Research Article

Assessment of *Phaseolus vulgaris* genotypes for resistance to *Meloidogyne incognita*[1]

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ABSTRACT

Several nematodes have been reported to damage common bean, being the main one the root-knot nematode (*Meloidogyne incognita*). This study aimed to evaluate the reaction of 20 common bean genotypes against the infestation of *M. incognita*, in an experiment conducted under greenhouse conditions, in a completely randomized design. The plants were inoculated with 4,000 eggs + J2 of *M. incognita* and, at 60 days after inoculation, they were removed for evaluation of root and shoot fresh mass, nematodes per plant and per gram of root, and reproduction factor. The BGF0011762, BGF0011854, BGF0011861, BGF0011862, BGF0011987, BGF0012533, BGF0013294, BGF0013355, BGF0013875 and BGF0013955 genotypes were classified as resistant. The principal component analysis explained 91.22 % of the variability among the nematode reproduction variables (nematode per plant and per gram of root, and reproduction factor) and the common bean phenotypic variables. The root and shoot fresh mass showed a high correlation between themselves, but also manifested an inverse relationship with nematodes per plant and nematode density population.

KEYWORDS: Common bean, root-knot nematode, genetic resistance.

RESUMO

Avaliação de genótipos de *Phaseolus vulgaris* quanto à resistência a *Meloidogyne incognita*

Vários nematoides têm sido relatados causando danos ao feijoeiro comum, sendo o principal deles o das galhas (*Meloidogyne incognita*). Objetivou-se avaliar a reação de 20 genótipos de feijão frente à infestação por *M. incognita*, em experimento conduzido em casa-de-vegetação e delineamento inteiramente casualizado. As plantas foram inoculadas com 4.000 ovos + J2 de *M. incognita* e, aos 60 dias após a inoculação, retiradas para avaliação da massa fresca da raiz e da parte aérea, nematoides por planta e por grama de raiz e fator de reprodução. Os genótipos BGF0011762, BGF0011854, BGF0011861, BGF0011862, BGF0011987, BGF0012533, BGF0013294, BGF0013355, BGF0013875 e BGF0013955 foram classificados como resistentes. A análise de componentes principais explicou 91,22 % da variabilidade entre as variáveis de reprodução do nematoide (nematoide por planta e por grama de raiz e fator de reprodução) e as variáveis fenotípicas do feijoeiro. A massa fresca da raiz e da parte aérea apresentaram alta correlação entre si, mas também relação inversa com os nematoides por planta e densidade populacional de nematoides.

PALAVRAS-CHAVE: Feijoeiro comum, nematoide das galhas, resistência genética.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most economically important vegetables cultivated worldwide (Machado et al. 2017), being an excellent source of important nutrients such as iron, phosphorus, magnesium, zinc, calcium, potassium and vitamins (Mojica & Mejía 2015). Dry bean is a vital food source for the Brazilian population, especially in low-income groups, where it is the main source of protein in the diet (Souza et al. 2016).

It is cultivated in nearly 36 million hectares worldwide, with a total annual production of about 28 million tons, and India, Brazil, USA and China being the major world producers (FAO 2022).

Brazil is one of the largest global producers and consumers of edible dry bean (USDA 2019), with a relatively constant production at around 2,966.9 thousand tons, distributed in three crops/seasons per year. A cultivated area of 2,743.7 thousand hectares of common bean is expected for the 2023/2024 harvest (Conab 2023).

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The crop is cultivated under different seasons and conditions in Brazil. The growing season is divided into first (rainy season, with sowing carried out between August and December), second (dry season, sowing between January and April) and third (winter season, sowing between May and July) (Mora & Brito 2015). The first one presents a greater productive potential due to the greater water availability, closer to the ideal temperature for the crop and higher radiation than in the dry season (Zanella et al. 2019).

P. vulgaris yield is compromised due to pests, diseases and weeds (Machado et al. 2017). Several nematodes have been reported to damage this species (Bonfim Junior et al. 2021). Under favourable conditions, nematodes attack leads to the development of common bean diseases; for example, the *Meloidogyne* genus can cause yield losses of up to 90 % (Santos et al. 2022).

