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Chickpea genotype resistance to *Meloidogyne javanica* and *Pratylenchus brachyurus* in field conditions

Abstract — The objective of this work was to evaluate the resistance of chickpea (Cicer arietinum) genotypes to the root-knot (Meloidogyne javanica) and root-lesion (Pratylenchus brachyurus) nematodes, as well as to verify how these species affect this crop in field conditions. The experiment was carried out in the Cerrado biome in the state of Goiás, Brazil, in a naturally infested area. The BRS Aleppo, Cícero, BRS Cristalino, BRS Toro, and BRS Kalifa cultivars and the Jamu 96 genotype were evaluated. The assessed variables were: nematode reproduction factor, fresh mass of the aerial part of the plant and of the root system, dry matter of the aerial part of the plants, total dry matter of the plants, chlorophyll index, height of the plants, and grain yield. Pratylenchus brachyurus multiplies more than M. javanica, decreasing root fresh matter, plant height, and grain yield. All genotypes are resistant to M. javanica, especially Jamu 96 and cultivar BRS Kalifa. Jamu 96 and cultivar Cícero are susceptible to P. brachyurus. BRS Kalifa is the cultivar most resistant to both nematodes and shows the highest grain yield.

Index terms: *Cicer arietinum*, pulses, root-knot nematode, root-lesion nematode.

Resistência de genótipos de grão-de-bico a *Meloidogyne javanica* e *Pratylenchus brachyurus* em condições de campo

Resumo — O objetivo deste trabalho foi avaliar a resistência de genótipos de grão-de-bico (*Cicer arietinum*) aos nematoides da galha (*Meloidogyne javanica*) e das lesões (*Pratylenchus brachyurus*), bem como verificar como estas espécies afetam essa cultura em condições de campo. O experimento foi realizado no bioma Cerrado, no estado de Goiás, Brasil, em área naturalmente infestada. Foram avaliados as cultivares BRS Aleppo, Cícero, BRS Cristalino, BRS Toro e BRS Kalifa e o genótipo Jamu 96. As variáveis avaliadas foram: fator de reprodução dos nematoides, massa fresca da parte aérea e do sistema radicular, matéria seca da parte aérea, matéria seca total das plantas, índice de clorofila, altura das plantas e rendimento de grãos. *Pratylenchus brachyurus* se multiplica mais do que *M. javanica* e diminui massa fresca da raiz, altura da planta e rendimento de grãos. Todos os genótipos são resistentes a *M. javanica*, principalmente Jamu 96 e a cultivar BRS Kalifa. Jamu 96 e a cultivar Cícero são suscetíveis a *P. brachyurus*. BRS Kalifa é a cultivar mais resistente a ambos os nematoides e apresenta maior rendimento de grãos.

Termos para indexação: *Cicer arietinum*, pulses, nematoide-das-galhas, nematoide-da-lesão-da-raiz.

Introduction

Chickpea (*Cicer arietinum* L.) is the fourth most important legume grown worldwide, behind the soybean (*Glycine max* L.), peanut (*Arachis hypogaea* L.), and bean (*Phaseolus vulgaris* L.) crops (FAO, 2019). It is used mainly for the production of flour, and its mature seeds have a high content of carbohydrates and proteins, with a very high digestibility (Arooj et al., 2021). There have been reports of its cultivation in 55 countries, with a total production of 14.25 million tons and an average yield of around 1.4 Mg ha⁻¹ (FAO, 2019).

In Brazil, the domestic consumption and production of chickpea are considered low. However, in recent years, due to the species nutritional characteristics and to changes/trends in the patterns of food consumption, the demand for the grain has grown (Avelar et al., 2018; Fonseca et al., 2020). Despite this, it is still necessary to import a large part of the quantity consumed in the domestic market (Fonseca et al., 2020), which reaches values close to 8,000 tons per year (Avelar et al., 2018). An economic option to increase national production is planting chickpea in the second summer harvest in the Brazilian Cerrado biome, using a small amount of rainfall (Silva et al., 2021), which is possible since the species shows drought tolerance (Arif et al., 2021).

However, the cultivation of the crop can be challenging due to the more than 50 pathogens that may affect it, and the main diseases are caused by fungi and nematodes, which often make production in infested areas unfeasible (Nathawat et al., 2020).

