Research Article

Reaction of common bean genotypes to plant parasitic nematodes[1]

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ABSTRACT

Among the efficient strategies to manage plant parasitic nematodes, the use of resistant cultivars stands out for being frequently the easiest and least expensive approach that can be adopted by farmers. However, for the common bean, in Brazil, few sources of resistance have been identified so far. This study aimed to assess the reaction of 81 common bean genotypes to the most abundant and harmful plant parasitic nematode species in Brazilian crop fields. Genotypes resistant to all tested nematodes were observed: 7 to *Heterodera glycines*, 2 to *Pratylenchus brachyurus*, 15 to *Meloidogyne incognita* and 8 to *M. javanica*.

KEYWORDS: *Phaseolus vulgaris*, root-lesion nematodes, root-knot nematodes, soybean cyst-nematodes, genetic resistance.

RESUMO

Reação de genótipos de feijoeiro a nematoides de plantas

Entre as estratégias eficientes de manejo de fitonematoides, o uso de cultivares resistentes se destaca por ser frequentemente a abordagem mais fácil e menos onerosa que pode ser adotada pelos agricultores. Entretanto, no caso do feijoeiro comum, no Brasil, poucas fontes de resistência foram identificadas até o momento. Objetivou-se avaliar a reação de 81 genótipos de feijão comum às espécies de fitonematoides mais abundantes e nocivas nas lavouras brasileiras. Foram observados genótipos resistentes a todos os nematoides testados: 7 a *Heterodera glycines*, 2 a *Pratylenchus brachyurus*, 15 a *Meloidogyne incognita* e 8 a *M. javanica*.

PALAVRAS-CHAVE: *Phaseolus vulgaris*, nematoides das lesões radiculares, nematoides das galhas, nematoides de cisto da soja, resistência genética.

INTRODUCTION

Brazil is the world's largest producer of common bean (*Phaseolus vulgaris* L.), a basic dietary protein food source in the diet of the Brazilian population (Ferreira & Barrigossi 2021). The consumer choice regarding grain color, grain type and culinary quality varies regionally, being the carioca bean (pinto bean) the type that predominates in 70 % of the Brazilian common bean market. This variety is produced mostly in the South, Southeast and Midwest regions.

To meet the demand, the crop is sown throughout the year, in a variety of cropping systems. In the 2020 harvest, 3,222 million tons of common bean were produced in an area of 2,927 million hectares, which account for an average yield of 1,101 kg ha^{-1} (Embrapa 2021).

Ecoregion characteristics and soil infestations by plant parasitic nematodes are among the main factors that interfere with the common bean yield and grain quality. The most harmful nematodes to common bean are the root-knot nematodes (*Meloidogyne incognita* and *M. javanica*) and root-lesion nematodes (*Pratylenchus brachyurus*) (Santini et al. 2016, Bernard et al. 2017). However, damages by other nematodes have also been reported: *Meloidogyne paranaenses* (Juliatti et al. 2010), *Pratylenchus jaehni* (Bonfim Junior & Inimoto 2012), *M. enterolobii* (sin. *M. mayaguensis*) (Guimarães et al. 2003) and the soybean cyst nematode *Heterodera glycines* (Yan et al. 2017, Jain et al. 2019).

The use of resistant cultivars is the most efficient strategy to control nematode populations, besides being appropriate to the producer. Nevertheless, few common bean genotypes that could serve as sources of resistance to nematodes have been identified in Brazil. Thus, this study aimed to assess the reaction of 81 common bean genotypes to the most abundant and harmful plant parasitic nematode species in Brazilian crop fields.

MATERIAL AND METHODS

The experiments were conducted in a greenhouse at the Embrapa Soja, in Londrina (Paraná state, Brazil), in the 2015/2016 crop season (October to March).

The reproduction of each nematode species on common bean genotypes was evaluated in separate experiments. Specifically for the soybean cyst nematode, the reactions of five soybean genotypes were added to assess the viability of the inoculant and to confirm the nematode race (Riggs & Schmitt 1988).

Initially, seeds of the bean and soybean genotypes were germinated in the sand. Two days after the emergence, one seedling was transplanted into a clay pot containing 1 kg of a mixture of soil and sand (1:3), previously autoclaved. Afterwards, the pots were placed in the greenhouse according to a completely randomized design, with six replications (pots). At two days after the transplant, the seedlings were inoculated by deposition of the suspension on the soil, close to the plant root, of 4,000 eggs (*H. glycines*), 500 juveniles and adults (*P. brachyurus*) or with 5,000 eggs (*M. incognita* and *M. javanica*). The incubation period varied according to the species, being 28 days for the soybean cyst nematode, 60 days for *P. brachyurus* and 50 days for the root-knot nematodes. The extraction of the nematodes produced in each bean or soybean root plant at the end of the experimental period was performed according to the methodologies by Dias et al. (2005) for the soybean cyst nematode, Coolen & D'Herde (1972) for the root-lesion nematodes, and Boneti & Ferraz (1981) and Bozbuga et al. (2015) for the root-knot nematodes. To quantify the population of nematodes, a pipette of the suspension was placed in Peters chambers, where the nematodes were counted with the aid of an optical microscope.

