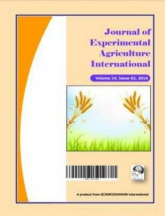

Journal of Experimental Agriculture International

20(3): 1-9, 2018; Article no.JEAI.39300
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Factors Influencing the Evaluation of the Reaction of Coriander Genotypes to Root-knot Nematodes: A Review

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2018/39300
Editor(s):
(1) Mohammad Reza Naroui Rad, Department of Seed and Plant improvement, Sistan Agricultural and Natural Resources Research Center, AREEO, Zabol, Iran.
Reviewers:
(1) Mahmoud M. A. Youssef, Egypt.
(2) Ade Onanuga, Canada.
Complete Peer review History: http://www.sciencedomain.org/review-history/23013

Review Article

Received 9th November 2017
Accepted 20th January 2018
Published 3rd February 2018

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 they give 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, which are those belonging to the genus *Meloidogyne* that cause damage to the root system of the plants affecting their production and productivity. Identification and indication of superior genotypes are extremely important, and it is necessary to use appropriate methodology to evaluate the coriander genotypes for the reaction to the root-knot nematodes, selecting the resistant genotypes.

Keywords: Coriandrum sativum L.; experimental plot; inoculum level; Meloidogyne incognita.

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1. GENERAL CONSIDERATIONS ON CORIANDER CULTURE

Coriander (*Coriandrum sativum* L.) is a species probably originated from the region between the eastern part of the Mediterranean basin and the Caucasus [1]. The plant is herbaceous and annual with a phenological cycle from three to four months. It has a very branched root system, and presents heterofilia, being leaves arranged in rosette where the inferior ones are usually ovate and the above ones cropped, and pale green colour [2]. 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, and of the umbels type, composed of three to ten rays that support umbeletas that have from ten to twenty flowers each. Those flowers are small with white or pinkish corolla [1] present inside the stamped umbeletas. The higher order umbeletas generally contain more staminate flowers than the first ones, and their flowering period is shorter [2]. The male flowers occur mixed with hermaphrodites throughout the coriander inflorescence. The differentiation of the two types of flowers is considered an epigenetic phenomenon [3].

The plant is allogamous and pollination is carried out by insects [4]. Similar to other species of the Umbelliferae family, coriander is diploid with a karyotype consisting of 11 pairs of homologous chromosomes, 2n = 22 [5].

The fruit is a striated globular schizocarp [1], composed of two seeds. Among its constituents, there are essential oils, with linalool has in the highest percentage, corresponding to 73.11% [6].

Coriander is one of the most cultivated vegetables in the states of Northeastern Brazil [7]. 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 [1]. It is also a source of raw material for the food, pharmaceutical and alternative medicine industries [8] as well as the perfumery industry [9].

In Brazil, coriander cultivation is traditionally carried out by small producers, in home, school and community vegetable gardens, in single crop or joined with other vegetables. The coriander consortium is agro-economically viable with beet [10], chives [11], lettuce [12] and kale. A great diversity of ladybug species contributed for this, promoting an increase of yield of fresh aerial biomass of 92% more in kale, being efficient in organic management in this type of consortium [13]. In the coriander-tomato consortium, there is favour of the natural biological control of the whitefly in an organic cultivation system [14].

The world market for vegetable seeds is around the 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 greater 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 [15].

In Brazil, the seed market is estimated at approximately US $ 10 billion, with a very solid and robust seed industry only behind the United States and China. This is 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 [16]. The market for the marketing of coriander seeds exceeded nine and a half million Brazilian real [17].

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 the final consumer [15].

In Brazil, cultivated varieties of coriander are divided into two groups, namely late and early. The latter is adapted to the subtropical or temperate climate, is indicated for the Southeast and South regions of the country, and have a longer vegetative phase generally from 50 to 60 days, is included in this group the Portuguese, Santo, Aztec, Giant American, Tapacura and others. The early cultivars are more adapted to the tropical climate, is indicated for the North and Northeast of the country, and have a vegetative phase in general from 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 [18].

