

# **Reaction of soybean genotypes to the nematodes** *Meloidogyne incognita* and *M. javanica*

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Abstract Brazil is the world's largest producer and exporter of soybean and studies show that production will continue to grow in the coming years. However, this increase is considered a major challenge due to the serious damage and losses caused by nematodes. The use of resistant materials presents a sustainable alternative for suppressing them. Therefore, the objective was to evaluate the reaction of soybean genotypes to populations of Meloidogyne incognita and M. javanica. Two experiments were carried out for each species, on different dates, under greenhouse conditions, in a randomized block design with ten and twenty-two treatments, respectively, and four replicates. Plants were inoculated ten days after planting with a suspension of 2500 eggs and second-stage juveniles of M. incognita and M. javanica. Evaluations took place sixty days after inoculation (DAI), determining plant height, stem diameter, Spad index, leaf area index, reproduction factor and reproduction factor reduction. For the species M. incognita all genotypes were susceptible. The UFUL 592 and UFUL 298 genotypes had greater vegetative development

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M. A. dos Santos e-mail: maria.dos@ufu.br and the UFUL 526 genotype behaved as a good host for the nematode. For *M. javanica*, the UFUL 172 and UFUL 592 genotypes showed good performance in terms of growth parameters, as well as nematological ones. In general, the UFUL 592 genotype performed well in the four trials.

**Keywords** Genetic improvement · *Glycine max* · Nematodes · Root-knot nematodes

### Introduction

Soybean is the most important oilseed crop currently cultivated, both in terms of economic value and nutritional benefits. The area under soybean production has been steadily growing in recent years. Globally, in the 2022/23 crop season, 136.0 million hectares were planted, resulting in a production of 369.0 million tons (Embrapa, 2023).

The productivity of the crop is affected by soil, climate, and phytosanitary factors. The phytosanitary issues are several and can have serious impact on the crop productivity depending on the region and the pathogenic organism involved. Furthermore, the impact of climate change on agriculture has influenced the occurrence of these pathogens and, consequently, their management.

These changes can have both direct and indirect effects on agricultural productivity and on the pathogen itself (Fao, 2021). Considering this, these

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harmful organisms pose a challenge to global food security as they directly impact the quantity and quality of soybeans (Hampf et al., 2021). According to the FAO, the estimated losses caused by these organisms annually range from 20 to 40% in global agricultural production, which translates to an economic loss of around 220 billion dollars per year (Fao, 2019).

Among the pathogens, plant-parasitic nematodes have been gaining importance, causing severe damage and losses in soybean fields, and even rendering some cultivation areas unviable (Grigolli and Asmus, 2014). Plant nematodes of the genus *Meloidogyne* are considered the most significant due to their widespread geographic distribution and a wide range of hosts. The integrated management of nematodes is one of the challenges in controlling plant-parasitic nematodes in agricultural systems (Dias-Arieira and Puerari, 2019).

In this context, the use of resistant material represents an alternative for suppressing nematodes. However, the low availability of materials resistant to these organisms, coupled with low productive potential and/or restricted adaptability to specific producing regions, hinders the widespread adoption of these genotypes (Corte et al., 2014; Mazzetti, 2017). Additionally, the soybean cultivars that are resistant or moderately resistant to Meloidogyne incognita and *M. javanica* have limited genetic diversity as they descend from a single source of resistance: the American cultivar Bragg (Batista, 2012). Thus, the objective of this study was to evaluate, under controlled conditions, the reaction of soybean genotypes from the germplasm program developed by the Laboratory of Mycology and Plant Protection at the Federal University of Uberlândia (LAMIP/UFU), to populations of Meloidogyne incognita and M. javanica, as well as the effect of these nematodes on plant development.

#### Materials and methods

Experiment location, climate, and timing

The experiments were conducted under greenhouse conditions at the experimental area of the Institute of Agricultural Sciences (ICIAG) of the Federal University of Uberlândia (UFU), Umuarama Campus, in the Municipality of Uberlândia/MG, at the geographical coordinates of  $18^{\circ}53'01''$  S and  $48^{\circ}15'42''$  W, at an altitude of 833 m.

For each *Meloidogyne* sp., two experiments were conducted. The trials with the *M. incognita* and *M. javanica* isolates were carried out in a randomized complete block design with ten and twenty-two treatments, respectively, and four replicates. The experiments were conducted between September 23, 2021, and January 3, 2022, during the period from spring to summer.

The soil used for the experiments has been analysed for chemical and physical properties and subsequently sterilized using Bunema. Soil analysis interpretation and fertilization recommendation followed the guidelines of Alvarez et al. (1999). Liming and fertilizers (MAP and KCl) were applied and incorporated before soybean sowing (Table 1).

# Nematode subpopulation acquisition and multiplication

The isolates of *M. incognita* and *M. javanica* were provided by Inova Genética LTDA. The subpopulations of each isolate were recovered and maintained in the greenhouse on Santa Cruz Kada tomato variety and okra plants. The species of *M. incognita* and *M. javanica* were previously identified through electrophoretic analysis (Carneiro and Almeida, 2001), conducted at the Laboratory of Phytopathological Diagnosis—Nemafito.

Nematodes were extracted from the soil and roots using the centrifugation flotation method (Jenkins, 1964). The roots were initially rinsed under running water to remove excess soil, weighed, and then processed using the technique described by Hussey and Barker (1973), modified by Bonetti and Ferraz (1981), before being subjected to the flotation method.

