotype–environment interactions and genetic parameters

The present study is the first to evaluate different N-response indices in common bean cultivars under contrasting N top-dressing environments. The significant effects of the interactions between cultivar \( \times N \) fertilization and cultivar \( \times \) locations on SY indicate that the differential behaviours of these cultivars depend on the level of N top-dressing fertilization and on their growing location. Genotype–environment interactions are often observed in common bean breeding programmes around the world and are considered a complicating factor in varietal selection (Bulyaba et al., 2020; Katuuramu et al., 2020; Pereira et al., 2018). In addition, N-utilization is affected by many environmental factors, especially in legume crops, such as the interaction between Rhizobium and N-fixation (Dong et al., 2020; Oldroyd & Leyser, 2020).

The broad-sense heritability \( (h^2) \) estimates observed in the present study were considered low for LNTI and LNAE and moderate for SY, SY\_LN, SY\_HN, and PCN. Heritability is the central
FIGURE 2  Adaptability and stability estimates (HMRPGV) for seed yield (SY, kg/ha), seed yield under low N (SY\textsubscript{LN}, kg/ha), seed yield under high N (SY\textsubscript{HN}, kg/ha), N-tolerance index (LNTI, %), low-N agronomic efficiency index (LNAE, kg/ha), N-agronomic efficiency index (NAE, kg/kg), and performance under contrasting N (PCN). The eight selected cultivars (selection intensity ~20%) are highlighted in green. Further information on the cultivars is presented in Table 1.

FIGURE 3  Biplot of the 39 carioca common bean cultivars selected for adaptability and stability (HMRPGV) based on seed yield (SY, kg/ha) (a), seed yield under low N (SY\textsubscript{LN}, kg/ha) (b), seed yield under high N (SY\textsubscript{HN}, kg/ha) (c), N-tolerance index (LNTI, %) (d), low-N agronomic efficiency index (LNAE, kg/ha) (e), N-agronomic efficiency index (NAE, kg/kg) (f), and performance under contrasting N (PCN) (g). Further information on the cultivars is presented in Table 1.
parameter for any breeding programme as it is used to estimate selection responses and to explain the proportion of phenotypic variation caused by genetic variations (Falconer & Mackay, 1996). According to Resende and Duarte (2007), the LNTI and LNAE indices are classified as having moderate $h^2$, whereas high $h^2$ was observed for SY, SY$_{LN}$, SY$_{HN}$, and PCN. Selective accuracy ($r$) is used to evaluate experimental quality and considers not only the number of repetitions and environmental variation, but also the relationship between genetic and residual variations. It is therefore considered the most important parameter in the context of selective evaluation (Henderson, 1984; Ribeiro et al., 2017). Thus, the PCN index can be considered the most promising N-response index due to the high $h^2$ and $r$ values found in the present study.

4.2 | N-response indices, adaptability, and stability

The use of the HMRPGV method allowed for cultivar selection based on N-response indices, and cultivars were chosen according to adaptability and penalized for instability between environments (Rosado et al., 2019; Silva et al., 2019). This method has been used for the simultaneous selection of yield, adaptability, and stability in several crops such as common bean (Delfini et al., 2018), wheat (Coan et al., 2018), corn (Pinto et al., 2019), soybean (Freiria et al., 2018), and papaya (Luz et al., 2018). However, there had previously been no studies using the HMRPGV method based on responses to N-response indices or in contrasting N top-dressing environments.

The LNTI index aims to select genotypes that exhibit small differences between stressful and non-stressful conditions, but without
considering SY as a selection criterion (Miti et al., 2010). Thus, even if this index selects genotypes tolerant to low N, it can result in selecting genotypes with low SY. This was confirmed in the present study as, although the LNTI index did select cultivars with high SY under both high- and low-N conditions (Pérola, IPR Quero-quero, and IAC Sintonia), it also selected cultivars (IPR Maracanã and TAA Goi) with SY values below the mean in both N top-dressing conditions.

The NAE index can be defined as assessing the efficiency of the cultivar in converting N into SY (Fageria, 2003). According to Wu et al. (2011), even if this index selects genotypes with higher N-agronomic efficiency, it also selects genotypes with lower performance under low-N conditions. A low SY under N-limiting conditions will increase its numerator (SY$_{HN}$ – SY$_{LN}$), consequently resulting in higher NAE. The difficulty of selecting cultivars with both properties was reported by some authors (Maia et al., 2011; Mendonça et al., 2017). In the present study, only the IPR Bem-te-vi and BRSMG Pioneiro cultivars were classified as low-N tolerant with high-N use efficiency because they presented LTNI and NAE values above the overall mean. On the other hand, most cultivars tolerant to low N showed low-N agronomic efficiency.