In several countries, the cultivation of coriander is carried out aiming at the extraction of the essential oil present in the fruits, is this a characteristic of interest of the improvement programs of the places where this activity is carried out. According to [19], olive grove planting 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, obtaining a longer period of post-harvest conservation, being this the focus of the breeding programs of coriander in Brazil. In addition, resistance to abiotic and biotic stresses are indispensable points in the selective processes of superior genotypes. As for the biotic stresses, they are diseases that affect coriander culture, such as leaf-burning (*Alternaria dauci*); cercosporiosis (*Cercospora* spp.); bacterial blight (*Xanthomonas* spp.); nematode (*Meloidogyne* spp.) [20], reniform nematode (*Rotylenchulus reniformis*), and anthracnose (*Colletotrichum gloeosporioides* Penz).

The cultivar Verdão [21], developed from the crossing of plants of Palmeira cultivar that is tolerant to anthracnose and alternaria, with plants collected from farmers in the states of Piauí, Maranhão and Pernambuco. The name Verdão (big green) was attributed due to the dark green colouration of its leaves [19]. Although market leader, the Verdão cultivar has undesirable characteristics as early swallowing and susceptibility to the root-knot nematodes. It is necessary to identify genotypes of coriander resistant to Meloidogyne spp. According to [22], search for genotypes resistant to the root-knot nematodes in the cultivated plant species is very important, mainly among commercialized cultivars, or from the development of new cultivars that are adapted to the diverse Brazilian conditions. This has aroused the attention of some researchers, especially from the early 1990s [22].

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

2. ROOT-KNOT NEMATODES

Phytonematodes are worms belonging to the Animalia kingdom and the Nemata phylum, which is composed of free life species in the soil, in fresh water and in the sea and the parasites of man, animals and plants [23]. In this phylum, the species are known as gall-forming nematodes of the genus *Meloidogyne* [24]. They are difficult-to-control pathogens that cause damages to cultivated species, causing losses of approximately 5% in crop worldwide in several crops [25].

Root-knot nematodes are obligate, sedentary endoparasites, and have sexual dimorphism, occurring in the post-embryonic development differentiation between the body of the females and males. Their life cycle is affected by environmental factors and host plant species, which will interfere with the sexuality differentiation, reproduction and fertility [26,23, 27]. Their 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 few hours after oviposition until the first stage juvenile formation (J1) in which a second juvenile ecdysis stage occurs (J2), breaks out and migrates into the soil searching for roots of host-susceptible plants [27].

After penetrating the root, J2 migrates to the vascular tissue and begins to feed, excreting substances in plant cells, which cause 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. This results in premature wilt and nutrient deficiency symptoms, leading to the plant underdevelopment. Inside the root, J2 suffers 3 more ecdises reaching

adulthood. At this stage, the male leaves the root, but the females remain from inside the gall and deposit the egg mass outside the root [28].

The main root-knot nematode species that attacks coriander culture is *Meloidogyne incognita* (Kofoid and White) Chitwood race 1. This species displays year-round activity in hot climates and moist soils [7]. In the coriander, the pathogen attack promotes the development of isolated and small-sized galls, which occur along the roots. In addition, only one female per gall, often exhibits egg mass outside 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 [29], is one of the most aggressive species of the genus. It has about 61 different enzymes extremely capable of degrading the cell wall, justifying the wide range host [25].

The control of the root-knot nematodes is difficult and costly to be raised, 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 [30].

In coriander culture, controlling the nematodes is not an easy task, since the microorganisms are soil dwellers and, in favourable conditions of temperature and humidity, multiply rapidly. Allied to this, the cultivation of coriander is usually carried out throughout the year and intensively in the same area, where some control measures should be taken such as use of organic matter, solarization, biological control and use of resistant cultivars [7].

There are more than 40 cultivars of coriander registered in MAPA in Brazil. However, little is known about their reaction to root-knot and reniform nematodes [7]. [29] evaluated the reaction of the cultivars Verdão, Palmeira and Portuguese as tolerant to *M. incognita* race 1 and all were susceptible to the pathogen. On the other hand, [31] 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 level 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.

3. TYPES OF CONTAINERS AND SUBSTRATE USED IN THE EVALUATION OF THE REACTION OF CULTIVATED SPECIES TO ROOT-KNOT NEMATODES

According to [32], among the difficulties of assessing the resistance of soybean to root-knot nematodes, there is variability of pathogens, limitation of sources of resistance, different criteria for the classification of genotypes and lack of standardization of methodologies, which may result in contradictory information.