# Soybean genotype response to *M. incognita* and *M. javanica*

For the *M. incognita* and *M. javanica* isolates, eight and twenty soybean genotypes were analyzed, respectively, for which no known reaction of resistance or susceptibility had been described. Two soybean varieties were used: BRS7980 and BMX Desafio, classified as resistant and susceptible, respectively (Table 2).

Table 1 Chemical and physical characteristics of the soil sample. Uberlândia-MG, 2021

Characteristi	cs Chemical								
pH (H <sub>2</sub> O)		Ca <sup>2+</sup>	Mg <sup>2+</sup>	Al <sup>3+</sup>	P-Mehlich	K <sup>+</sup>	H+Al	CTC	SB
1:2.5			$cmol_c dm^{-3}$		mg dm <sup></sup>	3		cmol <sub>c</sub> dm <sup>-3</sup>	
6.47	-	0.64	0.05	0.0	2.63	11.4	1.05	1.77	0.72
t	M.O	S	В	Cu	Fe	Mn	Zn	V	m
$\text{cmol}_{c} \text{ dm}^{-3}$	dag kg <sup>-1</sup>				mg dm <sup>-3</sup>			%	
0.72	0.52	28.6	0.38	0.27	46.6	4.60	0.58	40.68	0.0
Characteristi	cs physical								
Soil	Coarse sand	Fine sand	Silt			Clay		Textural Class	
				— g kg	g <sup>-1</sup>				
	594	225	62			119		Sandy-loam	

Water pH; Ca, Mg, Al = extractor (KCl 1 mol L<sup>-1</sup>); K = extractor (HCl 0.05 mol L<sup>-1</sup> + H<sub>2</sub>SO<sub>4</sub> 0.0125 mol L<sup>-1</sup>); P available = extractor Mehlich<sup>-1</sup>; S in calcium phosphate 0.01 mol L<sup>-1</sup>; H + Al = (Buffer solution – SMP a pH 7.5); Cu Fe, Mn, Zn = (DTPA 0.005 mol L<sup>-1</sup> + TEA 0.1 mol<sup>-1</sup> + CaCl<sub>2</sub> 0.01 mol L<sup>-1</sup> a pH 7.3). cmolc dm<sup>-3</sup> × 10 = mmolc dm<sup>-3</sup> / mg dm<sup>-3</sup> = ppm / dag kg<sup>-1</sup> = %; CTC a pH 7.0; V = Base saturation; m = Al saturation; M.O. = Organic matter. Source: EMBRAPA (2009) Source: LABAS, 2021

The genotypes under study were derived from crosses between BRS Caiapônia, IAC-100, BRS Santa Cruz, BRS Luziânia, Msoy 9350, UFUS Impacta, and Potenza genotypes in five different combinations (Table 2). These crosses were conducted in a greenhouse in 2007, resulting in the F1 generations. The advancement of generations continued over the years, ultimately leading to the use of the F8:9 generation in the present study.

The experiments were conducted in 770 mL plastic pots, filled with a soil-sand mixture in a 1:2 (v:v) ratio, previously sterilized with Bunema, and maintained in a greenhouse. Six seeds of each genotype were sown in each plastic pot for each experiment. After ten days of emergence, thinning was performed, leaving only one plant per pot. Subsequently, inoculation was carried out by putting an aqueous suspension containing 2500 eggs and juveniles second-stage (J2), using a pipette, in holes near the base of the stem at a depth of 2 cm for each plant in each pot. Throughout the experiment, plants were watered daily to maintain adequate soil moisture. In addition, ambient and soil temperatures were recorded using the ASKO thermohygrometer AK28 (see Fig. 1).

Evaluated agronomic parameters

Sixty days after inoculation, the following parameters were assessed: plant height (cm), stem diameter (cm), Spad index, leaf area, reproduction factor, and nematodes density per gram of root. Plant height measurement was conducted from the base to the apex of the plant. Stem diameter was determined at the height of the cotyledon node, in the opposite direction to their insertion. The Spad index (Soil Plant Analysis Development) was determined using the portable SPAD-502 Plus meter from Konica Minolta. The assessments were conducted in the morning (from 8:00 AM to 9:00 AM) and on each leaflet of the fully developed third trifoliate leaves.

For leaf area assessment, the length and width of the leaf were measured. To do this, the central leaflet was sampled, avoiding the main vein of the third fully open trifoliate leaf from the apex to the base of the plant. Using the width and length of the leaflets, the leaf area was estimated using the model proposed by Toebe et al. (2012):

 $Dfc = 0.7104 \times C \times L$ 

In which,

C-Maximum lenght;

L-Maximum width and

0.7104–Correction factor for the ovoid shape of the leaves.

