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Multi-trait selection of common bean lines resistant to *Meloidogyne incognita*

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ABSTRACT

Meloidogyne incognita (root-knot nematode) is one of the most damaging soilborne pathogens affecting the common bean. Control relies primarily on resistant cultivars, making accurate resistance phenotyping a key component of breeding programs. Here, we developed an integrated phenotyping approach to identify resistant genotypes in a recombinant inbred line (RIL) population. For initial screening, 361 RILs were evaluated with three replications for galling index (GI), number of galls (NG), and egg masses (EM) at 60 days after inoculation (DAI). A subset of 24 segregating RILs was further assessed in a second trial for GI, NG, EM, and reproduction factor (RF), with seven replications at 30 and 60 DAI. A multi-trait factor analytic mixed model was used to derive an overall resistance index (ORI) for genotype classification into moderately resistant (MR), intermediate (I) and susceptible (S) classes. We also assessed the potential of a qPCR-based phenotyping protocol using two contrasting RILs from the segregating subset. High heritability (> 0.8) and strong genotypic correlations among resistance components were observed in the RIL segregants, indicating a robust genetic basis for selection. MR genotypes consistently exhibited reduced GI, NG, EM, and RF, and transgressive segregants were identified within the MR group, confirming that the ORI framework effectively distinguished resistance levels. Moreover, later evaluation improved genotype classification and revealed resistance shifts. qPCR-based phenotyping consistently discriminated MR and S lines in agreement with classical phenotyping, supporting its use as a complementary evaluation tool. Overall, our results validate an integrative multi-trait strategy for more precise resistance phenotyping and genotype selection.

Keywords: *Phaseolus vulgaris*, root-knot nematode, resistance phenotyping, multi-trait analysis, factor analytic mixed model

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is a staple food crop of major socioeconomic importance, particularly in developing countries, where it represents an essential source of dietary protein (Myers and Kmiecik 2017). Global production reached 28.5 million tons in 2023, with Brazil, the second largest producer worldwide, harvesting close to 3 million tons (FAO 2022; IBGE 2023). Common bean diversity is structured into two major gene pools, known as Mesoamerican and Andean (Schmutz et al. 2014; Castro-Guerrero et al. 2016; Assefa et al. 2019). In Brazil, the Mesoamerican Carioca type accounts for around 70% of all beans consumed. Currently, the Brazilian breeding programs are focused on improving and releasing new Carioca cultivars (Almeida et al. 2020). Despite its importance, the common bean is strongly affected by biotic stresses, among which phytonematodes are especially damaging (Parker et al. 2022; Mtonga and Maruthi 2024).

Nematodes represent one of the major challenges to agriculture worldwide, causing substantial economic losses estimated at nearly US$157 billion per year (Bernard et al. 2017; Sikandar et al. 2023;

Afzal and Mukhtar 2024). Among them, one of the most damaging species affecting the common bean in Brazil is *Meloidogyne incognita*, a sedentary endoparasite (Mota et al. 2013). According to the official pest risk assessment, *M. incognita* is one of the most frequent and significant phytosanitary threats nationwide (MAPA, 2022). In common bean fields, *Meloidogyne* spp. have been detected in 45% of surveyed root samples (Bonfim Junior et al. 2021). Under favorable temperature range of 25-30 °C, the nematode completes its life cycle within 3-4 weeks (Perry and Moens 2013). Parasitism is established when second stage juveniles (J2) induce the formation of specialized feeding cells in the host, resulting in gall formation. These alterations impair water and nutrient uptake and ultimately reduce yield (Perry and Moens 2013; Mota et al. 2013). Such vulnerability is particularly relevant in the common bean, whose roots are mainly concentrated in the upper soil layers, increasing exposure to plant nematodes that are most abundant in the topsoil (Lynch and Brown 2001; Perry and Moens 2013).

Control of RKN is particularly challenging given their polyphagous nature and broad host range, further aggravated by the low adoption of non-host crop rotations (Abad et al. 2003; Ralmi et al. 2016). Chemical control with nematicides is limited by toxicity and residual effects, driving the demand for sustainable strategies, including the use of cultivars that limit nematode reproduction in the field (Desaeger et al. 2020; Machado et al. 2022; Yaseen et al. 2023). The use of resistant genotypes is one of the most effective strategies for controlling nematodes. In the common bean, several Brazilian genotypes have shown partial or full resistance to *M. incognita* under greenhouse conditions. In one study, only 15 out of 81 genotypes from Brazilian germplasm exhibited partial resistance (Dias et al. 2023). Similarly, 11 resistant genotypes among 20 Mesoamerican accessions, representing diverse commercial classes, including black and *Carioca* types, were reported in another study (Nasiru et al. 2024). A collection of 180 common bean genotypes, representing the genetic diversity of the Instituto Agronômico de Campinas (IAC) germplasm repository, was evaluated for resistance to *M. incognita.* Wide phenotypic variation was found for nematode-related traits, and moderate resistance was also identified (Giordani et al., 2022). Despite these advances, genotypes exhibiting complete resistance are scarce, and their integration into commercial cultivars remains limited.

One factor that impairs breeding for RKN resistance is the phenotyping complexity, since this trait is quantitatively inherited and influenced by diverse host-pathogen-environment interactions (Castagnone-Sereno 2002; Fuller et al. 2008; Yüksel and Bozbuğa 2025). Effective selection strategies should combine phenotypic traits that capture distinct stages of host-nematode interaction, enabling more accurate identification of resistant genotypes. In addition, quantitative PCR has emerged as a complementary phenotyping approach, as it allows the quantification of pathogen DNA within plant tissues (Hodson et al., 2023; Ruiz et al., 2023). Although still exploratory in breeding programs, qPCR-based phenotyping may support future selection of contrasting host responses.

Given these challenges, we propose an integrative phenotypic approach for identifying superior segregants with enhanced nematode resistance within a recombinant inbred line (RIL) population. Early infection traits, including galling index and number of galls, were integrated with reproductive parameters (egg masses and reproduction factor) into a single phenotyping pipeline. The integration of multiple phenotypic traits into a unified resistance index was hypothesized to improve the identification and selection of resistant genotypes. In addition, we explored the potential of qPCR as a complementary

phenotyping tool in two contrasting genotypes. In this scenario, our objectives were to: (i) characterize the response of a common bean RIL population to *M. incognita*; (ii) identify segregating genotypes within the RIL population and classify their resistance response using integrated phenotypic traits evaluated at 30 and 60 days after inoculation (DAI); and (iii) quantify the nematode DNA by qPCR to distinguish a resistant from a susceptible RIL and support selection in breeding programs.

MATERIAL AND METHODS

Plant material

A recombinant inbred line (RIL) population consisting of 361 lines was developed from a cross between a moderately resistant genotype (MR, 'IAC-Tybatã') and a susceptible one (S, 'Branquinho'). These two Mesoamerican Carioca-type common bean accessions from the Instituto Agronômico de Campinas (IAC, Brazil) core collection were previously characterized for their contrasting response to *Meloidogyne incognita* race 3 (Giordani et al., 2022). The RILs, along with the parental lines, and two black bean checks, the moderately resistant 'Puebla-152-CIAT' and the susceptible 'Jamapa-CNF-1671' were evaluated. The trial followed a randomized complete block design with three replications.

Plant phenotyping for the response to *Meloidogyne incognita*

Phenotyping of the RIL population was carried out in a controlled-environment chamber (26 °C, 69% relative humidity, and a 16/8 h light-dark cycle) from March to June 2024. Seeds were germinated in 400 mL plastic containers filled with an autoclaved 2:1 sand:soil mixture, irrigated with distilled water, and supplemented weekly with Hoagland's nutrient solution (Hoagland and Arnon 1950).The *M. incognita* race 3 inoculum was originally collected from cotton (*Gossypium spp.*) fields in Mato Grosso state, Brazil (15°32'48" S 55°10'08" W) (Giordani et al. 2022b), maintained on susceptible wild passion fruit (*Passiflora nitida* Kunth.), and subsequently multiplied on the 'Santa Clara' tomato (*Solanum lycopersicum* L.). Egg extraction from infected roots was performed 60 days after inoculation (DAI), following Hussey and Barker (1973). Roots were washed free of soil particles, cut into approximately 1.0 cm pieces, immersed in 0.5% NaOCl solution, and shaken for 4 minutes. The suspension was passed through 90 and 25 µm sieves and rinsed to remove remaining NaOCl, after which eggs were collected. Egg solution concentration was standardized by microscope counts using a Peter's slide. Seedlings were inoculated 10 days after germination with an initial population of 2,000 eggs per plant. Plants were evaluated 60 DAI for three traits: galling index (GI, 0 to 10 scale) (Bridge and Page 1980), total number of galls (NG), and total number of egg masses (EM) (Taylor and Sasser 1978). After assessing GI and NG, roots were stained with acid fuchsin to facilitate EM visualization. Roots were submerged for 10 min in a dye solution (75 mL distilled water, 25 mL glacial acetic acid, and 0.35 g acid fuchsin), rinsed in tap water, and the EM were enumerated (Byrd et al. 1983).

Selection and evaluation of RIL segregants

Based on the initial population screening, 24 segregating lines (12 moderately resistant and 12 susceptible) were selected to assess the consistency of their phenotypic performance, including the parental lines and

the black bean checks. This trial was conducted from December 2024 to February 2025 and followed a randomized complete block design with seven replicates. Non-inoculated controls of the parental lines were also included to validate experimental conditions. Inoculum preparation, growth conditions, and phenotyping procedures were as described above. The same phenotypic traits (GI, NG, and EM) were recorded, with evaluations performed at 30 and 60 DAI, totaling 392 experimental units. The early assessment (30 DAI) was conducted based on findings by Orsi et al. (2025), which demonstrated that this time point corresponds to the completion of the first nematode life cycle, confirmed by the presence of adult females and egg masses. The later evaluation (60 DAI) was included to assess phenotypic responses at a more advanced infection stage, corresponding to approximately two nematode life cycles.

Additionally, the final nematode population (PF) was assessed to estimate the reproduction factor (RF), calculated as RF= PF/PI, where the initial population consisted of 2,000 eggs per plant (Taylor and Sasser 1978). After staining and quantification of EM, roots were immersed in a 0.5% NaOCl solution for 4 minutes, and the suspension was poured through 200 and 500 mesh sieves (Hussey and Barker 1973). Eggs and J2 juveniles retained on the sieves were collected in a beaker. Samples were fixed in 40% formaldehyde solution for subsequent counting using a Peter's slide. Two subsamples (0.5 mL each) were counted to determine the number of nematodes per mL, which was multiplied by the total sample volume to obtain the final population for each genotype.

