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RESISTANCE OF INCASOY-36 (*Glycine max* (L.) Merrill.) CULTIVAR TO CUBAN POPULATION OF *Meloidogyne incognita* (Kofoid and White) Chitwood

Resistencia del genotipo INCASoy-36 (*Glycine max* (L.) Merrill.) a población cubana de *Meloidogyne incognita* (Kofoid y White) Chitwood

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ABSTRACT. To determine the resistant/ susceptibility behavior of the soybean (*Glycine max* L.) cuban cultivar INCASoy-36 to *M. incognita* and the damage that different population levels may cause. The inoculum was preparing using NaOCl and four treatments were established: control (whiteout nematodes) and three population levels (1.5; 2.5 and 5 eggs-juveniles per gram of soil^{-1}), with complete random design and 7 replicas. The Root Galling Index (IA), Reproduction Factor (FR), and with these data, the categories as host or not and Resistant / susceptible were establish at 35 days. The parameters plant high;leaf, flowers and legume numbers were determined; beside steam diameter and fresh weigh. The data were analyzed by Simple Variance Analysis and the media were compere (p<0.05). For determine the relation between nematode population levels and some develop parameters the regression analysis PROC GLM, using SAS (Version 9.0 SAS) were used. INCASoy-36 do not was host for *M. incognita* race 2, with reproduction factors between 0.1 and 0.9, showing it resistant behavior because the nematode can't reproduce; nevertheless, the high ($R^2 = 0.751$) and fresh weigh ($R^2 = 0.859$) were significantly affected; beside the leaf, flowers and legumes number. According to the results, the cultivar is classified as resistant and intolerant to *M. incognita.*

Key words: soybean, root knot nematodes, legumes, hosts

RESUMEN. Los objetivos del estudio fueron establecer el comportamiento resistente/susceptible del cultivar cubano de soya (*Glycine max* L.) INCASoy-36 frente a *M. incognita* y determinar el daño que pueden causar poblaciones de diferente número de individuos del nematodo en indicadores seleccionados de su desarrollo. El inoculo se preparó utilizando NaOCl y se establecieron cuatro tratamientos: testigo (sin nematodos) y tres niveles poblacionales: 1,5; 2,5 y 5 huevos-juveniles por gramo de suelo^{-1}, siguiendo un diseño completamente aleatorizado, con siete repeticiones. A los 35 días se determinó el Índice de Agallamiento (IA) y el Factor de Reproducción (FR) para determinar las categorías de hospedante o no y resistencia/ susceptibilidad. Se evaluaron altura de las plantas, número de hojas, flores y legumbres; diámetro del tallo y masa fresca de biomasa aérea. Los datos se sometieron a Análisis de Varianza Simple y las medias se compararon mediante la prueba de rangos múltiples de Duncan (p<0,05). Las relaciones entre niveles poblacionales y los parámetros de desarrollo, se determinaron a través de un análisis de regresión, según PROC GLM del Paquete Estadístico SAS 9.0. INCASoy-36 no resultó buen hospedante de *M. incognita* raza 2, con factores de reproducción entre 0,1 y 0,9; acreditando su resistencia, al no propiciar la reproducción del nematodo; sin embargo, al incrementar el nivel de inoculo se afectaron, de manera significativa, los parámetros altura (R^2=0,751) y masa fresa de las plantas (R^2=0,859); así como el número de hojas, flores y legumbres. La respuesta de este cultivar, en base a los resultados del estudio, es catalogada como resistente e intolerante a *M. incognita.*

Palabras clave: soya, nematodos agalleros, leguminosas, hospederos

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INTRODUCTION

The legumes constitute a region of agricultural importance on a world scale and among them soy (*Glycine max* L.) stands out, as it is a high protein content food, oligosaccharide content, high concentration of unsaturated fatty acids, high levels of vitamin E, tocopherol, lecithin and other compounds, universally used in the human and animal diet. In the world, soybean production has grown over the last 10 years, more than any other food species, with 44%, going from 222 to 320 million tons in 2015-2016 (1). In Cuba, there is no official data for the soy area; the National Bureau of Statistics (2), reports that some129,911 ha are used in the production of legumes; a small part of them is soybean, cultivation present in areas of the west, center and east of the country, in productive areas or as part of studies of introduction and extension of cultivars, in the local seed sector used by thousands of farmers (3 ,4).