The reaction of a genotype to a nematode species was inferred from an index named relative reproduction factor, which consists of the ratio between the reproduction factor of that nematode species on the genotype and the corresponding reproduction factor on the susceptible standard. The reproduction factor consisted of the ratio between the estimated mean of individuals recovered from the plant and the number of individuals inoculated. The susceptible standards were defined as the genotype that presented the maximum reproduction factor, namely: Macanudo (*H. glycines*), WAF 75 (*M. javanica*), CNFC 11962 (*M. incognita*) and BRSMG Pioneiro (*P. brachyurus*). The estimated mean of soybean cyst nematodes accounted only for the females, while the root-knot nematodes were considered the eggs and second-stage juveniles, and adults and juveniles for the root-lesion nematode. For this reason, the relative reproduction factor of the soybean cyst nematode is referred to as the female index (FI).

To meet the criteria established by the International Union for Protection of New Varieties of Plants (UPOV 1996), of which Brazil is a member, the reaction of a bean genotype to a nematode has to be classified in one of these three categories: resistant (R), moderately resistant (MR) and susceptible (S). In the present study, the classification of all cultivars followed the criterion used by Riggs & Schimitt (1988), which defined intervals to classify the reaction of soybean cultivars to soybean cyst nematode as FI < 10 % (R), 10 % ≤ FI ≤ 30 % (MR) and FI > 30 % (S). Instead of the FIs, for the root-knot and the root-lesion nematodes, the relative reproduction factor values were used. The population showing the highest reproduction index was considered a reference for susceptibility. Soon afterwards, the reproduction index of the reference was compared with that of the other populations, calculating the reduction percentage of each one. Based on these values, the levels of resistance of each cultivar were defined according to the following reproduction criterion established by Moura & Régis (1987), which classifies the cultivar according to the reduction percentage in the nematode inhibition (RI): HS - highly susceptible (RI of 0-25 %); S - susceptible (RI of 26-50 %); NVR - not very resistant (RI of 51-75 %); MR - moderately resistant (RI of 76-95 %); R - resistant (RI of 96-99 %); HR - highly resistant/immune (RI of 100 %).

When the F-test evidenced the genotype effect, the Tukey multiple comparison test was applied to

indicate genotypes with similar reproduction means. Since the statistical distribution of nematode counting is typically non-normal, the statistical analyses using generalized linear mixed model (GLMM) technics was conducted.

To conduct the analysis, in-house scripts encompassing several procedures from the SAS/STAT® software, version 9.4. (Copyright© 2016, SAS Institute Inc.), were used. Initially, analyses of variance (Anova) equations by the glimmix procedure were fitted, assuming the default variance-covariance structure, the genotype as the only fixed effect, and one of the following distributions: normal, lognormal, gamma, Poisson, negative binomial and generalized Poisson. The link function for the normal and lognormal distributions was the identity, while the logarithm was the link function for the other distributions. To assess the goodness-of-fit of these models, the Pearson's residuals were analyzed, observing the resemblance of their distribution to the normal distribution, the dispersion of the residuals throughout the predicted values, and the QQ-plots.

To adjust the estimates of the residual variance-covariance matrices, a non-observed factor that served as a blocking factor for that matrix was defined, hereby referred to as the blocking factor. The levels of this factor indicated groups of genotypes, which had within-group homoscedasticity. This task was performed in three steps. First, the analysis of variance under the data normality assumption and heterogeneous residual variances was ran. Second, using the cluster procedure, the standard errors using the hierarchical algorithm flexible-beta were sorted, setting the parameter NONNORM to suppress the normalization in the computation of the distances, since the algorithm took only one variable. Finally, the cluster hierarchy obtained in the previous step using the tree procedures was cut, setting the parameter H equal to the RSQ (R^2, proportion of the variance accounted by the clusters) and different values of the parameter LEVEL (0.7 to 0.99 by 0.01). The combination of these parameters resulted in the lowest number of clusters that yields an R^2 value of at least the value set in the parameter LEVEL. The cluster membership of each genotype became the levels of the blocking factor.

RESULTS AND DISCUSSION

The soybean cultivar Lee 74 (susceptibility standard to *H. glycines* in soybean) exhibited a mean of females equal to 197 (Table 1), certifying the inoculum viability and that the environmental conditions in the experiment were favorable to the parasitism exerted by the nematode. From the four soybean genotypes used to differentiate soybean cyst nematode races, only the PI 88788 behaved as susceptible (FI ≥ 10.0 %) (Table 1). This pattern would classify the soybean cyst nematode race as 1, according to Riggs & Schmitt (1988). Race change may occur in greenhouses due to environmental factors (Riggs et al. 1988).

The mean of females on common bean cultivars ranged from 10.9 (CNFP 10103) to 147.3 (Macanudo) (Table 2). These results in common bean and in the susceptible standard of soybean (Lee 74), as well as those in Abawi & Jacobsen (1984) and Becker & Ferraz (2000), certify that the reproduction capacity of *H. glycines* is similar in susceptible common bean and soybean cultivars (Yan et al. 2017). However, seven common bean cultivars investigated in this study produced FIs lower than 10.0 % (Figure 1), being, therefore, classified as resistant (CNFP 10103, BRSMG Majestoso, CNFC 11954, BRSMG Pioneiro, BRS Notável, Xamego and BRS Esteio), and 33 cultivars behaved as moderately resistant (FI between 10 and 30 %). These findings differ from those of Becker & Ferraz (2000), who found only moderately resistant cultivars (FI between 26 and 50 %). Among the seven cultivars classified as

Table 1. Mean of females, female index and reaction of five soybean genotypes to the *Heterodera glycines* nematode.

