Review Paper

Factors influencing the evaluation of the reaction of coriander genotypes to the ~~gill~~ nematode

ABSTRACT

Coriander is among the most hardwood vegetables produced and consumed in Brazil, because it is part of the national cuisine, mainly in the Northeast and North regions of the country. Its leaves and seeds are much appreciated because of the aroma and flavor that ~~it gives~~ to the dishes in which they are incorporated. The culture is traditionally exploited by small farmers, having great socioeconomic importance. Among the factors limiting the crop production are the nematodes, among them those belonging to the genus *Meloidogyne* that ~~promote~~ damage to the root system of the plants affecting ~~the~~ production and productivity ~~of the crops~~. The identification and indication of superior genotypes is extremely important, and it is necessary to use appropriate methodology to evaluate the coriander genotypes for the reaction to the ~~gill~~ nematodes, selecting the resistant ~~individuals.~~

Key words: *Coriandrum sativum* L., experimental plot, inoculum ~~concentration,~~ *Meloidogyne incognita*.

GENERAL CONSIDERATIONS ON CORIANDER CULTURE

~~The coriander~~ (*Coriandrum sativum* L.) is a species probably originated from the region between the eastern part of the Mediterranean basin and the Caucasus [3]. The plant is herbaceous and annual with phenological cycle of three to four months, has a very branched root system, presents heterofilia being leaves arranged in rosette where the inferior ones are usually ovate and the ~~superior~~ ones are cropped, pale green color [14]. In the vegetative phase, the plant is 30 to 50 cm ~~high~~ and can reach up to one meter. In the reproductive phase, there is the development of the cylindrical, hollow and branched stem from which the inflorescences appear, of the umbels type, composed of three to ten rays that support umbeletas that have from ten to twenty flowers each, ~~whose~~ flowers are small with white corolla or rosada [3] being present inside the stamped umbeletas. The higher order umbeletas generally contain more staminate flowers than the first ones, and their flowering period is shorter [14]. The male flowers occur mixed hermaphrodites throughout the coriander inflorescence, ~~the~~ differentiation of the two types of flowers being considered an epigenetic phenomenon [45].

The plant is allogenic and pollination is carried out by insects [3]. Similar to other species of the Umbelliferae family, the coriander is diploid with a karyotype consisting of 11 pairs of homologous chromosomes, 2n = 22 [10].

The fruit is a striated globular schizocarp [3], composed of two seeds, which among the constituents are the essential oils, where linalool has the highest percentage, corresponding to 73.11% [56].

Coriander is one of the most cultivated vegetables in the states of Northeastern Brazil [39]. It involves a large number of producers in its exploitation throughout the year, making it of great social and economic importance. It usually has fresh leaves and dried fruits used in the composition of dishes and seasonings that are part of the cuisine of several countries [3]. It is also a source of raw material for the food, pharmaceutical and alternative medicine industries [41] and perfumery industry [33].

In Brazil the cultivation is traditionally carried out by small producers, in home gardens, school and community, in single crop or consorciado with other vegetables. The coriander consortium is agroeconomically viable with beet [23], chives [55], lettuce [34] and cabbage, for this, contributed with greater diversity of species (Eucalyptus spp.), which is responsible for the increase of yield of fresh aerial biomass of 92% more in the cabbage, being efficient in organic management in this type of consortium [40]. In the coriander-tomato consortium, there is favor of the natural biological control of the whitefly in an organic cultivation system [49].

The world market for vegetable seeds is around US $ 4.6 billion, consisting of the chain of research companies, international traders, multipliers, distributors and resellers. More than 70% of the commercialization of vegetable seeds takes place in Asia and Europe, which can be attributed to the population in Asia and the use of the seeds with the greatest genetic potential in Europe. This market is normally divided into six major segments: Solanaceae, bulbs and roots, brassicas, large seeds, cucurbitaceous and hardwood, whose main ones are spinach, lettuce and coriander [1].

In Brazil the seed market is estimated at approximately ~~R~~ $ 10 billion, with a very solid and robust seed industry behind only the United States and China~~. As~~ a result of the growth and professionalization of a segment of great importance, such as the market of forage seeds and oilseeds, which currently represent 11% and 6% of the total seed market, respectively [2]. ~~And the~~ market for the marketing of coriander seeds exceeded nine and a half million Brazilian real.