To determine the Reproduction Factor (RF) and the number of nematodes per gram of roots, nematode extraction followed the technique proposed by Hussey and Barker (1973), modified by Bonetti and Ferraz (1981). After extraction, nematodes were

Treatment	Genotypes	Crossing that gave rise to the genotype or commercial lineage	Generation
Experiment of the	he M. incognita		
T1	UFUL 157	BRS Caiapônia x IAC100 planta 14	F8:9
T2	UFUL246	BRS Santa Cruz x Msoy 9350 planta 3	F8:9
Т3	UFUL298	BRS Caiapônia x IAC 100 planta 110.1.1–9	F8:9
T4	UFUL457	BRS Santa Cruz x IAC 100 planta 8.3.2	F8:9
T5	UFUL511	BRS Luziânia x Potenza planta 173.2	F8:9
Т6	UFUL525	BRS Caiapônia x IAC 100 planta 87.1–9.1	F8:9
T7	UFUL526	BRS Caiapônia x IAC 100 planta 87.1–9.2	F8:9
Т8	UFUL592	BRS Caiapônia x IAC 100 planta 23.1.17.1	F8:9
Treatment	Genotypes	Crossing that gave rise to the genotype or commercial lineage	Generation
Experiment of the	he M. javanica		
T1	UFUL154	BRS Caiapônia x IAC planta1-3	F8:9
T2	UFUL157	BRS Caiapônia x IAC100 planta 14	F8:9
Т3	UFUL172	BRS Luziânia x Potenza planta 134.1	F8:9
T4	UFUL173	BRS Luziânia x Potenza PL 134.5	F8:9
T5	UFUL195	BRS Luziânia x Potenza planta 32.2.5	F8:9
Тб	UFUL218	BRS Luziânia x UFUS Impacta planta 25.1.4	F8:9
T7	UFUL246	BRS Santa Cruz x Msoy 9350 planta 3	F8:9
Т8	UFUL259	BRS Caiapônia x IAC 100 planta 110.1.7.1	F8:9
Т9	UFUL261	BRS Caiapônia x IAC 100 planta 110.1.7.2	F8:9
T10	UFUL280	BRS Caiapônia x Potenza planta 3.1	F8:9
T11	UFUL294	BRS Caiapônia x IAC 100 planta 23.2	F8:9
T12	UFUL298	BRS Caiapônia x IAC 100 planta 110.1.1-9	F8:9
T13	UFUL456	BRS Santa Cruz x IAC 100 planta 8.3.1	F8:9
T14	UFUL457	BRS Santa Cruz x IAC 100 planta 8.3.2	F8:9
T15	UFUL511	BRS Luziânia x Potenza planta 173.2	F8:9
T16	UFUL525	BRS Caiapônia x IAC 100 planta 87.1–9.1	F8:9
T17	UFUL526	BRS Caiapônia x IAC 100 planta 87.1–9.2	F8:9
T18	UFUL528	BRS Caiapônia x IAC 100 planta 87.1–9.3	F8:9
T19	UFUL592	BRS Caiaponia x IAC 100 planta 23.1.17.1	F8:9
T20	UFUL611	BRS Luziânia x Potenza planta 5	F8:9

 Table 2
 Characterization of genotypes. Uberlândia-MG, 2021

Source: Juliatti, 2021

quantified through counting using a Peters counting chamber under a light microscope.

The resistance level of each genotype was estimated using the Moura and Régis (1987) criterion. In this classification, the percentage reduction of the Reproduction Factor (RF) is calculated by the formula: RFR=[(RF of susceptible standard—RF of treatment) / RF of susceptible standard]×100, where 0 to 25%=highly susceptible (AS); 25.1 to 50%=susceptible (S); 50.1 to 75%=moderately susceptible (MS); 75.1 to 90%=moderately resistant (MR); 90.1 to 95%=resistant (R); 95.1 to 100%=highly resistant (AR). The data collected in the experiments were subjected to analysis of variance using the statistical program R Core Team (2020), version 4.0.2. In cases where the assumptions for ANOVA were not met at a significance level of 0.05, the data were transformed using  $\sqrt{x}$  for growth variables and log (x+1) for nematological variables, and then subjected to a new analysis. Then the data were subjected to the F-test of analysis of variance (F=0.05) using the R Core Team program (2020), and means were compared using the Scott-Knott test (p ≤0.05).



Fig. 1 Maximum, minimum, and average soil and ambient temperatures in the greenhouse during the experiments. Uberlândia-MG, 2021. Notes: A- Temperature of the soil; B- Greenhouse ambient temperature. Source: Gontijo, 2021

### **Results and discussion**

The isoenzyme profiles of the esterase from electrophoretic analyses confirmed that the populations under study belong to the species M. incognita and *M. javanica* (Carneiro and Almeida, 2001). The esterase phenotypes for the two species of Meloidogyne spp. characterized in this study are illustrated in the gels (Fig. 2).

## Soybean genotypes reaction to the nematode M. incognita

The studied genotypes exhibited differences in vegetative parameters (Table 3). At 60 days after inoculation with the *M. incognita* isolate, genotypes UFUL 298 and UFUL 511 showed the largest stem diameter.

For plant height, genotypes UFUL 298, UFUL 457, and UFUL 592 showed greater above-ground development.

The Leaf Area Index (LAI) ranged from 17.12  $cm^2$  to 7.92  $cm^2$ , demonstrating that UFUL 592 had a larger LAI compared to the susceptibile standard (Table 3). LAI is an important parameter in plant growth and development, as it affects the interception of solar radiation and shading of leaves near the ground (Board and Harville, 1992). In soybean cultivation, the higher the LAI, the greater the light absorption, consequently leading to increased production of photosynthates and higher yields.

In the second assay, the genotypes exhibited different behaviors compared to the first assay (Table 3). For stem diameter, genotypes UFUL 157, UFUL 525, and UFUL 592 had the smallest stem diameters.

