

Methodology for the Genetic Improvement of Coriander Aiming for Resistance to the Root Knot Nematode

ABSTRACT

Coriander is among the most cultivated hardwoods in Brazil, but its production can be compromised due to damage caused by phytonematodes in the growing areas. Although there are several coriander cultivars in the market, none exhibit resistance to nematodes of the genus *Meloidogyne*. Thus, the present work aims at proposing 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 coriander cultivars, handled in three types of containers and three types of substrates, inoculated 15 days after sowing with 1,200 eggs per plant, were evaluated 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 nematode reproduction factor for each cultivar. The use of a 128-cell tray with Basaplant® substrate and experimental plot consisting of eight plants, a value obtained from estimation by the maximum modified curvature method, 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 plot, 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 leafy vegetables in the world market for vegetable seeds, being losses in post-harvest capable of reaching up to 100% of production [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] and the name of the color in Portuguese. This cultivar was developed from the selection of plants of the cultivar Palmeira, which presented anthracnose tolerant stains and would alternate and cross with accessions of coriander criollo collected in properties in the States of Piauí, Maranhão and Pernambuco.

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

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

One of the symptoms of the presence of the **root knot nematode** in the host is the formation of galls in the root system with consequent reduction in nutrient absorption and translocation of water. **This results** 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 **root knot nematodes** (*Meloidogyne spp.*) becomes the focus of programs for the genetic improvement of coriander in the country. In addition, selection for agronomic characteristics, such as those cited by [3]: leaf/stem ratio, greater leaf size and thickness, and attainment of a period of more extensive post-harvest conservation are indispensable.

According to [7], nematodes are among the pathogens that cause damage to cultivated plant species, causing losses of approximately 5% in crop worldwide in various crops. Besides, they are difficult to control. Among its genus, *M. incognita* is an extremely aggressive species, with 61 distinctive enzymes capable of degrading the cell wall, justifying the wide range of hosts. [8]. These authors pointed out that identifying sources of resistance to the genus *Meloidogyne*, mainly among commercialized cultivars or even 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 genotypes to **root knot** nematodes. However, there is variation in the methodologies applied mainly in relation to the types of containers: pots, 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 **root knot** nematode is a fundamental step for breeding programs, considering that the use of inadequate methodology can limit the aggression of the nematode, 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 container and/or substrate more favorable to the selection of coriander genotypes for resistance to *M. incognita* race 1, by means of the reaction of coriander cultivars to the root knot nematode. In order to identify the optimum size of the plot that can optimize the selective process of phytonematodes resistant genotypes, the modified maximum curvature method was performed.**

2. MATERIAL AND METHODS

2.1. Study Location and Site Description

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 temperatures during the work were 21.7°C for the minimum temperatures and 29.5°C for the maximum, according to data obtained from the Recife meteorological station [14].

2.2. Experimental Field and Experimental Design

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 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 2,700 individuals evaluated.

For evaluating the reactions of Verdão, Tabocas, Tapacurá, Palmeira and HTV Dom Luiz coriander cultivars, they were inoculated with *M. incognita* race 1 and handled in three types of containers: 400 ml beaker, 116 ml tube and 128-cell tray with approximate volume of 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 (Yellow Latosol) was sterilized by autoclaving at 120 °C for two intermittent hours.

Physical characteristics and chemical properties of the substrates, such as porosity, aeration space, density, pH and electrical conductivity (EC). The vessel method was used to determine the porosity and aeration space. Densities of wet substrates and of their air dried samples were obtained by the autocompaction method. 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, a plot was established with tomato Santa Clara cultivar (*Solanum lycopersicum* L.) in each replication which is susceptibility standard to root knot nematodes.

Sowing was performed at 1 cm depth by placing three coriander fruits or three tomato seeds per container, where thinning was carried out after the germination when the plants produced the first definitive leaf, leaving only one plant.

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

At 15 days after sowing, with the aid of a veterinary syringe, a 1,200 egg-inoculation per container was carried out for three week days, 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 Santa Clara cultivar.

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. Next, the galls were counted again followed by egg extraction of each cultivar separately, according to methodology proposed by [17] and modified by [18]. These eggs were stored with water in labeled containers and placed in cold chamber until the counting of the number of eggs per plant. In the quantification of the number of eggs, a Peter's counting slide and a microscope with a magnification of 40X were used.