The annual losses caused by nematodes worldwide are estimated to be around 14% (Zwart et al., 2019). After nematode infestation, roots are damaged, losing their functionality, a process that leads to poor plant growth and a reduction in yield, also decreasing the ability of the plants to cope with abiotic stresses, such as drought (Zwart et al., 2019), and to absorb nutrients from the soil (Gilarte et al., 2020).

Many species of nematodes can damage chickpea around the world. However, the main problems are caused by: root-knot nematodes, such as *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, and *Meloidogyne artiellia* Franklin, 1961; the cyst nematode, *Heterodera ciceri* Vovlas, Greco & Di Vito, 1985; and the root-lesion nematode, *Pratylenchus* spp., especially *Pratylenchus brachyurus* (Godfrey, 1929)

Filipjev & Schuurmans Stekhoven, 1941 (Zwart et al., 2019).

Among the most widespread methods for the control of plant-parasitic nematodes are nematicides, crop rotation, antagonistic plants, plant leaf extracts, fallow, soil fumigation, solarization, and genetic resistance (Kumar et al., 2020). For chickpea, crop rotation with nonhost crops for a period that allows the decomposition of infested crop residues reduces pathogen viability and association, especially under no-tillage, also enhancing the population and activity of beneficial soil organisms and minimizing the impact of root diseases (Gilarte et al., 2020; Kumar et al., 2020). However, none of these practices are fully effective when used isolated, showing that the association of more than one control method is most often the best option.

Another key method is the use of cultivars with genetic resistance, which has grown in the last decade, particularly aiming reductions in the risk of environmental contamination and in costs and time for the implementation of other management techniques (Kumar et al., 2020; Lala et al., 2020). To obtain genetically resistant varieties, it is necessary to use accessions that may contain resistance genes for later introgression (Behmand et al., 2020; Coyne et al., 2020). However, although many characterization studies have been carried out in several parts of the world, it is still difficult to find sources of resistance, as most genotypes are normally susceptible (Zwart et al., 2019; Lala et al., 2020). Despite these constraints, there are a few reports of chickpea genotypes with nematode resistance (Ansari et al., 2004; Chakraborty et al., 2016; Sumita, 2017; Lala et al., 2020), justifying the continuity of research in this area. In this context, it is important to select high yield and resistant cultivars (Kumar et al., 2020; Gautam et al., 2021) that can be adopted by the production sector.

Obtaining uniformly inoculated field areas is also a challenge, which explains why most of the studies are carried out in pots under controlled conditions (Lala et al., 2020). However, this type of environment does not allow observing the plant's reaction to nematodes associated with its potential grain yield.

The objective of this work was to evaluate the resistance of chickpea genotypes to the root-knot and root-lesion nematodes, as well as to verify how these species affect this crop in field conditions.

Materials and Methods

The experiment was carried out in the Cerrado biome, in the municipality of Urutaí, in the state of Goiás, Brazil, during the second growing season of 2018, in a field area (17°29'10"S, 48°12'38"W, at 697 m of altitude) naturally infested with *M. javanica* and *P. brachyurus*. The climate of the region, according to the classification of Köppen-Geiger, is of the Cwb type, tropical, with a dry winter and a rainy summer. During the experiment, the average temperature was 25°C and the average annual precipitation was 1,677 mm. The soil of the area is classified as a Latossolo Vermelho ácrico típico, according to the Brazilian system of soil classification (Santos et al., 2018), i.e., an Oxisol.

The BRS Aleppo, Cícero, BRS Cristalino, BRS Toro, and BRS Kalifa chickpea cultivars, as well as the Jamu 96 genotype, all from the germplasm bank of Embrapa Hortaliças, were evaluated. The experiment was conducted in a randomized complete block design, with four replicates. Each plot was composed of six planting rows, with 3 m of length, spaced at 0.5 m from each other, with 13 seeds per linear meter. Initially, the soil chemical analysis was carried out 15 days before the infurrow application of 28 kg ha⁻¹ N, 70 kg ha⁻¹ P₂O₅, 63 kg ha⁻¹ K₂O, 6.8 kg ha⁻¹ Cu, 16 kg ha⁻¹ S, and 0.7 kg ha⁻¹ Zn. The area was prepared for planting by plowing and disk harrowing, followed by sowing on March 14, 2018.