Root-knot nematodes stand out as the main plant pathogens, and one of the most important among the plant parasitic genera (Ravichandra 2018). *Meloidogyne* spp., which has over 100 species, is the most damaging in agriculture soils (Trinh et al. 2019), causing an estimated loss of US$ 100 billion per year worldwide (Singh et al. 2015). This group has a high reproductive potential and an extensive range of host plants, what makes their control difficult (Onkendi et al. 2014). Therefore, a range of alternative techniques are needed to replace chemical control, such as the use of resistant genotypes (Hegazy et al. 2019, Heflish et al. 2021).

The main control measures for plant pathogenic nematodes are the use of resistant varieties, cultural practices, nematicides and biological control (Dutta et al. 2019, Lopes et al. 2019). Obtaining bean varieties resistant to root-knot nematodes is of fundamental importance for the sustainability of their production chain (Machado et al. 2017). Forghani & Hajihassani (2020) complement that resistant cultivars, when available, are the most promising tool in managing nematodes. However, Oliveira et al. (2018) emphasize that resistant sources must be identified, measured and quantified to produce new cultivars that can keep the population at low levels.

Considering the importance of common bean for food security in several countries, and the impact of root-knot nematode on this crop, some studies have reported the reaction of common bean genotypes to *M. incognita* [Alves et al. (2011), Stirling et al. (2011), Santos et al. (2012), Machado et al. (2017), Oliveira et al. (2018), Waceke (2018), Costa et al. (2019), Kgabo et al. (2019), Brida et al. (2020), Bhuiyan & Garlick (2021), Dias et al. (2023)]. Thus, this study aimed to phenotypically evaluate 20 common bean accessions widely genotyped for resistance to *M. incognita*.

MATERIAL AND METHODS

The experiment was carried out under greenhouse conditions at the Universidade Federal de Goiás, in Goiânia, Goiás state, Brazil (16º35'52.1"S and 49º16'53.5"W), from January to March 2022, testing 20 common bean genotypes of Mesoamerican origin belonging to the Embrapa Arroz e Feijão core collection (16º30'20.4"S and 49º16'55.4"W) of the commercial classes carioca, preto, rajado, branco, jalo, mulatinho, cranberry, dark red kidney, purple, dalima, pink and red, for reaction to *M. incognita* nematode (Table 1).

Substrate composed of a mixture of soil and sand in a 1:1 (v/v) ratio was autoclaved (120 ºC, for 40 min) and distributed in plastic containers with capacity of 500 mL, where the bean seeds were sown. The trial was installed in a completely randomized design. The average minimum and maximum temperatures in the greenhouse and the soil temperature were 22, 39 and 29 ºC, respectively.

Embrapa has a group of 340 bean accessions of Mesoamerican origin that are part of the Embrapa's Brazilian Common Bean Core Collection genotyped with approximately 17,000 single nucleotide polymorphisms (Valdisser et al. 2017). These genotypes have not been investigated before for reaction against infestation of plant parasitic nematodes. The selection of the 20 genotypes was done by random selection, based on seeds availability. The majority of the selected genotypes has a characteristic indeterminate stem growth, with only one showing a determinate stem growth habit (Table 1). The genotypes were replicated seven times.

The *M. incognita* inoculum was produced in a greenhouse by multiplying in tomato plant. The identification and confirmation of the nematode species were performed through analysis of esterase phenotypes (Carneiro & Almeida 2001), while the extraction of nematodes to prepare the inoculum was

Table 1. Description of common bean genotypes from the Embrapa Arroz e Feijão core collection tested for resistance to *Meloidogyne incognita*.

Number	Genotype	Accession cod	Stem habit	Grain color	M100[a]
1	Guapo Brilhante	BGF0011743	Indeterminate	Black	14.50
2	Roxinho e Chumbinho	BGF0011762	Indeterminate	Pink	17.50
3	Roxo	BGF0011772	Indeterminate	Purple	20.93
4	Preto	BGF0011854	Indeterminate	Black	19.83
5	Rosa	BGF0011861	Indeterminate	Pink	26.49
6	Preto	BGF0011862	Indeterminate	Black	21.43
7	Mulatinho	BGF0011932	Indeterminate	Brown	21.97
8	Paraná	BGF0011987	Indeterminate	Brown	24.69
9	Café	BGF0012054	Determinate	Yellow	22.82
10	Roxinho	BGF0012148	Indeterminate	Pink	25.27
11	Mulatinho	BGF0012533	Indeterminate	Beige	27.73
12	Cafezinho	BGF0012685	Indeterminate	Yellow	26.68
13	Rajadinho	BGF0012734	Indeterminate	Beige	21.68
14	Catarina	BGF0013010	Indeterminate	Black	21.74
15	Chumbinho	BGF0013294	Indeterminate	Black	24.74
16	Paranazinho	BGF0013343	Indeterminate	Pink	23.61
17	Carioca	BGF0013355	Indeterminate	Beige	29.73
18	DE	BGF0013561	Indeterminate	Beige	31.80
19	Rajado	BGF0013875	Indeterminate	Purple	26.39
20	Carioca	BGF0013955	Indeterminate	Beige	29.14