2.3. Response Variables

The score scale proposed by [19] was used on assessments with coriander genotypes as for the following characteristics: incidence of galls, number of galls, and number of eggs. For the incidence of galls, score 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, score 1 = number of galls ≤ 10;

139 2 = number of galls ≥ 11 and ≤ 20 ; 3 = number of galls ≥ 21 and ≤ 30 ; 4 = number of galls $\geq$
 140 31 and ≤ 40 and 5 = number of galls ≥ 41 . For the number of eggs, score 1 = number of
 141 eggs <1200 ; 2 = number of eggs 1200 - 2400; 3 = number of eggs 2401 - 3600; 4 = number
 142 of eggs 3600 - 4800 and 5 = number of eggs ≥ 4801 .

143

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

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149 2.4. Statistical Analysis

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151 Data were transformed into $\sqrt{x}$ and submitted to analysis of variance, where the means of
 152 the treatments were grouped by the Scott-Knott test to 5% significance. Analyzes were
 153 performed by the SISVAR software [21].

154

155 In order to obtain the optimal size of the experimental plot, we used the data from the
 156 originally installed plots to perform 2,000 samplings applied in simulations in order to
 157 estimate the CV% and for the sizes of the plots, starting with two plants and increment of
 158 one plant, up to 20 plants per plot. For this process, the statistical software GENES [22] was
 159 used. The optimal size of the experimental plot was determined by the modified maximum
 160 curvature method proposed by [23]. In this method, the relationship between the coefficient
 161 of variation and plot size with X basic units is explained by the regression model $CV = aX^b$,
 162 where "a" and "b" are the parameters to be estimated. From the curvature function given by
 163 this model, the value of the abscissa was determined where the point of maximum curvature
 164 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
 165 point of maximum curvature, which corresponds to the estimation of the optimal size of the
 166 experimental plot [24].

167

168 3. RESULTS AND DISCUSSION

169

170 There was a significant difference for triple interaction, cultivars X containers X substrates, at
 171 the level of 5% probability for incidence of galls and number of galls in the root system and
 172 1% probability for egg number and reproduction factor (Table 1). The coefficients of
 173 experimental variation ranged from 13.52%, for incidence of galls, to 24.68% for number of
 174 eggs. Cultivars Verdão, Tabocas, Tapacurá, Palmeira and HTV Dom Luiz responded to the
 175 treatments used, expressing differences in the means obtained for all the variables under
 176 study.

177

178 Table 1. Summary of analysis of variance of the incidence of galls (IG), number of
 179 galls in the root system (NGRS), number of eggs (NE) and reproduction factor (RF) in
 180 coriander cultivars inoculated with *Meloidogyne incognita* race 1. Recife, UFRPE,
 181 2016.

VF	GL	QM			
		IG	NGRS	NE	RF
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 ($P = 0.05$); ** Significant ($P = 0.01$).

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 test for galls incidence (IG), number of galls in the root system (NGRS), number of eggs (NE) and reproduction factor (RF) for the evaluation of coriander cultivars in the greenhouse 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
NGRS	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
NE	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
RF	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
	B	1.69 aBα	2.42 cCβ	1.59 aAα	2.01 bBβ	1.54 aAα
Tray	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 ($P = 0.05$). (J2-100 g⁻¹ soil). 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 Verdão cultivar, for galls incidence, the use of a tray with any substrate presented the highest averages, while in the root system number of galls variable the treatments did not differ among one another. For number of eggs, the tray treatments with Basaplant® + coconut fiber and the 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 Verdão cultivar, the Basaplant® substrate can be used with any of the three containers.

In the Tabocas cultivar, for incidence of galls, the use of tray with any of the substrates presented the highest averages, whereas in the number of galls in the root system variable, the tray treatments with Basaplant®, the tray with soil + sand + humus and the cup with Basaplant® presented the highest averages. For number of eggs, the tray treatments with Basaplant® + coconut powder, the tray with Basaplant® and the 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 Tabocas cultivar, 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 for galls incidence within the cultivar Tapacurá, 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 of the treatments did not differ among one another, except for the compounds with the Basaplant® tube + coconut powder and soil + sand + humus. The number of eggs had their highest averages in the treatment cup with Basaplant®, cup with soil + sand + humus and the tray with Basaplant® + coconut powder. For the reproduction factor, the tray treatments with Basaplant®, the tray with soil + sand + humus, the tube with Basaplant® and the 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 Palmeira cultivar, in the incidence of galls variable, 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, the tray with Basaplant® or soil + sand + humus were the treatments with higher averages. Thus, the use of a tray with Basaplant® has the highest averages obtained for the studied variables.