Genotype	Mean of females	Female index	Reaction
Pickett (race-differential soybean line)	5.8	3.0	R
Peking (race-differential soybean line)	1.7	0.8	R
PI 88788 (race-differential soybean line)	23.7	12.0	S
PI 90763 (race-differential soybean line)	0.3	0.2	R
Lee 74 (soybean susceptibility standard to *H. glycines*)	197.0	100.0	S

R: resistant; S: susceptible.

Table 2. Mean of females (MF) of *Heterodera glycines* on 81 common bean genotypes.

Genotype	MF	SE	MRT	Genotype	MF	SE	MRT
Aporé	82.7	15.1	ABCDEFG	CNFP 10103	10.9	4.3	H
BRS 7762 Supremo	22.2	3.9	FGH	CNFP 10794 (BRS FP403)	46.9	4.3	EFGH
BRS 9435 Cometa	31.7	8.7	EFGH	CNFP 11979	25.5	3.9	FGH
BRS Agreste	111.7	8.7	ABCD	CNFP 11984	24.5	3.9	FGH
BRS Ametista	30.7	3.9	EFGH	CNFP 11995	74.0	3.9	BCDEFGH
BRS Campeiro	67.3	8.7	CDEFGH	Corrente	131.3	8.7	AB
BRS Embaixador	43.2	3.9	EFGH	Diamante Negro	49.2	8.7	EFGH
BRS Esplendor	21.0	3.9	FGH	EMGOPA Ouro	105.0	8.7	ABCD
BRS Esteio	14.3	3.9	H	Guapo Brilhante	28.0	4.8	EFGH
BRS Estilo	29.2	3.9	EFGH	IAC Alvorada	16.2	3.9	GH
BRS Executivo	27.8	8.7	EFGH	IAC Diplomata	49.5	3.9	EFGH
BRS Expedito	42.7	3.9	EFGH	IAC Formoso	93.7	8.7	ABCDE
BRS Grafite	29.7	8.7	EFGH	IAC Harmonia	59.3	8.7	DEFGH
BRS Horizonte	47.8	3.9	EFGH	IAPAR 81	124.3	8.7	AB
BRS Madrepérola	87.5	15.1	ABCDEF	IPR 139	34.1	9.5	EFGH
BRS Marfim	75.3	3.9	BCDEFGH	IPR Campos Gerais	108.5	8.7	ABCD
BRS Notável	13.8	3.9	H	IPR Eldorado	49.5	8.7	EFGH
BRS Pitanga	114.2	8.7	ABC	IPR Garça	79.7	3.9	ABCDEFG
BRS Pontal	28.2	3.9	EFGH	IPR Gralha	41.0	4.8	EFGH
BRS Radiante	36.8	8.7	EFGH	IPR Graúna	104.9	16.6	ABCDE
BRS Requinte	53.0	3.9	EFGH	IPR Juriti	26.7	4.3	FGH
BRS Sublime	23.5	3.9	FGH	IPR Saracura	30.8	3.9	EFGH
BRS Timbó	107.8	8.7	ABCD	IPR Siriri	76.9	16.6	ABCDEFGH
BRS Valente	28.8	3.9	EFGH	IPR Tangará	144.5	15.1	AB
BRS Vereda	32.5	3.9	EFGH	IPR Tiziu	35.0	3.9	EFGH
BRSMG Majestoso	12.5	3.9	H	IPR Tuiuiu	54.7	3.9	EFGH
BRSMG Pioneiro	13.5	3.9	H	IPR Uirapuru	36.3	9.5	EFGH
BRSMG Realce	87.7	8.7	ABCDE	Jalo Precoce	54.7	3.9	EFGH
BRSMG Talismã	31.7	3.9	EFGH	Light Red Kidney	68.5	15.1	BCDEFGH
BRSMG Tesouro	100.0	8.7	ABCDE	Macanudo	147.3	3.9	A
BRSMG União	37.8	3.9	EFGH	Macotaço	84.3	10.7	ABCDEF
CAL 96 (BRS FS305)	54.2	3.9	EFGH	Minuano	98.3	8.7	ABCDE
CNFC 10431	25.5	3.9	FGH	Pérola	21.5	3.9	FGH
CNFC 10432	26.3	4.3	FGH	Princesa	45.2	8.7	EFGH
CNFC 10467	28.3	3.9	EFGH	RP 1	142.7	15.1	AB
CNFC 10729	23.5	3.9	FGH	Rudá	37.7	3.9	EFGH
CNFC 10762	25.3	4.8	FGH	Varre	58.0	3.9	EFGH
CNFC 11948 (BRS FC402)	48.7	3.9	EFGH	VP 22	32.5	3.9	EFGH
CNFC 11954	13.2	3.9	H	WAF 75 (BRS Ártico)	43.3	8.7	EFGH
CNFC 11962	17.3	3.9	GH	WAF 141	33.0	8.7	EFGH
-	-	-	-	Xamego	13.8	3.9	H

SE: standard errors; MRT: Tukey multiple range test.

resistant in the present study, Xamego presented the most contrasting behavior. In that study, Xamego was classified as susceptible, while, in the present study, it behaved as resistant. Discrepancies between reactions obtained in different studies are common and usually explained in terms of the origin of seeds or nematode populations, variations in environmental conditions during the experiments, susceptibility standards and uncertainties in estimates.