Fig. 2 Esterase phenotypes of Meloidogyne spp. populations from tomato roots. Uberlândia-MG, 2021. Notes: P- Standard phenotype of the *M. javanica*. R- Repetitions. A- M. incognita. **B**- M. javanica. Source: Nemafito, 2021





Treatments	Experiment	Experiment 1 <sup>1</sup>								Experiment 2 <sup>1</sup>						
	Spad index	Stem Diam- eter		Plant height		LAI		Spad index		Stem Diam- eter		Plant height		LAI		
Desafio	30.80	3.30	b	24.02	b	7.92	b	31.4	а	3.27	b	20.42	b	6.19	b	
BRS7980	29.19	3.20	b	28.17	b	10.03	b	27.96	b	3.21	b	27.67	b	8.27	a	
UFUL 157	30.99	3.37	b	26.90	b	10.51	b	31.11	а	3.33	b	25.30	b	7.55	b	
UFUL 246	25.71	3.48	b	21.70	b	11.03	b	31.83	а	3.71	а	23.80	b	7.91	а	
UFUL 298	29.08	4.31	а	33.83	а	12.38	b	29.61	а	3.93	а	33.90	а	9.65	а	
UFUL 457	29.09	3.73	b	35.77	а	8.63	b	28.27	а	3.80	а	35.30	а	7.07	b	
UFUL 511	29.78	4.17	а	27.87	b	12.31	b	30.28	b	3.96	а	25.60	b	7.23	b	
UFUL 525	25.65	2.98	b	24.70	b	10.24	b	25.64	b	3.33	b	25.82	b	4.73	b	
UFUL 526	27.96	3.16	b	23.67	b	10.63	b	26.64	b	3.78	а	27.10	b	6.16	b	
UFUL 592	27.85	3.56	b	36.17	а	17.12	а	28.95	b	3.37	b	31.25	а	9.87	а	
CV (%)	12.83	12.97		22.73		19.12		7.96		10.52		14.35		23.21		
pvalor	0.44865 <sup>ns</sup>	0.00466*		0.02401*		0.00018*		0.00965*		0.03660*		0.0003*		0.00757*		
$^{\circ}L$ / SW <sup>2</sup>	0.0975 <sup>2</sup>	0.7305		0.868		0.7537		$0.0579^2$		0.7258		0.3339		0.7156		
F lev	0.642	0.0033		0.3417		0.4195		0.2641		0.7158		0.8972		0.629		
F adit	0.3217	0.6453		0.6215		0.2223		0.3182		0.00251		0.1515		0.9557		

**Table 3** Means of Spad index, stem diameter (mm), plant height (cm), and Leaf Area Index (LAI) for soybean materials evaluatedin the greenhouse at 60 days after *Meloidogyne incognita* inoculation. Uberlândia-MG, 2021

<sup>1</sup>Means followed by different letters in the column differ significantly according to the Scott-Knott test at 0.05 significance level. \* Significant and <sup>ns</sup> non-significant according to the F-test at 0.05 significance level. °L / SW<sup>2</sup>, F lev, F adit: statistics for the Lilliefors (Kolmogorov–Smirnov)/ Shapiro-Wilk<sup>2</sup>, Levene, and Tukey tests, respectively; values in bold indicate normal distribution of residuals, homogeneous variances, and block additivity at 0.05 significance level

Plants that are subject to attack by root-knot nematodes have compromised root systems, which hinders their development. This would explain the smaller stem diameter observed in these genotypes.

Genotypes UFUL 298, UFUL 457, and UFUL 592 showed a growth increase of 66.01%, 75.80%, and 53.03%, respectively, compared to the susceptible control (Desafio cultivar) (Table 3). In soybean cultivation, plant height is of utmost importance in grain production, as it is closely related to the number of nodes, which will give rise to branches and reproductive structures (Buzzello, 2010).

Genotypes UFUL 246, UFUL 298, and UFUL 592 exhibited greater leaf development (Table 3). According to Porras et al. (1997), this Leaf Area Index reflects plant growth and yield through the interception of solar radiation and accumulation of photosynthates.

Regarding the Spad index, it's possible to observe the formation of two groups with distinct behaviors. Genotypes UFUL 157, UFUL 246, UFUL 298, and UFUL 457 showed a high Spad index, indicating an increase in chlorophyll content as the plant enhances its ability to absorb nutrients from the soil. In contrast, genotypes UFUL 511, UFUL 525, UFUL 527, and UFUL 592 exhibited a low value (Table 3).

According to Zotarelli et al. (2002), it is possible to estimate leaf chlorophyll content through the Spad index. Therefore, it is important to highlight that the parasitism of *M. incognita* in the soybean plant's root system can indirectly affect chlorophyll levels in the leaves, consequently influencing plant development. This can be observed in genotypes UFUL 511, UFUL 525, UFUL 527, and UFUL 592 (Table 3).

In 1982, Ferraz, while studying the effect of *M. incognita* on the absorption and translocation of nutrients in black pepper plants and its influence on the total chlorophyll content of the plants, observed that the nematode reduced plant growth and the chlorophyll content in the leaves due to a lower rate of nutrient absorption and translocation.