In the cultivar HTV Dom Luiz, for galls incidence, the use of a 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 have better performance. As for number of eggs and reproduction factor, the treatments that presented lower means 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 that which 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 galls incidence variable, there was no significant difference for most treatments, except for cup with soil + sand + humus where the cultivar Tapacurá presented an average higher than the others did. Due to its low ability to identify differences between genotypes, this variable is not a good option for selection among cultivars. In the number of galls in the root system variable, there was no significant difference between the cultivars only in the cup treatments with Basaplant® + coconut powder and tray with Basaplant®. The Verdão cultivar showed higher averages than the others, where the cup with Basaplant® treatment was the one that was able to identify greater difference between the cultivars evaluated. For the number of eggs and reproduction factor variables, in the treatments with tube, there was no significant difference between the cultivars, while all other treatments were able to identify them, being the cup with Basaplant® + coconut powder and tray with Basaplant® treatments those that presented greater capacity to identify the genotypes under study.

The evaluation of the resistance to *Meloidogyne* requires the adoption of an efficient, rapid and practical method 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], the greater the heritability the greater the gain. Based on the exposed, the cup with Basaplant® and the tray with Basaplant® treatments 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 $RF < 1$. In the same way, the cup treatments with Basaplant® + coconut powder and soil + sand + humus in the Verdão cultivar also presented $RF < 1$. These results were not due to the genetic resistance of the cultivars, but to the effect of the environment that possibly disadvantaged the nematode, interfering in the selective process carried out by the breeder (Table 2).

[27] verified variation of RF as a function of substrate type, even under low population densities (2,000 eggs). Under these conditions, banana plants 'Prata Anã' presented rickety size. 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. These authors verified that the plastic vessel of 3 L was the most impracticable because of the dispersion of eggs in their large volume, caused 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 to climatic conditions [28,29]. Thus, the type of substrate used in an experiment evaluating plant genotypes for resistance nematodes, influence the

development of the nematode and the infection ability. Thus, 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. This allowed 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 nematode, possibly because it was not integrated into the soil system.

In the results obtained for the physical analysis of the substrate, 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 nematode. 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 mainly due 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 Basaplant substrates. 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 movement. 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 storage of water and/or nutritive solution, not limiting the movement and consequent ability to parasitize the plant roots, because both the supply of moisture and oxygenation are important, as discussed by [33]. This emphasizes for 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 nematodes, 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, where pH values above 7.0 are a limitation to the development of **phytonematodes**. Thus, the substrate soil + sand + humus treatment was the only one that presented 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.

TABLE 4. Regressions, coefficients of determination (R²) and estimates of optimal plot sizes (X₀) 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
					2.14
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
					1.83
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
					3.16

IG - incidence of galls; NGRS - number of galls in the root system; NE - number of eggs.
(J2·100 g⁻¹ soil).

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 (R²) 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 incidence of galls variable, the lowest value was obtained for the Verdão cultivar (X₀ = 1.76) and the highest was observed in Tapacurá (X₀ = 4.58). Among the three characteristics assessed namely incidence of galls, number of galls in the root system, and number of eggs, the latter presented the highest values for X₀, 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 X₀ = 4.74 ≈ 5.00 plants per plot.

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, using 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.

4. CONCLUSION

The methodology with better efficiency in differentiating Verdão, Tabocas, Tapacurá, Palmeira and HTV Dom Luiz cultivars, aiming for resistance to *M. incognita*, consisted in using a 128-cell expanded polystyrene tray with commercial Basaplant® substrate, with experimental plot consisting of eight plants. This methodology is able to identify cultivars with experimental reliability, without underestimating or overestimating their values as the incidence of galls, number of galls in the root system, number of eggs and reproduction factor variables.

COMPETING INTERESTS

Authors have declared that there are no competing interests.

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