The reproduction factors of *P. brachyurus* in the common bean genotypes ranged from 0.3 (IPR Tangará) to 5.9 (BRS Campeiro and BRSMG Pioneiro) (Table 3). The corresponding relative reproduction factor varied between 4.5 (IPR Tangará) and 90.9 (WAF 75) (Figure 1), and, therefore, according to the adopted criteria, the genotypes IPR Tangará and Light Red Kidney were classified as resistant, and 18 other genotypes as moderately
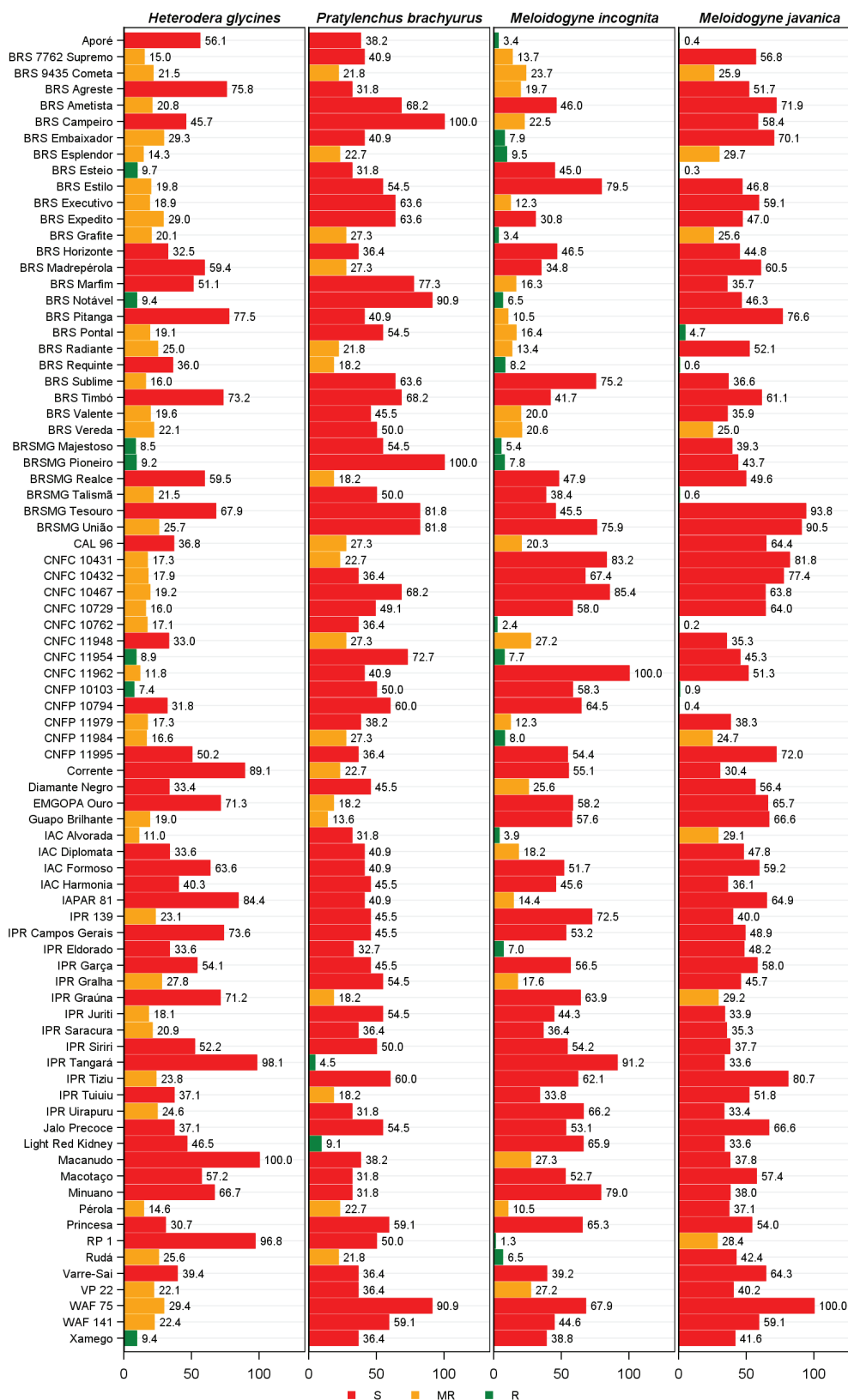
Figure 1. Female index (*Heterodera glycines*), relative reproduction factor (*Pratylenchus brachyurus*, *Meloidogyne incognita* and *Meloidogyne javanica*) and reactions of 81 common bean genotypes to the aforementioned nematodes species. Susceptibility standards have a female index or relative reproduction factor equal to 100. S: susceptible; MR: moderately resistant; R: resistant.

Table 3. Reproduction factor (RF) of *Pratylenchus brachyurus* on 81 common bean genotypes.

