

Original Research Article

Methodology for the Genetic Improvement of Coriander Aiming Resistance to the Galls Nematode

ABSTRACT

The coriander is among the most cultivated hardwoods in Brazil, but its production can be compromised due to the occurrence of phytonematoids in the growing areas. Although there are several cultivars of coriander in the market, none presents resistance to nematodes of the genus *Meloidogyne*. Thus, the present work aims to propose a methodology to be used in coriander breeding programs for resistance to *M. incognita*. The experiment was carried out in a greenhouse at the Federal Rural University of Pernambuco. Five cultivars of coriander, handled in three types of containers and three types of substrates, inoculated 15 days after sowing with 1,200 eggs per plant, were conducted in a randomized block design with three replications in a subdivided plot arrangement. At 45 days after inoculation the evaluation was started, quantifying the incidence of galls, number of galls in the root system, number of eggs and estimation of the pathogen reproduction factor for each plant. The use of a 128-cell tray with the Basaplant® substrate and experimental plot consisting of eight plants, a value obtained from the estimation by the mean of the maximum modified curvature, was the methodology that presented better results, being able to be used in the selection of coriander genotypes for resistance to *M. incognita*.

Keywords: *Coriandrum sativum*, *Meloidogyne incognita*, experimental portion, plant breeding.

1. INTRODUCTION

In Brazil, especially in the Northeast region, the cultivation of coriander (*Coriandrum sativum*) is carried out by a large number of producers throughout the year, making it a culture of great social and economic importance. The crop fields are intended to produce green biomass or seed. Along with spinach and lettuce, coriander is among the most important leaflets in the world market for vegetable seeds [1].

The most commonly used coriander cultivar in Brazil is Verdão, such denomination is due to the dark green coloration that their leaves present [2,3]. This cultivar was developed from the selection of plants of the cultivar Palmeira, which presented tolerant anthracnose and would alternate, and crosses with accesses of coriander crioulo collected in properties in the States of Piauí, Maranhão and Pernambuco.

Among the biotic factors that can compromise the production of coriander, there are phytopathogens, highlighting the nematodes belonging to the genus *Meloidogyne*, especially the species *M. incognita*. This pathogen has juvenile second stage as the infecting form that penetrates the root by the meristematic region [4], migrates to the periphery of the central

cylinder, where it establishes its feeding site in the vascular parenchyma beginning a complex relationship with the plant [5].

One of the symptoms of the presence of the pathogen in the host is the formation of galls in the root system with consequent reduction in nutrient absorption and translocation of water, resulting in lower development of the aerial part of the plant [6], impacting on the production of coriander crops which causes significant losses to producers. In this way, resistance to nematodes of galls (*Meloidogyne spp.*) becomes the focus of programs for the genetic improvement of coriander in the country, being also, indispensable the selection for agronomic characteristics, such as those cited by [3]: leaf/stem ratio, greater leaf size and thickness and obtaining a period of more extensive post-harvest conservation.

According to [7], nematodes are among the pathogens that cause damage to cultivated species, causing losses of approximately 5% in crop worldwide in various crops, besides, they are difficult to control. The *M. incognita* is a species within its genre, extremely aggressive, has 61 distinct enzymes capable of degrading the cell wall, justifying the wide range of host. [8], pointed out that identifying sources of resistance to the genus *Meloidogyne* mainly among commercialized cultivars, or from the development of new cultivars that are adapted to the different Brazilian conditions, has been a concern of some researchers since the early 1990s.

The development of breeding programs for resistance to phytonematodes is a process that demands adequate resources and methodology, that allow to obtain gains with the selection of superior genotypes. In the literature there are several works evaluating the reaction of the genotypes to the galls nematodes, however, there is variation in the methodologies applied mainly in relation to the types of receptions: vessels, tubes and expanded polystyrene trays; and of substrates: mixtures of soil and sand (1:1); soil, sand and humus (3:1:1) and commercial substrate [9,10,11,12]. In addition, there is variation in the area of the experimental plot and in the number of plants, being constituted from 1-10 plants to cultivated area of 1 m² [9,12,13].

The search for a methodology that is able to identify the genetic variation among the genotypes under study for resistance to the galls nematode is a fundamental step for breeding programs. Considering that the use of inadequate methodology can limit the aggression of the pathogen, leading to errors and consequently compromising the selective process carried out by the breeder, thus causing, loss of resources and time. In view of the above, the present work had as objective to evaluate the recipient and / or substrate more favorable to the selection of coriander genotypes for resistance to *M. incognita* race 1, by means of the reaction of cultivars of coriander to the pathogen. In order to identify the optimum size of the plot that can optimize the selective process of phytonematoid resistant genotypes, the modified maximum curvature method was performed.