After the area was prepared for planting, samples were collected to quantify the initial population of M. javanica and P. brachyurus in the soil. Three simple soil samples were collected from each plot at a depth of 0-20 cm and placed in a container, from where a 400 g soil sample was taken. This sample was packed in plastic bags and sent to the Nematology Laboratory of Instituto Federal Goiano, located in the state of Goiás, Brazil, where it was identified, extracted, and quantified. For the identification of M. javanica, the perineal region of adult females was cut, based on the patterns described by Eisenback & Triantaphyllou (1991). For the analysis of the phenotype of the esterase isoenzyme, the technique proposed by Carneiro & Almeida (2001) was used. The nematode species that caused root lesions was identified as P. brachyurus according to Gonzaga et al. (2016). Nematodes were extracted using the centrifugal fluctuation technique in sucrose solution, following the methodology of Jenkins (1964). After their extraction, nematodes were quantified using Peters counting chamber and visualized under a stereoscopic microscope.

For all genotypes and plots, at 90 days after sowing (DAS), soil samples and roots were collected, constituting the final population. Three plants were collected per plot for this quantification, totaling 12 plants per genotype. Nematodes were extracted from the root samples according to the methodology of Coolen & D'Herde (1972) and from the soil samples according to Jenkins (1964). The calculation of the reproduction factor (RF) of M. javanica and P. brachyurus was performed using the equation: (RF = Fp / Ip), where Fp is the final population in the soil + final population in the root system, and Ip is the initial population in the soil. The grading scale described by Oostenbrink (1966) was used to classify the genotypes, as: immune (RF = 0), resistant (RF < 1), and susceptible (RF > 1). The percentage of control for each plant-parasitic nematode was obtained by the arithmetic mean of the RF in the soil and in the roots relative to RF = 1.

The fresh mass of the aerial part of the plant, the fresh mass of the roots, and the dry matter of the aerial part of the plant were also evaluated at 90 DAS. For this, plants were placed in paper bags, which were taken to an oven, at 60°C, until reaching a constant mass. The percentage of plant dry matter was calculated using the following equation: (APDM/APFM)×100, where APDM is the dry matter of the aerial part of the plant, and APFM is the fresh mass of the aerial part of the plant. The height (cm) of each genotype, an important characteristic associated with mechanical harvest, was measured from the ground level up to the top of the main stem of ten representative plants per plot, totaling 40 plants per genotype.

The chlorophyll index was also determined at 90 DAS, using the electronic meter ClorofiLOG CFL 1030 (Falker Automação Agrícola Ltda., Porto Alegre, RS, Brazil); three frequency ranges of light were analyzed in the measurement, through absorption ratios of different frequencies (Barbieri Junior et al., 2012). The evaluation was carried out by sampling leaves located in the middle portion of the canopy of three plants from each plot/replicate, totaling 12 plants per genotype. That variable is important to analyze the development of genotypes and their response related to their photosynthetic potential.

At 110 DAS, when the 200 remaining plants in each plot in the field reached physiological maturity, yield (Mg ha⁻¹) was evaluated by weighing the grains at 13% humidity; a total of 800 plants per genotype were assessed.

The data were subjected to the analysis of variance and grouping of treatment means by Scott-Knott's test, at 5% probability, using the Genes statistical software (Cruz, 2016). To verify the possible effect of nematodes on plant development and the relationship between plant characters, a simple correlation analysis was performed between characters.

Results and Discussion

All evaluated genotypes were resistant to *M. javanica*, showing a reproduction factor (RFMJ) lower than 1 (Oostenbrink, 1966), with an average value of 0.58 (Table 1). *Pratylenchus brachyurus* multiplied better in chickpea, with an average reproduction factor (RFPB) of 0.68. Jamu 96 was the genotype with the greatest susceptibility to *P. brachyurus*, with a RFPB of 1, which enabled the initial population of the nematode to remain stable. On cultivars BRS Kalifa and BRS Toro, the population of this nematode showed a greater reduction, mainly in the soil with a lower final population of *P. brachyurus*. The opposite was verified for the Cícero cultivar, which was the most susceptible to *M. javanica* (RFMJ = 0.84) and also one of the most susceptible to *P. brachyurus* (RFPB = 0.95) (Table 1).