Data analysis

All linear mixed models described below were solved using restricted maximum likelihood (REML, Patterson and Thompson 1971) with the algorithm implemented in the ASReml-R package, v. 4.2 (The VSNi Team 2023). The analytical process was divided into two stages. In the first stage, data from both trials were utilized to estimate broad-sense heritability and obtain the best linear unbiased predictions (BLUPs; Henderson 1975) for each line. Based on these estimates, a subset of RILs was selected for the second trial. This second stage focused on characterizing the correlation between phenotypic traits at different time points (DAI) to enable a more informed selection of superior RILs. To obtain estimates for each trait, a linear mixed model was fitted using data from both trials as follows:

$$y_{l(j)i} = \mu + e_l + b_{l(j)} + g_i + \varepsilon_{l(j)i} \quad (1)$$

where $y_{l(j)i}$ is the phenotypic observation of line $i \in \{1, \dots, m\}$ in block $j \in \{1, \dots, q\}$ within trial $l \in \{1,2\}$, μ is the intercept, e_l is the fixed effect of the trial l, $b_{l(j)}$ is the random effect of block j within trial l $[b \sim N(0, \sigma_b^2)]$, g_i is the random effect of line i $\left[g \sim N\left(0, \sigma_g^2\right)\right]$, and $\varepsilon_{l(j)i}$ is the residual $[\varepsilon \sim N(0, \sigma_\varepsilon^2)]$. σ_b^2, σ_g^2 and σ_ε^2 are the block and genotypic variances. The significance of the genetic variance component ($\sigma_g^2 > 0$) was tested using a likelihood ratio test at a 5% significance level ($\alpha = 0.05$).

The broad-sense heritability was computed as follows:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{\bar{q}}} \quad (2)$$

To account for differences in the number of replicates resulting from missing data, the harmonic mean ($\breve{q}$) of the replications per line was utilized. To conclude the first stage of the analysis, the reliabilities of the BLUPs were calculated using the following expression:

$$r_i^2 = 1 - \frac{PEV_i}{\sigma_g^2} \tag{3}$$

where PEV_i is the prediction error variance of line i.

Regarding the selected RIL dataset, single-DAI, single-trait models were initially fitted. These models followed the structure of Eq. 1, excluding the trial effect and treating the replicate effect as fixed. For these analyses, the significance of the genotypic variance component was assessed, and broad-sense heritability, BLUPs, and their respective reliabilities were estimated according to Eqs. 2 and 3. Thereafter, for each DAI, the following second-order factor analytic (Piepho 1997; Bančič et al. 2023) multi-trait model was fitted:

$$\ddot{\boldsymbol{y}} = \boldsymbol{Xb} + \boldsymbol{Z}(\boldsymbol{\Lambda} \otimes \boldsymbol{I}_m)\boldsymbol{f} + \boldsymbol{Z\delta} + \boldsymbol{\varepsilon} \tag{4}$$

where $\ddot{\boldsymbol{y}}$ is a $np \times 1$ vector of stacked scaled phenotypic observations, with n and p being the number of plots and traits, respectively; $\boldsymbol{b}$ a $(p + q + 1) \times 1$ vector of fixed effects (intercept, traits and replicates), $\boldsymbol{\Lambda}$ a $p \times 2$ matrix of loadings, $\boldsymbol{f}$ a $2m \times 1$ vector of scores, $\boldsymbol{\delta}$ a $mp \times 1$ vector of specific effects, and $\boldsymbol{\varepsilon}$ the residual. These effects are distributed as $(\boldsymbol{\Lambda} \otimes \boldsymbol{I}_m)\boldsymbol{f} \sim N(\boldsymbol{0}, \boldsymbol{\Lambda D \Lambda'} \otimes \boldsymbol{I}_m)$, $\boldsymbol{\delta} \sim N(\boldsymbol{0}, \boldsymbol{\Psi} \otimes \boldsymbol{I}_m)$, and $\boldsymbol{\varepsilon} \sim N(\boldsymbol{0}, \boldsymbol{I}_n \otimes \boldsymbol{\Sigma}_\varepsilon)$, where $\mathbf{D}$ is a 2×2 matrix of scores variance, $\boldsymbol{\Psi}$ a diagonal matrix of order m with specific variances, $\boldsymbol{\Sigma}_\varepsilon$ a $p \times p$ unstructured residual variance-covariance matrix, $\boldsymbol{I}$ identity matrices whose order are indicated by their subscripts, and $\otimes$ the Kronecker product. The capital letters represent incidence matrices. A final detail is that, due to constraints on the estimation process, $\boldsymbol{\Lambda}$ was rotated using singular value decomposition. This guarantees the interpretability of factor loadings, which are important for the selection process described later. For reference on rotation, see Smith et al. (2021).

Two factors were enough to explain almost 100% of the underlying variance in the dataset. The percentage of explained variance per trait and factor were calculated as follows (Smith et al. 2015):

$$v_{tk} = \frac{\lambda_{tk} d_k}{\sum_{k=1}^{2} \lambda_{tk} d_k + \psi_t} \times 100 \tag{5}$$

where λ_{tk} is the k-th factor loading of trait t, d_k is the k-th diagonal element of $\boldsymbol{D}$, and ψ_t is the t-th diagonal element of $\boldsymbol{\Psi}$.

To obtain REML-estimates of genotypic correlation, the factor loadings were used, as follows (Cullis et al. 2010):

$$\rho_{g_{tt'}} = \frac{\sum_{k=1}^{2} \lambda_{kt}^2 \lambda_{kt'}^2}{\sqrt{\sigma_{g_t}^2 \, \sigma_{g_{t'}}^2}} \tag{6}$$

The residual correlations were also computed. In this case, the residual variances and covariances were used:

$$\rho_{\varepsilon_{tt'}} = \frac{\sigma_{\varepsilon_{tt'}}}{\sqrt{\sigma_{\varepsilon_t}^2 \, \sigma_{\varepsilon_{t'}}^2}} \tag{7}$$

where $\sigma_{\varepsilon_{tt'}}$ is the residual covariance between traits t and t'.

The first factor explained a substantial portion of the total variance. Consequently, this latent variable was utilized as the basis for selection. First, a factorial regression was employed to provide an overview of the behavior of the lines across all traits. In this setting, the scores and loadings of the first factor represented the slopes and the predictor, respectively, while the second factor represented the lack-of-fit (Cullis et al. 2014). Subsequently, following Smith and Cullis (2018), the information provided by the first factor was incorporated into an index, designated the overall resistance index (ORI), a parallel to the overall performance (OP) index proposed by those authors. This index was constructed as follows:

$$ORI_i = \frac{1}{p}\sum_{t=1}^{p} \lambda_{1t} f_{1i}$$

Based on the ORI thresholds were defined, with lines classified as moderately resistant, intermediate and susceptible to root-knot nematode, as follows:

$$\text{Classes} = \begin{cases} ORI_i < ORI_i - \tau \times sd(\boldsymbol{ORI}) \rightarrow \text{Moderately resistant} \\ ORI_i - \tau \times sd(\boldsymbol{ORI}) < ORI_i < ORI_i + \tau \times sd(\boldsymbol{ORI}) \rightarrow \text{Intermediate} \\ ORI_i > ORI_i + \tau \times sd(\boldsymbol{ORI}) \rightarrow \text{Susceptible} \end{cases}$$

where $sd()$ denotes the function used to calculate the standard deviation of the **ORI** vector, and τ represents a weighting factor, defined as **0.8** in this study. Notably, τ is a tunable parameter, allowing future researchers to adjust the classification threshold based on specific experimental requirements or breeding objectives. All plots were built using the ggplot2 package (Wickham 2016), with add-ins from the packages ggpubr (Kassambara 2023), ggnewscale (Campitelli 2025) and ggalluvial (Brunson 2020).

Quantifying *M. incognita* in contrasting RILs at early stage of interaction using qPCR

Two RILs were randomly selected from each contrasting bulk: T_B_266 (MR) and T_B_294 (S). Two independent experiments (EXP1 and EXP2) were conducted in a growth chamber under the above conditions. The egg suspension concentration was calibrated, and plants inoculated with 2,000 eggs of *M. incognita* ten days after germination. Four biological replicates per genotype were inoculated, and four additional non-inoculated plants included as controls, totaling 16 experimental units per experiment. Nematode quantification was performed at 30 DAI.

Total genomic DNA was extracted from root tissues (5 g), ground to a fine powder in liquid nitrogen and transferred to 2.0 mL microcentrifuge tubes using a modified CTAB method (Oliveira et al. 2020). The procedure included an additional purification step using ammonium acetate (7.5 M) and absolute ethanol to improve DNA quality. DNA concentration was determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and samples were standardized to 500 μL^{-1}.

A preliminary PCR assay was carried out using the species-specific primers RKNf (5'-GCT GGT GTC TAA GTG TTG CTG ATA C-3') and RKNr (5'-GAG CCT AGT GAT CCA CGA TAA G-3') (Toyota et al. 2008) to confirm amplification of the expected 185 bp fragment from the *M. incognita* ITS region. PCR products were verified by agarose gel electrophoresis. Quantitative PCR (qPCR) reactions were performed in 48-well plates using 20 µL reaction mixtures containing 10 µL of SYBR Green Master Mix, 1 µL of each RKN-f and RKN-r primer (10 µM), 6 µL of nuclease-free water, and 2 µL of DNA (1:10 dilution, 50 ng μL^{-1}). Samples were analyzed in duplicate, including non-template controls (NTC).

Amplifications were run in a StepOne™ Real-Time PCR System (Applied Biosystems) with the following cycling parameters: 95 °C for 10 s; 45 cycles of 95 °C for 5 s and 60 °C for 30 s, following the protocol described by Oliveira et al. (2020). qPCR efficiency was determined using the LinReg PCR Software (Ruijter et al. 2009). Threshold cycle (Ct) values were corrected for efficiency and converted to quantification cycle (Cq) values for the analysis of amplification curves.

RESULTS

Evaluations of the RILs

The BLUP distributions revealed broad variation among RILs for all traits associated with nematode resistance (EM, GI, and NG) (Fig. 1). BLUP estimates varied from 6.14 to 58.55 for EM, 1.13 to 3.72 for GI, and 12.39 to 122.14 for NG, indicating wide segregation in the population. The susceptible parent showed BLUP values of 54.7 for EM, 3.3 for GI, and 106.8 for NG, while the moderately resistant showed consistently lower estimates (EM = 8.2, GI = 1.3, NG = 15.3), reinforcing their contrasting phenotypic responses across traits. The susceptible control 'Jamapa' exhibited the expected galling symptoms and number of egg masses (EM = 51.3, GI = 2.6, NG = 63.1), while the moderately resistant 'Puebla' showed less pronounced symptoms (EM = 6.4, GI = 1.3, NG = 26.5).