2. MATERIAL AND METHODS

The experiment was conducted under greenhouse conditions, in the Department of Agronomy of the Federal Rural University of Pernambuco (UFRPE), located at 8° 54' 47" S, 34° 54' 47" W, 6 m high, in the period from August to October 2015. The average during driving the work to the minimum temperatures were of 21.7°C and maximum of 29.1°C, according to data obtained from the Recife meteorological station [14]. The design was of randomized blocks with three replications in plot arrangement subdivided in the space, whose parcel was composed by the types of container and the subplot the factorial between substrates and cultivars, resulting in 45 treatments (5 cultivars X 3 substrates X 3

containers), where the experimental plot consisted of 20 plants, totaling 2700 individuals evaluated.

~~Were evaluated~~ the reactions of Verdão, Tabocas, Tapacurá, Palmeira and HTV Dom Luiz coriander cultivars inoculated with *M. incognita* race 1 and handled in three types of receptions: 400 ml beaker, 116 ml tube and 128 cell tray with approximate volume ~~per~~ 29 ml cell; and three types of substrates: Basaplant® (pinus bark, turf, charcoal, vermiculite, initial fertilization with NPK and micronutrients), mixture of Basaplant® + coconut powder (1:1) and the soil + sand + humus mixture (3:1:1). The soil was sterilized by autoclaving at 120 ° C for two intermittent hours.

~~Were determined physical~~ characteristics and chemical properties of the substrates, such as porosity, aeration space, density, pH and electrical conductivity (CE). ~~the vessel method~~ was used to determine the porosity and aeration space. The densities ~~the wet substrates~~ and their air dried samples were obtained by the autocompaction method. The pH and CE were obtained from the reading, with potentiometer, of the solutions obtained by mixing 20 mL of each substrate with 50 mL of deionized water [15].

To verify the efficiency of the inoculum, in each repetition a plot was established with tomato cultivar Santa Clara (*Solanum lycopersicum* L.), in which is the standard of susceptibility to the ~~galls~~ nematodes.

The sowing was performed at 1 cm depth, placing three coriander fruits or three tomato seeds per container, ~~being the~~ thinning carried out after the germination when the plants ~~presented~~ the first definitive leaf, being only left a plant.

Irrigation was performed manually, depending on the water requirement of the crop. After the germination, ~~per three days of the week was carried out~~ fertigation with adapted nutrient solution from the proposal by [16], composed of macro and micronutrient.

At 15 days after sowing, with the aid of a veterinary syringe, It was carried out the inoculation of 1200 eggs per container at a distance of approximately 0.5 cm from the plant lap, directly on the substrate. The inoculum was obtained from sources kept in tomatoes of cultivar Santa Clara.

The evaluation of the plants started at 45 days after inoculation, determining the incidence of galls in the clods, which were later washed in standing water. ~~In followed~~ the galls were counted again and ~~we~~ proceeded to the extraction of eggs of each plant separately, according to the methodology proposed by [17] and modified by [18], being stored with water in identified containers and placed in the cold chamber until counting the number of eggs per plant. In the quantification of the number of eggs ~~was used~~ Peters sheet and microscope with the increase of 40X.

Were used the scales of notes proposed by [19], on assessments with coriander genotypes, how many the characteristics incidence of galls, number of galls and number of eggs. For the incidence of galls, note 1 = zero galls; 2 = one or two galls; 3 = three to ten galls; 4 = 11 to 30 galls and 5 = more than 30 galls. For the number of galls, note 1 = number of galls ≤ 10; 2 = number of galls ≥ 11 and ≤ 20; 3 = number of galls ≥ 21 and ≤ 30; 4 = number of galls ≥ 31 and ≤ 40 and 5 = number of galls ≥ 41. For the number of eggs, note 1 = number of eggs <1200; 2 = number of eggs 1200 - 2400; 3 = number of eggs 2401 - 3600; 4 = number of eggs 3600 - 4800 and 5 = number of eggs ≥ 4801.

The reproduction factor (FR) was used to verify the reaction of coriander cultivars to *M. incognita* race 1, according to the scale proposed by [20] being considered resistant the progenies with $FR < 1$ and those susceptible present $FR > 1$, where the reproduction factor is the quotient between the final population (Pf) and the initial nematode (Pi).

The data were transformed into $\sqrt{x}$ and submitted to analysis of variance, being the means of the treatments grouped by the test of Scott-Knott to 5% of significance. The analyzes were performed by the SISVAR computational application [21].