Genotype	RF*	SE	Genotype	RF*	SE	Genotype	RF*	SE
Aporé	2.2	0.6	BRSMG Realce	1.1	0.7	IPR 139	2.7	1.3
BRS 7762 Supremo	2.4	0.9	BRSMG Talismã	2.9	0.9	IPR Campos Gerais	2.7	0.9
BRS 9435 Cometa	1.3	0.6	BRSMG Tesouro	4.8	0.9	IPR Eldorado	1.9	0.6
BRS Agreste	1.9	0.9	BRSMG União	4.8	0.7	IPR Garça	2.7	0.7
BRS Ametista	4.0	1.6	CAL 96 (BRS FS305)	1.6	0.5	IPR Gralha	3.2	1.3
BRS Campeiro	5.9	1.3	CNFC 10431	1.3	0.5	IPR Graúna	1.1	0.5
BRS Embaixador	2.4	0.7	CNFC 10432	2.1	0.7	IPR Juriti	3.2	0.9
BRS Esplendor	1.3	0.3	CNFC 10467	4.0	0.9	IPR Saracura	2.1	0.7
BRS Esteio	1.9	0.3	CNFC 10729	2.9	0.7	IPR Siriri	2.9	0.9
BRS Estilo	3.2	1.7	CNFC 10762	2.1	0.7	IPR Tangará	0.3	0.3
BRS Executivo	3.7	1.3	CNFC 11948 (BRS FC402)	1.6	0.3	IPR Tiziu	3.5	1.4
BRS Expedito	3.7	0.3	CNFC 11954	4.3	0.9	IPR Tuiuiu	1.1	0.5
BRS Grafite	1.6	0.7	CNFC 11962	2.4	0.3	IPR Uirapuru	1.9	0.5
BRS Horizonte	2.1	0.7	CNFP 10103	2.9	0.9	Jalo Precoce	3.2	0.9
BRS Madrepérola	1.6	0.9	CNFP 10794 (BRS FP403)	3.5	0.6	Light Red Kidney	0.5	0.5
BRS Marfim	4.5	0.9	CNFP 11979	2.2	0.3	Macanudo	2.2	0.7
BRS Notável	5.3	1.6	CNFP 11984	1.6	0.9	Macotaço	1.9	0.9
BRS Pitanga	2.4	0.7	CNFP 11995	2.1	0.7	Minuano	1.9	0.9
BRS Pontal	3.2	0.9	Corrente	1.3	0.7	Pérola	1.3	0.5
BRS Radiante	1.3	0.3	Diamante Negro	2.7	0.9	Princesa	3.5	0.9
BRS Requinte	1.1	0.5	EMGOPA Ouro	1.1	0.5	RP 1	2.9	1.6
BRS Sublime	3.7	1.3	Guapo Brilhante	0.8	0.3	Rudá	1.3	0.6
BRS Timbó	4.0	1.3	IAC Alvorada	1.9	0.9	Varre-Sai	2.1	1.3
BRS Valente	2.7	0.9	IAC Diplomata	2.4	0.7	VP 22	2.1	0.5
BRS Vereda	2.9	1.3	IAC Formoso	2.4	0.9	WAF 75 (BRS Ártico)	5.3	1.6
BRSMG Majestoso	3.2	0.7	IAC Harmonia	2.7	1.3	WAF 141	3.5	0.9
BRSMG Pioneiro	5.9	2.1	IAPAR 81	2.4	0.7	Xamego	2.1	0.7

* No significant differences were found between the differences of the reproduction factors by the Tukey multiple range test. SE: standard errors.

resistant. However, the statistical analyses did not support the hypothesis of genotype effect on the reproduction factor (RF) means of the root-lesion nematode (Table 3), being the variations observed in those RFs most likely due to environmental factors and uncertainties on the estimates. Such a statement is also based on our experience with soybean cultivars (data not shown), in which, in a first experiment, considered the bean cultivars BRS Campeiro, FT Soberano, IPR Chopim, IPR 139, BRS Estilo, BRS Radiante, IAPAR 81 and IPR Tuiuiú as being resistant (RF < 1.0) to *P. brachyurus*. However, when repeating the study, it was found that all the genotypes showed susceptibility reactions (RF between 5.38 and 9.22). Currently, there are few studies to elucidate the genetic control of the common bean resistance, as well as for soybean, to the root-lesion nematode. It is only from experimental information that bean breeders can decide on the viability, and, if so, create strategies to develop resistant cultivars (Abadiyah et al. 2016).

For the *M. incognita* race 3, in which the reproduction factors ranged from 0.4 (RP 1) to 34.3 (CNFC 11962), 15 resistant bean genotypes and 21 moderately resistant were found (Table 4; Figure 1). In the case of *M. javanica* (Table 5), the reproduction factors ranged between 0.2 (CNFC 10762) and 123.0 (WAF 75), what led to the classification of eight genotypes as resistant and eight as moderately resistant (Figure 1), according to the adopted criteria. Therefore, as 81 genotypes in this study represent a large sample of the Brazilian common bean germplasm, it is likely that the frequency of resistance to *M. javanica* is lower than to *M. incognita* race 3. This fact has also been observed in soybean, a leguminous plant that is genetically very close to common bean. Ferreira et al. (2010) also reported the existence of genetic variability in the Brazilian bean germplasm, regarding genetic resistance to the race 3 of *M. incognita* and *M. javanica*. Among the 81 bean genotypes evaluated in the present study, only three (Aporé, BRS Requinte and CNFC 10762)

Table 4. Reproduction factor (RF) of *Meloidogyne incognita* on 81 common bean genotypes.