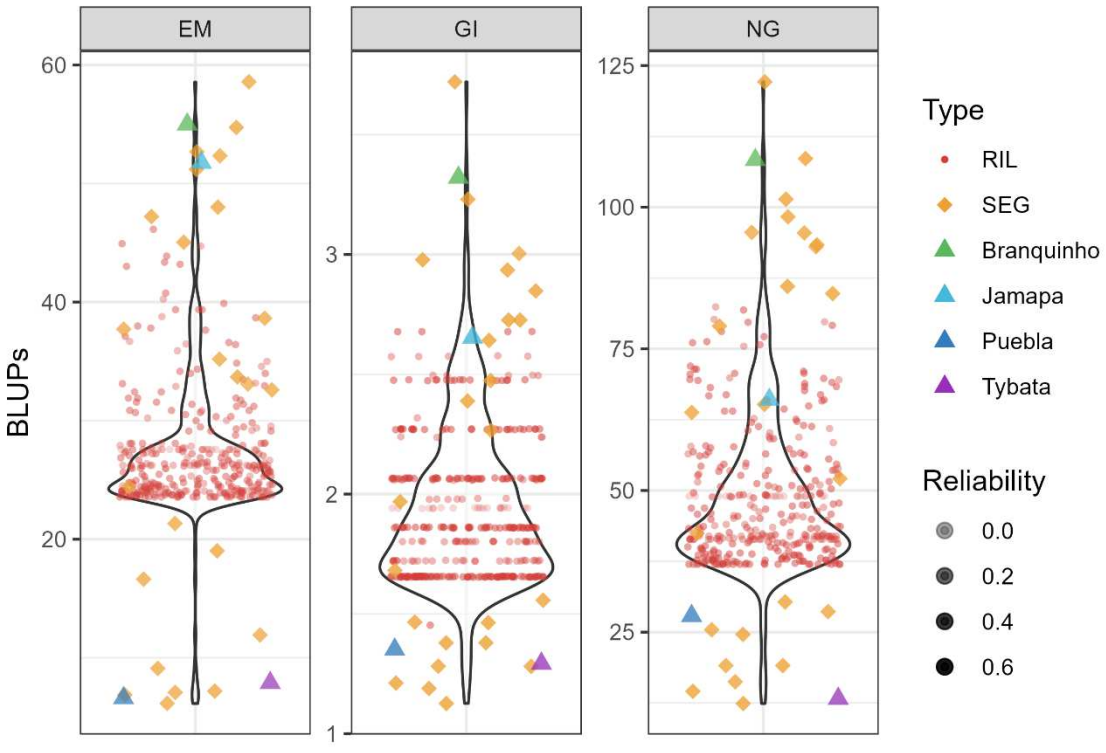

Figure 1. Distribution of BLUP estimates for egg masses (EM), galling index (GI), and number of galls (NG) in the recombinant inbred line (RIL) population at 60 days after inoculation, including the selected segregating genotypes (SEG). Parental lines 'Branquinho' (susceptible, S) and 'Tybatã' (moderately resistant, MR) and black bean controls 'Jamapa' (S) and 'Puebla'(MR) are indicated by colored triangles. Individual points represent RIL genotypes and color intensity reflects the reliability of BLUP estimates.

Selected segregants were predominantly located at the upper and lower tails of the distributions, supporting their use as contrasting genotypes for subsequent analyses. Some segregating genotypes exceeded parental performance, especially for EM and GI traits, suggesting transgressive variation within the population (Fig. 1). Together, these results indicate that EM, GI, and NG are informative for discriminating genotypes for nematode resistance in this common bean genetic background.

Evaluation of the selected RILs

For segregating genotypes, estimates of coefficient of variation (CV) ranged from 0.35 to 0.80 across traits and showed a slight reduction over time for RF and NG traits (Fig. 2), indicating lower experimental variability at 60 days after inoculation (DAI). Broad-sense heritability remained moderate to high, ranging from 0.80 to 0.90 for both evaluation times, with higher estimates observed for RF, GI, and NG at 60 DAI.

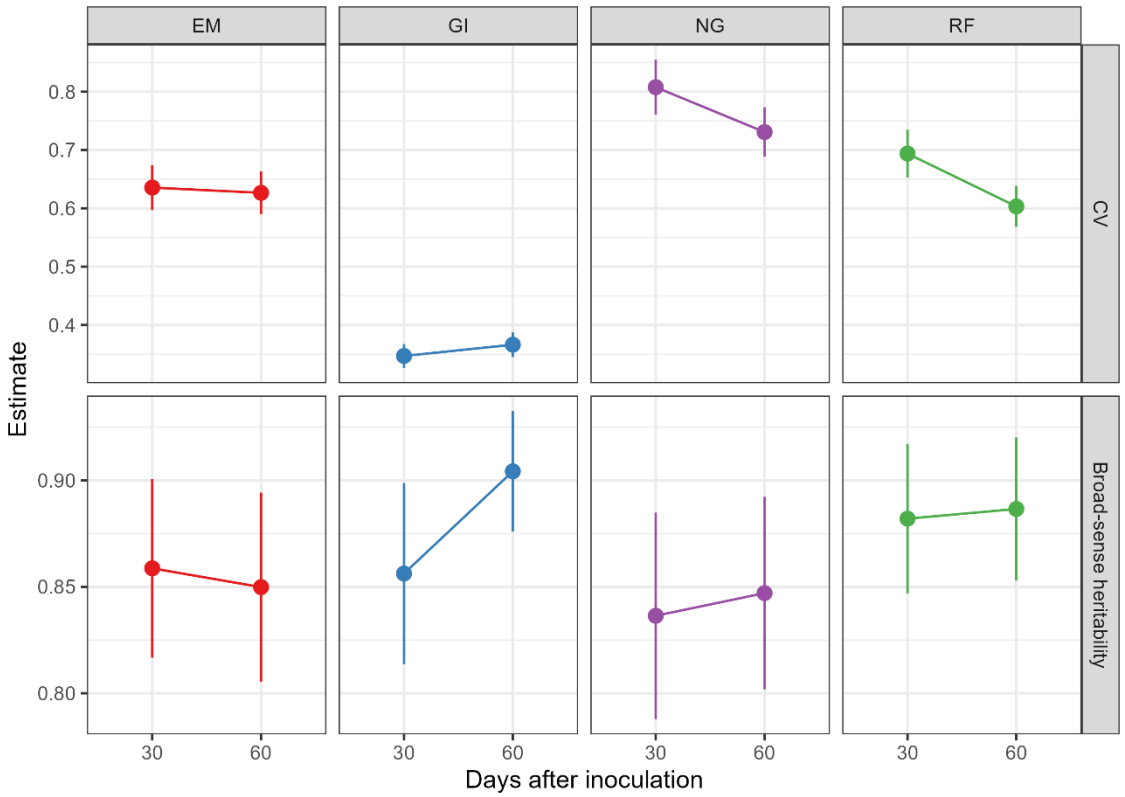

Figure 2. Estimates of coefficient of variation (CV) and broad-sense heritability for egg masses (EM), galling index (GI), number of galls (NG), and reproduction factor (RF) across segregating genotypes of the recombinant inbred line (RIL) population at 30 and 60 days after inoculation. The vertical lines represent standard errors estimated using the Delta method.

Strong genotypic associations among traits were observed at both evaluation times (Fig.S1). Genotypic correlations remained high between RF and EM (0.994 at 30 DAI and 0.986 at 60 DAI), indicating a stable genetic relationship between nematode reproduction and egg mass production. Genotypic correlations involving GI-RF and NG-RF were slightly reduced at 60 DAI, showing partial separation between galling severity and reproductive performance after two nematode life cycles. Residual correlations were consistently lower than genotypic correlations and increased for specific trait

combinations, particularly NG-EM at 60 DAI, reflecting greater non-genetic variability affecting both traits at later stages of infection. Overall, the strong genotypic correlations combined with lower residual correlations highlight the predominance of genetic over non-genetic variations among traits, as previously suggested by the broad-sense heritability values (Fig. S1).

The relationship between genotype scores and the first factor loadings revealed a consistent resistance gradient across segregating lines. Parental references and black bean checks aligned with their expected phenotypic responses (Fig. S2). Integration of traits into the overall resistance index (ORI) enabled genotype classification at both evaluation times (Fig. 3). The classification of genotypes according to the Temporal shifts in ORI rankings were observed between 30 and 60 DAI. Some lines initially categorized as intermediate at 30 DAI exhibited increased susceptibility by 60 DAI, while others demonstrated stable or improved resistance profiles (Fig. S3). Most class transitions occurred among intermediate genotypes. A detailed representation of class transitions for each segregating genotype between evaluation times is provided in Supplementary Figure S4.

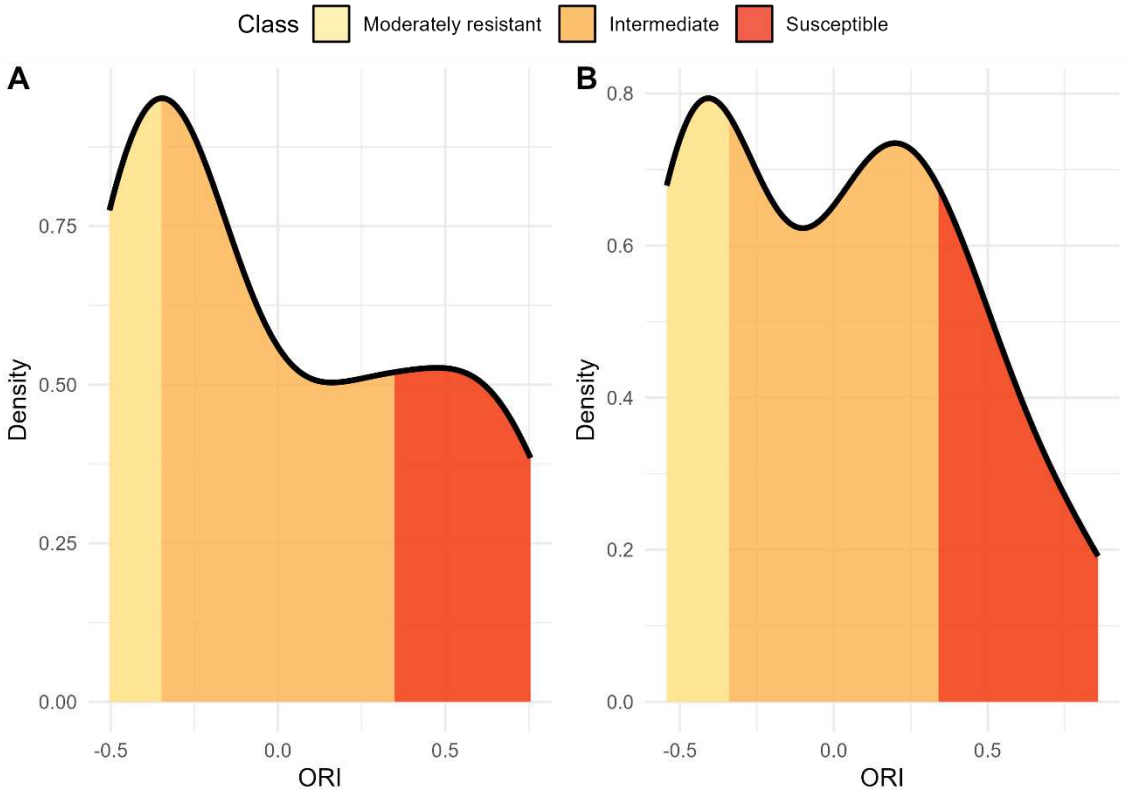

Figure 3. Distribution of the overall resistance index (ORI), derived from multi-trait factor analytic linear mixed models, showing classification into intermediate, moderately resistant and susceptible classes at 30 (A) and 60 (B) days after inoculation. See Material and Methods for the adopted thresholds for classification

The ORI is built upon first factor loadings and scores. This latent variable explained a major portion of total variation, 98.24% in 30 DAI and 94.26% in 60 DAI (Fig. 4). Trait loadings showed a clear separation between galling-related traits (GI and NG) and reproduction traits (RF and EM), with EM behaving as an intermediate trait between these components. RF showed a high loading along Factor 1,

indicating a strong contribution. Genotypes with similar resistance classification clustered together, whereas intermediate genotypes were distributed between these extremes.