About one hundred companies and institutions are involved in research on vegetable breeding. These include Monsanto, Syngenta and Bayer; but there are also several other large companies with state-of-the-art technology~~,~~ institutes such as universities in the United States, Israel and China, and dozens of small and medium-sized private companies. Many of these companies are located in major vegetable growing regions such as the United States, Italy, France, China and Turkey, but also in places with a tradition of research, such as Israel and Japan, or in international trade, such as the Netherlands. These companies perform breeding research on dozens of vegetable and fruit species with a short cycle, with the aim of developing new varieties that are better adapted to the different growing conditions in the world and meet the expectations of the entire production chain: farmer, processor, supermarket and final consumer [1].

In Brazil the cultivated varieties of coriander are divided into two groups, late and early. The latter are adapted to the subtropical or temperate climate, being indicated for the Southeast and South regions of the country, and have a longer vegetative phase generally of 50 to 60 days, being included in this group the Portuguese, Santo, Aztec, Giant American, Tapacura and others. The early cultivars are more adapted to the tropical climate, being indicated for the North and Northeast of the country, have a vegetative phase in general of 30 to 45 days. Included in this group ~~are~~ the cultivars Verdão, Palmeira and Tabocas, which correspond to more than 80% of the area cultivated with coriander [51].

In several countries the cultivation of coriander is carried out aiming the extraction of the essential oil present in the fruits, being this a characteristic of interest of the programs of improvement of the places where this activity is carried out. According to [35], the planting of ~~this~~ olive grove is aimed at the production of green mass, promoting the development of cultivars that meet this demand, showing a high leaf / stem ratio, larger leaf size and thickness and obtaining a longer period of post-harvest conservation, being these focus of the breeding programs of coriander in Brazil. In addition, the resistance to abiotic and biotic stresses are indispensable points in the selective processes of superior genotypes. As for the biotic stresses are the diseases that affect the coriander culture, such as leaf-burning (*Alternaria dauci*); cercosporiosis (*Cercospora* spp.); bacterial blight (*Xanthomonas* spp.); nematode (*Meloidogyne* spp.) [24] and (*Rotylenchulus reniformis*) and anthracnose (*Colletotrichum gloeosporioides* Penz).

The cultivar Verdão [7], developed from the crossing of plants of the Palmeira cultivar, tolerant to anthracnose and alternaria, with plants collected from farmers in the states of Piauí, Maranhão and Pernambuco. Being the name Verdão attributed due to the dark green coloration

~~that~~ its leaves ~~present~~ [35]. Although market leader, the Verdão cultivar ~~presents~~ undesirable characteristics as early swallowing and susceptibility to the nematodes ~~of the galls~~. It is necessary to identify genotypes of coriander resistant to Meloidogyne spp., ~~Which~~ according to [19], the search for genotypes resistant to the nematodes ~~of the galls~~ in the cultivated species is very important, mainly among commercialized cultivars, or from the development of new cultivars that are adapted to the diverse Brazilian conditions, which has aroused the attention of some researchers, especially from the early 1990s [19].

In order to optimize the selective process of *Meloidogyne* resistant coriander genotypes, it is necessary to use adequate methodology in breeding programs which can be obtained from the identification of the best container, substrate, inoculum ~~concentration~~, inoculation time and size of experimental plot, in order to allow evaluation time and increase selective efficiency.

THE ~~GILL~~ NEMATODES

Phytonematoids are worms belonging to the Animalia kingdom and the Nemata phylum, which is composed of species of free life in the soil, in fresh water and in the sea and the parasites of man, animals and plants [22]. In this phylum ~~are~~ the species known as ~~gill~~-forming nematodes of the genus *Meloidogyne* [20], difficult-to-control pathogens that cause damages to cultivated species, causing losses of approximately 5% in crop worldwide in several crops [30].

~~Gill~~ nematodes are obligate, sedentary endoparasites and ~~present~~ sexual dimorphism, occurring in the post-embryonic development differentiation between the body of the females and males, having the life cycle affected by environmental factors and host plant species, which will interfere in the differentiation sexuality, reproduction and fertility [32, 22, 47]. The life cycle consists of six stages of development: eggs, juveniles (J1, J2, J3, J4) and adult, where the egg is deposited by the female in a gelatinous matrix with development a few hours after oviposition until juvenile ~~formation~~ (J1) in which a second stage juvenile ecdysis occurs (J2) ~~that explodes~~ and migrates in the soil ~~the search~~ for roots of plants ~~susceptible to host them~~ [47].