Genotype	RF	SE	MRT	Genotype	RF	SE	MRT
Aporé	1.2	0.7	NOP	CNFP 10103	20.0	3.2	ABCDE
BRS 7762 Supremo	4.7	0.7	BCDEFGHIJKLMNO	CNFP 10794 (BRS FP403)	22.1	5.4	ABCDE
BRS 9435 Cometa	8.1	2.9	ABCDEFGHIJKL	CNFP 11979	4.2	0.7	DEFGHIJKLMNO
BRS Agreste	6.7	1.6	ABCDEFGHIJKLMN	CNFP 11984	2.7	1.6	FGHIJKLMNO
BRS Ametista	15.8	2.9	ABCDEFGH	CNFP 11995	18.6	5.4	ABCDEF
BRS Campeiro	7.7	1.6	ABCDEFGHIJKLM	Corrente	18.9	4.9	ABCDE
BRS Embaixador	2.7	0.7	FGHIJKLMNOP	Diamante Negro	8.8	1.6	ABCDEFGHIJK
BRS Esplendor	3.3	0.7	EFGHIJKLMNO	EMGOPA Ouro	19.9	4.9	ABCDE
BRS Esteio	15.4	4.9	ABCDEFGH	Guapo Brilhante	19.7	6.9	ABCDE
BRS Estilo	27.2	2.9	ABC	IAC Alvorada	1.4	0.7	LMNOP
BRS Executivo	4.2	1.7	CDEFGHIJKLMNO	IAC Diplomata	6.2	2.9	ABCDEFGHIJKLMN
BRS Expedito	10.6	2.9	ABCDEFGHIJK	IAC Formoso	17.7	4.9	ABCDEFG
BRS Grafite	1.2	0.7	MNOP	IAC Harmonia	15.6	4.9	ABCDEFGH
BRS Horizonte	15.9	1.6	ABCDEFGH	IAPAR 81	4.9	0.7	BCDEFGHIJKLMNO
BRS Madrepérola	11.9	2.9	ABCDEFGHIJK	IPR 139	24.8	2.9	ABCD
BRS Marfim	5.6	1.6	ABCDEFGHIJKLMN	IPR Campos Gerais	18.2	4.9	ABCDEF
BRS Notável	2.2	0.7	JKLMNOP	IPR Eldorado	2.4	0.7	HIJKLMNOP
BRS Pitanga	3.6	1.6	EFGHIJKLMNO	IPR Garça	19.4	3.2	ABCDE
BRS Pontal	5.6	1.6	ABCDEFGHIJKLMN	IPR Gralha	6.0	0.7	ABCDEFGHIJKLMN
BRS Radiante	4.6	1.6	CDEFGHIJKLMNO	IPR Graúna	21.9	2.9	ABCDE
BRS Requinte	2.8	0.7	FGHIJKLMNO	IPR Juriti	15.2	3.2	ABCDEFGHIJ
BRS Sublime	25.8	4.9	ABCD	IPR Saracura	12.5	2.9	ABCDEFGHIJK
BRS Timbó	14.3	4.9	ABCDEFGHIJ	IPR Siriri	18.6	2.9	ABCDEF
BRS Valente	6.8	1.6	ABCDEFGHIJKLMN	IPR Tangará	31.2	6.9	A
BRS Vereda	7.0	1.7	ABCDEFGHIJKLMN	IPR Tiziu	21.3	5.4	ABCDE
BRSMG Majestoso	1.8	0.7	KLMNOP	IPR Tuiuiu	11.6	2.9	ABCDEFGHIJK
BRSMG Pioneiro	2.7	0.7	GHIJKLMNOP	IPR Uirapuru	22.7	2.9	ABCD
BRSMG Realce	16.4	5.4	ABCDEFGH	Jalo Precoce	18.2	2.9	ABCDEF
BRSMG Talismã	13.2	2.9	ABCDEFGHIJ	Light Red Kidney	22.6	6.9	ABCD
BRSMG Tesouro	15.6	2.9	ABCDEFGH	Macanudo	9.3	1.6	ABCDEFGHIJK
BRSMG União	26.0	6.9	ABCD	Macotaço	18.1	5.4	ABCDEFG
CAL 96 (BRS FS305)	7.0	0.7	ABCDEFGHIJKLMN	Minuano	27.1	12.2	ABC
CNFC 10431	28.5	2.9	ABC	Pérola	3.6	0.7	EFGHIJKLMNO
CNFC 10432	23.1	6.9	ABCD	Princesa	22.4	5.4	ABCDE
CNFC 10467	29.3	6.9	AB	RP 1	0.4	0.7	P
CNFC 10729	19.9	4.9	ABCDE	Rudá	2.2	0.7	IJKLMNOP
CNFC 10762	0.8	0.7	OP	Varre-Sai	13.4	4.9	ABCDEFGHIJ
CNFC 11948 (BRS FC402)	9.3	2.9	ABCDEFGHIJK	VP 22	9.3	2.9	ABCDEFGHIJK
CNFC 11954	2.6	0.7	GHIJKLMNOP	WAF 75 (BRS Ártico)	23.3	4.9	ABCD
CNFC 11962	34.3	4.9	A	WAF 141	15.3	3.2	ABCDEFGHI
-	-	-	-	Xamego	13.3	3.2	ABCDEFGHIJK

SE: standard errors; MRT: Tukey multiple range test.

showed resistance to both the root-knot nematodes. These results, as well as those by Ferreira et al. (2010), indicate that, in common bean, the genes of resistance to *M. incognita* race 3 and to *M. javanica* are not the same.