[a] M100: mass of 100 grains (g) at 12-14 % of moisture content.

carried out according to Coolen & D'Herde (1972). The inoculum suspension contained 4,000 eggs + J2 plant^{-1}, and the seedlings inoculation took place at 7 days after their emergence.

At 60 days after inoculation, the plants were removed from the plastic containers for evaluation. Each plant's root system was washed, dried on paper towel, weighted and recorded as fresh root mass. Similarly, the shoot of each plant was weighted and recorded as fresh shoot mass. The roots were then cut into pieces of 2.0 cm of length and grinded in a blender for 45 sec, in a 0.5 % sodium hypochlorite solution (Coolen & D'Herde 1972). The suspension was poured over two stacked sieves [100 mesh (0.074 mm) and 500 mesh (0.028 mm)] and washed with abundant tap water. The material caught on the 500-mesh sieve, consisting of an egg suspension and some small particles, was separated by centrifugal floatation. First, 5 cm^3 of kaolin were added to each tube, which was calibrated with water on a scale (0.001 g precision), and then centrifuged for 5 min at 1,800 rpm. After removal from the centrifuge, the liquid was discarded from each tube. Then, a sucrose solution (454 g of sugar completed with 1 L of water) was added to the precipitate, calibrated and weighted on the scale. They were then centrifuged for 1 min at 1,800 rpm. The supernatant was poured into a 500-mesh sieve and washed thoroughly with water several times to remove the sucrose excess (Coolen & D'Herde 1972). Finally, the eggs found in each root system were counted under an optical microscope (40x magnification), with the aid of a Peter's chamber. The total number of nematodes found in the total root volume corresponded to the final population.

The reproduction factor (RF) was calculated by dividing the final population (nematodes plant^{-1}) by the initial population (inoculated nematodes population). The genotypes were classified according to Oostenbrink (1966), and the genotypes with RF ≥ 1 were considered susceptible, whereas those with RF < 1 were considered resistant. The nematodes population density (nematodes g^{-1} of root) was also calculated by dividing the final population (nematodes plant^{-1}) by the root fresh mass.

The means of the variables root and shoot fresh mass, nematodes final population and population density were transformed into Box Cox equation, and analysis of variance was conducted for each one. The averages were grouped by the Scott-Knot test at 5 % of probability, using the statistical software RStudio version 4.1.3 (R Core Team 2016). The principal component analysis (PCA) was conducted to obtain the visual correlations among the evaluated variables.

RESULTS AND DISCUSSION

Regarding the phenotypic variables evaluated (Figure 1), it was observed that the BGF0012734 genotype recorded the highest fresh mass (11.0 g), followed by BGF0012054 (9.53 g). BGF0013875 exhibited the lowest root fresh mass (2.16 g).

BGF0012734 had the highest value for shoot fresh mass (10.8 g), followed by BGF0011861 (8.62 g) and BGF0013010 (8.04 g), while BGF0013875 recorded the lowest shoot fresh mass (3.02 g), followed by BGF0013355 (3.05 g). The values were separated into two groups (Figure 2).

Analysing the nematological variables, it was possible to observe that all common bean genotypes hosted *M. incognita*. BGF0012734 recorded the highest number for nematode final population, followed by BGF0013561 and BGF0012685, while BGF0011854 recorded the lowest number of eggs + J2 plant^{-1}, followed by BGF0013875 and BGF0011861. For this variable, three groups were formed according to the Scott-Knott test (Figure 3).