In Cuba, soybean genotypes were introduced since the early twentieth century and there are currently more than 20 cultivars adapted to the conditions of the country; of them, almost half of the varieties (5) are obtained in the country; however, the productions are still low, in comparison with the potential that the island has for its production, since climatic, technological, socio-economic and biological factors affect the crop, among them an important group of pests (6)

Gill nematodes (*Meloidogyne* spp.) represent limiting factors in the production of soybean on an international scale, causing stunting, chlorosis and premature senescence, depending on the initial population levels of the nematode (7).

In Cuba, species of genera *Helicotylenchus Longidorus*, *Meloidogyne*, *Pratylenchus* and *Rotylenchulus* were reported parasitizing soybeans (8); however, there are no recent reports on the impact of phytonematodes on crop health and yield. However, there is a Cuban cultivar, INCASoy-36, tolerant to *Meloidogyne* spp. (9), which suggests that it is little affected by these nematodes, but it is not known if it manifests or does not resist.

Tolerance is not a type of resistance, it is the term used to quantify the damage that the plant suffers or does not suffer from populations of nematodes (10). Tolerance and its opposite, intolerance, are used to describe the ability of the plant to withstand nematode infestation, while intolerant plants are damaged and grow less or die when infested; the tolerant plants, generally, are less damaged before populations of the nematode.

The cultivar INCASoy-36 can produce good crops in the winter, summer or spring, has a good cutting height and produces at least 60 pods per plant (9), which gives it qualities for commercial exploitation. Since 2008 it is a commercial variety; In addition, it was selected as the parent of transgenic varieties resistant to herbicide, obtained and registered in Cuba (5,6) (SCIGBL1 and Yaguajay L7 CIGB).

The species *Meloidogyne incognita* (Kofoid and White) Chitwood race 2 is the species / race of gill nematode most distributed throughout the country, in vegetable crops (11,12). In the production of food in Cuba, gall nematodes are one of the main pests of soil and it is necessary to address the spread of this pest, the use of resistant varieties, with the use of biofumigation, biological control agents, and the application of chemical fumigants and establish learning methods in action in a participatory way, to know their management.

The objectives of this study were to establish the resistant / susceptible behavior of the cultivar INCASoy-36 against *M. incognita* race 2 and to verify its status as tolerant genotype, defining the damage that populations of different magnitude (number of individuals) of the nematode can cause in selected indicators of its development.

MATERIALS AND METHODS

The study was carried out in facilities of the Plant Health Directorate of the National Center for Agricultural Health (CENSA), San José de las Lajas municipality, Mayabeque province, Cuba and it is a research project developed in the framework of the “Diagnosis and management of pests in grains with emphasis on the development and use of bioactive products “, funded by the National Animal and Plant Health Program, Cuba (2013-2016).

The research was carried out in the period between December 10, 2013 and January 16, 2014. The temperatures in the period were measured with a Loger TESTO® digital hydro-thermometer and ranged between 21-25 ºC.

A population of *M. incognita* race 2 was used, maintained in the biological isolators of CENSA, using as host tomato (*Solanum lycopersicum* L. cv Campbell 28). This population belongs to the collection of pure populations and was previously identified by morphological, physiological and molecular techniques (13).

To obtain enough juvenile and nematode eggs for the trials, the population was multiplied, previously, in galvanized zinc boxes of 50 x 50 cm, with a mixture of soil and organic fertilizer (ratio 1: 1), sterilized in an autoclave (121 °C, 30 min.), where aubergine (*Solanum melongena* L.), madama (*Impatiens balsamina* L.) and tomato (*S. lycopersicum* var. Campbell 28) plants grew.

The experiment was carried out in semi-controlled conditions (biological isolators) and pots of 1 kg capacity were used, where a mixture of soil and organic fertilizer was placed in proportion and sterilized.

The soybean genotype seeds INCASoy-36, supplied by the National Institute of Agricultural Sciences (INCA), of the certified basic seed produced in that center, were placed to germinate in polyethylene trays with alveoli (cepellones), containing a mixture of soil and organic matter (as described above). After 21 days, the seedlings were transferred to the pots and inoculated one week after the transplant.

The inoculum was prepared using NaOCl (14) and introduced into the soil through four holes made on the root system and in the area close to the stem. Four treatments were established: control (without nematodes) and three population levels: 1.5; 2.5 and 5 eggs-J2 per gram of soil^{-1} (equivalent to 1500, 2500 and 5000 juvenile eggs per pot).