The phenotypic resistance of common bean genotypes to both the root-knot nematodes has already been explored (Abadiyah et al. 2016). None of the three bean genotypes classified here as resistant to both species of root-knot nematodes showed resistance to *M. incognita* in the evaluations where only the BRS Requinte genotype behaved as resistant to *M. javanica*. From those three genotypes, Ferreira et al. (2010) corroborates the resistance of the Aporé cultivar to *M. incognita* race 3, while Juliatti et al. (2010) classified this cultivar as susceptible. Finally, the BRSMG Talismã genotype, classified here as susceptible to *M. incognita* and resistant to

Table 5. Reproduction factor (RF) of *Meloidogyne javanica* on 81 common bean genotypes.

Genotype	RF	SE	MRT	Genotype	RF	SE	MRT
Aporé	0.5	0.2	F	CNFP 10103	1.1	0.4	EF
BRS 7762 Supremo	69.8	8.5	ABCD	CNFP 10794 (BRS FP403)	0.5	0.2	F
BRS 9435 Cometa	31.9	5.5	BCD	CNFP 11979	47.1	7.9	ABCD
BRS Agreste	63.6	8.0	ABCD	CNFP 11984	30.4	5.2	D
BRS Ametista	88.4	12.2	ABC	CNFP 11995	88.5	12.2	ABC
BRS Campeiro	71.8	12.2	ABCD	Corrente	37.4	8.7	ABCD
BRS Embaixador	86.2	15.6	ABCD	Diamante Negro	69.4	15.3	ABCD
BRS Esplendor	36.6	8.5	ABCD	EMGOPA Ouro	80.8	7.9	ABCD
BRS Esteio	0.3	0.1	F	Guapo Brilhante	81.9	12.3	ABCD
BRS Estilo	57.5	10.3	ABCD	IAC Alvorada	35.7	9.4	ABCD
BRS Executivo	72.7	12.3	ABCD	IAC Diplomata	58.8	10.1	ABCD
BRS Expedito	57.8	10.3	ABCD	IAC Formoso	72.9	12.3	ABCD
BRS Grafite	31.5	5.4	CD	IAC Harmonia	44.4	7.3	ABCD
BRS Horizonte	55.1	10.3	ABCD	IAPAR 81	79.8	14.7	ABCD
BRS Madrepérola	74.4	12.6	ABCD	IPR 139	49.2	8.3	ABCD
BRS Marfim	43.9	7.2	ABCD	IPR Campos Gerais	60.2	8.3	ABCD
BRS Notável	56.9	10.2	ABCD	IPR Eldorado	59.2	8.2	ABCD
BRS Pitanga	94.2	13.0	AB	IPR Garça	71.3	12.1	ABCD
BRS Pontal	5.8	1.2	E	IPR Gralha	56.2	8.3	ABCD
BRS Radiante	64.1	8.1	ABCD	IPR Graúna	35.9	7.2	ABCD
BRS Requinte	0.8	0.2	F	IPR Juriti	41.6	6.7	ABCD
BRS Sublime	45.0	7.4	ABCD	IPR Saracura	43.4	8.0	ABCD
BRS Timbó	75.2	12.7	ABCD	IPR Siriri	46.4	7.7	ABCD
BRS Valente	44.1	7.3	ABCD	IPR Tangará	41.4	7.5	ABCD
BRS Vereda	30.8	5.3	CD	IPR Tiziu	99.3	8.7	A
BRSMG Majestoso	48.3	8.1	ABCD	IPR Tuiuiu	63.7	8.5	ABCD
BRSMG Pioneiro	53.7	7.1	ABCD	IPR Uirapuru	41.1	9.8	ABCD
BRSMG Realce	61.0	8.4	ABCD	Jalo Precoce	81.8	8.0	ABCD
BRSMG Talismã	0.7	0.2	F	Light Red Kidney	41.3	7.5	ABCD
BRSMG Tesouro	115.4	13.0	A	Macanudo	46.5	7.7	ABCD
BRSMG União	111.3	6.1	A	Macotaço	70.6	6.6	ABCD
CAL 96 (BRS FS305)	79.3	6.8	ABCD	Minuano	46.7	6.7	ABCD
CNFC 10431	100.6	8.8	A	Pérola	45.7	7.5	ABCD
CNFC 10432	95.1	14.3	AB	Princesa	66.4	8.3	ABCD
CNFC 10467	78.5	6.7	ABCD	RP 1	35.0	8.1	ABCD
CNFC 10729	78.6	14.5	ABCD	Rudá	52.1	6.8	ABCD
CNFC 10762	0.2	0.1	F	Varre-Sai	79.0	6.8	ABCD
CNFC 11948 (BRS FC402)	43.4	7.2	ABCD	VP 22	49.5	8.3	ABCD
CNFC 11954	55.7	9.9	ABCD	WAF 75 (BRS Artico)	123.0	16.4	A
CNFC 11962	63.1	7.9	ABCD	WAF 141	72.7	12.3	ABCD
-	-	-	-	Xamego	51.2	10.0	ABCD

SE: standard errors; MRT: Tukey multiple range test.

M. javanica, was classified by Ferreira et al. (2010) as highly resistant to *M. incognita* and susceptible to *M. javanica*. These intriguingly opposite reactions have yet to be addressed in future studies.

Either the reaction class of a genotype obtained through relative reproduction factor intervals or the statistical grouping of reproduction factors has its drawbacks. The main limitation of the first approach is to exclude from the criteria the uncertainties involved in the entire process of quantification of the nematode reproduction, which can be high and heterogeneous. The adequacy of the adopted intervals would be less problematic if the experimental error variances were homoscedastic and restricted to a confidence interval of length of 10 %, which is very hard to achieve, as it can be observed in Tables 2-5. Another limitation is the difficulty to compare the reaction classes throughout different studies,

particularly due to the use of different susceptibility standards and reproduction rates of the nematodes. The latter is particularly hard to control, since it results of a combination of multiple factors, including the origin of the nematode inoculum, the resistance level of the genotypes and environmental conditions.

