RESEARCH/INVESTIGACIÓN

EVALUATION OF COWPEA (*VIGNA UNGUICULATA* L.) GERMPLASM FOR THE SOURCE OF RESISTANCE TO ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*

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ABSTRACT

Khan, Z., N. K. Gautam, B. H. Gawade, and S. C. Dubey. 2018. Evaluation of cowpea (*Vigna unguiculata* L.) germplasm for the source of resistance to root-knot nematode, *Meloidogyne incognita*. Nematropica 48:27-33.

Screening experiments were conducted during 2013 to 2015 to assess the host status of 350 accessions of cowpea (*Vigna unguiculata*) for resistance to *Meloidogyne incognita*. Based on the number of root galls induced by *M. incognita* among the tested accessions, six (EC0517140, EC0528391, EC0723686, EC0724523, EC0725122, and IC0253277) were resistant, and the rest were susceptible. The resistant accessions reduced root gall formation by 96-99% as compared to highly susceptible accession EC0724571. Nematode penetrations were also lower in roots of all resistant cowpea accessions compared to the susceptible accession EC0724571 ($P<0.05$). Similarly, egg-mass formation was retarded in the resistant accessions when observed 45 d after inoculation ($P<0.05$). These resistant accessions may be useful in plant breeding programs to develop nematode resistant cowpea cultivars.

Key words: cowpea, resistance, root-knot nematode, screening

RESUMEN

Khan, Z., N. K. Gautam, B. H. Gawade, y S. C. Dubey. 2018. Evaluación del germoplasma de frijol (*Vigna unguiculata* L.) como fuente de resistencia al nematodo agallador, *Meloidogyne incognita*. Nematropica 48:27-33.

Los experimentos se llevaron a cabo durante 2013 a 2015 para evaluar 350 variedades de caupí (*Vigna unguiculata*) para la resistencia a *Meloidogyne incognita*. En base al número de agallas de raíz inducidas por *M. incognita*, entre las variedades probadas, seis (EC0517140, EC0528391, EC0723686, EC0724523, EC0725122, e IC0253277) fueron resistentes y el resto fueron susceptibles a *M. incognita*. Las variedades resistentes redujeron la formación de agallas en un 96-99% en comparación con el altamente susceptible EC0724571. Las penetraciones de nematodos también fueron más bajas en las raíces de todas los caupí resistentes en comparación con el susceptible EC0724571 ($P <0,05$). De forma similar, la formación de masa de huevos se retrasó en las variedades resistentes cuando se observó 45 días después de la inoculación ($P <0,05$). Los resistentes pueden ser útiles en programas de mejoramiento de plantas para desarrollar cultivares de caupí resistente al nematodo.

Palabras claves: caupí, cribado, nematodo agallador, Resistencia

INTRODUCTION

Cowpea (*Vigna unguiculata* L.) is an important food legume crop, an essential component of cropping systems in the drier regions of the tropics and subtropics, and important to the livelihood of millions of people (Quin, 1997). Root-knot nematodes, *Meloidogyne* spp., are the major constraint in the production of cowpea in tropical and subtropical regions (Sikora and Greco, 1990; Sikora *et al*., 2005). Root galling induced by *Meloidogyne* spp. interferes with water and nutrient-conducting abilities of the roots and suppresses Rhizobium nodulation. Intensive root galling often results in permanent wilting, premature defoliation, and eventually plant death.

Nematodes can be managed by adopting different methods either singly or in combination (Ferris, 1992; Verdejo-Lucas *et al*., 2013). Genetic components to nematode management include identification of resistance through screening method(s) and utilization of resistance in the breeding programs (Narayanasamy, 2002). The nematode-resistant cultivars are an eco-friendly and economically feasible means for the management of root-knot nematodes. As the information regarding resistant cowpea against root-knot nematodes in India is scanty (Mishra, 1992; Subramaniyan *et al*., 1997), therefore, the present study was undertaken to identify source of resistance in cowpea germplasm to *M. incognita*.