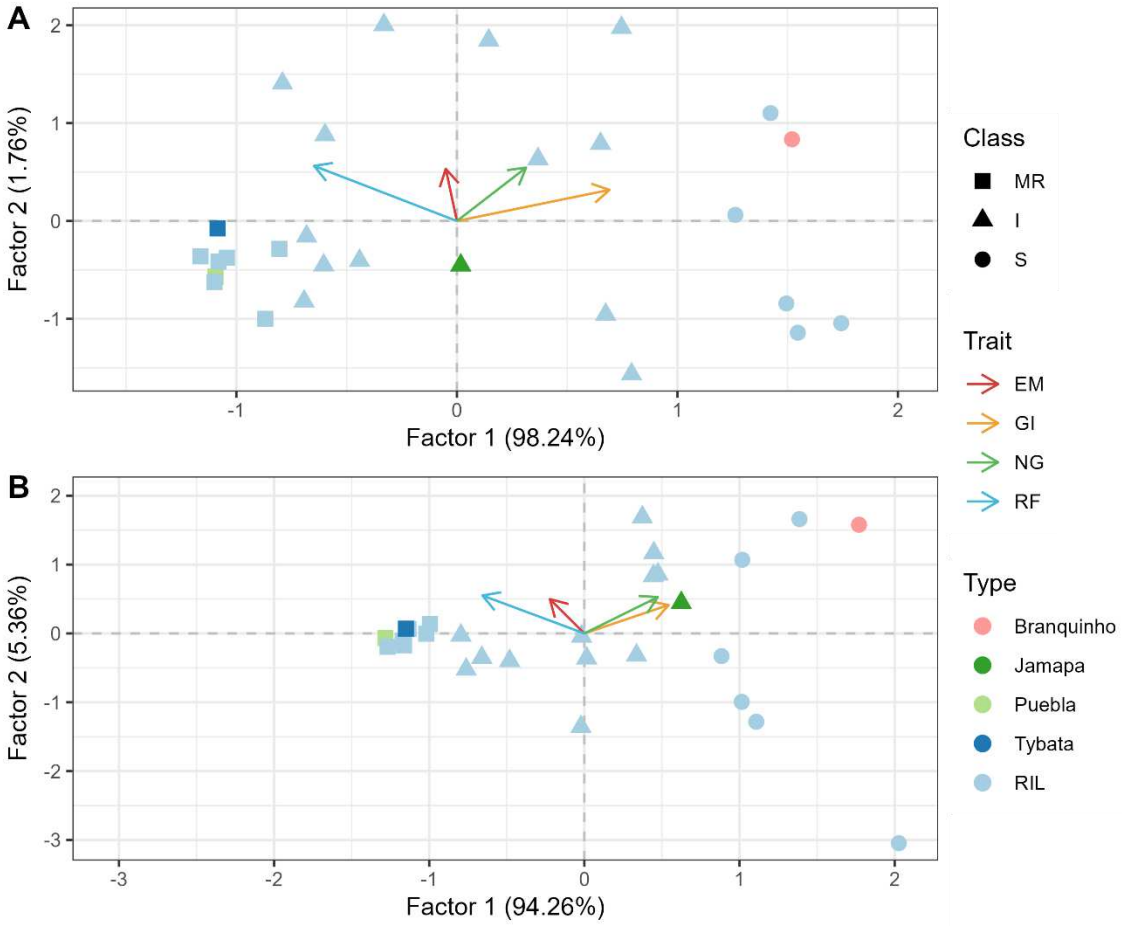

Figure 4. Factorial biplot showing the distribution of segregating genotypes at 30 (A) and 60 (B) days after inoculation. Arrows represent trait loadings for egg masses (EM), galling index (GI), number of galls (NG), and reproduction factor (RF), while symbols indicate segregating genotypes of the recombinant inbred line (RIL) population classified as moderately resistant (MR), intermediate (I), or susceptible (S). The direction of arrows reflects the association between traits and factor components. The arrow length indicates the contribution of each trait.

The BLUP means across resistance classes demonstrated a clear genotypic distribution, validating the effectiveness of the integrated ORI classification (Fig. 5). Across evaluation times, MR genotypes consistently showed lower values for EM, RF, GI, and NG compared to intermediate and susceptible classes (Fig. 5A). Within the MR group, individual segregating genotypes exhibited variable responses across traits, including transgressive individuals relative to the parental references (Fig. 5B). These patterns indicate that the integrated framework captured both overall class differentiation and within-class variation and enabled identification of superior recombinants.

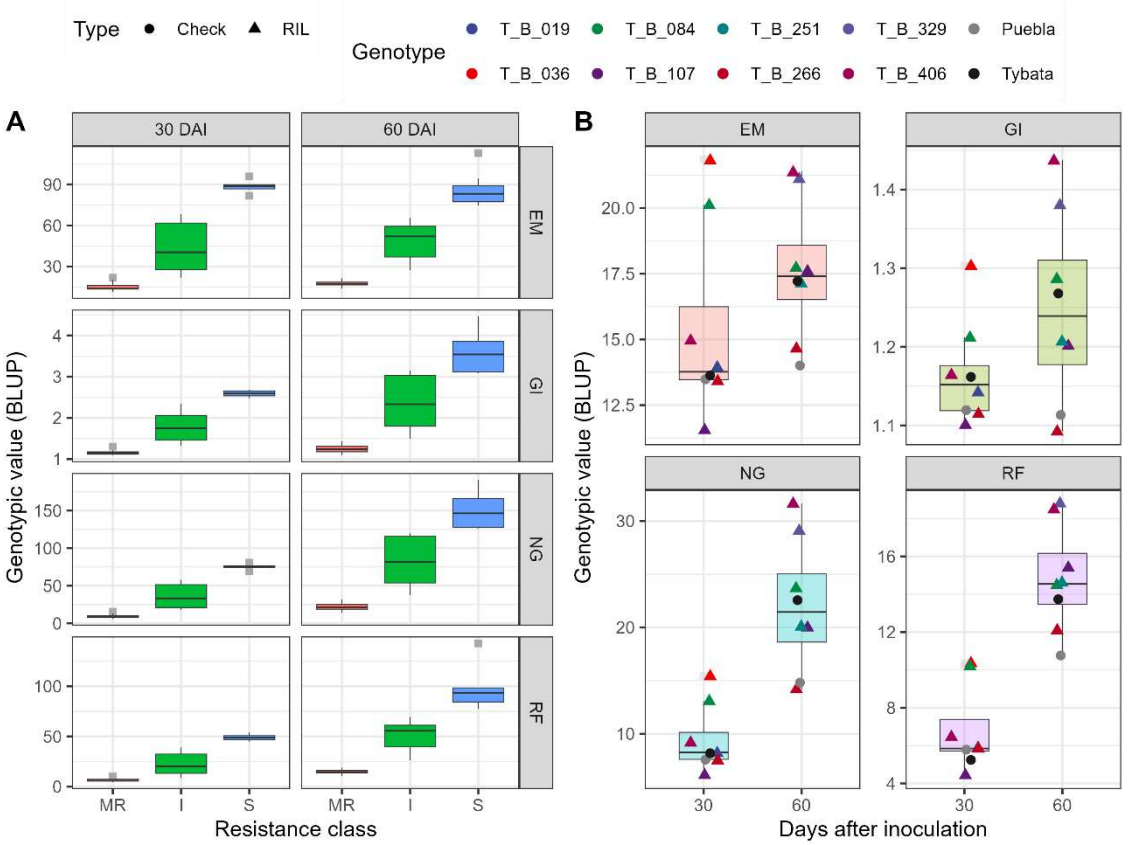

Figure 5. Distribution of BLUP means for segregating genotypes of the recombinant inbred line (RIL) population across moderately resistant (MR), intermediate (I), and susceptible (S) resistance classes (A) and within the MR class (B) at 30 and 60 days after inoculation for egg masses (EM), reproduction factor (RF), galling index (GI), and number of galls (NG). Point shapes distinguish recombinant inbred lines (RILs, triangles) and checks (parents and checks *per se*, circles). In B, the colors distinguish MR RILs, including transgressive segregants relative to the parental MR line 'Tybatã', with the MR control 'Puebla' also included as reference.

Quantification of *M. incognita* in contrasting genotypes using qPCR

Across both experiments, the susceptible genotype consistently showed lower Cq values than the MR genotype, indicating higher *M. incognita* DNA accumulation in susceptible roots at 30 DAI (Supplementary Table 1; Fig. 6A). Mean Cq values were 26.78 (MR) and 23.81 (S) in EXP 1, and 26.32 (MR) and 23.04 (S) in EXP2. These differences corresponded to a 5.77 (EXP1) and 6.76 times (EXP2) higher qPCR signal in the S genotype relative to the MR. This contrast was consistent with final nematode population estimates (Supplementary Table 1; Fig. 6B). The qPCR at 30 DAI reliably discriminates contrasting resistance genotypes using a small amount of root tissue, closely mirroring classical phenotyping based on final population.