BGF0011743 showed the highest population density (1,275 nematodes g^{-1} of root), followed by BGF0012685, BGF0012148 and BGF0011932, while

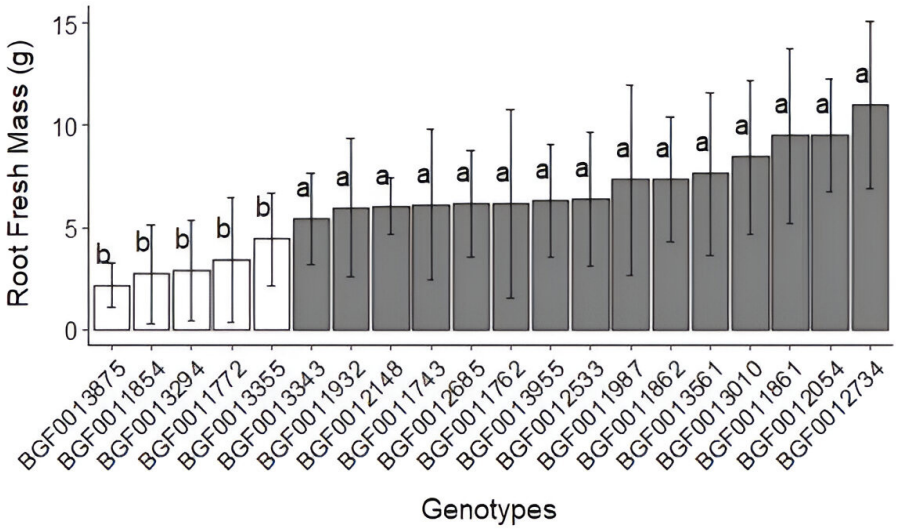

Figure 1. Root fresh mass of 20 common bean genotypes inoculated with *Meloidogyne incognita*. Means followed by the same letter do not differ by the Scott-Knot test at 5 % of probability.

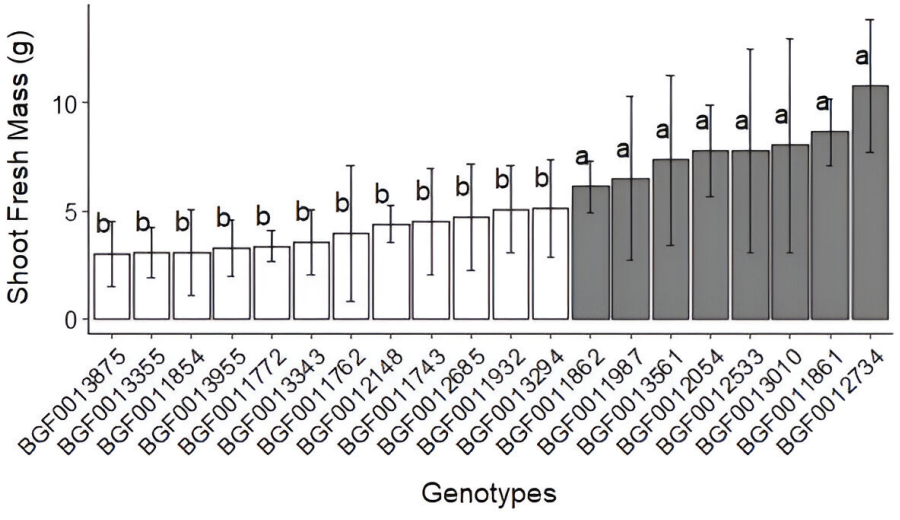

Figure 2. Shoot fresh mass of 20 common bean genotypes inoculated with *Meloidogyne incognita*. Means followed by the same letter do not differ by the Scott-Knot test at 5 % of probability.

BGF0011861 recorded the lowest population density (333 nematodes g^{-1} of root) (Figure 4).

In relation to the reproduction factor, according to the classification criteria proposed by Oostenbrink (1966), BGF0011772, BGF0011932, BGF0012054, BGF0012148, BGF0012685, BGF0012734, BGF0013010, BGF0013343 and BGF0013561 were classified as susceptible, with a reproduction factor greater or equal to 1 (Figure 5), whereas BGF0011762, BGF0011854, BGF0011861, BGF0011862, BGF0011987, BGF0012533, BGF0013294, BGF0013355, BGF0013875, BGF0011743 and BGF0013955 were classified as resistant, each one with a reproduction factor lower than 1 (Figure 5).