The results in Table 4 demonstrate that in the experiments, all tested soybean genotypes had  $RF \ge 1.0$  and therefore were classified as susceptible (Oostenbrink's criteria, 1966). The cultivar BRS 7980, used as a resistance standard, showed

Treatments	Experimen	nt 1 <sup>1</sup>				Experiment 2 <sup>1</sup>							
	Nematode g <sup>-1</sup>		RF		RFR <sup>3</sup>	Classification <sup>3</sup>	Nematode g <sup>-1</sup>		RF		RFR <sup>3</sup>	Classification <sup>3</sup>	
Desafio	5156.44 b		8.18 a -		-	-	1657.00 b		3.40		-		
BRS7980	1245.94	а	1.87	а	76.91	MR	649.89	а	1.05	а	69.12	MS	
UFUL 157	1698.06	а	4.70	а	41.98	S	894.93	a	2.18	а	35.88	S	
UFUL 246	4840.33	b	10.9	b	-34.57	AS	1421.28	b	4.24	а	-24.71	AS	
UFUL 298	1871.01	а	6.07	а	25.06	S	603.52	а	2.03	а	40.29	S	
UFUL 457	2013.42	а	7.53	а	7.04	AS	1048,17	a	3.54	а	-4.12	AS	
UFUL 511	1896.41	а	5.66	а	30.12	S	689.16	a	2.90	а	14.71	AS	
UFUL 525	5103.46	b	9.94	b	-22.72	AS	1134.55	а	3.31	а	2.65	AS	
UFUL 526	6007.33	b	13.6	b	-67.90	AS	1815.13	b	5.10	а	-50.00	AS	
UFUL 592	1846.13	а	4.27	a	47.28	S	1276.02	b	3.11	а	8.53	AS	
CV (%)	60.65	38.51			-	-	46.84		45.53		-	-	
pvalor	0.00390*	0.00009*			-	-	0.02574*		0.02121*		-	-	
°L	0.1915	0.9064			-	-	0.6166		0.9193		-	-	
F lev / F OM <sup>2</sup>	0.0646 <sup>2</sup>	0.2233			-	-	$0.0132^2$		0.0901		-	-	
F adit	0.3247	0.0626			-	-	0.5669		0.4785		-	-	

**Table 4** Nematodes per gram of root (Nematode  $g^{-1}$ ), Reproduction Factor (RF), and classification of soybean genotypes inoculated with *Meloidogyne incognita*. Uberlândia-MG, 2021

<sup>1</sup>Means followed by different letters in the column differ significantly by the Scott-Knott test at 0.05 significance level. \* Significant and <sup>ns</sup> non-significant by the F test at 0.05 significance level. °L, F lev/ F OM<sup>2</sup>, F adit: statistics for Lilliefors tests (Kolmogorov–Smirnov), Levene / Oneillmathews<sup>2</sup>, and Tukey, respectively; values in bold indicate residues with normal distribution, homogeneous variances, and additivity of blocks at 0.05 significance level. <sup>3</sup> Classification proposed by Moura and Régis (1987): *AS* highly susceptible (0 to 25%); *S* susceptible (25.1 to 50%); *MS* moderately susceptible (50.1 to 75%); *MR* moderately resistant (75.1 to 90%); *R* resistant (90.1 to 95%); *AR* highly resistant (95.1 to 100%)

RF values of 1.8 and 1.05 in the first and second trials, respectively, which were the lowest RF values in magnitude.

Analyzing the parasitism of *M. incognita* in soybean cultivation, in the first assay (Table 4), it can be observed that all genotypes had their roots parasitized. The average values of the juvenile population in the root system varied between 1200 to 6000 specimens per gram of root, demonstrating a significant difference among the evaluated materials. The genotype UFUL 526 showed the highest number of nematodes in the root system, consequently obtaining a higher reproduction factor.

The increase in the population of this microorganism in the soybean root system leads to changes in the absorption flow of water and nutrients, obstruction of the vascular tissue due to gall formation, which hinders physiological processes such as photosynthesis and respiration, consequently affecting the plant's development (Ferraz, 1982). In the second trial, there was also a variation in the average population per gram of root. However, the variation in the number of specimens was lower compared to the first trial. This can be attributed to the influence of temperature on the nematodes' development (Table 4 and Fig. 1). As for the reproduction factor, the cultivar BRS 7980 and the genotype UFUL 298 showed the lowest factors, while the genotype UFUL 526 exhibited the highest factor.

The reduction in RF values in the materials in the second trial compared to the first did not affect the nematode's development. This means that the nematode infected the genotypes, completed its development without a significant production of eggs and juveniles, likely due to the influence of temperature.

According to Dickson and De Waele (2005), temperature is one of the abiotic factors that most influence the survival and parasitism of *Meloidogyne* sp. During the experiment, the average temperatures in the soil and the greenhouse were above 28°C (Fig. 1), which could have affected the nematode's development.

Furthermore, studies have demonstrated that during the reproduction phase, in countries with tropical and subtropical climates, the range minimum temperatures for survival for *Meloidogyne* species is between 5 to 10°C, the normal range for biological activities is between 15 and 22°C, and above 30°C, the nematode begins to experience limitations in its activities (Ferraz and Brown, 2016).

The results in Table 4 clearly demonstrate that in both experiments, when using the Moura and Régis (1987) criterion, no genotype was classified as resistant or highly resistant to *Meloidogyne incognita* under greenhouse conditions. In both experiments, the cultivar BRS 7980, classified as resistant by Embrapa (2012), behaved as moderately resistant and moderately susceptible, respectively. It is also noteworthy that the high susceptibility of the genotype UFUL 526 to *M. incognita* was confirmed by the excellent multiplication of the inoculum in soybean roots and the restriction of plant growth (Tables 3 and 4).