In order to obtain the optimal size of the experimental plot, we used the data from the originally installed plots to perform 2000 sampling applied in simulations in order to estimate the CV% and for the sizes of the plots, starting with two plants and increment of one plant, up to 20 plants per plot. For this process, the statistical program GENES [22] was used. The optimal size of the experimental plot was determined by the modified maximum curvature method proposed by [23]. In this, the relationship between the coefficient of variation and plot size with X basic units is explained by the regression model $CV = aX - b$, where a and b are the parameters to be estimated. From the curvature function given by this model, the value of the abscissa was determined where the point of maximum curvature occurs, given by: $X_0 = [a.b(2.b+1)/b+2]^{1/2+2b}$, where X_0 is the value of the abscissa at the point of maximum curvature, which corresponds to the estimation of the optimal size of the experimental plot [24].

3. RESULTS AND DISCUSSION

There was a significant difference for triple interaction, cultivars X containers X substrates, at the level of 5% probability for incidence of galls and number of galls in the root system and 1% probability for egg number and reproduction factor (Table 1). The coefficients of experimental variation ranged from 13.52%, for incidence of galls, to 24.68% for number of eggs. The cultivars Verdão, Tabocas, Tapacurá, Palmeira and HTV Dom Luiz responded to the treatments used, expressing differences in the means obtained for all the variables under study.

Table 1. Summary of analysis of variance of the incident characters of galls (IG), number of galls in the root system (NGSR), number of eggs (NO) and reproduction factor (FR) in coriander cultivars inoculated with *Meloidogyne incognita* race 1. Recife, UFRPE, 2016.

FV	GL	QM			
		IG	NGSR	NO	FR
Block	2	0.07	1.64	1.12	0.20
Container	2	20.73**	5.70**	12.81**	2.76**
Error 1	4	0.40*	0.90	0.16	0.03
Substrate	2	3.70**	1.76*	8.76**	3.11**
Substrate * Container	4	2.58**	0.95*	0.74	0.52**
Grow crops	4	0.27	14.73**	2.50**	0.86**
Cultivate * Container	8	0.31*	0.90*	1.87**	0.37**
Cultivate * Substrate	8	0.06	0.91*	2.44**	0.45**
Cultivate * Container * Substrate	16	0.28*	0.72*	2.24**	0.28**
Error 2	88	0.13	0.38	0.38	0.04
CV (%)		13.52	17.53	24.68	14.14
Average		2.69	3.53	2.52	1.50

* Significant at 5% probability; ** Significant at 1% probability

Table 2 shows the results obtained by the Scott-Knott test for the incidence of galls, number of galls in the root system, number of eggs and reproduction factor of the five cultivars evaluated.

Table 2. Scott-Knott's test for galls incidence (IG), number of galls in the root system (NGSR), number of eggs (NO) and reproduction factor (FR) for the evaluation of coriander cultivars in the home of vegetation for resistance to *M. incognita* race 1. Recife. UFRPE. 2016.