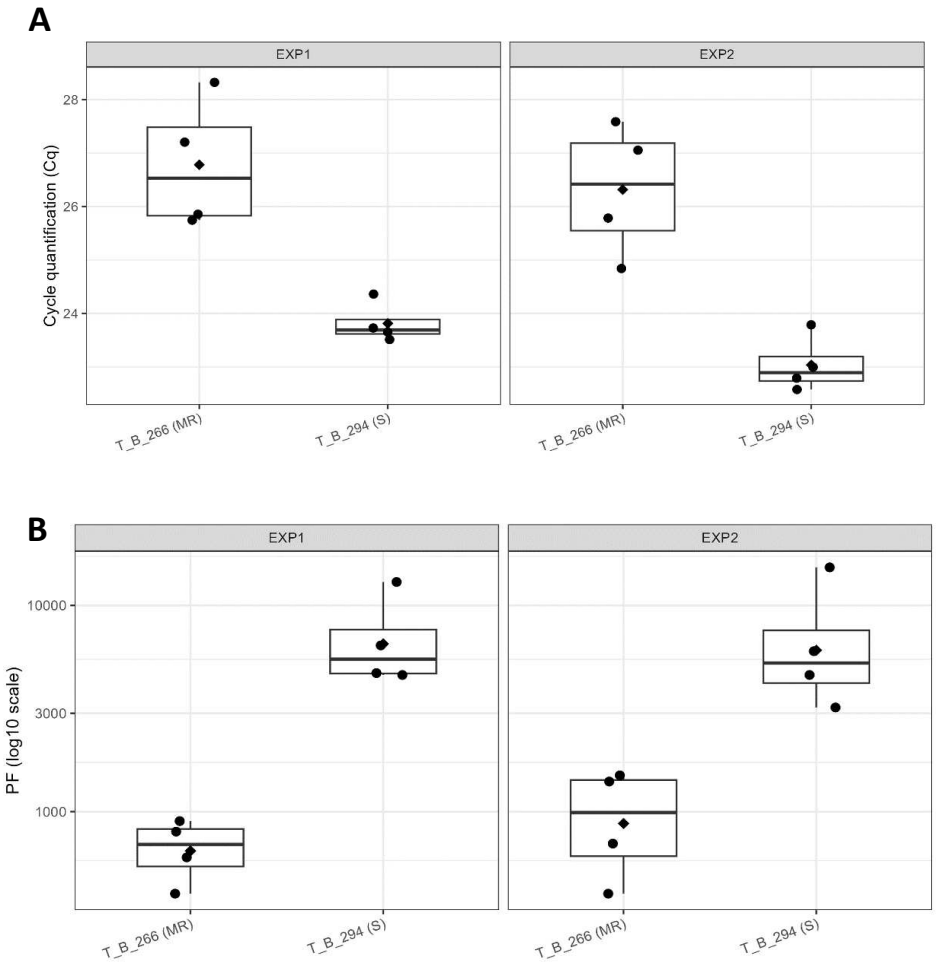

Figure 6. qPCR quantification and phenotyping in contrasting recombinant inbred lines at 30 days after inoculation (DAI). **A.** DNA quantification of *M. incognita* by qPCR (Cq values) in moderately resistant (MR) and susceptible (S) genotypes, evaluated in two independent experiments (EXP1 and EXP2). **B.** Final nematode population (PF) quantified from the remaining root tissues of the same plants (n = 4). Values are shown on a log10 y-axis for visualization. Black circles represent individual biological replicates, and diamonds indicate the mean.

DISCUSSION

Quantitative resistance to *M. incognita* involves distinct but genetically correlated mechanisms

In the present study, we evaluated the nematode resistance in a set of RILs of the common bean using a soil-based phenotyping workflow. Previously, our group assessed resistance using a soil-free seedling approach on pouches, J2 inoculation, and evaluation at 30 DAI (Giordani et al. 2022; Floriani et al. 2025). Here, we adopted a refined phenotyping designed to better approximate field conditions e.g. a soil-based assay, egg inoculation to enable large-scale screening, extending evaluation to 60 DAI (Costa et al. 2019; Nasiru et al. 2024). Importantly, the broad phenotypic variation in the RILs was consistent with patterns reported in our previous studies, including a diversity panel (Giordani et al. 2022) and an F_2 biparental population derived from the same cross (Floriani et al. 2025). Studies involving RIL-based phenotyping and multi-trait evaluation in common bean-*M. incognita* interaction are still limited.

In our dataset, parental lines contrasting for EM, GI and NG indicate that resistance affects both infection-related traits and nematode multiplication, as measured by egg mass formation. At population level, BLUP distributions for EM, GI, and NG showed a predominance of RILs clustered within the lower to intermediate portion of genotypic values distribution (Fig. 1), while segregating lines extended toward the upper extremes of the distributions.

To further refine the analysis, a subset of segregants was reassessed with additional biological replicates at 30 and 60 DAI using EM, GI, NG, and reproduction factor (RF). Reproduction-based traits are widely used for screening resistance, as nematologists traditionally distinguish host response to infection from the capacity of the plant to support nematode reproduction (Oostenbrink 1966; Taylor and Sasser 1978; Starr et al. 2002). Importantly, this proposed approach enabled the integrated evaluation of multiple components underlying the resistance response.

According to Corwin and Kliebenstein (2017) quantitative resistance generally involves a continuous distribution ranging from susceptible to resistant phenotypes. In our study, both RILs phenotypes and BLUP-derived genotypic estimates exhibited a continuous spectrum of responses, consistent with this resistance pattern. The complex genetic architecture of resistance to *M. incognita* in the common bean has previously been reported (Giordani et al. 2022), with evidence of control by quantitative trait loci (QTL) with moderate to large effects (Floriani et al. 2025). Notably, the application of an integrated overall resistance index (ORI) enabled the statistical stratification of this continuous variation into moderately resistant (MR), intermediate (I), and susceptible (S) classes. Such an integrative pipeline for resistance evaluation is particularly suitable for traits controlled by multiple genetic components, as selection based on complementary parameters enhances genotype discrimination. The ORI was derived using the factor-analytic selection framework proposed by Smith and Cullis (2018). This approach assumes that the first factor explains a substantial proportion of the total genotypic variance and captures most of the variance shared among traits. As shown in Figure 4, this assumption was satisfied in the present study.

Genotypic correlations among EM, GI, NG, and RF were consistently high across evaluation times ($\geq$ 0.80) and exceeded residual correlations, indicating a predominance of genetic over non-genetic variation and reinforcing the multicomponent structure of resistance. Despite these strong associations, factor analysis clearly separated galling-related traits (GI and NG) from nematode multiplication traits (EM and RF) at early and later stages of interaction. Although galling and reproduction were positively correlated overall, this structural separation suggests that partially distinct resistance components may regulate these responses, consistent with a quantitative resistance. Remarkably, a slight reduction in the genotypic correlations between NG-RF and GI-RF, from 30 to 60 DAI, further supports this differentiation. Similar patterns have been observed in the common bean and Lima bean, where independent genetic control of root galling and *M. incognita* reproduction was identified. In these cases, nematode reproduction occurred independently of severe galling (or was dissociated from), suggesting distinct resistance mechanisms. (Roberts et al. 2008; Pesqueira et al. 2025). Although root galling and nematode reproduction are both used to measure resistance in the common bean, they represent distinct host responses (Mullin et al. 1991; Fuller et al. 2008).

Within this multi-trait framework, RF emerged as a major determinant of resistance in our study, and the correlation between RF and EM was among the strongest at both evaluation times. This is consistent

with soybean greenhouse screenings against *M. incognita*, where RF is frequently used as a primary criterion for resistance classification, since nematode reproduction differs markedly among genotypes (Teixeira et al. 2017; Rashidifard et al. 2025). This strong association is biologically supported by the reproductive strategy of *Meloidogyne* spp., in which each gelatinous egg mass typically harbors hundreds to over a thousand eggs (Handoo et al. 2025), indicating that population growth is largely driven by egg production per mass. Accordingly, a classical study evaluating common bean genotypes under *M. incognita* infection at 30 DAI reported stronger phenotypic correlations between RF and EM (r = 0.85) than between EM and GI (r=0.45), reinforcing the biological relevance of reproduction-based traits in resistance evaluation (Omwega 1988).

Integrated selection reveals transgressive segregants

ORI-based classification enabled the identification of promising lines, while BLUP estimates confirmed the presence of transgressive segregants within the MR class. Eight lines showed improved resistance profiles under the integrated framework, and two to three genotypes surpassed the moderately resistant parent in at least three of the four evaluated traits, including both galling and reproduction parameters. This pattern supports the recombination of distinct resistance components, consistent with the multicomponent structure of our dataset.

Transgressive segregation is commonly associated with quantitative traits, where favorable alleles dispersed between parental lines recombine to generate progeny exceeding the parental phenotype, providing strong material for selection (Rieseberg et al. 1999; Mackay et al. 2021). Similar mechanisms have been reported in QTL mapping studies of cotton resistance to *M. incognita*, where individual loci showed limited effects on galling index and egg production, but combinations of favorable alleles across multiple loci resulted in transgressive resistance (Wang et al. 2012).

These findings demonstrate the importance of integrated resistance phenotyping for uncovering superior genotypes that may remain undetected under single-trait evaluation.

Later evaluation improves discrimination of resistance classes

The reduction in experimental variation for NG and RF, together with consistently high broad-sense heritability (0.80-0.90) for GI, NG, and RF at 60 DAI, provided more favorable conditions for genotype discrimination, supporting later evaluation as a more informative time point for resistance phenotyping. This improvement is expected for root-knot nematode assays, where reproductive traits often exhibit substantial experimental variability, thereby impacting the estimation of genotypic parameters (Ndeve et al. 2019).

Previous studies from our research group have reported moderate to high heritability for nematode resistance traits in common bean, with estimates of 0.49 and 0.553 for EM and GI respectively (Giordani et al. 2022), and 0.78 and 0.82 for EM and GI (Floriani et al. 2025). Interestingly, the high variability typical of nematode assays can be mitigated by increasing the number of biological replicates. This is evidenced by the higher broad-sense heritability estimates for all traits in our study, which increased from ~ 0.3-0.4 with three replicates in the RIL population to ~0.8-0.9 with seven replicates in the segregating genotypes

(Figure S5). The present study is the first to include NG and RF as phenotyping traits, and the high heritability estimates obtained reinforce the reliability of the multi-trait selection.

Shifts in genotype classification between 30 and 60 DAI further highlighted the role of evaluation time. While early assessments are useful for eliminating highly susceptible lines, later evaluations better capture differences in nematode multiplication (Costa et al. 2019; Nasiru et al. 2024) and reduce misclassification among intermediate genotypes. In our dataset, three lines classified as intermediate showed increased nematode multiplication at 60 DAI and were reclassified as susceptible. Conversely, some genotypes maintained stable performance across evaluation times, suggesting delayed responses that restrict nematode multiplication, as illustrated in Figures S3 and S4.

The FA biplot provides visual support for these patterns, presenting a more compact clustering of genotypes and clearer separation of resistance classes at 60 DAI along Factor 1. In addition, the FA structure provided further insight into how traits contribute to genotype discrimination. Remarkably, EM occupied an intermediate position in the biplot, which is biologically consistent since egg mass formation reflects the successful establishment of feeding sites followed by the transition to reproduction (Jones et al. 2013). Although it does not replace the integrated multi-trait approach, EM may serve as a practical proxy for rapid resistance screening.

Given that common beans are typically harvested around 90 days after planting, evaluations conducted after two nematode life cycles, combined with an increased number of replicates, provide a more realistic assessment of resistance and may better predict field performance.

qPCR-based phenotyping as a complementary tool for resistance evaluation

In addition to the integrated selection, our findings suggest that qPCR-based quantification can provide accurate molecular detection of *M. incognita* multiplication in roots of contrasting genotypes. Real-time PCR has also been explored as a molecular phenotyping approach in breeding contexts. For instance, Ruiz et al. (2023) developed a root-based qPCR method for phenotyping resistance to the citrus nematode (*Tylenchulus semipenetrans*) and support screening in rootstock-breeding programs. The qPCR assay based on cycle quantification (Cq) was developed to detect and quantify *M. incognita* in soil and root samples, associating Cq values to nematode amounts (Toyota et al. 2008; Oliveira et al. 2020). This approach can be useful as a complementary tool in breeding pipelines, since it detects nematode DNA from mixed life stages and can capture differences consistent with final population estimates. In our study, even using a small amount of root tissues at 30 DAI, qPCR consistently discriminated between a moderately resistant and a susceptible RIL across two independent experiments, supporting the use of qPCR in the common bean for phenotyping.