Response of soybean genotypes to the nematode *M*. *javanica* 

In the first assay, it was observed that the nematode had an influence (p < 0.05) on all evaluated parameters. Regarding plant height, the average values ranged from 4.77 to 6.99 cm. Genotypes UFUL 259 and UFUL 592 exhibited greater shoot growth in relation to the susceptibility and resistance standards (Table 5).

The low growth of the genotypes may be attributed to nematode infestations. These attacks caused disruptions in the mechanisms of mineral absorption and translocation through cell rupture, resulting in low concentrations of nutrients available for plant development (Chitwood et al., 1952).

The highest Leaf Area Index (LAI) was observed in genotypes UFUL 172, UFUL 259, UFUL 261, UFUL 298, and UFUL 592. With the increase in LAI, light interception also increases, leading to higher net photosynthesis and, consequently, greater plant growth (Müller, 1981).

Genotypes UFUL 218, UFUL 261, UFUL 457, UFUL 526, and UFUL 592 obtained lower Spad index, while the other genotypes showed similar behavior. Regarding stem diameter, the mean values

ranged from 2.77 to 3.87, with the resistance standard BRS 7980 numerically presenting the smallest diameter (Table 5).

Genotypes UFUL 172, UFUL 259, UFUL 298, and UFUL 592 performed well in the evaluated parameters (Table 5). This indicates that the studied genotypes likely possess some level of resistance to the root-knot nematode. Genotype UFUL 172 is derived from the F8:9 generation of the cross between BRS Luziânia and Potenza (PL 134.1). The parent Luziânia has proven resistance to *M. javanica* (Embrapa, 2012). On the other hand, the other genotypes have BRS Caiapônia x IAC-100 as their parental lines.

The parental BRSGO Caiapônia does not possess resistance to *M. javanica* (Embrapa, 2012). On the other hand, the parental IAC-100 has in its genealogy the cross of the American cultivars BRAGG x Pi 229,358 (Veiga et al., 1999), with the cultivar BRAGG originating from the cross Jackson x D49-2491. Jackson, in turn, descends from Palmetto x Volstate, both of which have resistance to *M. javanica* and *M. incognita* (Silva, 2001). This could explain the behavior of genotypes UFUL 259, UFUL 298, and UFUL 592.

In the second experiment, there was also interference from the population density of *M. javanica* in the evaluated parameters. The results show that genotypes UFUL 172 and UFUL 511 exhibited higher Spad index, stem diameter, and greater above-ground growth. Genotype UFUL 592 showed a higher leaf area index and vegetative development (Table 5).

According to Marschner (1995), plants well-supplied with nutrients are more vigorous and consequently exhibit greater development. However, studies have shown that plants infected by nematodes have low nutrient concentrations (Board et al., 1994), which would explain the lower development of the other genotypes.

Due to the similarity between the data obtained in the two experiments, it is observed that the vast majority of the analyzed genotypes presented similar mean values for nematodes per gram of root (Table 6). The quantity of nematodes per gram of root was higher in genotypes UFUL 246, UFUL 456, UFUL 526, and UFUL 528. Additionally, a higher density of nematodes per gram of root was observed in the susceptible standard.

Furthermore, it is possible to observe that in the evaluations conducted in both trials, genotypes UFUL

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**Table 5** Means of the Spad index, stem diameter (mm), plant height (cm), and leaf area index (LAI) for soybean materials evaluated in a greenhouse 60 days after inoculation with *Meloidogyne javanica*. Uberlândia-MG, 2021