The pots were arranged following a completely randomized design, with four treatments (population levels), with seven repetitions each (pots).

The plants were maintained for 35 days in the isolators, they were irrigated every other day and the health status of the experiments was monitored weekly, so that if pests (fungi, arthropods, others) appeared, the appropriate measures were taken. After this period, the plants were extracted, moved to the Laboratory of Agricultural Nematology of CENSA, their roots were washed to remove the surrounding soil, they were placed to dry on filter papers and then processed.

Host capacity of INCASoy-36 to *M. incognita* race 2

The reproductive factor (FR) was determined, where FR = final population (Pf)/initial population (Pi) (initial population levels).

The final population of nematodes was established by the sum of the population extracted from the roots, using the method of Hussey and Barker and the specimens obtained from the soil. The latter were extracted from three sub-samples (five grams each), from the soil of each replica, which were processed by the Baermann funnel method. The solutions, obtained by both methods, were quantified through direct counting of the J2-eggs in a Zeiss® stereoscope microscope with 160 magnifications.

To establish the capacity of the genotype as host of the nematode, the established categories were used (15), where: excellent host (FR>10); good host (10 <FR <1); maintenance host (FR=1) and poor or non-host (0<FR<1).

The resistance or susceptibility of the cultivar was evaluated using established criteria (16), which indicates that the FR≥1 indicates susceptible host; FR<1, host resistant and FR = 0, immune host.

Study of the damage produced by progressive population levels of *M. incognita* race 2 in development indicators of soybean cultivar INCASoy-36

At the end of the experiment the following parameters were determined: height of the plants, using tape measure (cm); counting the number of leaves, flowers and legumes; diameter of the stem at the level of the root neck with a vernier caliper (mm); Fresh mass of aerial biomass, using a KERN® electronic technical balance (e = 0.01g) and Grazing Index (AI) using the 11-degree scale from 0 to 10 (17).

The data obtained in each parameter were submitted to Analysis of Simple Variance and the means were compared through Duncan's Multiple Range Compcimal (p <0.05). The relationships between the population levels and the development parameters of the genotypes were determined through regression analysis, according to PROC GLM of the SAS Statistical Package, Version 9.0.

RESULTS AND DISCUSSION

Host capacity of INCASoy-36 to *M. incognita* race 2

The national soybean cultivar INCASoy-36 was not a good host of *M. incognita* race 2, with reproduction factors of 0.9; 0.1 and 0.1 in the presence of initial populations (Pi) of 1.5; 2.5 and 5 J2-eggs g of soil^{-1}, respectively, proving that this cultivar is resistant, since it did not favor the reproduction of the nematode. This commercial cultivar is generalized in different provinces and was selected as a progenitor in the soy improvement program transformed in Cuba for its satisfactory productive results of its progenies and for being efficient in the necessary *in vitro* process (6,18,19).

It has been pointed out that resistance to phytonematodes (20), is the term used in Nematology in the non-epidemiological sense, to describe the effect of the plant on the reproduction of the nematode. However, INCASoy-36 showed galls in the root system, with AI of 4 and 5 (depending on Pi), which confirms that the presence or absence of galls in the roots is not indicative of susceptibility or resistance; In this regard, it is pointed out that the galling does not represent a satisfactory indicator of resistance to nematodes (21).

When the initial inoculum level (Pi) was increased, decreases in the RF resulted, resulting from lower final population levels (Pf). In this regard, numerous authors pointed out that high values of Pi can cause competition among the nematodes and as a result, low levels of Pf will be obtained, due to the deterioration of the sites of infestation and the accumulation of metabolic waste of these organisms, which would affect the development of the nematode (11,22).

In the absence of varieties that show resistance to nematodes, cultivars with slight resistance to the gall nematode may be recommended in batches infested with this pest, in combination with the rotation of non-host crops and the treatment of seed and soil, can be effective (23-25). In the presence of high populations of the nematode, the best management alternatives are the use of non-host crops in rotation with soy (corn, sunflower and sorghum) and the use of resistant varieties, chemical or biological control of the seed, prevention of infestation and direct sowing (26,27).