Lack of standardization is found in the evaluation of the reaction to root-knot 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 [33,34,35].

In the evaluation of the soybean genotypes, TMG 115 RR (susceptible standard) and BRS 316 RR (resistant standard), were seeded in three types of plastic containers: 290 mL, 1-L and 2-L pots of a 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 (reproductive factor) for the two cultivars was observed when they were cultivated in the 1-L pot [32].

Evaluating the 'Prata Anã' banana 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 grass gram and higher reproductive factor [36].

As for beans (*Phaseolus vulgaris* L.), it was observed that the type of vessel influences the expression of the reaction of genotypes inoculated with *Meloidogyne incognita.* The 3-L

plastic vessel was most unviable when compared to the polystyrene box "Plantagil 1477" of 128 cells of 70 cm^3 and plastic cups of 200 cm^3. This fact was attributed to the dispersion of eggs due to the large volume of the container [37].

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 [29,31,38].

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

4. INOCULUM LEVEL

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

In the first experiment, the inoculation of approximately 5,000 eggs and juveniles of the second stage was carried out, while in the second, plants were inoculated with 1,500 eggs and juveniles. No difference was verified in the selection of resistant cultivars. However, it is not necessary to use a high level of inoculum to evaluate the resistance of lettuce plants to root-knot nematodes [34].

In the banana tree, when evaluating different levels of inoculum (0, 2,000, 10,000 and 50,000 eggs and infective juveniles) of *Meloidogyne incognita* race 2, it was verified that, regardless of the substrate used, the highest RF (reproduction factor) was observed in plants inoculated with 2,000 eggs and juveniles [36].

The study of the inoculum level to be used in the evaluation of coriander plants in relation to the resistance reaction to root-knot nematodes is a fundamental step in crop breeding programs in order to classify and select plants that are actually resistant to the pathogens.

5. NUMBER OF PLANTS PER EXPERIMENTAL PLOT

The size of the experimental plot is of fundamental importance in the evaluation of the reaction of plants to infections caused by root-knot nematodes. According to a research carried out with different cultures under conditions of stress caused by *M. incognita*, a variation on the size of the experimental plot with the following plants was observed: a lettuce plant [35]; six sweet potato [39]; 1 m^2 area in carrot [40]; five pepper plants [41]; two soybean plants [42]; [29], 10 coriander plants [31], 16 plants [31] and eight plants [38].

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 [43]. Such size depends on the species under study, the environment and the characters evaluated [44].

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 the Farifield Smith method, visual inspection method of maximum curvature, modified maximum curvature method, linear model of plateau response (LMPR), 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:

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

Where:

$V_{\bar{x}_i}$ is the variance of the average yield per basic unit for plots of X_i units;

V_1 is the variance of plot yield with a basic unit;

The number of basic units of size i (i = 1, 2, ..., n); and

B is 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 [46] 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 plot size (X) the coefficient of variation is calculated (CV (x)). The set of ordered pairs (X, CV (x)) are plotted on a graph and the curve formed by joining the dots. 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 [47] aims to extract the greatest amount of information from a unit of area. If 100% of relative information (RI) is assigned to plots whose size is equal to a basic unit (BU) and in the other plots (with different size of the BU), the RIs are obtained from the division of the variance of the BU by the comparable variances $CV_{(x)} = V_{(x)}/X$, which consists of the quotient between the variances of a given plot size by the number of BUs of the respective size; where the obtained RI was = $V_1 / CV_{(X)}$.

The method proposed by [48] is indicated for experimentation with arboreal species, based on the minimization of the variance of the treatment mean V (m), which is a function of the number of useful trees per plot (k) and a number of useful rows by the 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):

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

Where:

k is the number of neighboring subplots, the value 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 [49] 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:

CV_i is the coefficient of variation observed experimentally among plot totals of size of X_i basic units; X_i is the size of the plot in clustered basic units; X_c 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; ε_i 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 β_0 β_1, and P are estimates of β_0 β_1, and P parameters of the regression model, obtained using non-linear regression methods, using the nls function of R.

The method of maximum modified curvature proposed by [50] 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 [51], 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 = regression constant; 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 [52]. A number of authors have reported the efficiency of this method in estimating the number of plants per experimental plot, as in eucalyptus [52], coffee [53], papaya [54], and lettuce [55].

6. FINAL CONSIDERATIONS

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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