MATERIALS AND METHODS

Seed materials

The seeds of 350 accessions (Table 1) of cowpea germplasm were obtained from the national gene back of ICAR-National Bureau of Plant Genetic Resource, New Delhi, India. The experiments were conducted during 2013-2015 under net house conditions to screen the host status of cowpea accessions to the root-knot nematode, *M. incognita*.

Preliminary screening in naturally infested soil

A preliminary screening was conducted in root-knot nematode-infested soil collected from a greenhouse/polyhouse of Indian Agricultural Research Institute farm, New Delhi, India, which has been continuously cultivated with tomato and cucumber and was heavily infested with *M. incognita*. Infested soil was thoroughly mixed and the population density of second-stage juveniles (J2) of *M. incognita* in the soil was determined by extracting nematodes from 10, 200-g subsamples of soil using sieving and decantation (Southey, 1986). Nematode population density was estimated as 3 J2/g soil or 1,500 J2/pot. Seeds of each cowpea accession were sown in 10-cm diam. pots containing 500 g nematode-infested soil. Each cowpea accession was sown in five pots (replication), and pots were watered when required to maintain moisture at field capacity. Seven weeks after sowing, plants were uprooted from the pots, and adhering soil was removed gently by washing in tap water. Galls per root system were counted and a gall index (GI) of 0-5 was assigned where: 0=no galls, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5 = >100 galls per root system (Taylor and Sasser, 1978). Host status of cowpea germplasm accessions, designated as Immune (I) when GI = 0.0, resistant (R) GI ≤ 2.0 or susceptible (S) >2.0, was determined using GI (Sasser *et al*., 1984) (Table 2).

Nematode inoculum and extraction

A single egg mass was used to culture *M. incognita* on 4-wk-old nematode susceptible tomato cv. Pusa Ruby. The plants were carefully uprooted from pots 45 d after inoculation. The root systems were gently washed with tap water to remove adhering soil and debris. Egg masses of *M. incognita* were handpicked with the aid of forceps and placed on Baermann funnel for 3 d to allow for J2 to hatch out (Southey, 1986). Hatched J2 were counted and adjusted to maintain a uniform rate of inoculation.

Confirmatory screening with artificial inoculation

In this experiment, six accessions of cowpea germplasm viz., EC0517140, EC0528391, EC0723686, EC0724523, EC0725122 and IC0253277, which were found resistant during preliminary screening, and one susceptible accession (EC0724571) were selected. Seeds were sown in 10-cm-diam. plastic pots filled with 500-g steam-sterilized soil. Two weeks after germination, each plant was artificially inoculated with 1,000 freshly hatched J2 of *M. incognita*. The J2 suspension was dispensed in 5 ml of water around the cowpea root zone with a pipette, and the pots were then lightly watered. Three-week-old tomato cv. Pusa Ruby susceptible to *M. incognita* were also planted in pots and inoculated at the same level to verify inoculum viability. Plants were watered as needed to maintain the soil moisture at field capacity. Each accession was replicated five times. Pots were arranged in randomized complete block design in a net house. Forty-five days after inoculation, plants were carefully uprooted from pots and processed for galling and host status designation as mentioned above. The experiment

Table 1. Cowpea germplasm accessions (350) used for preliminary screening for resistance to *Meloidogyne incognita* and their host reaction/gall formation.