Conclusions

Our integrative framework effectively distinguished resistance levels and enabled the identification of transgressive genotypes within the RIL population. Later evaluation time points improved genotype discrimination and revealed resistance shifts not detectable at early stage, highlighting its importance for reliable phenotyping. Importantly, increasing the number of replicates improved the estimation of

heritability, which reached high values across traits, reinforcing the robustness of the phenotyping approach. qPCR-based evaluation consistently discriminated MR and S lines, in agreement with classical phenotyping, supporting its use as a complementary evaluation tool. Overall, our results validate an integrative phenotyping strategy that combines complementary resistance components for more precise genotype selection.

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REFERENCES

Abad P, Favery B, Rosso M, Castagnone-Sereno P (2003) Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. Mol Plant Pathol 4:217–224. https://doi.org/10.1046/j.1364-3703.2003.00170.x

Afzal A, Mukhtar T (2024) Revolutionizing nematode management to achieve global food security goals - An overview. Heliyon 10:e25325. https://doi.org/10.1016/j.heliyon.2024.e25325

Almeida CPD, Paulino JFDC, Morais Carbonell SA, et al (2020) Genetic Diversity, Population Structure, and Andean Introgression in Brazilian Common Bean Cultivars after Half a Century of Genetic Breeding. Genes 11:1298. https://doi.org/10.3390/genes11111298

520 Assefa T, Assibi Mahama A, Brown AV, et al (2019) A review of breeding objectives, genomic resources,
521 and marker-assisted methods in common bean (Phaseolus vulgaris L.). Mol Breed 39:20.
522 https://doi.org/10.1007/s11032-018-0920-0

523 Bančič J, Ovenden B, Gorjanc G, Tolhurst DJ (2023) Genomic selection for genotype performance and
524 stability using information on multiple traits and multiple environments. Theor Appl Genet
525 136:104. https://doi.org/10.1007/s00122-023-04305-1

526 Bernard GC, Egnin M, Bonsi C (2017) The Impact of Plant-Parasitic Nematodes on Agriculture and
527 Methods of Control. In: Shah MM, Mahamood M (eds) Nematology - Concepts, Diagnosis and
528 Control. InTech

529 Bonfim Junior MF, Inomoto MM, Araújo Filho JVD (2021) Phytonematodes infesting common bean fields
530 in Brazil, and pathogenicity tests with Pratylenchus brachyurus. Arq Inst Biológico 88:e00312020.
531 https://doi.org/10.1590/1808-1657000312020

532 Bridge J, Page SLJ (1980) Estimation of Root-knot Nematode Infestation Levels on Roots Using a Rating
533 Chart. Trop Pest Manag 26:296–298. https://doi.org/10.1080/09670878009414416

534 Brunson J (2020) ggalluvial: Layered Grammar for Alluvial Plots. J Open Source Softw 5:2017.
535 https://doi.org/10.21105/joss.02017

536 Byrd DW, Kirkpatrick T, Barker KR (1983) An improved technique for clearing and staining plant tissues
537 for detection of nematodes. J Nematol 15:142–143

538 Campitelli E (2025) _ggnewscale: Multiple Fill and Colour Scales in 'ggplot2'_

539 Castagnone-Sereno P (2002) Genetic variability of nematodes: a threat to the durability of plant resistance
540 genes? Euphytica 124:193–199

541 Castro-Guerrero NA, Isidra-Arellano MC, Mendoza-Cozatl DG, Valdés-López O (2016) Common Bean:
542 A Legume Model on the Rise for Unraveling Responses and Adaptations to Iron, Zinc, and
543 Phosphate Deficiencies. Front Plant Sci 7:. https://doi.org/10.3389/fpls.2016.00600

544 Corwin JA, Kliebenstein DJ (2017) Quantitative Resistance: More Than Just Perception of a Pathogen.
545 Plant Cell 29:655–665. https://doi.org/10.1105/tpc.16.00915

546 Costa JPGD, Soares PLM, Vidal RL, et al (2019) Reaction of common bean genotypes to the reproduction
547 of Meloidogyne javanica and Meloidogyne incognita. Pesqui Agropecuária Trop 49:e54008.
548 https://doi.org/10.1590/1983-40632019v4954008

549 Cullis BR, Jefferson P, Thompson R, Smith AB (2014) Factor analytic and reduced animal models for the
550 investigation of additive genotype-by-environment interaction in outcrossing plant species with
551 application to a Pinus radiata breeding programme. Theor Appl Genet 127:2193–2210.
552 https://doi.org/10.1007/s00122-014-2373-0

553 Cullis BR, Smith AB, Beeck CP, Cowling WA (2010) Analysis of yield and oil from a series of canola
554 breeding trials. Part II. Exploring variety by environment interaction using factor analysis.
555 Genome 53:1002–1016. https://doi.org/10.1139/G10-080

556 Desaeger J, Wram C, Zasada I (2020) New reduced-risk agricultural nematicides - rationale and review. J
557 Nematol 52:1–16. https://doi.org/10.21307/jofnem-2020-091

558 Dias WP, Wendland A, Souza Carneiro GED, et al (2023) Reaction of common bean genotypes to plant
559 parasitic nematodes. Pesqui Agropecuária Trop 53:e74717. https://doi.org/10.1590/1983-
560 40632023v5374717

561 FAO (2022) FAOSTAT: Crops and Livestock Products. Food and Agriculture Organization of the United
562 Nations

Floriani TDO, Gama HC, Moreno BM, et al (2025) Developing a model to implement marker-assisted selection for root-knot nematode resistance in common bean. G3 Genes Genomes Genet 15:jkaf221. https://doi.org/10.1093/g3journal/jkaf221

Fuller VL, Lilley CJ, Urwin PE (2008) Nematode resistance. New Phytol 180:27–44. https://doi.org/10.1111/j.1469-8137.2008.02508.x

Giordani W, Gama HC, Chiorato AF, et al (2022a) Genetic mapping reveals complex architecture and candidate genes involved in common bean response to *Meloidogyne incognita* infection. Plant Genome 15:e20161. https://doi.org/10.1002/tpg2.20161

Giordani W, Gama HC, Chiorato AF, et al (2022b) Genetic mapping reveals complex architecture and candidate genes involved in common bean response to *Meloidogyne incognita* infection. Plant Genome 15:e20161. https://doi.org/10.1002/tpg2.20161

Handoo Z, Hunt DJ, Habteweld A (2025) Root-knot Nematodes. In: Manzanilla-López RH, Hunt D, Marbán-Mendoza N, Infante-Gil S (eds) Practical Plant Nematology, 2nd edn. CABI, GB, pp 266–306

Henderson CR (1975) Best Linear Unbiased Estimation and Prediction under a Selection Model. Biometrics 31:423. https://doi.org/10.2307/2529430

Hoagland DR, Arnon d. i. (1950) The water-culture method for growing plants without soil (Revised ed.). California Agricultural Experiment Station, Berkeley, CA: University of California

Hussey RS;, Barker KR (1973) A comparison of methods of collecting inocula of Meloidogyne spp., including a new technique. Plant Dis Report 57:1025–1028

IBGE (2023) Produção de Feijão no Brasil

Jones JT, Haegeman A, Danchin EGJ, et al (2013) Top 10 plant-parasitic nematodes in molecular plant pathology. Mol Plant Pathol 14:946–961. https://doi.org/10.1111/mpp.12057

Kassambara A (2023) ggpubr: ggplot2 Based Publication Ready Plots

Livak KJ, Schmittgen TD (2001) Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262

Lynch JP, Brown KM (2001) Topsoil foraging - an architectural adaptation of plants to low phosphorus availability. Plant Soil 237:

Machado ACZ, Lemes CFC, Silva SA, et al (2022) Variant Meloidogyne javanica populations from Brazil could impact the breeding programs and the management in soybean fields. Trop Plant Pathol 47:442–449. https://doi.org/10.1007/s40858-022-00492-2

Mackay IJ, Cockram J, Howell P, Powell W (2021) Understanding the classics: the unifying concepts of transgressive segregation, inbreeding depression and heterosis and their central relevance for crop breeding. Plant Biotechnol J 19:26–34. https://doi.org/10.1111/pbi.13481

Mota FC, Alves GCS, Giband M, et al (2013) New sources of resistance to *Meloidogyne incognita* race 3 in wild cotton accessions and histological characterization of the defence mechanisms. Plant Pathol 62:1173–1183. https://doi.org/10.1111/ppa.12022

Mtonga A, Maruthi MN (2024) Diseases of Common Bean. In: Elmer WH, McGrath M, McGovern RJ (eds) Handbook of Vegetable and Herb Diseases. Springer International Publishing, Cham, pp 1–52

Mullin BA, Abawi GS, Pastor-Corrales MA (1991) Modification of Resistance Expression of Phaseolus vulgaris to Meloidogyne incognita by Elevated Soil Temperatures. J Nematol 23:182–187

Myers JR, Kmiecik K (2017) Common Bean: Economic Importance and Relevance to Biological Science Research. In: Pérez De La Vega M, Santalla M, Marsolais F (eds) The Common Bean Genome. Springer International Publishing, Cham, pp 1–20

Nasiru BS, Bernardes Neto JF, Souza TLPOD, et al (2024) Assessment of Phaseolus vulgaris genotypes for resistance to Meloidogyne incognita. Pesqui Agropecuária Trop 54:e79475. https://doi.org/10.1590/1983-40632024v5479475

Ndeve AD, Santos JRP, Matthews WC, et al (2019) A Novel Root-Knot Nematode Resistance QTL on Chromosome Vu01 in Cowpea. G3 GenesGenomesGenetics 9:1199–1209. https://doi.org/10.1534/g3.118.200881

Oliveira CMD, Souza Júnior ITD, Almeida NO, et al (2020) Development of quantitative detection method for Meloidogyne incognita by qPCR. Biosci J 36:78–86. https://doi.org/10.14393/BJ-v36n1a2020-42265

Omwega CO (1988) A Nondestructive Technique for Screening Bean Germ Plasm for Resistance to Meloidogyne incognita. Plant Dis 72:970. https://doi.org/10.1094/PD-72-0970

Oostenbrink M (1966) Major characteristics of the relation between nematodes and plants. H. Veenman & Zonen, Wageningen

Orsi N, Marques JPR, Bibiano LBJ, et al (2025) Genotype-Specific Responses of Common Bean to *Meloidogyne incognita*. Phytopathology® 115:535–547. https://doi.org/10.1094/PHYTO-10-24-0313-R

Parker TA, Gallegos JA, Beaver J, et al (2022) Genetic Resources and Breeding Priorities in Phaseolus Beans: Vulnerability, Resilience, and Future Challenges. In: Goldman I (ed) Plant Breeding Reviews, 1st edn. Wiley, pp 289–420

Patterson HD, Thompson R (1971) Recovery of inter-block information when block sizes are unequal. Biometrika 58:545–554. https://doi.org/10.1093/biomet/58.3.545

Perry RN, Moens M (eds) (2013) Plant nematology, 2nd edn. CABI, UK

Pesqueira AM, González AM, Barragán-Lozano T, et al (2025) Insights into the Genetics Underlying the Resistance to Root-Knot Nematode Reproduction in the Common Bean Ouro Negro. Plants 14:1073. https://doi.org/10.3390/plants14071073