Cultivar Cícero showed the highest value for the chlorophyll index, and Jamu 96, the lowest (Table 2). Jamu 96 was also grouped among the genotypes with the lowest plant height, root fresh mass, and fresh mass

of the aerial part of the plant; however, it presented the highest percentage of plant total dry matter. Genotypes with a higher plant height also showed a greater fresh root weight, a coincidence that was not observed for the fresh mass of the aerial part of the plant. BRS Kalifa was the only cultivar that had, at the same time, a higher plant height and a greater fresh weight of roots and aerial part, being the most productive, with a yield of 2.29 Mg ha⁻¹ (Table 2).

In the Brazilian Cerrado biome, studies regarding the yield of chickpea genotypes are still scarce, especially in areas infested by nematodes. Artiaga et al. (2015) evaluated the average yield of 15 chickpea genotypes under drought conditions in a nonirrigated area in Brasília, in Distrito Federal, over three planting seasons from January to March, and reported lower average yields of 0.44 and 0.21 Mg ha⁻¹, respectively, for Jamu 96 and cultivar Cícero, besides a maximum yield of 1.34 Mg ha⁻¹ for accession FLIP03-109C. However, both of these average grain yields and the one obtained in the present study are lower than those of other works also carried out in the Brazilian Cerrado, but in the state of Minas Gerais. Hoskem et al. (2017) found an average yield of 2.79 Mg ha⁻¹ for the Cícero cultivar in two locations in the state of Minas Gerais, between May and July, with higher yields in June. Likewise, Avelar et al. (2018) assessed the productivity of cultivar BRS Aleppo in two locations, also in the

Table 1. Evaluation of chickpea (*Cicer arietinum*) genotypes for resistance to the *Meloidogyne javanica* and *Pratylenchus brachyurus* nematodes in the second growing season of 2018, in the Cerrado biome, in the municipality of Urutaí, in the state of Goiás, Brazil⁽¹⁾.

Genotype	IPMJS	FPMJS	FPMJR	RFMJ ⁽²⁾	CPMJ ⁽³⁾	IPPBS	FPPBS	FPPBR	RFPB ⁽²⁾	CPPB(3)
'BRS Aleppo'	317.75	101.25a	126.50b	0.72b	56.82e	373.00	44.25a	193.25a	0.65c	67.59d
'Cícero'	317.75	33.50b	236.25a	0.84a	58.32d	373.00	60.50a	292.00a	0.95b	52.76e
'BRS Cristalino'	317.75	85.00a	123.75b	0.66c	66.95c	373.00	56.50a	173.00a	0.61c	69.33c
Jamu 96	317.75	65.50a	96.75b	0.50e	74.96a	373.00	89.50a	279.50a	1.00a	49.97f
'BRS Kalifa'	317.75	54.50b	46.25c	0.32e	84.23a	373.00	16.00b	153.00a	0.44c	87.88a
'BRS Toro'	317.75	83.25a	59.75c	0.45d	77.81b	373.00	28.25b	127.75a	0.43c	75.69b
General mean	317.75	70.50	114.91	0.58	69.85	373.00	49.17	203.19	0.68	67.20
CV (%)	-	15.24	15.49	19.31	15.21	-	19.46	21.25	15.23	11.50

(1) Means followed by equal letters, in the columns, do not differ by Scott-Knott's test, at 5% probability. Data were transformed by x+0.5. (2) Reproduction factor (RF) = final population/initial population, used to classify the genotypes as immune (RF = 0), resistant (RF < 1), and susceptible (RF > 1) (Oostenbrink, 1966). (3) Control percentage, obtained by the arithmetic mean of the RF in the soil and in the roots relative to RF = 1. IPMJS, initial population of *M. javanica* in the soil; FPMJR, final population of *M. javanica* in the roots; RFMJ, reproduction factor of *M. javanica*; CPMJ, control percentage of *M. javanica*; IPPBS, initial population of *P. brachyurus* in the soil; FPPBR, final population of *P. brachyurus* in the soil; FPPBR, final population of *P. brachyurus*; and CPPB, control percentage of *P. brachyurus*. CV, coefficient of variation.