Variables	Containers	Substrates	Cultivars				
			Verdão	Tabocas	Tapacurá	Palmeira	HTV
IG	Cup	B	2.15 aAα	2.68 aBα	2.60 aAα	2.17 aAα	2.27 aAα
		B + PC	1.93 aAα	2.37 aBα	2.42 aAα	2.17 aAα	2.85 aBα
		S + A + H	2.20 aAβ	1.82 aAα	3.33 bBβ	2.47 aAβ	2.17 aAβ
	Tube	B	2.85 aBβ	2.50 aBα	2.82 aBα	2.63 aBα	2.50 aBα
		B + PC	2.67 aBβ	2.65 aBα	2.78 aBα	3.00 aBβ	2.37 aBα
		S + A + H	1.22 aAα	1.48 aAα	1.42 aAα	1.40 aAα	1.30 aAα
	Tray	B	3.62 aAγ	3.45 aAβ	3.47 aAβ	3.78 aAβ	3.43 aAβ
		B + PC	3.90 aAγ	3.28 aAβ	3.63 aAβ	3.55 aAβ	3.35 aAβ
		S + A + H	3.38 aAγ	3.53 aAβ	3.20 aAβ	3.40 aAγ	3.15 aAγ
NGSR	Cup	B	4.95 dAα	3.55 cAβ	4.03 cAα	2.82 bAα	1.72 aAα
		B + PC	4.45 aAα	3.63 aAα	3.38 aAα	3.94 aBα	4.40 aBβ
		S + A + H	4.70 bAα	3.02 aAβ	3.75 aAβ	3.02 aAα	3.62 aBβ
	Tube	B	4.97 cAα	2.40 aAα	3.75 bBα	2.97 aAα	2.32 aAα
		B + PC	5.00 bAα	2.87 aAα	2.83 aAα	3.40 aAα	2.52 aAα
		S + A + H	4.97 bAα	2.02 aAα	2.25 aAα	2.27 aAα	2.32 aAα
	Tray	B	4.77 aAα	3.95 aAβ	3.48 aAα	4.15 aAβ	3.95 aAβ
		B + PC	4.69 bAα	3.32 aAα	3.63 aAα	3.97 aAα	3.28 aAα
		S + A + H	4.92 bAα	3.17 aAβ	3.33 aAβ	3.10 aAα	3.17 aAβ
NO	Cup	B	2.33 aBα	3.00 bAα	3.33 bBβ	2.33 aAα	2.00 aAα
		B + PC	1.00 aAα	4.33 dBβ	2.33 bAα	3.00 cAα	3.33 cAα
		S + A + H	1.00 aAα	2.00 aAα	3.67 bBγ	1.67 aAα	2.67 bAβ
	Tube	B	2.33 aBα	2.00 aBα	2.00 aBα	2.33 aAα	2.33 aBα
		B + PC	2.33 aBβ	3.00 aBα	2.33 aBα	2.33 aAα	2.67 aBα
		S + A + H	1.00 aAα	1.00 aAα	1.00 aAα	2.00 aAα	1.00 aAα
	Tray	B	2.67 aAα	5.00 cBβ	2.33 aAα	3.67 bBβ	2.33 aAα
		B + PC	4.00 bBγ	4.00 bBβ	3.67 bBβ	2.00 aAα	2.67 aAα
		S + A + H	2.00 aAα	1.67 aAα	2.33 aAβ	4.67 bBβ	2.67 aAβ
FR	Cup	B	1.37 aBα	1.80 bBα	1.88 bAβ	1.47 aAα	1.28 aAα
		B + PC	0.76 aAγ	2.44 cCγ	1.61 bAα	1.72 bAα	1.75 bBα
		S + A + H	0.63 aAα	1.20 bAβ	1.94 cAγ	1.18 bAα	1.67 cBβ
	Tube	B	1.35 aBα	1.47 aBα	1.46 aBα	1.50 aAα	1.49 aBα
		B + PC	1.51 aBβ	1.68 aBα	1.58 aBα	1.49 aAα	1.61 aBα
		S + A + H	0.42 aAα	0.57 aAα	0.74 aAα	1.32 bAα	0.47 aAα
	Tray	B	1.69 aBα	2.42 cCβ	1.59 aAα	2.01 bBβ	1.54 aAα
		B + PC	1.97 bBα	2.05 bBβ	1.94 bBα	1.50 aAα	1.56 aAα
		S + A + H	1.21 aAβ	1.29 aAβ	1.42 aAβ	2.28 bBβ	1.63 aAβ

Averages followed by the same lowercase letter on the line, upper case for substrate and Greek for column vessel do not differ statistically by the Scott-Knott test at 5% probability. B-Basaplant®; PC-Coco Powder; S-Soil; A-Sand; H-Humus.

Within the cultivars, there was a wide variation among the treatments used (Table 2). In the cultivar Verdão, for galls incidence, the use of tray with ~~any of the~~ substrates presented the highest averages, while in the variable number of galls in the root system, the treatments did not differ among them. For number of eggs the tray treatments with Basaplant® + coconut fiber and cup or tube with Basaplant® presented the highest mean values, while for the reproduction factor, the use of Basaplant® with ~~any of the~~ containers promoted the highest averages. Thus, to carry out the selection within the cultivar Verdão, Basaplant® substrate can be used with ~~any~~ of the three containers.

In the cultivar Tabocas, for incidence of galls, the use of tray with ~~any of the~~ substrates presented the highest averages, whereas in the variable number of galls in the root system, tray treatments with Basaplant®, tray with soil + sand + humus and cup with Basaplant® presented the highest averages. For number of eggs, tray treatments with Basaplant® + coconut powder, tray with Basaplant® and cup with Basaplant® + coconut powder showed the highest mean values, while for the reproduction factor only the tray with Basaplant® and the glass with Basaplant® + coconut powder promoted the highest averages. With this, to carry out the selection within the cultivar Tabocas the best treatment to be used is the tray with Basaplant®, for being the one that expresses the best results for the evaluated variables.

Observing the performance within the cultivar Tapacurá for galls incidence, it is noticed that the treatments composed by the tray with ~~any~~ one of the three substrates expressed the highest averages. As for number of galls in the root system, most treatments did not differ among them, except for the compounds with the Basaplant® tube + coconut powder and soil + sand + humus. The number of eggs had the highest averages in the treatments cup with Basaplant®, cup with soil + sand + humus and tray with Basaplant® + coconut powder. For the reproduction factor, tray treatments with Basaplant®, tray with soil + sand + humus, tube with Basaplant® and tube with soil + sand + humus expressed lower averages. Taking into account the four variables under study, the cup treatments with Basaplant® or soil + sand + humus promoted the achievement of superior results allowing selection within the cultivar Tapacurá.