Treatments	Experiment 1 <sup>1</sup>								Experiment 2 <sup>1</sup>							
	Spad index		Stem Diam- eter		Plant height <sup>+</sup>		LAI <sup>+</sup>		Spad index	Stem Diameter			Plant height		LAI	
Desafio	33.64	a	3.10	b	4.88	с	2.56	b	34.6	a	3.03	с	21.77	b	5.36	с
BRS7980	23.57	b	2.77	b	5.28	c	2.62	b	22.53	b	2.83	c	29.72	b	7.56	c
UFUL154	30.02	а	3.45	а	5.71	b	2.63	b	31.14	а	3.10	c	25.85	b	6.35	c
UFUL157	29,55	a	3.70	a	5.28	c	2.66	b	30.34	а	3.43	c	28.25	b	6.51	c
UFUL172	30.74	а	3.87	а	5.68	b	3.17	а	31.31	а	4.08	а	38.02	а	6.98	c
UFUL173	29.09	а	3.56	а	5.38	c	2.78	b	28.12	b	3.53	b	27.45	b	6.64	c
UFUL195	31.16	а	3.33	b	5.96	b	2.77	b	31.35	а	3.37	c	34.65	а	7.06	c
UFUL218	27.73	b	3.65	а	6.00	b	2.96	b	31.10	а	3.07	c	24.52	b	6.63	c
UFUL246	33.21	а	3.26	b	4.94	c	2.52	b	33.23	а	3.28	c	24.25	b	6.29	c
UFUL259	29.67	а	3.85	а	6.67	а	3.56	а	24.61	b	3.73	b	35.57	а	10.99	b
UFUL261	26.50	b	3.45	а	5.55	b	3.16	а	25.55	b	3.52	b	30.22	b	10.23	b
UFUL280	29.75	а	3.72	а	5.78	b	2.69	b	30.80	а	3.80	b	34.32	а	5.92	c
UFUL294	31.59	а	3.51	а	5.69	b	2.91	b	31.16	а	3.38	c	30.05	b	7.00	c
UFUL298	28.94	а	3.73	а	5.32	c	3.33	а	30.19	а	3.80	b	27.35	b	8.79	b
UFUL456	30.10	а	3.15	b	5.09	c	2.58	b	27.21	b	3.63	b	29.00	b	8.30	b
UFUL457	28.40	b	3.76	а	5.51	b	2.69	b	25.69	b	3.42	c	28.67	b	7.94	c
UFUL511	31.81	а	3.68	а	5.69	b	2.68	b	30.97	а	4.27	a	33.50	а	8.82	b
UFUL525	32.23	а	2.97	b	5.22	c	2.38	b	30.05	а	3.21	c	27.30	b	5.65	c
UFUL526	27.95	b	2.95	b	5.13	c	2.35	b	27.49	b	3.17	c	26.67	b	5.24	c
UFUL528	29.32	а	3.32	b	4.77	c	2.17	b	29.91	а	2.93	c	20.70	b	4.41	c
UFUL592	25.99	b	3.52	а	6.99	а	3.75	а	27.45	b	3.70	b	38.75	а	13.76	a
UFUL611	30.04	а	3.32	b	5.56	b	2.58	b	25.58	b	3.31	c	29.32	b	9.03	b
CV (%)	9.26		10.63		7,83		13.54		12.76	9.39			17.08		27.43	
pvalor	0.00045*		0.00072*		0.00000*		0.00000*		0.00167*	0.0000*			0.00003*		0.0000*	
°L	0.4499		0.6939		0.0631		0.0790		0.0589	0.144			0.519		0.0732	
F lev / F OM <sup>2</sup>	0.0934 <sup>2</sup>		<b>0,1068<sup>2</sup></b>		$3.14e^{-06}$		$5.687e^{-05}$		0.1951	0.1518			0.1605		$0.014^{2}$	
F adit	0.7473		0.8203		0.0009		0.3994		0.7960	0.2579			0.6600		0.6617	

<sup>1</sup>Means followed by different letters in the column differ from each other according to the Scott-Knott test at 0.05 significance level. \* Significant and <sup>ns</sup> non-significant by the F test at 0.05 significance level. °L, F lev / F OM<sup>2</sup>, F adit: statistics of the Lilliefors (Kol-mogorov–Smirnov), Levene / Oneillmathews<sup>2</sup>, and Tukey tests respectively; values in bold indicate normal distribution of residuals, homogeneous variances, and additivity of blocks at 0.05 significance level. <sup>+</sup>Values transformed into  $\sqrt{x}$  to perform statistical analysis

172 and UFUL 592 showed good performance in both growth and nematological parameters. These observations indicate that the genotype may have resistance to the root-knot nematode.

The UFUL 592 genotype comes from the F8:9 line from the cross between BRSGO Caiapônia and IAC-100 (PL 23.1.17.1). The parental BRSGO Caiapônia is not resistant to *M. javanica*, but rather to *M. incognita* (Embrapa, 2012). Meanwhile, the parental IAC-100 presents in its genealogy the

crossing of the American cultivars BRAGG x Pi 229,358, with the BRAGG cultivar being resistant to *M. javanica* and *M. incognita* (Veiga et al., 1999).

Given this, it is possible that during the selection process in the breeding program, alleles conferring resistance to the root-knot nematode may have been transferred to the genotype UFUL 592. This could potentially explain the genotype's behavior. However, this assumption needs to be further verified.

Treatments	Experimer	nt 1 <sup>1</sup>				Experiment 2 <sup>1</sup>						
	Nematode g <sup>-1</sup>		RF	RFR <sup>4</sup>	Classification <sup>4</sup>	Nematode g <sup>-1+</sup>		RF <sup>+</sup>	RFR <sup>4</sup>	Classification <sup>4</sup>		
Desafio	1485.66 b		1.90	-	-	1166.62	а	0,31	-	-		
BRS7980	838.76	b	0.87	54,21	MS	650.08	a	0,19	38,71	S		
UFUL154	734.27	а	1.21	36,32	S	728.44	а	0,29	6,45	AS		
UFUL157	381.15	а	1.15	39,47	S	463.05	а	0,23	25,81	S		
UFUL172	517.74	а	1.02	46,32	S	130.50	а	0,12	61,29	MS		
UFUL173	726.69	а	1.43	24,74	AS	398.12	а	0,21	32,26	S		
UFUL195	627.00	а	1.66	12,63	AS	193.64	а	0,16	48,39	S		
UFUL218	610.20	а	1.57	17,37	AS	693.85	а	0,31	0,00	AS		
UFUL246	1059.68	b	1.90	0,00	AS	574.27	а	0,29	6,45	AS		
UFUL259	686.84	а	1.79	5,79	AS	345.01	а	0,20	35,48	S		
UFUL261	690.76	а	1.44	24,21	AS	376.72	а	0,23	25,81	S		
UFUL280	455.79	а	1.15	39,47	S	521.61	а	0,26	16,13	AS		
UFUL294	456.32	а	1.48	22,11	AS	239.23	а	0,19	38,71	S		
UFUL298	533.99	а	1.17	38,42	S	606.09	а	0,30	3,23	AS		
UFUL456	869.30	b	1.67	12,11	AS	721.53	а	0,40	-29,03	AS		
UFUL457	607.01	а	1.46	23,16	AS	334.40	а	0,21	32,26	S		
UFUL511	446.81	а	1.57	17,37	AS	216.41	а	0,20	35,48	S		
UFUL525	667.62	а	1.34	29,47	S	445.01	а	0,26	16,13	AS		
UFUL526	1038.24	b	1.95	-2,63	AS	533.29	а	0,28	9,68	AS		
UFUL528	1213.43	b	2.57	-35,26	AS	918.68	а	0,32	-3,23	AS		
UFUL592	233.40	а	0.62	67,37	MS	134.92	а	0,12	61,29	MS		
UFUL611	323.71	а	0.90	52,63	MS	318.25	а	0,19	38,71	S		
CV (%)	59.2		53.52	-	-	16,15		59,58	-	-		
pvalor	0.00983*		0.22781 <sup>ns</sup>	-	-	0.00811*		0.57223 <sup>ns</sup>	-	-		
$^{\circ}L$ / SW <sup>2</sup>	$0.0552^2$		0.1149	-	-	0.7112		0.0675	-	-		
F lev / F OM <sup>3</sup>	0.152		0.366	-	-	0,0679 <sup>3</sup>		0.1691 <sup>3</sup>	-	-		
F adit	0.080		0.0574	-	-	0.1567		0.1316	-	-		