Piepho H-P (1997) Analyzing genotype-environment data by mixed models with multiplicative terms. Biometrics 53:761–766. https://doi.org/10.2307/2533976

Ralmi NHAA, Khandaker MM, Mat N (2016) Occurrence and control of root knot nematode in crops: A review. Aust J Crop Sci 10:1649–1654. https://doi.org/10.21475/ajcs.2016.10.12.p7444

Rashidifard M, Berner J, Fourie H (2025) Confirmation of resistance in different soybean cultivars to Meloidogyne incognita using greenhouse screenings, enzymes and histopathology approaches. J Plant Dis Prot 132:141. https://doi.org/10.1007/s41348-025-01139-5

Rieseberg LH, Archer MA, Wayne RK (1999) Transgressive segregation, adaptation and speciation. Heredity 83:363–372. https://doi.org/10.1038/sj.hdy.6886170

Roberts PA, Matthews WC, Ehlers JD, Helms D (2008) Genetic Determinants of Differential Resistance to Root-Knot Nematode Reproduction and Galling in Lima Bean. Crop Sci 48:553–561. https://doi.org/10.2135/cropsci2007.07.0384

Ruijter JM, Ramakers C, Hoogaars WMH, et al (2009) Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. Nucleic Acids Res 37:e45–e45. https://doi.org/10.1093/nar/gkp045

Ruiz M, Vo AD, Becker JO, Roose ML (2023) Real-Time PCR to Phenotype Resistance to the Citrus Nematode Tylenchulus semipenetrans Cobb. Plants 12:2543. https://doi.org/10.3390/plants12132543

Schmutz J, McClean PE, Mamidi S, et al (2014) A reference genome for common bean and genome-wide analysis of dual domestications. Nat Genet 46:707–713. https://doi.org/10.1038/ng.3008

Sikandar A, Jia L, Wu H, Yang S (2023) Meloidogyne enterolobii risk to agriculture, its present status and future prospective for management. Front Plant Sci 13:1093657. https://doi.org/10.3389/fpls.2022.1093657

Smith AB, Cullis BR (2018) Plant breeding selection tools built on factor analytic mixed models for multi-environment trial data. Euphytica 214:143. https://doi.org/10.1007/s10681-018-2220-5

Smith AB, Ganesalingam A, Kuchel H, Cullis BR (2015) Factor analytic mixed models for the provision of grower information from national crop variety testing programs. Theor Appl Genet 128:55–72. https://doi.org/10.1007/s00122-014-2412-x

Smith AB, Norman A, Kuchel H, Cullis BR (2021) Plant variety selection using interaction classes derived from factor analytic linear mixed models: models with independent variety effects. Front Plant Sci 12:1857. https://doi.org/10.3389/fpls.2021.737462

Starr JL, Bridge J, Cook R (2002) Resistance to plant-parasitic nematodes: history, current use and future potential. In: Starr JL, Cook R, Bridge J (eds) Plant resistance to parasitic nematodes, 1st edn. CABI Publishing, UK, pp 1–22

Taylor AL, Sasser J (1978) Biology, identification and control of root-knot nematodes (Meloidogyne species). North Carolina State University Graphics, North Carolina

Teixeira RA, Barbosa KAG, Rocha MRD (2017) Reaction of soybean cultivars to Meloidogyne incognita race 3. Científica 45:31–40. https://doi.org/10.15361/1984-5529.2017v45n1p31-40

The VSNi Team (2023) asreml: Fits Linear Mixed Models using REML

Toyota K, Shirakashi T, Sato E, et al (2008) Development of a real-time PCR method for the potato-cyst nematode *Globodera rostochiensis* and the root-knot nematode *Meloidogyne incognita*. Soil Sci Plant Nutr 54:72–76. https://doi.org/10.1111/j.1747-0765.2007.00212.x

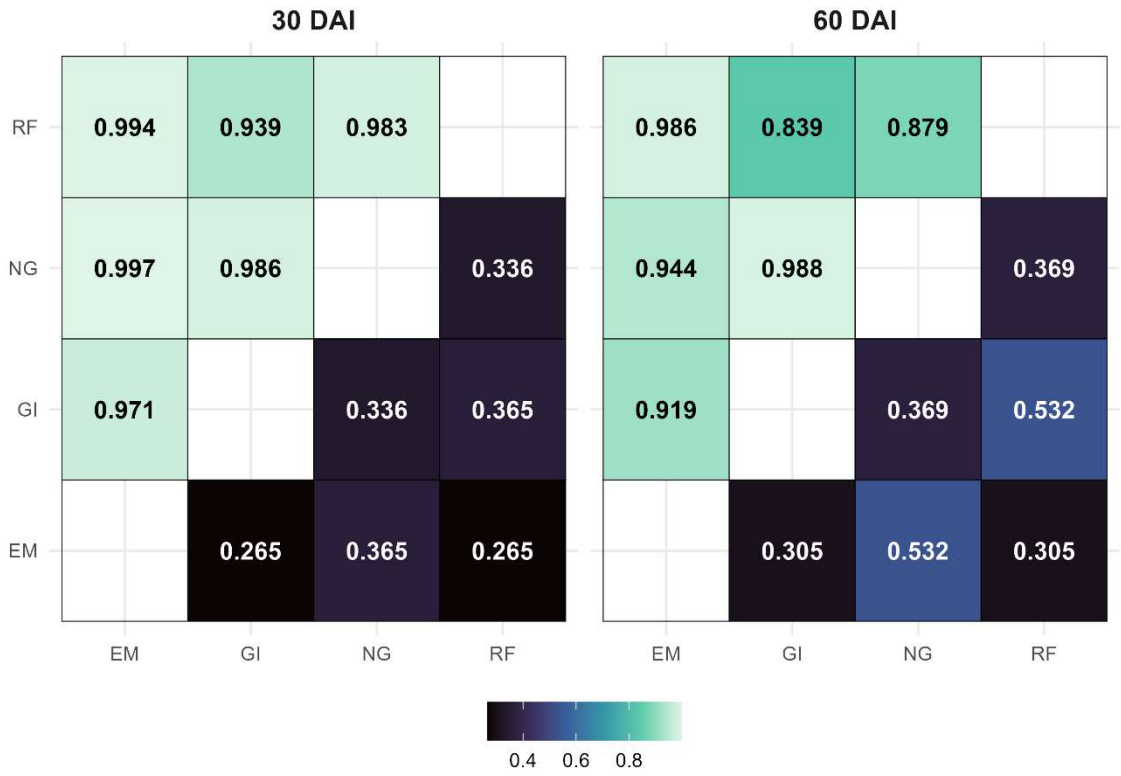
Wang C, Ulloa M, Mullens TR, et al (2012) QTL Analysis for Transgressive Resistance to Root-Knot Nematode in Interspecific Cotton (Gossypium spp.) Progeny Derived from Susceptible Parents. PLoS ONE 7:e34874. https://doi.org/10.1371/journal.pone.0034874

Wickham H (2016) ggplot2: Elegant graphics for data analysis, 2nd edn. Springer, Cham

Yaseen I, Mukhtar T, Kim H-T, Arshad B (2023) Quantification of resistance to Meloidogyne incognita in okra cultivars using linear and nonlinear analyses of growth parameters and nematode infestations. Bragantia 82:e20230114. https://doi.org/10.1590/1678-4499.20230114

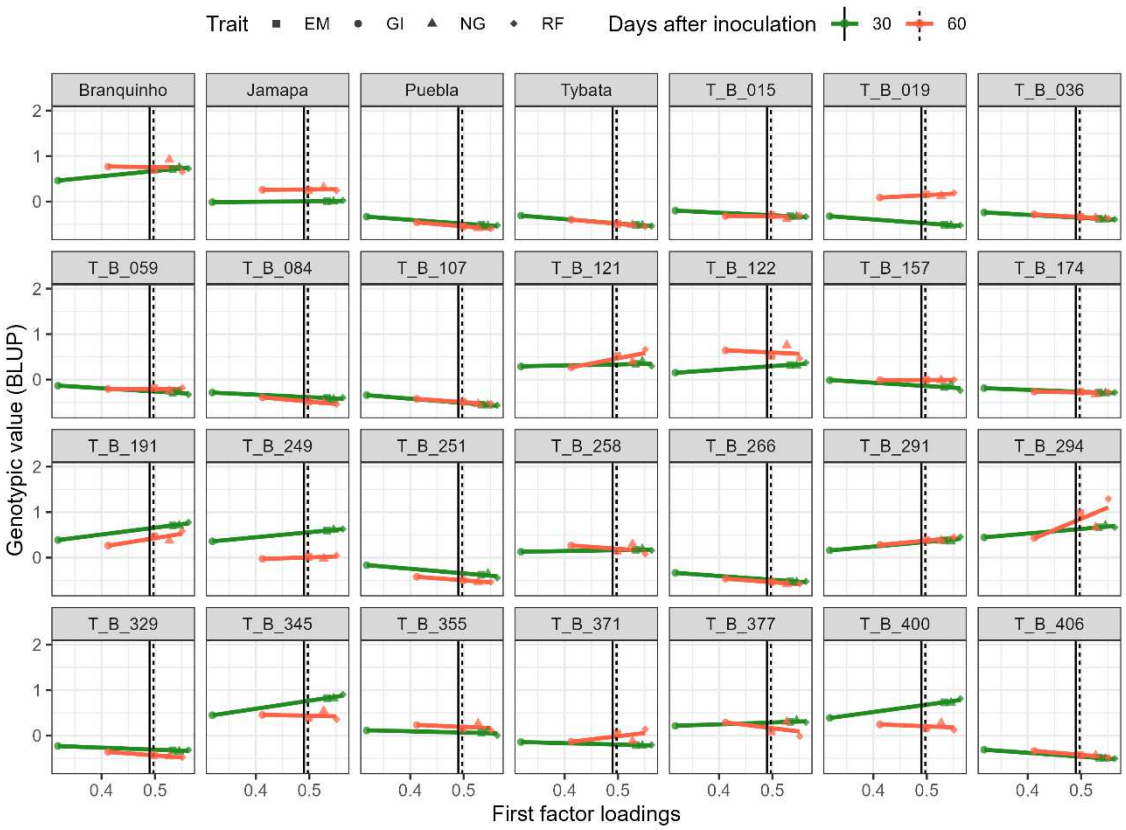
Yüksel D, Bozbuğa R (2025) Plant resistance genes against nematodes: Mechanisms and genetic resources in crop protection. Physiol Mol Plant Pathol 140:102962. https://doi.org/10.1016/j.pmpp.2025.102962

686 **SUPPLEMENTARY MATERIAL**



687

688 **Figure S1.** REML-estimates of genotypic (upper triangle) and residual (lower triangle) correlations among
689 egg masses (EM), galling index (GI), number of galls (NG), and reproduction factor (RF) within
690 segregating genotypes of the recombinant inbred line (RIL) population at 30 and 60 days after inoculation.