For the cultivar Palmeira, in the variable incidence of galls the treatments composed by tray with ~~any~~ of the substrates and tube with Basaplant® + coconut powder presented the highest averages. As for the number of galls in the root system, the tray with Basaplant® presented an average higher than the others. While for the number of eggs and reproduction factor treatments with higher averages were tray with Basaplant® or soil + sand + humus. In this way, the use of tray with Basaplant® ~~is the treatment in which~~ the highest averages ~~are~~ obtained for the study variables.

In the cultivar HTV Dom Luiz for galls incidence, the use of tray with ~~any~~ of the three substrates obtained the highest averages. For number of galls in the root system, the tray treatments with Basaplant® or soil + sand + humus and cup with soil + sand + humus ~~were the treatments with~~ better performance. As for number of eggs and reproduction factor treatments that presented inferior mean were tube with soil + sand + humus, for both variables, and cup with Basaplant® only for the reproduction factor. The tray treatment with Basaplant® or soil + sand + humus ~~is what~~ presented good results for the four evaluated variables, being thus indicated for the selection within the cultivar HTV Dom Luiz.

Among the cultivars, for the variable galls incidence, there was no significant difference for most treatments, except for cup with soil + sand + humus where the cultivar Tapacurá presented an average superior to the others. Due to its low ability to identify differences between genotypes, this variable is not a good option for selection among cultivars. In the

variable number of galls in the root system, there was no significant difference between the cultivars only in the cup treatments with Basaplant® + coconut powder and tray with Basaplant®. The cultivar Verdão showed higher averages than the others, being the treatment cup with Basaplant® which was able to identify greater difference between the cultivars evaluated. For the variables number of eggs and reproduction factor, in the treatments with tube there was no significant difference between the cultivars, while all other treatments were able to differentiate them, being the treatments cup with Basaplant® + coconut powder and tray with Basaplant® those that presented greater capacity to differentiate the genotypes under study.

The evaluation of the resistance to *Meloidogynose* requires the adoption of an efficient method, rapid and practical so that large populations of plants can be analyzed in a short time [25]. Therefore, the choice of container interaction X substrate that expresses the greatest (genetic) variability is fundamental to increase heritability ($h^2 = \sigma_g / \sigma_t$) and, consequently, gain with selection. Because, heritability influences directly on the gain with the selection ($G = i \cdot \sigma_p \cdot h^2$) [26], in this way, the greater the heritability the greater the gain. Based on the exposed, cup treatments with Basaplant® and tray with Basaplant® presented the highest averages to carry out the selection among the cultivars, making possible the differentiation of the genotypes (Table 2).

Although the five cultivars evaluated are susceptible to the nematode, the results indicated that the use of the tube factor X soil + sand + humus for most cultivars, except Palmeira, would mask the genotypes under study, which would be classified as resistant for obtaining $FR < 1$. In the same way, the cup treatments with Basaplant® + coconut powder and soil + sand + humus in the cultivar Verdão also presented $FR < 1$. These results were not due to the genetic resistance of the cultivars, but to the effect of the environment that possibly disadvantaged the pathogen, interfering in the selective process carried out by the breeder (Table 2).

[27] verified variation of FR as a function of substrate type, even under low population densities (2,000 eggs), under these conditions, the banana plants 'Prata Anã' presented rickety. The type of container also influences the expression of the reaction of genotypes inoculated with *Meloidogyne incognita*, fact evidenced by [25] in *Phaseolus vulgaris* L., comparing the effect of three containers (Styrofoam box "Plantagil 1477" of 128 cells of 70 cm³, plastic cups of 200 cm³ and plastic pots of 3000 cm³) in the expression of bean resistance verified that the plastic vessel of 3 L was the most impracticable, due to the dispersion of eggs in their large volume, made by irrigation water. With this, depending on the type of substrate and container used there may be variation of the reproduction factor, leading to erroneous classification of genotypes, being fundamental the identification treatment (container X substrate) able to identify the real genetic potential of the evaluated genotypes.

The losses caused by phytonematodes are directly related to their populations, to the age of the plant, to soil type, and climatic conditions [28,29]. Thus, the type of substrate used in experiment evaluating plant genotypes for resistance to nematodes, influence in development of the pathogen and the infection capacity, thus, the Basaplant® stood out as the best option among the substrates used, due to its practicality, ease of removal of the root system and ability to express the potential of the genotypes for resistance to the pathogen, allowing the quantification of galls and extraction of nematode eggs.

The combination of Basaplant + coconut powder presented higher averages in some of the treatments, however, the coconut powder adheres to the root system in such a way that it makes it difficult to count galls and to extract eggs, making the use of this substrate

impossible. In addition, [30] highlights the potential of coconut fiber in the composition of soil conditioners with nematicidal characteristics, however, in the present work the use of coconut powder associated with Basaplant® did not affect the infestation of the pathogen, possibly because it was not integrated into the soil system.