Accession number	Number of galls per root system
EC0517140, EC0528391, EC0723686, EC0724523, EC0725122, IC0253277	<10
EC0149444, EC0528391, IC0202790, IC0202803, IC0202824, IC0253279, IC0402090	11-30
EC0106357, EC0107173, EC0341722, EC0366776, EC0390207, EC0390213, EC0390215, EC0390217, EC0390220, EC0390224, EC0390226, EC0390228, EC0390230, EC0390235, EC0390254, EC0390257, EC0390264, EC0390266, EC0390267, EC0390268, EC0390278, EC0390283, EC0390285, EC0390294, EC0397692, EC0454025, EC0514422, EC0528382, EC0528393, EC0528404, EC0528406, EC0528411, EC0723657, EC0723674, EC0723677, EC0723690, EC0723692, EC0723693, EC0723780, EC0723782, EC0723800, EC0723840, EC0723868, EC0723871, EC0723984, EC0723996, EC0724303, EC0724393, EC0724413, EC0724422, EC0724488, EC0724510, EC0724519, EC0724536, EC0724547, EC0724707, EC0724740, EC0724762, EC0725103, EC0725116, EC0725118, EC0725163, EC0725180, IC0202777, IC0202859, IC0257428, IC0257453, IC0257471, IC0259064, IC0259100, IC0398083, IC0548288, IC0559398	31-50
EC0116902, EC0101070, EC0101943, EC0101949, EC0107121, EC0107124, EC0107127, EC0107164, EC0107190, EC0107193, EC0107340, EC0109112, EC0112972, EC0170604, EC0232352, EC0240642, EC0240715, EC0240825, EC0244018, EC0328650, EC0343008, EC0343037, EC0343057, EC0367703, EC0367706, EC0367707 EC0367708 EC0367710, EC0367711, EC0367713, EC0390204, EC0390222, EC0390223, EC0390225, EC0390231, EC0390232, EC0390233, EC0390237, EC0390238, EC0390239, EC0390242, EC0390246, EC0390247, EC0390249, EC0390250, EC0390253, EC0390256, EC0390258, EC0390259, EC0390260, EC0390261, EC0390263, EC0390269, EC0390277, EC0390280, EC0390282, EC0390287, EC0390293, EC0394708, EC0454027, EC0472250, EC0472261, EC0472270, EC0514421, EC0528333, EC0528381, IC0202640, EC0528392, EC0528405, EC0528407, EC0528408, EC0528414, EC0528416, EC0528420, EC0528423, EC0528693, EC0528697, EC0528699, EC0723651, EC0723659, EC0723660, EC0723683, EC0723688, EC0723699, EC0723733, EC0723739, EC0723747, EC0723755, EC0723777, EC0723779, EC0723781, EC0723786, EC0723791, EC0723804, EC0723808, EC0723812, EC0723815, EC0723823, EC0723826, EC0723870, EC0723887, EC0723894, EC0723899, EC0723908, EC0723911, EC0723964, EC0723980, EC0723982, EC0724054, EC0724307, EC0724324, EC0724344, EC0724369, EC0724382, EC0724405, EC0724408, EC0724417, EC0724420, EC0724428, EC0724429, EC0724448, EC0724455, EC0724458, EC0724462, EC0724465, EC0724471, EC0724486, EC0724497, EC0724504, EC0724517, EC0724524, EC0724529, EC0724564, EC0724570, EC0724577, EC0724590, EC0724596, EC0724597, EC0724602, EC0724606, EC0724615, EC0724644, EC0724680, EC0724681, EC0724751, EC0724760, EC0724761, EC0724764, EC0724775, EC0724778, EC0724780, EC0725119, EC0725147, EC0725153, EC0725164, EC0725166, EC0725167, EC0725178, EC0725186, EC0725211, IC0 116922, IC0528288, IC0020584, IC0033267, IC0058905, IC0201083, IC0202762, IC0202779, IC0202781, IC0202784, IC0202789, IC0202797, IC0202800, IC0202829, IC0202849, IC0202926, IC0204844, IC0208337, IC0208618, IC0214753, IC0219484, IC0219550, IC0219574, IC0219592, IC0219594, IC0219639, IC0247435, IC0249141, IC0249583, IC0249586, IC0249588, IC0249591, IC0253255, IC0253268, IC0253273, IC0257406, IC0257407, IC0257424, IC0257427, IC0257437, IC0257438, IC0257441, IC0257446, IC0257449, IC0257452, IC0257461, IC0257463, IC0257469, IC0257472, IC0257473, IC0257478, IC0257480, IC0257483, IC0257485, IC0259058, IC0259078, IC0259081, IC0259104, IC0259105, IC0259106, IC0259159, IC0266776, IC0276933, IC0326807, IC0338832, IC0347189, IC0371749, IC0396744, IC0397577, IC0397847, IC0397896, IC0398097, IC0398755, IC0402098, IC0402104, IC0402164, IC0402166, IC0402172, IC0402174, IC0536543, IC0536638	51-100
EC0101959, EC0149438, EC0367702, EC0390212, EC0390241, IC0390252, EC0528397, EC0572717, EC0723738, EC0723813, EC0723883, IC0724048, EC0724384, EC0724556, EC0724571, EC0725151, EC0725169, IC0201098, IC0249132, IC0257445, IC0330950, IC0330977, IC0396667	>100