The principal component analysis explained 90.14 % of the variability, where the first and second components explained 58.57 and 31.57 % of the variances, respectively (Figure 6). A high correlation was observed between the variables root and shoot fresh mass, and these variables did not present correlations with nematode reproduction variables. However, BGF0012054, BGF0012734, BGF0013010 and BGF0013561

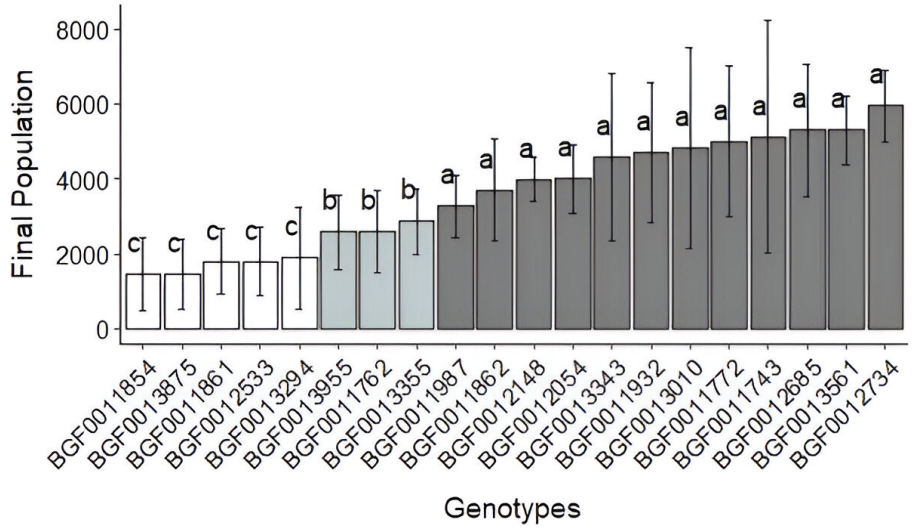

Figure 3. Final population (nematodes $plant^{-1}$) of 20 common bean genotypes inoculated with *Meloidogyne incognita*. Means followed by the same letter do not differ by the Scott-Knot test at 5 % of probability.

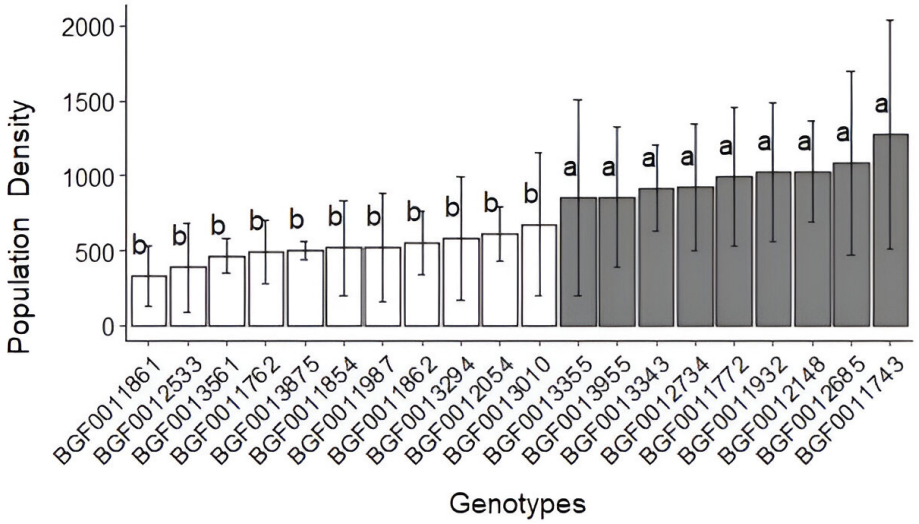

Figure 4. Population density (nematodes g^{-1} of root) of 20 common bean genotypes inoculated with *Meloidogyne incognita*. Means followed by the same letter do not differ by the Scott-Knot test at 5 % of probability.

showed correlation, concerning root and shoot fresh mass. BGF0011854, BGF0013294, BGF0013355, BGF0013875 and BGF0013955 showed an inverse correlation with root and shoot fresh mass. Nematode reproduction variables (final population, population density and reproduction factor) were highly correlated positively, but presented an inverse correlation with the classification of genotypes, considering resistance. BGF0011743, BGF0011772, BGF0011932, BGF0012148, BGF0012685 and BGF0013343 showed correlation with nematode reproduction variables (nematodes plant^{-1} and nematodes g^{-1} of root) and were also classified as susceptible. However, BGF0011762, BGF0011861, BGF0011862, BGF0011987 and BGF0012533 showed an inverse correlation with nematode