After penetrating the root, J2 migrates to the vascular tissue and begins to feed, ~~introducing~~ substances in plant cells, which causes morphological and physiological changes promoting the increase of size and division of nuclei without division of the cell wall, accelerating the metabolism, promoting gall formation and the physical obstruction of water and mineral conducting vessels, resulting in premature wilt and nutrient deficiency symptoms, leading to underdevelopment of the plant. Inside the root J2 suffers 3 more ecdises reaching adulthood. At this stage, the male leaves the root, but, the females remain inside the ~~gill~~ and ~~place~~ the egg mass outside the root [21].

The main ~~gill~~ nematode species that attacks coriander culture is ~~the~~ *Meloidogyne incognita* (Kofoid and White) Chitwood race 1, which displays year-round activity in hot climates and moist soils [39]. In the coriander, the pathogen attack promotes the development of isolated and small-sized galls, which occur along the roots and usually ~~present~~ only one female per ~~gill~~ and often exhibit egg mass ~~external to~~ the plant tissue, due to the caliber of the roots that are almost capillary. The cultivars Palmeira, Verdão and Portuguese are good hosts of *M. incognita* race 1 [8], being one of the most aggressive species of the genus, ~~extremely aggressive,~~ has about 61 different enzymes capable of degrading the cell wall, justifying the wide range ~~of host~~ [30].

The control of the ~~gall~~ nematodes is difficult and costly to raise, and several forms of control are proposed, ranging from biological control to the use of pesticides. In some crops, control is generally achieved through the application of nematicides, which will be progressively excluded due to adverse effects on human health and the environment [52].
In the coriander culture, the control of the nematoids is not an easy task, since the microorganisms are soil dwellers and in favorable conditions of temperature and humidity multiply rapidly, allied to this, the cultivation of coriander is usually carried out yearly intensively in the the use of manure, the use of organic matter, ~~the use of organic matter~~, ~~the use of organic matter,~~ ~~the use of organic matter,~~ solarization, biological control and the use of resistant cultivars [39].

There are more than 40 cultivars of coriander registered in MAPA in Brazil, however, little is known about their reaction to ~~gall~~ and reniform nematodes [39]. [8] evaluated the reaction of the cultivars Verdão, Palmeira and Portuguese as ~~tolerance~~ to *M. incognita* race 1 and all were susceptible to the pathogen. Already, [15] evaluated the cultivars Portuguese, Tabocas, Tapacurá, Verdão, Palmeira for both *M. incognita* race 1 and ~~M. javanica~~, verifying that all cultivars were susceptible to *M. incognita* race 1 and all were resistant to *M. incognita* race 3 and *M. javanica*.

In the evaluation of coriander genotypes, it is fundamental to use a methodology capable of expressing the most accurate variability and data information, and it is necessary to select the recipient, substrate, inoculum ~~concentration~~ and size of the plot to provide the expression of the pathogen and to differentiate the genotypes regarding the reaction of infection caused by the worm.

TYPES OF CONTAINERS AND SUBSTRATE USED IN THE EVALUATION OF THE REACTION OF CULTIVATED SPECIES TO THE ~~GALL~~ NEMATODES

According to [4], among the difficulties of assessing the resistance of soybean to ~~gnath~~ nematodes, there is variability of pathogens, limitation of sources of resistance, different criteria for classification of genotypes and lack of standardization of methodologies, which may result in contradictory information.

The lack of standardization is ~~present~~ in the evaluation of the reaction to the ~~gill~~ nematodes in several species of economic importance. In lettuce, there are experiments conducted in: expanded polyethylene trays with 128 cells and commercial Plantmax substrate; vessels containing 2 L of substrate obtained by the mixture of soil, sand and organic matter (1: 2: 1), previously autoclaved, plastic cups containing 700 cm^3 of substrate formed by the mixture of soil and sand (1: 1), previously autoclaved [43, 53, 17].