In the results obtained for the physical analysis of the substrates, the highest densities were obtained for the substrate soil + sand + humus, resulting in lower porosity and aeration space, important characteristics for the plant and the pathogen. The difference between the wet density and the dry air sample, was higher for Basaplant® + coconut powder, being this one a substrate with good retention of humidity due mainly to the presence of coconut powder. For both porosity and airing space, the Basaplant® X cell (tray) presented the highest values (Table 3).

TABLE 3. Physical and chemical characteristics of substrates Basaplant. Basaplant + coconut fiber and soil + sand + humus. Recife. UFRPE. 2016.

Substrates X Containers	Characteristics					
	Wet density	Density of dried sample in air	Porosity%	Aeration space%	pH	CE mS/cm
Cell x S+A+H	1.25	1.12	11	21	7.8	1.33
Cell x B+PC	0.61	0.33	39	27	5.7	0.57
Cell x B	0.63	0.59	41	29	5.3	1.32
Tube x S+A+H	1.25	1.31	24	10	7.8	1.33
Tube x B + PC	0.61	0.33	20	4	5.7	0.57
Tube x B	0.63	0.59	23	4	5.3	1.32
Cup x S+A+H	1.25	1.31	29	5	7.8	1.33
Cup x B + PC	0.61	0.33	24	4	5.7	0.57
Cup x B	0.63	0.59	28	4	5.3	1.32

B-Basaplant®; PC-Coco Powder; S-Soil; A-Sand; H-Humus.

Several factors, such as temperature, humidity, pH and soil texture, influence the life span of species of the genus *Meloidogyne* [31] being fundamental to the maintenance of water films in the soil to allow the movement of the nematodes [32], where excess drainage is a limitation to the pathogen displacement. Although the Basaplant® X cell (tray) has presented greater aeration space, corresponding to 29% of the porosity, there is 71% of the porous space destined to the storage of water and/or nutritive solution, not limiting the displacement, and consequent ability to parasitize the roots of the plant, because it is important both the supply of moisture and oxygenation, as discussed by [33] that emphasizes low aeration as a factor that significantly reduces the survival of entomopathogenic nematodes. Regardless of the substrate used, the interaction of the cup and the tube, with these, resulted in lower aeration values than those presented for tray, which is the container with the best capacity for aeration and retention of water and/or nutrient solution.

The chemical analyzes of the substrates, present in table 4, presented pH of 7.8 (soil + sand + humus), 5.7 (Basaplant® + coconut powder) and 5.3 (Basaplant). According to [34] the majority of nematodes develop best at pH ranging from 5.5 to 6.0, with pH values above 7.0 a limitation to the development of phytonematoids. In this way, the substrate soil + sand + humus was the only one that presented the pH above the favorable range for the nematode. CE values ranged from 0.57 (Basaplant® + coconut powder) to 1.33 (soil + sand + humus), all inferior to those used by [35] which were between 2.55 and 12.34 dS.m-1, where it was observed that the CE increase promoted a linear reduction for growth and the production of

coriander. Thus, none of the substrates evaluated had CE that could limit the development of the culture.

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TABLE 4. Regressions, coefficients of determination (R^2) and estimates of optimal plot sizes (X_0) for coriander cultivars in a greenhouse inoculated with *M. incognita* race 1. Recife. UFRPE. 2016.

Variables	Cultivars				
	Verdão	Tabocas	Tapacurá	Palmeira	HTV Dom Luiz
IG	Regression	$y = 22,03x^{-0,117}$	$y = 23,74x^{-0,153}$	$y = 19,93x^{-0,3}$	$y = 20,49x^{-0,174}$
	$R^2(\%)$	94	95	98	96
	X_0	1,76	2,57	4,58	2,50
NGSR	Regression	$y = 26,45x^{-0,105}$	$y = 27,34x^{-0,107}$	$y = 27,88x^{-0,103}$	$y = 28,40x^{-0,078}$
	$R^2(\%)$	91	93	93	92
	X_0	1,92	2,03	1,99	1,52
NO	Regression	$y = 33,57x^{-0,18}$	$y = 23,65x^{-0,241}$	$y = 29,26x^{-0,145}$	$y = 27,52x^{-0,162}$
	$R^2(\%)$	96	97	95	96
	X_0	4,74	4,43	3,09	3,28

IG - incidence of galls; NGSR - number of galls in the root system; NO - number of eggs.