The statistical analysis is particularly valuable to test the hypothesis of genetic effect on the observed nematodes means. Shortly, a GLMM framework consists of choosing a link function that allows the predicted values to be described by a statistical distribution in the exponential family and defining one or more random factor(s). The latter is not necessarily a variable in the experiment, being usually adopted to produce a valid residual variance-covariance matrix. Ideally, a suitable GLMM model is expected to produce predicted values of the response variable that are linear on the explanatory variables, have standardized residuals that are homoscedastic and independent and are normally distributed.

Under these assumptions, classical inference methods, such as the F-test and the multiple comparison tests, are valid. Nevertheless, none of these models produced residuals that seemed either independent or normally distributed. Moreover, the homoscedasticity hypothesis was discarded for all models. Under the normal distribution, the models with the blocked variance matrix produced a better fit for the *H. glycines* and *P. brachyurus* data than models assuming other distributions, for both default and heterogeneous variance matrices. For the root-knot nematodes data, the best fit was obtained under the gamma distribution, with a default variance matrix for the *M. glycines* data and a blocked matrix for the *M. javanica*. The superior fit of models using the blocked variance matrix is likely due to the plant-nematode interaction: the higher the susceptibility of a genotype to a nematode species, the higher the standard error of the mean. On the other hand, given the high number of genotypes, it is plausible to assume that many genotypes had similar standard errors, making either the assumption of full homoscedasticity or heteroscedasticity unreasonable. Interestingly, the correlation between means and standard errors for the *M. incognita* data, for which the default variance provided a better fit, was 82 %, while, for *H. glycines*, *P. brachyurus* and *M. javanica*, they were 65, 71 and 62 %. Therefore, we believe that higher correlations between means and variances could work as an indirect indicator

of the likelihood of the blocked variance matrix to enhance the goodness-of-fit measures for Anova models of nematode counting. The major advantage of using the blocked matrix, if compared to the heterogeneous structure, was the possibly more accurate estimation of the standard errors under higher degrees of freedom.

Regarding the statistical grouping, the lack of a well-defined statistical distribution for the relative reproduction factor restrains the application of multiple comparison tests to the means of recovered nematodes, which is then used to compute the reproduction factors. Therefore, as this criterion does not require a susceptibility standard, the decision consists in choosing genotypes that have no significant difference in the group of minimum reproduction factors. The main benefit of the statistical analysis is to confirm or refute the hypothesis of the genotype effect, as was the case for *P. brachyurus*. On the other hand, the results might not be as useful when the residual variances are heterocedastic and high for some genotypes, as shown in Table 2. In this table, the minimum mean of females (10.9) was not significantly different from the mean of females recovered from the other 61 genotypes. Among them, there are 37 classified as moderately resistant and 18 classified as susceptible by the relative reproduction factor criteria.

In Tables 4 and 5, which show the results of the root-not nematodes, the number of genotypes whose reproduction factors were not significantly different from the minimum value was close to the number of genotypes classified as resistant in both species through the criterion of the relative reproduction factor intervals. The latter indicated 15 genotypes resistant to *M. incognita* and 8 to *M. javanica*, while the former put 12 and 7 genotypes in the groups that had the minimum reproduction factors (P and F). Based on these results and on the correlations between estimated mean and standard errors for *M. incognita* (81 %) and for *M. javanica* (79 %), it is plausible to conjecture that the major factor devaluing the worth of the statistical grouping is not the heteroscedasticity, but the low correlation between reproduction factors and standard errors. Corroborating this conjecture are the corresponding correlations in Tables 2 (60 %) and 3 (67 %).

It is important to point out that the statistical analysis of reproduction factors and the classification of the genotypes based on the relative reproduction

factor intervals are relevant to the understanding of the structure of the data, as was the case for *P. brachyurus*. Both approaches provide different lenses that allow a better interpretation and decision-making, particularly when drawing strategies for population management in systems cultivating common beans. The obtained results allowed selecting some strains with high joint resistance to two or more nematode species. These resistant strains can be combined and indicated for planting in areas with a high incidence of nematodes, and even more importantly, they can be used as sources of resistance to the four different species in common bean breeding programs.

CONCLUSIONS

Among 81 common bean genotypes, 7 were classified as resistant to *Heterodera glycines* (BRS Esteio, BRS Notável, BRSMG Majestoso, BRSMG Pioneiro, CNFC11954, CNFP10103, Xamego), 15 to *Meloidogyne incognita* (Aporé, BRS Embaixador, BRS Esplendor, BRS Grafite, BRS Notável, BRS Requinte, BRSMG Majestoso, BRSMG Pioneiro, CNFC 10762, CNFC 11954, CNFP 11984, IAC Alvorada, IPR Eldorado, RP 1, Rudá) and 8 to *M. javanica* (Aporé, BRS Esteio, BRS Pontal, BRS Requinte, BRSMG Talismã, CNFC 10762, CNFP 10103, CNFP 10794), remaining unclear if the two genotypes found to be resistant to *Pratylenchus brachyurus* (IPR Tangará and Light Red Kidney) are in fact resistant.

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