**Table 6** Nematodes per gram of root (Nematode  $g^{-1}$ ), reproduction factor (RF), and classification of soybean genotypes inoculated with *Meloidogyne javanica*. Uberlândia-MG, 2021

<sup>1</sup>Means followed by different letters in the column differ from each other by the Scott-Knott test at 0.05 significance level. \* Significant and <sup>ns</sup> non-significant by the F test at 0.05 significance level. °L / SW<sup>2</sup>, F lev / F OM<sup>3</sup>, F adit: statistics of the Lilliefors tests (Kolmogorov–Smirnov)/ Shapiro–Wilk (SW)<sup>2</sup>, Levene / Oneillmathews<sup>3</sup>, and Tukey, respectively; values in bold indicate residues with normal distribution, homogeneous variances, and block additivity at the 0.05 significance level. <sup>4</sup>Classification proposed by Moura and Régis (1987): *AS* highly susceptible (0 to 25%); *S* susceptible (25.1 to 50%); *MS* moderately susceptible (50.1 to 75%); *MR* moderately resistant (75.1 to 90%); *R* resistant (90.1 to 95%); AR = highly resistant (95.1 to 100%). <sup>+</sup>Values transformed into log x + 1 to perform statistical analysis

Another cross that favored the reduction of *M. javanica* was BRSGO Luziânia with Potenza. It is noteworthy that the genotype UFUL 172 derived from this cross showed a lower quantity of nematodes per gram of root (Table 6). According to data from Embrapa (2012), the parental BRSGO Luziânia shows resistance to the nematode under study.

The cultivar BRSGO Luziânia originated from the cross of Braxton x {FT x [Dourados-1 (5) x SS-1]}

(Gianluppi et al., 2004), where the cultivar Braxton stems from the cross between F59-1505 and {(Bragg (3) x D60-7965)}. The F59-1505, in turn, has Jackson x D49-2691 (S-100×CNS) as its parents, and the D60-7965 resulted from the cross of D55-4090 (Ogden x CNS) with D55-4159 (Ogden x Biloxi) (Bernard et al., 1988). Both parents of Jackson exhibit resistance to *M. javanica* and *M. incognita* (Silva, 2001).

Based on the results obtained using the Moura and Régis criteria (1987), only three genotypes in the first trial were classified as moderately susceptible to *M. javanica*. In the second experiment, it is observed that the genotypes exhibited different behavior (Table 6). According to Tihohod and Ferraz (1986), the variation in pathogen aggressiveness is a factor that can lead to differences in resistance classification results, as observed in the conducted trials.

Furthermore, it is important to highlight that in experiments that seek to evaluate the reaction of genotypes though a more complete analysis, two parameters must be considered, the first parameter being the reproduction factor and the second the reduction of this reproduction factor (Moura, 1997). As one can observe in the two tests done for *M. javanica* some genotypes were considered moderately susceptible and susceptible even though their RF is below one. Such classification can occur when using the criteria of Moura and Régis (1987).

The most evident observation in this study was the variation in genotype behavior when infested by both nematodes. In both trials with *M. incognita* and *M. javanica*, the tested soybean genotypes exhibited different reactions to parasitism. Although almost all evaluated materials were classified as susceptible, there was variation in the average values of the analyzed parameters. Therefore, further studies are needed to identify soybean materials resistant to these pathogens.

#### Conclusions

In the trials with *M. incognita*, all genotypes exhibited a reproduction factor greater than 1.0, classifying them as highly susceptible or susceptible. Genotypes UFUL 592 and UFUL 298 showed greater vegetative development. Soybean genotype UFUL 526 had a higher number of nematodes per gram of root and a higher reproduction factor for *M. incognita* in both trials.

For the experiments with *M. javanica*, genotypes UFUL 172 and UFUL 592 demonstrated good performance in both growth and nematological parameters.

Overall, genotype UFUL 592 exhibited strong performance in all four trials.

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#### Declarations

**Conflict of Interest** The authors declare that they have no conflict of interest.

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