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The estimates of the points of maximum curvature were obtained from algebraically rounded values (opting to round up), from the regressions whose values determination coefficient (R^2) were higher than 90% (Table 4). These values indicate that the regressions are able to explain the values obtained for the optimal plot size. There was a variation between the variables and cultivars in relation to the estimated optimum size. In the variable incidence of galls, the lowest value was obtained for the cultivar Verdão ($X_0 = 1.76$) and the highest was observed in Tapacurá ($X_0 = 4.58$). Among the three characteristics assessed incidence of galls, number of galls in the root system and number of eggs, this latter presented the highest values for X_0 , being therefore chosen to determine the optimal size that can be used in coriander breeding programs that aim to evaluate the same characters used in the present study. Thus, the highest value obtained was $X_0 = 4.74 \approx 5.00$ plants per plot.

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The point of maximum curvature should be interpreted as a lower limit of plot size, rather than the optimal size of the experimental plot [36]. Thus, the smallest size to be used is 5 plants per plot, however, the use of 8 plants would be the most adequate, according to the methodology of the present study, since the trays of 128 cells are composed in each vertical line by 8 cells. Soon, each vertical line will represent a progeny or cultivar under evaluation, facilitating the experimental management. In addition, the plot represented by eight plants allows the evaluation of a greater number of individuals within each progeny, being interesting for the recurrent selection of half-siblings, commonly used in breeding programs for coriander.

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4. CONCLUSION

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The methodology with better efficiency in differentiating the cultivars Verdão, Tabocas, Tapacurá, Palmeira and HTV Dom Luiz, aiming resistance to *M. incognita*, consisted in

using of 128-cell expanded polystyrene tray with the commercial Basaplant® substrate, with experimental plot consisting of eight plants. This methodology is able to differentiate the cultivars with experimental reliability, without underestimating or overestimating their values as the variable incidence of galls, number of galls in the root system, number of eggs and reproduction factor.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. ABRASEM. Brazilian Seed and Seed Association. Yearbook. Brasília: Abrasem; 2014. Available: <http://www.abrasem.com.br>.
2. Barros Júnior AP, Bezerra Neto F, Negreiros MZ, Oliveira EQ, Silveira LM, Câmara MJT. Agronomic performance of commercial cultivars of coriander in single crop under conditions of high temperature and wide luminosity. *Caatinga*. 2014;17(1):82-86.
3. Oliveira NS. Genetic parameters of progenies of coriander tolerant to heat. Recife, Rural Federal University of Pernambuco; 2013.
4. Cordeiro MJZ, Matos AP, Kimati H. Diseases of Banana. In: Kimati H, Amorim L, Rezende JAM, Bergamin Filho A, Camargo LEA. Manual of Phytopathology: Diseases of Cultivated Plants. 4th ed. São Paulo: Publisher Agronomic Ceres; 2005.
5. Taylor DT, Sasser JN. Biology, identification and control of root nodule nematodes (*Meloidogyne species*). North Carolina State University and USAID; 1983.
6. Tihohod D. Applied agricultural nematology. 2th ed. Jaboticabal: Funep. 2000.
7. McCarter JP. The genome sequence of the plant-parasitic roundworm *Meloidogyne incognita* opens new avenues to boosting food production. *Nat. Biot.* 2008;26(1):882-884.
8. Fiorini AVA, Gomes LAA, Maluf WR, Fiorini IVA, Duarte RPF, Licursi V. Evaluation of F2 populations of lettuce on nematode resistance of galls and tolerance to early flowering. *Hort. Braz.* 2005;23(1):299-302.
9. Biondi CM, Prado MDC, Medeiros JE, Pedrosa EMR, Moura RM. Coriander tolerance parasitic nematode *Meloidogyne incognita* Race 1. *Nem. Braz.* 2001;25(1):239-241.
10. Paiva TCG, Santos MA. Reaction of pea cultivars to *Meloidogyne incognita* and *M. javanica*. *Bios. Jour.* 2004;20(1):73-76.
11. Carvalho Filho JLS, Gomes LAA, Silva RR, Ferreira S, Carvalho RRC, Maluf WR. Population parameters and correlation between resistance characteristics of gnats nematodes in lettuce. *Rev. Braz. of Sci. Agrar.* 2011;6(1):46-51.
12. Fernandes AM, Kulczynski SM. Reaction of lettuce cultivars to *Meloidogyne incognita*. *Agrar.* 2009;2(1):143-148.