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same state, observing yields of 4.2 Mg ha⁻¹ for sowing in May, 3.6 Mg ha⁻¹ in June, and 2.3 Mg ha⁻¹ in July. Grain yield is an important factor for the acceptance of cultivars by the production sector, as is resistance to nematodes; however, that trait varies according to region or environment conditions. Although, in the present study, the BRS Kalifa cultivar stood out with a yield of 2.29 Mg ha⁻¹, considered very good, all other cultivars presented an average yield lower

than that of those analyzed in other studies in Brazil, which is an indicative that higher yields can probably be achieved in other environments.

In general, there were no significant correlations between the higher reproduction of the *M. javanica* nematode and characters of the aerial part of the plants, such as chlorophyll index, dry or fresh matter, and grain yield. However, there were significant effects of *P. brachyurus* (Table 3), including reductions of -0.57

Table 2. Traits and grain yield of chickpea (*Cicer arietinum*) genotypes grown in soil naturally infested with the *Meloidogyne javanica* and *Pratylenchus brachyurus* nematodes in the second growing season of 2018, in the Cerrado biome, in the municipality of Urutaí, in the state of Goiás, Brazil⁽¹⁾.

Genotype	Chlorophyll index	Height (cm)	RFM (g)	APFM (g)	APDM (g)	DM (%)	GY (kg ha ⁻¹)
'BRS Aleppo'	28.46b	58.15a	4.22a	57.01b	17.31a	30.15b	1,200.83b
'Cícero'	63.16a	43.70b	3.29b	93.36a	26.30a	28.11b	1,464.29b
'BRS Cristalino'	29.71b	58.60a	5.02a	56.97b	16.72a	29.15b	998.96b
Jamu 96	17.00c	41.55b	2.15b	43.45b	15.96a	37.35a	1,524.88b
'BRS Kalifa'	33.57b	60.25a	5.46a	72.78a	22.93a	30.58b	2,293.11a
'BRS Toro'	31.52b	55.10a	5.38a	66.15b	18.87a	28.54b	1,661.33b
General mean	33.90	52.89	4.25	64.95	19.68	30.65	1,523.90
CV (%)	21.41	6.00	27.98	24.62	27.91	9.87	26.83

⁽¹⁾ Evaluated at 90 days after sowing: chlorophyll index; Height, plant height; RFM, root fresh mass; APFM, fresh mass of the aerial part of the plant; APDM, dry matter of the aerial part of the plant; and DM, total dry matter percentage. Evaluated at 110 days after sowing, i.e., at the end of the plant cycle: GY, grain yield. CV, coefficient of variation.

Table 3. Simple correlations among the characters evaluated in six chickpea (*Cicer arietinum*) genotypes grown in soil naturally infested with the *Meloidogyne javanica* and *Pratylenchus brachyurus* nematodes in the second growing season of 2018, in the Cerrado biome, in the municipality of Urutaí, in the state of Goiás, Brazil⁽¹⁾.

	FPMJR	RFMJ	CPMJ	FPPBS	FPPBR	RFPB	СРРВ	CI	Height	RFM	APFM	APDM	DM%	GY
FPMJS	-0.31	-0.03	-0.17	-0.01	-0.39	-0.37	0.39	-0.42	0.34	0.42	-0.20	-0.24	-0.07	-0.27
FPMJR		0.38	-0.42	0.30	0.39	0.36	-0.37	0.41	-0.42	-0.41	0.19	0.07	-0.23	-0.33
RFMJ			-0.97*	0.39	0.31	0.25	-0.23	0.37	0.00	-0.24	-0.12	-0.20	-0.15	-0.06
CPMJ				-0.38	-0.15	-0.34	0.23	-0.32	-0.03	0.19	0.11	0.18	0.13	0.07
FPPBS					0.47*	0.57*	-0.46*	-0.08	-0.57*	-0.41	-0.25	-0.15	0.34	-0.61*
FPPBR						0.85*	-0.80*	0.11	-0.35	-0.47*	-0.18	-0.12	0.27	-0.35
RFPB							-0.94*	0.11	-0.24	-0.55*	-0.27	-0.24	0.19	-0.28
CPPB								-0.15	0.37	0.55*	0.24	0.21	-0.21	0.34
CI									-0.20	-0.07	0.56*	0.40	-0.47*	0.15
Height										0.52*	-0.11	-0.16	-0.32	0.43*
RFM											0.35	0.27	-0.27	0.44*
APFM												0.94*	-0.22	0.36
APDM													0.10	0.23
DM														-0.45*