In the evaluation of the soybean genotypes TMG 115 RR (susceptible standard) and BRS 316 RR (resistant standard), seeded in three types of plastic containers: 290 mL ~~pots and~~ 1L and 2L pots of substrate, composed of (1: 3), it was found that the type of recipient did not interfere with the GI of the cultivars, however, the highest RF for the two cultivars was observed when they were cultivated in the 1-L pot [4].

Evaluating the banana 'Prata Anã' on different substratum types and inoculum levels, there was a significant interaction between these two sources of variation on the dry mass of the banana shoots, with high nematode multiplication rates observed in all substrates, mainly in 4 and 5, which presented higher number of nematodes per gram grass and higher reproductive factor [25].

In the bean (*Phaseolus vulgaris* L.), it was observed that the type of vessel influences the expression of the reaction of genotypes inoculated with *Meloidogyne incognita*, the plastic vessel being 3 L most unviable when compared to the polystyrene box "Plantagil 1477". 128 cells of 70 cm3 and plastic cups of 200 cm3, fact attributed the dispersion of eggs due to the large volume of the container [11].

In coriander, there is also a variation regarding the container and substrate used, being verified from the use of soil, sand and humus (3: 1: 1), Plantmax and Basaplant; with the cultivation carried out on 128-cell expanded polyethylene trays or 2-L pot [8, 15, 42].

In view of the above, it is evident that depending on the type of substrate and container used, variation of the expression of the evaluated genotypes may occur as regards the reaction to the ~~gill~~ nematodes, which can lead to results that erroneously classify the individuals, being necessary ~~the~~ previous study of the best interaction vessel X substrate that allows the classification and differentiation between the genotypes ~~and~~ resistance to the pathogen.

~~CONCENTRATION~~ OF INOCULUM

The ~~concentration~~ of the inoculum used in an experiment to verify the reaction of cultivated species to ~~the gill~~ nematodes is also an important factor, and it is essential to study the concentration most indicated, according to the efficiency in characterizing the genotypes in resistant or susceptible, for each plant species.

In the first experiment ~~the~~ inoculation of approximately 5000 eggs and juveniles of the second stage was carried out while in the second the plants were inoculated with 1500 eggs and juveniles, no difference was verified in the selection of resistant cultivars~~, because [53]~~. However, it is not necessary to use high ~~concentrations~~ of the inoculum to evaluate the resistance of lettuce plants to the ~~gill~~ nematodes [53].

In the banana tree, when evaluating different levels of inoculum (0, 2000, 10000 and 50000 eggs and infective juveniles) of *Meloidogyne incognita* race 2, it was verified that, regardless of the substrate used, the highest FR was verified in plants inoculated with 2000 eggs and juveniles [25].

The study of the ~~concentration~~ of inoculum to be used in the evaluation of coriander plants in relation to the resistance reaction to ~~gall~~ nematodes is a fundamental step in crop breeding programs in order to classify and select the individuals that actually ~~present resistance~~ to the pathogens.

NUMBER OF PLANTS PER EXPERIMENTAL PLOT

The size of the experimental plot is of fundamental importance in the evaluation of the reaction of plants the infections caused by ~~the~~ nematodes ~~of the galls.~~ According to research carried out with different cultures under conditions of stress caused by M. incognita, a variation on the size of the experimental plot with a lettuce plant is observed [17]; six plants in sweet potato [28]; area of 1 m^2 in carrot [12]; five pepper plants [35]; two soybean plants [47]; [8], 16 plants [15] and eight plants [41].

In plant genetic improvement, the minimum sample size is the minimum number of elements required to estimate the mean and variance of the characters under study [53]. Such size depends on the species under study, the environment and the characters evaluated [6].

The estimation of the appropriate number of plants per plot can be performed by different methods, based on the relation between plot size and residual variation; such as Farifield Smith method, visual inspection method of maximum curvature, modified maximum curvature method, linear model of plateau response (MLRP), relative information method, Pimentel Gomes method, among others.

The method presented by [45] is based on the empirical relationship between plot size and variance, establishing a relation by the expression:

$$V_{\bar{x}_i} = \frac{V_1}{x_i^b},$$

at where: the variance of the average yield per basic unit for plots of Xi units; I saw the variance of $V_{\bar{x}_i}$ plot yield with a basic unit; the number of basic units of size i (i = 1, 2, ..., n) and b the coefficient of soil heterogeneity. The author calculates the optimal plot size by associating the soil heterogeneity coefficient with the costs of the experiment.