- 419 13. Charchar JM, Gonzaga V, Vieira JV, Oliveira VR, Moita AW, Aragão FAS. Effect of crop
420 rotation on the control of *Meloidogyne* spp. in carrot in the northern region of the state of
421 Minas Gerais. *Nem. Braz.* 2007;31(1):173-179.
- 422
- 423 14. INMET. National Institute of Meteorology. Climate. Brasília: Inmet. 2016.
424 Available: <http://www.inmet.gov.br/portal/index.php?r=bdmep/bdmep>.
- 425
- 426 15. Fermino MH. Substrates: composition, characterization and method of analysis. Guaíba:
427 Agrolibers; 2014.
- 428
- 429 16. Furlani PR, Silveira LCP, Bolonhezi D, Faquin V. Hydroponic plant cultivation.
430 Campinas: Agronomic Institute of Campinas (IAC); (1999).
- 431
- 432 17. Hussey RS, Barker KR. A comparasion of methods of collecting inocula of *Meloidogyne*
433 spp. including a new technique. *Plant Dis. Rep.* 1973;57(1):1025-1028.
- 434
- 435 18. Bonetti JIS, Ferraz S. Modifications of the Hussey and Barker method for extracting eggs
436 of *Meloidogyne exigua* in coffee roots. *Fit. Braz.* 1981;6(1):553.
- 437
- 438 19. Diniz GMM. Resistance of coriander (*Coriandrum sativum* L.) to *Meloidogyne incognita*
439 (Race 1 and 3) and *Meloidogyne javanica*. Recife, Rural Federal University of Pernambuco;
440 2012.
- 441
- 442 20. Oostenbrink M. Major characteristics of the relation between nematodes and plants.
443 *Med. Van De Land. Te Wag.* 1966;66(4):1-46.
- 444
- 445 21. Ferreira DF. Sisvar: a computer statistical analysis system. *Sci. and Agrot.*
446 2011;35(1):1039-1042.
- 447
- 448 22. Cruz CD. GENES - a software package for analysis in experimental statistics and
449 quantitative genetics. *Act. Sci. Agro.* 2013;35(1):271-276.
- 450
- 451 23. Lessman KJ, Atkins RE. Optimum plot size and relative efficiency of lattice designs for
452 grain sorghum yield test. *Crop Sci.* 1963;3(1):477-481.
- 453
- 454 24. Meier VD, Lessman RJ. Estimation of optimum field plot shape and size for testing yield
455 in *Crambe abyssinica* Hochst. *Crop Sci.* 1971;11(1):648-645.
- 456
- 457 25. Carneiro RG, Ferraz S, Regazzi AJ. Studies of resistance evaluation methods in
458 common bean varieties. *Nem. Braz.* 1992;16(1):53-63.
- 459
- 460 26. Borém A, Miranda GV. Plant Breeding. 6th ed. Viçosa: Publishing UFV; 2013.
- 461
- 462 27. Jesus AM, Wilcken SRS, Kano C, Grassi Filho H. Pathogenicity of *Meloidogyne*
463 *incognita* Race 2 the Banana 'Prata Anã' in Different Substrates. *Nem. Braz.* 2009;33(1):37-
464 44.
- 465
- 466 28. Quénéhervé P. Population of nematodes in soils under banana cv. Poyo in the Ivory
467 Coast, 2. Influence of soil texture, pH and organic matter on nematode populations. *Rev. of*
468 *Nem.* 1988;1(1):245-251.
- 469

- 470 29. Davide RG. Influence of cultivars, age, soil texture, and pH on *Meloidogyne incognita*
471 and *Radopholus similis* in banana. Los Banos: Phil. Agric. and Res. Res. Found.
472 1992;1(1):65-70.
473
- 474 30. Carrijo OA, Liz RS, Makishima N. Fiber of the green coconut shell as an agricultural
475 substrate. Hort. Braz. 2002;20(1):533-535.
476
- 477 31. Ribeiro RCF, Costa CC, Xavier AA, Figueiredo FP, Oliveira FG, Campo VP, Dias-Arieira
478 CR, Mizobutsi EH. Effect of different irrigation slides on the population of *Meloidogyne*
479 *javanica* and banana productivity in northern Minas Gerais. Rev. Braz. Frut. 2009;31(1):090-
480 095.
481
- 482 32. Lewis EE, Campbell JF, Sukhdeo MVK. The behavioural ecology of parasites. 1th ed.
483 Publishing CABI; 2002.
484
- 485 33. Kung SP, Gaugler R, Kaya HK. Soil type and entomopathogenic nematodes persistence.
486 ~~Jour. of Inv. Pat.~~ 1990;55(1):401-406.
487
- 488 34. Massaroto JA, Yamashita OM. Soil properties related to flooding for control of
489 nematodes. Rev. of Sci. Agro-Env. 2011;9(1):153-163.
490
- 491 35. Rebouças JRL, Ferreira Neto M, Dias NS, Souza Neto ON, Diniz AA, Lira RB.
492 Hydroponic cultivation of coriander ~~with salt reject use, Irriga~~, 2013;18(4):624-634.
493
- 494 36. Henriques Neto D, Sediyaama T, Souza MA, Cecon PR, Yamanaka CH, Sediyaama MAN,
495 Viana AES. Size of plots in experiments with irrigated wheat under no-tillage and
496 conventional tillage. Braz. Agric. Res. 2004;39(6):517-524.
497
498