Wu et al., (2011) developed the LNAE index by dividing SY under high N by SY under low N and then multiplying this ratio by SY under low N. Thus, the main function of the LNAE index is to select genotypes that are low-N tolerant but maintain high SY under low-N conditions (Santos et al., 2019; Wu et al., 2011). When calculating this index, the multiplication by SY under low N leads to the selection of genotypes with better performance under low-N conditions instead of selecting genotypes that are superior under both N conditions. On the other hand, the division of SY under low N by SY under high N may cause the LNAE index to select genotypes that are not responsive to N. For example, in this study, the IPR Sabiá cultivar was ranked as the third most productive genotype under low N but was not selected by the LNAE index due to the large SY difference under low- and high-N conditions.

In addition to having the highest $h^2$ and $\hat{r}$ values, the PCN index presented the highest concordance and correlation among SY, SY$_{LN}$, and SY$_{HN}$ values. In general, the PCN index leads to the selection of more productive and stable genotypes under contrasting N conditions and does not favour a specific condition (Santos et al., 2019). Another advantage of the PCN index is the possibility of considering more than two N availability scenarios, enabling the selection of genotypes under stress gradients (Mendonça et al., 2017).

### 4.3 Genetic variability and crossing recommendations

AFLP markers have been widely used to detect DNA polymorphisms in common bean genotypes (Beebe et al., 2001; Hanai et al., 2010; Kumar et al., 2008; Maciel et al., 2003; Pallottini et al., 2004). However, the use of SNP markers to measure genetic diversity in beans has been reported more recently (Ariani et al., 2018; Blair et al., 2013; Cortés et al., 2011; Raatz et al., 2019; Wu et al., 2019). The joint use of these markers has the advantage of combining the codominance and multi-loci characteristics of the SNP and AFLP markers, respectively (Nadeem et al., 2018). Moreover, both markers have the advantage of being highly reproducible (Amom & Nongdam, 2017).

According to Pereira et al. (2019), the recommendation of crosses in breeding programmes should take into account genetic dissimilarity and the per se potential of parents, which increases the probability of obtaining transgressive segregants for the traits of interest. In this sense, the following crossings can be recommended: IPR Quero-quero × IAC Sintonia, IPR Quero-quero × BRS Notável, IPR Sabiá × IAC Sintonia, and IPR Sabiá × BRS Notável. The IPR Quero-quero and IPR Sabiá cultivars were allocated to group 2 (yellow) in the molecular analyses and have the highest PCN index values. Thus, these cultivars can be crossed with the IAC Sintonia and BRS Notável cultivars, which both belonged to group 1 (orange). Besides belonging to a genetically distinct group, the IAC Sintonia and BRS Notável cultivars also presented high PCN index values.

### 5 Conclusion

The results obtained in this study indicated the presence of large genetic variability among *carioca* common bean cultivars in terms of N-response indices and SNP and AFLP markers. Furthermore, these results confirm the difficulty of selecting for both low-N tolerant and N-use efficient genotypes, as there is a conceptually inverse relationship between the LNTI and NAE indices. It can be concluded that the PCN index is the most appropriate for genotype selection under contrasting N conditions considering that this index produced the highest $h^2$ and $\hat{r}$ estimates. The cultivars highlighted in this study are recommended for cultivation under N-limiting conditions, as well as to be used as parents in crossbreeding to develop new cultivars less dependent on N fertilizers.

### Authors Contributions

Douglas Mariani Zeffa, Vânia Moda-Cirino, Paul Gepts, Carlos Alberto Scapim, and Leandro Simões Azeredo Gonçalves conceived and designed the experiments. Douglas Mariani Zeffa, Alison Fernando Nogueira, Jéssica Delfini, Isabella Medeiros de Arruda, and José dos Santos Neto were involved in the data collection, statistical analysis and interpretation of results. Douglas Mariani Zeffa wrote the original draft. Vânia Moda-Cirino, Paul Gepts, Carlos Alberto Scapim, and Leandro Simões Azeredo Gonçalves read and edited the manuscript. All authors approved the submitted version.

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CONFLICT OF INTEREST
The authors declare that there is no competing interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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