691

692 **Figure S2.** Factorial regression of recombinant inbred lines (RILs), parental references 'Branquinho'
693 (susceptible) and 'Tybatã' (moderately resistant) and black bean controls 'Jamapa' (susceptible) and
694 'Puebla' (moderately resistant) at 30 (green) and 60 (red) days after inoculation. Each panel represents an
695 individual regression with genotype scores as slopes and first-factor loadings as predictors. EM=egg
696 masses, GI= galling index, NG= number of galls, and RF=reproduction factor.

697

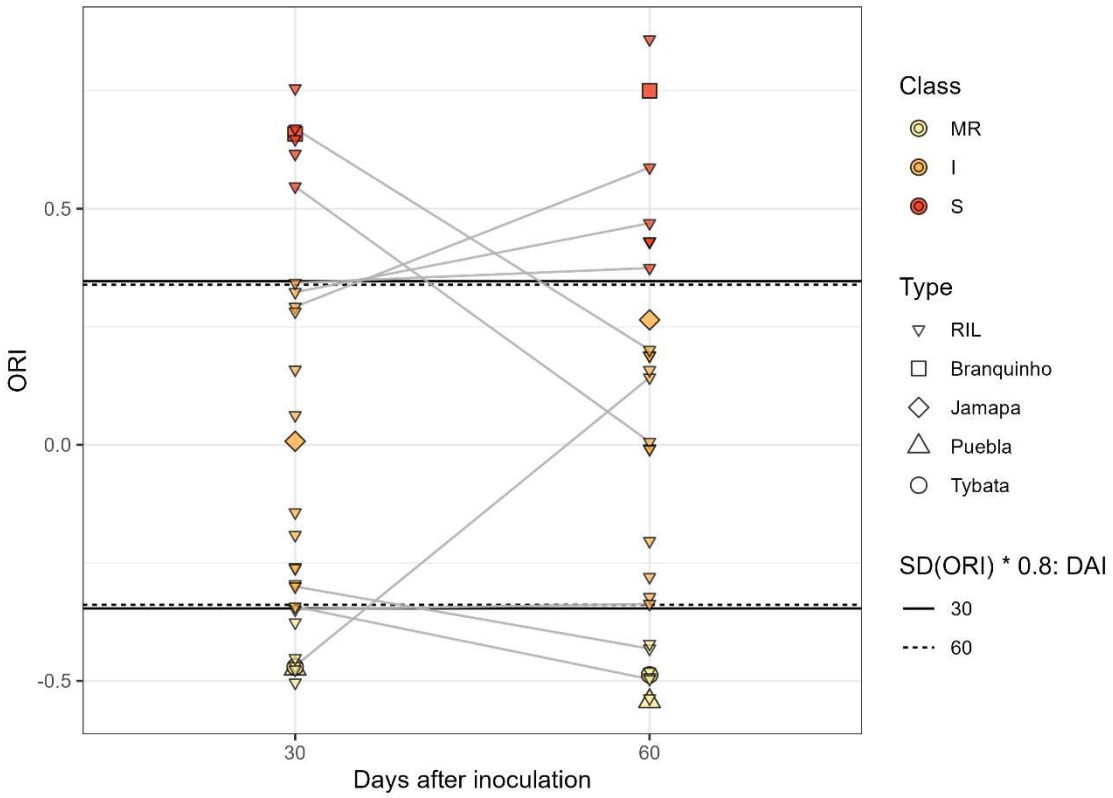


Figure S3. Classification of segregating genotypes based on the overall resistance index (ORI) at 30 and 60 days after inoculation (DAI). Point shapes distinguish recombinant inbred lines (RIL), parents (Branquinho and Tybatã) and checks (Jamapa and Puebla). Colors indicate resistance classes: moderately resistant (MR), intermediate (I), and susceptible (S). Horizontal lines (solid 30 DAI and dashed 60 DAI) represent classification thresholds (± 0.8*SD of ORI). Grey solid lines connecting points illustrate shifts in genotype classification between evaluation times.

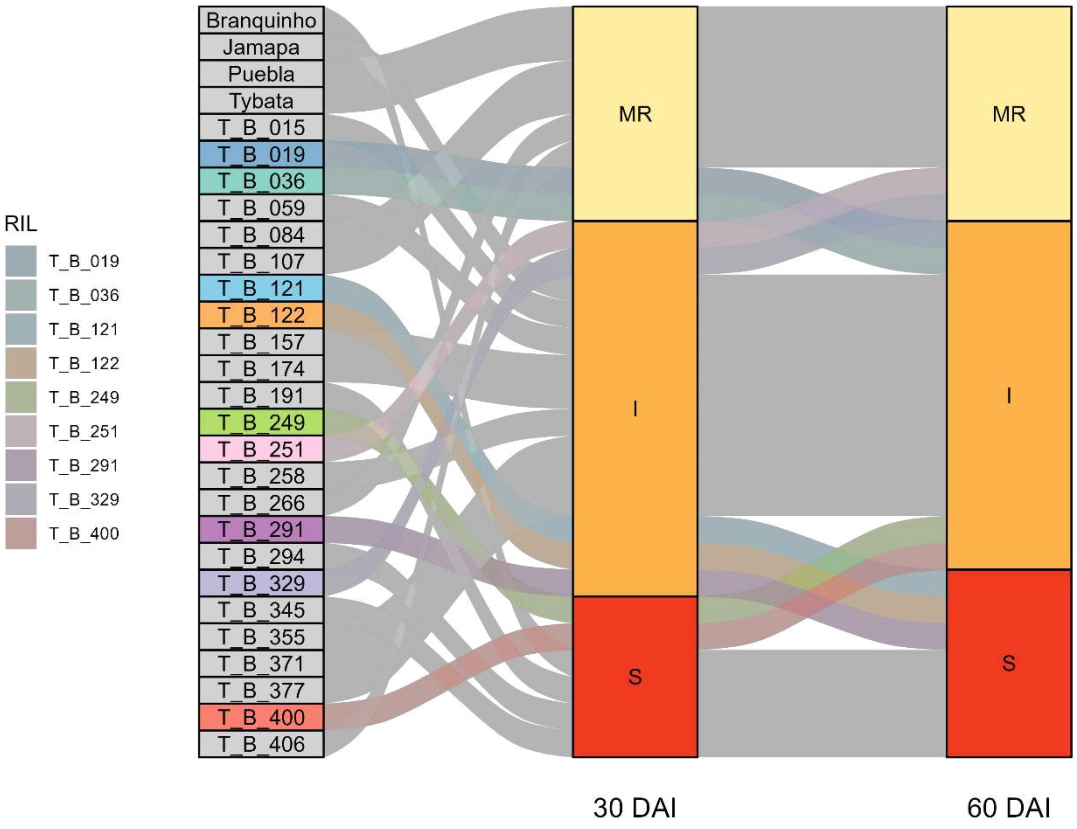


Figure S4. Segregating genotypes of the recombinant inbred line (RIL) population class shifts based on the overall resistance index (ORI) between 30 and 60 days after inoculation (DAI). Parental references 'Branquinho' (susceptible) and 'Tybatã' (moderately resistant), black bean conrols 'Jamapa' (susceptible) and 'Puebla' (moderately resistant) were also included.

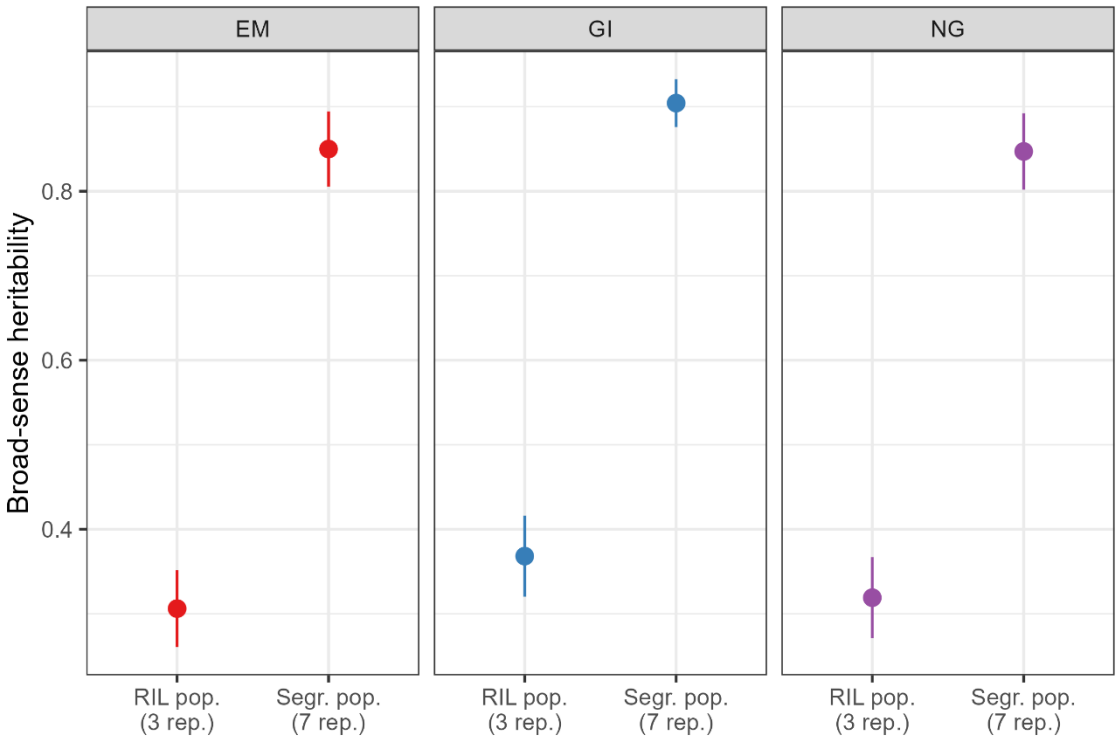


Figure S5. Broad-sense heritability estimates for egg masses (EM), galling index (GI), and number of galls (NG) at 60 days after inoculation, comparing the RIL population evaluated with three replicates (3 rep.) and the segregating genotypes evaluated with seven replicates (7 rep.).

Supplementary Table 1. Summary of qPCR (Cq) and final population (PF) in two contrasting RILs at 30 days after inoculation-DAI (n = 4; per genotype per experiment)

Experiment	Genotype (class)	Cq (mean ± SD)	PF (mean ± SD)	ΔCq (MR − S)	qPCR fold (S/MR) [a]	PF fold (S/MR)
EXP1	T_B_266 (MR)	26.78 ± 1.22	675 ± 222	2.97	5.77x	10.63x
EXP1	T_B_294 (S)	23.81 ± 0.38	7175 ± 3970	—	—	—
EXP2	T_B_266 (MR)	26.32 ± 1.24	1000 ± 535	3.28	6.76x	7.28 x
EXP2	T_B_294 (S)	23.04 ± 0.53	7275 ± 5471	—	—	—

[a] qPCR fold (S/MR) was calculated using PCR efficiency (EXP1= 1.8049 and EXP2 = 1.7908) and the formula E^(ΔCq), where ΔCq = Cq(MR) − Cq(S) (Livak and Schmittgen 2001).