The method of visual inspection of the maximum curvature was proposed by [16] to determine the optimal size of the experimental plot, using data from an experiment in which the values are obtained in a basic experimental unit of size X. For each size of plot (X) ~~is~~ calculated the coefficient of variation (CV (x)). The set of ordered pairs (X, CV (x)) are plotted on a graph and the curve formed by the union of the points. The optimum plot size is the point of maximum curvature of the curve, which is determined by visual inspection.

The relative information method proposed by [25] aims to extract the greatest amount of information from a unit of area. If 100% of relative information (IR) is assigned to parcels whose size is equal to a basic unit (UB) and in the other parcels (with different size of the UB) the IRs are obtained from the division of the variance of the UB palas comparable variances VC (x) = V (x) / X, which consists of the quotient between the variances of a given parcel size by the number of UBs of the respective size; IR being obtained IR = V1 / VC (X).

The method proposed by [37] is indicated for experimentation with arboreal species, based on the minimization of the variance of the mean of treatments V (m), which is a function of the number of useful trees per plot (k) and number of rows useful by plot (n). The optimal plot size consists of a combination of the values of (n) and (k) which makes the value of V (m) minimum. The estimation of such size is based on the interclass correction coefficient (ρ) and the soil heterogeneity index (b):

$$\rho = \frac{K^{1-b} - 1}{K - 1} \qquad (K > 1)$$

on what:
k is the number of neighboring subplots, the value of which must be fixed;
b is the soil heterogeneity index.
For b = 0, ρ = 1 and when b = 1, ρ = 0.

The linear model of plateau response was used by [36] as a new method for the calculation of optimal plot size and uses the following regression model:

$$CV_i = \begin{cases} \beta_0 + \beta_1 X_i + \varepsilon_i & \text{se} \quad X_i \le X_c \\ P + \varepsilon_i & \text{se} \quad X_i > X_c \end{cases};$$

where CVi is the coefficient of variation observed experimentally among plot totals of size of Xi basic units; Xi is the size of the plot in clustered basic units, Xc is the optimal size of plots for which the linear model becomes a plateau, relative to abscissa; P is the coefficient of variation at the point corresponding to the plateau; i ε is the error associated with the CV (x) considered normally and independently distributed with mean 0 and variance 2 σ ε constant. The optimal plot size was estimated by the expression:

$$X_c = \left(\hat{P} - \hat{\beta}_0\right) / \hat{\beta}_1 ;$$

Where β0 β1, and P are estimates of β0 β1, and P parameters of the regression model, obtained using non-linear regression methods, according to Bates and Watts (1988), using the nls function of the R.

The method of maximum modified curvature proposed by [26] was to represent the relationship between the coefficient of variation (CVexp) and plot size, using the regression equation of type Y = aX-b (in that Y represents the coefficient of experimental variation, and X corresponds to the plot size). From the curvature function, the abscissa value is determined where the point of maximum curvature occurs, as presented by [30], by means of:

$$X_{MC} = \left[\frac{a^2 b^2 (2b-1)}{(b-2)}\right]^{\frac{1}{2-2b}}$$

where XMC = abscissa at the point of maximum curvature, which corresponds to the estimate of the optimal size of the experimental plot; a = constant of the regression; and; b = regression coefficient.

Even using the principle of the maximum curvature method, the modified maximum curvature method is more precise than this, by establishing a regression equation between the number of plants per plot and the coefficient of variation in order to explain the relationship between these parameters [49]. A number of authors ~~have~~ reported the efficiency of this method in estimating the number of plants per experimental plot, as in eucalyptus [42], coffee [13], papaya [9, 27].

FINAL CONSIDERATIONS

In view of the above, it can be ~~seen~~ that different factors are fundamental for the evaluation of the reaction of coriander genotypes to infection caused by ~~the nematodes of the galls~~, being essentially the use of adequate methodology, which ranges from the type of container to the size of the experimental plot, being these~~,~~ primordial factors for greater efficiency of the selective process aiming at obtaining resistant genotypes.

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