⁽¹⁾FPMJS, final population of *M. javanica* in the soil; FPMJR, final population of *M. javanica* in the roots; RFMJ, reproduction factor of *M. javanica*; CPMJ, control percentage of *M. javanica*; FPPBS, final population of *P. brachyurus* in the soil; FPPBR, final population of *P. brachyurus* in the roots; RFPB, reproduction factor of *P. brachyurus*; CPPB, control percentage of *P. brachyurus*; CI, chlorophyll index; Height, plant height (cm); RFM, root fresh mass (g); APFM, fresh mass of the aerial part of the plant (g); APDM, dry matter of the aerial part of the plant (g); DM, percentage of plant total dry matter; and GY, grain yield (kg ha⁻¹).

and -0.61 in plant height and grain yield, respectively, with a higher final population of the nematode in the soil and a reduction of -0.47 in root fresh mass with a higher final population in the roots. There was also a correlation between the higher RFPB and the lower root fresh mass of -0.55, as well as between the higher control percentage of this species of nematodes and the higher root fresh mass of 0.55, indicating a possible cause and effect relationship between the severity of this nematodes species and the development of plant roots, height, and grain yield.

A higher root fresh mass was also positively related to a higher plant height. The genotypes with the highest fresh mass in the aerial part and the highest values for the chlorophyll index presented the lowest percentages of plant total dry matter. Therefore, a higher grain yield was related to a higher plant height and a greater root fresh mass, but negatively related to the percentage of plant total dry matter (Table 3).

Similarly, Artiaga et al. (2015) reported a significant correlation of 0.61 between a higher grain yield and taller plants. The authors also found a correlation between the greater weight of the plant and a higher grain yield of 0.86, an association that was observed in the present work, but was not statistically significant. Thakur & Sirohi (2009) also verified a correlation of 0.43 between plant height and grain yield, as in the present study.

The correlation analysis does not consider separately the differential effects of the genotypes that are more resistant or more susceptible to nematodes. However, differences observed between cultivars Cícero and BRS Kalifa for the characters plant height, grain yield, and root fresh mass are an indicative that nematodes, mainly *P. brachyurus*, directly influence the development of plants.

A reduction in the fresh mass of the roots of the genotypes that are more susceptible to *M. javanica* was not observed in the analysis of correlations, probably because, although these nematodes damage roots, the greater formation of galls can increase root weight (Lala et al., 2020), as noted here. The effect of chlorosis on the leaves of the most susceptible genotypes (Zwart et al., 2019) was also not verified in the present study according to the analysis of the chlorophyll index.

Lala et al. (2020), when evaluating 60 chickpea genotypes in a greenhouse, concluded that none were highly resistant to *M. incognita* race 2, and that root

length, shoot length, fresh shoot weight, and dry shoot weight were negatively correlated with the root-knot index (r = -0.68, -0.77, -0.65, and -0.70, respectively), while fresh root weight and dry root weight were positively correlated with the index (r = 0.71 and 0.29, respectively). This indicates that the root-knot nematode decreased plant grow, but not root weight.

The lack of significant correlations of nematodes with the evaluated plant characters may have been due to the fact that most of the genotypes behaved as resistant to nematodes, since the final population of nematodes was generally smaller than the initial one, mainly for *M. javanica*.

Conclusions

- 1. The nematode species *Pratylenchus brachyurus* multiplies better in chickpea (*Cicer arietinum*) than *Meloidogyne javanica*, decreasing plant root fresh matter, plant height, and grain yield under field conditions in the Cerrado biome in the state of Goiás, Brazil.
- 2. All the evaluated chickpea genotypes cultivars BRS Aleppo, Cícero, BRS Cristalino, BRS Toro, and BRS Kalifa and genotype Jamu 96 are resistant to *M. javanica*, mainly Jamu 96 and BRS Kalifa.
- 3. Jamu 96 and cultivar Cícero are susceptible to *P. brachyurus*, with a reproduction factor equal to or close to 1.
- 4. BRS Kalifa is the most resistant cultivar to both studied nematodes and has the highest grain yield, being considered an option for the management of these species or as a source of resistance to be used in chickpea breeding programs.

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