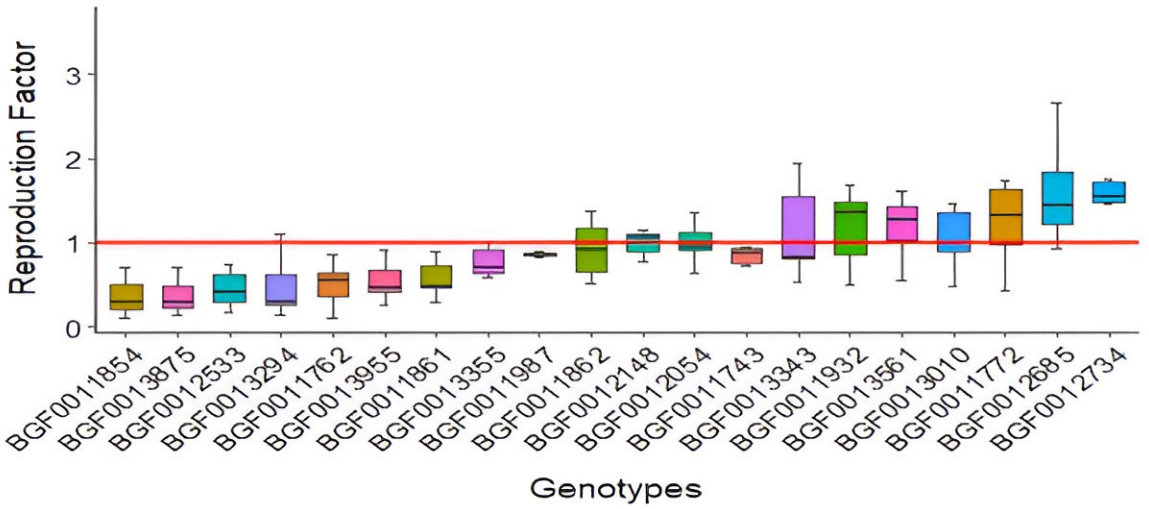

Figure 5. Reproduction factor of 20 common bean genotypes inoculated with *Meloidogyne incognita.*