Soybeans can be used in crop rotations, so it would be advisable to continue the study of the INCASoy-36 genotype, so that its behavior can be verified with respect to the other species that make up the poly-specific Meloidogyne communities that are present in Cuba and offer, to the producers, the pertinent suggestions for their rational use in crop rotations. This is necessary, since the host capacity of the cultivars differs from one population or another, which complicates the use of plants, poor or non-host, in the management of *M. incognita* (28).

Study of the damage produced by progressive population levels of *M. incognita* race 2 in development indicators soybean cultivar INCASoy-36

In the soybean cultivar, strawberry height and mass parameters of the plants were affected (Figure 1), with the highest values of both parameters in the plants without nematodes (controls) and the lowest ones in those that were developed in the presence of the population levels older, with significant differences.

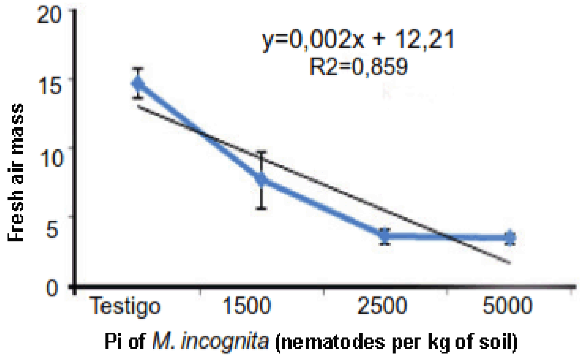

Figure 1. Effect of increasing population levels of *M. incognita* on the parameters height and fresh mass of soybean plants to grow INCASoy-36

The effect of nematodes on the development of legumes was documented in other studies (8), which is why we can expect that the yields of the cultivar will be affected, since in the presence of a generation of *M. incognita* there were affectations, in addition of the height and fresh mass of the plants, in the number of leaves, flowers and legumes (Table 1), it being convenient to develop this study in micro-plot conditions in the future, to reach the end of the crop and evaluate the yields.

Table 1. Vegetative and reproductive parameters values of INCASoy-36 cultivar in the presence of increasing levels of Cuban population of *M. incognita* race 2

Population level (number of juveniles and eggs x g of soil^{-1})	Stem diameter (mm)	No. leaves	No. flowers	No. vegetables
0 (control)	4,71a	9,71 a	3,43 a	9,14 a
1,5	3,51 a	6,14 b	1,57 b	3,85 b
2,5	2,97 a	5,28 b	2 b	1,71 b
5	2,50 a	4,71 b	1 b	1,71 b
ESx	0,22	0,46	0,31	0,73

Different letters, in the same column, indicate significant difference ($p < 0.05$)

The response of this cultivar, based on the results of the study, is cataloged as resistant and intolerant to *M. incognita*, since it did not allow the reproduction of the nematode (FR <1); however, its development and formation of flowers and legumes are affected, in the presence of populations of different magnitude.

In Cuba, it was reported that soy genotype H-19 was susceptible to *M. incognita* races 2 and 3, *Meloidogyne arenaria*, *Meloidogyne javanica* and *Meloidogyne hapla* (9); hence, having a genotype that is resistant is an important element in the development of the improvement program carried out by scientific institutions in the country. However, the manifest susceptibility to damage of the INCASoy-36 genotype is an element that is important to bear in mind, since its development and, possibly, its production will be affected by the presence of certain population levels of the nematode in the soils that should be the subject of future research.

Possessing a national soybean cultivar, with resistance to the most distributed species/race in the country, makes it possible to look for varieties with levels of resistance in the improvement program and represents an important option for the management of gill nematodes, which is why it should be emphasized in the disclosure of this positive fact; however, it must be taken into account that the cultivar is intolerant and the infestations of gill nematodes in the soil should be monitored, because they can affect its development.

The use of cultivars resistant to nematodes, is an efficient tactic for the management of gill nematodes when used together with other management measures, in the cultivation of soy, the generality of the varieties are susceptible and have a cultivar that has resistance, contributes to maintaining nematode populations at low levels, since it is known that resistance can be lost due to high levels of nematode populations, the appearance of virulent populations or environmental factors (28).

CONCLUSIONS

The results have relevance, from the theoretical-practical point of view and are the basis for the development of future research, to establish the mechanism of resistance of INCASoy-36 cultivar and improve its tolerance to gill nematodes to achieve satisfactory exploitation in infested fields by *M. incognita*.

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