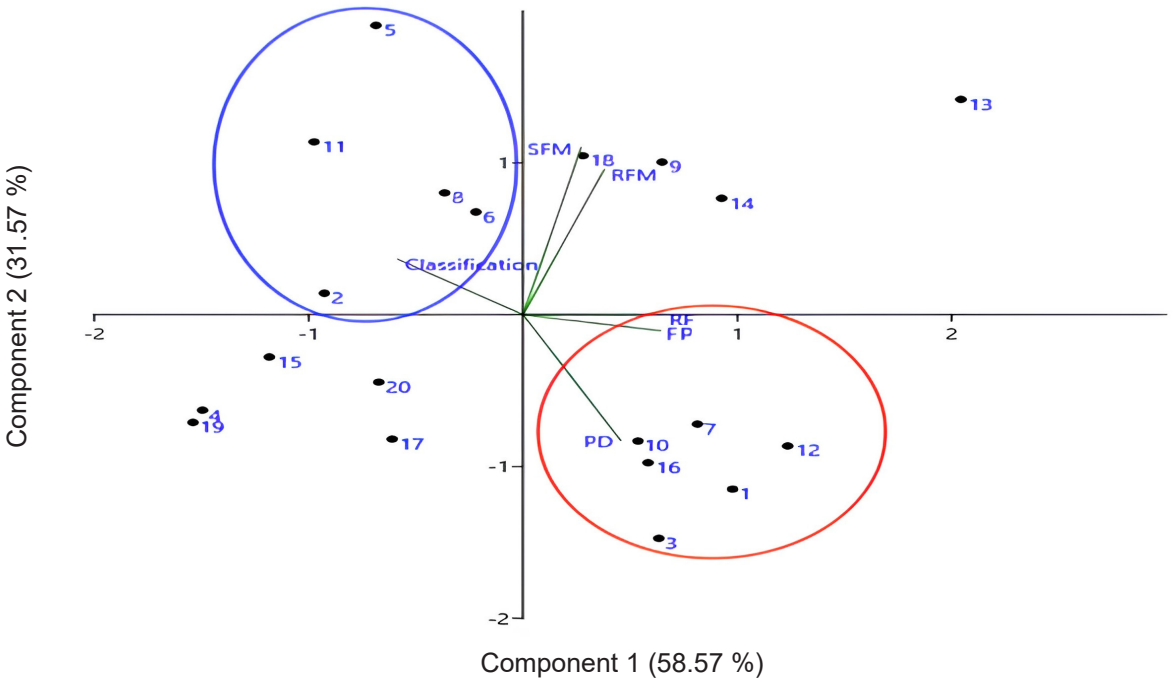

Figure 6. Principal component analysis showing the correlation among nematode reproduction variables (FP: final population; PD: population density; RF: reproduction factor) and common bean phenotypic variables (RFM: root fresh mass; SFM: shoot fresh mass).

reproduction variables, and were classified as resistant genotypes.

Some bean genotypes exhibited significantly high root and shoot weight, even in the presence of the root-knot nematode, agreeing with Stirling et al. (2011), who observed that shoot and root biomass can be used to assist the comparison between accessions. The same was observed for the phenotypic variable final nematode population, showing that the isolates do not respond in the same way, regarding nematode reproduction. Machado et al. (2017) reported that all genotypes tested for *M. incognita* were susceptible, although great phenotypic variations were observed. This result agrees with Bhuiyan & Garlick (2021), who stated that nematode eggs plant^{-1} have been widely used to measure the reproductive capabilities of nematodes on plant resistance screening trials. Regarding the number of nematodes g^{-1} of root, with half of the evaluated genotypes exhibiting a lower population density, Bhuiyan & Garlick (2021) also related this observation to the resistance of the bean genetic material.

As previously mentioned, studies evaluating the genotype resistance of dry bean plants are extremely necessary for management and genetic breeding programs for root-knot nematodes that can cause major damage to the crop. Following the resistance classification proposed by Oostenbrink (1966), 11 of the 20 evaluated genotypes were considered resistant to the root-knot nematode. This investigation corroborates Alves et al. (2011), that indicated a variation in responses of 33 individual bean genotypes against *M. incognita* race 1 population from Brazil. Waceke (2018) and Kgabo et al. (2019) also reported that common bean lines varied in reaction to root-knot nematode infestation. More recently, Dias et al. (2023) evaluated 81 common bean accessions against phytonematodes, describing that only 15 are resistant to *M. incognita*. On the other hand, Santos et al. (2012), evaluating six bean genotypes, reported that none behaved as resistant to root-knot nematode. Costa et al. (2019) observed that 26 bean genotypes challenged with *M. incognita* were susceptible. Finally, two of seven evaluated bean genotypes of *P. vulgaris* were described as resistant to this pathogen by Brida et al. (2020).

The principal component analysis revealed no significant correlation of nematode (*M. incognita*) reproduction variables (nematode final population, population density and reproduction factor) with the evaluated phenotypic variables (root and shoot fresh mass). Bhuiyan & Garlick (2021) observed non-significant linear correlations for nematode final population (eggs plant^{-1}) in sugarcane accession lines in all their trials. Similarly, Oliveira et al. (2018) reported no significant linear correlation between nematode eggs plant^{-1} and shoot mass among common bean genotypes infested with *M. javanica*. However, it was observed that some genotypes manifested a high root and shoot fresh mass, while others showed an inverse correlation with root and shoot fresh mass. These findings corroborate Oliveira et al. (2018), who observed that some common bean cultivars have a high shoot mass and low root-knot nematode reproduction, while some manifested low shoot mass and high nematode reproduction. According to them, this divergence between shoot mass and nematode reproduction leads to the idea of a possibility of non-relationship between these variables.

CONCLUSIONS

The BGF0011762, BGF0011854, BGF0011861, BGF0011862, BGF0011987, BGF0012533, BGF0013294, BGF0013355, BGF0013875, BGF0011743 and BGF0013955 *Phaseolus vulgaris* genotypes are resistant to *Meloidogyne incognita*. The phenotypic variables presented inverse correlation with knot-root nematode reproduction variables. However, varying responses were visible among the evaluated common bean genotypes.

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