Table 2. Gall formation in selected accessions of cowpea by *Meloidogyne incognita* during confirmatory screening with artificial inoculation.

Accession number	Number of galls per root system (mean±SD)	Gall index[w]	Percent reduction[x]	Host status[y]
EC0517140	1±1a[z]	2	99.19	R
EC0528391	5±2a	2	96.38	R
EC0723686	3±1a	2	97.85	R
EC0724523	1±1a	2	99.09	R
EC0725122	3±1a	2	97.85	R
IC0253277	5±1a	2	96.51	R
EC0724571	149±11b	5	0.00	S

[w]Number of galls per root system were rated on a scale of 0-5 where: 0= No galls, 1= 1-2, 2= 3-10; 3=11-30, 4=31-100, 5= >100 galls per root system (Taylor and Sasser, 1978).
[x]Percent reduction of gall formation in resistant accessions as compared to more susceptible accession.
[y]Host status of cowpea accessions was determined using root gall index (GI) where: GI ≤1=Highly resistant (HR); GI ≤ 2 resistant (R); and GI > 2= susceptible (Sasser et al., 1984).
[z]Means followed by different letters in the column are different according to Duncan Multiple Range Test (P < 0.05).

was repeated once with the same materials and methods. Similarity between experiments was tested by analysis of variance using experimental runs as factor. This allowed combining data from both experiments to determine the host status of tested cowpea germplasm accessions.

Observation on nematode penetration, development, and egg-mass formation

In this experiment, one highly susceptible and six resistant cowpea accessions (Table 3) were selected to determine nematode penetration in roots or their development of egg mass formation and were sown separately in 10-cm diam. pots containing steam-sterilized soil. Two weeks after sowing, each accession was inoculated with 1,000 J2 of *M. incognita*. All selected accessions were replicated five times for each experiment. At 2, 5, 7, and 10 days after inoculation (DAI), plants were uprooted from pots, and the roots were carefully washed and fixed with formalin-acetic acid-alcohol overnight (formalin: glacial acetic acid: 95% ethanol: distilled water= 2:1:10:7). The fixed roots were cleared in 2% NaOCl for 10 min and stained with 0.07% bromophenol blue for 8 hr, and rinsed in 50% ethanol (Kim *et al*., 1986). The number of nematodes in an infection site was counted using a stereoscopic binocular microscope. To examine egg-mass formation, plants were carefully uprooted 45 DAI. The root systems were washed gently with running tap water and stained with phloxine B (0.15 g/l tap water) for 15 min to stain egg masses. Egg masses were examined with naked eyes as well as with the aid of a magnifying glass. Means were separated using Duncan's Multiple Range Test ($P < 0.05$) using SPSS software version 17.0.

RESULTS

The cowpea germplasm accessions (350) screened and analyzed against nematode infection contained genotypes that were resistant and susceptible to *M. incognita*. Six accessions (Table 1 & 2) were found resistant with less than 10 galls per root system. The remaining 344 accessions (Table 1) were susceptible with a GI >2.0 because of the formation of large number of root galls. Susceptible accessions vary in their host reaction or in number of gall formed. The majority of the accessions, 241, showed 51-100 galls per root system, whereas 23 accessions showed more than 100 galls per root system (Table 1.). Root galls induced by *M. incognita* in roots of resistant accessions were few (1-5) and smaller in size, whereas in susceptible accessions severe root galling was observed (Fig. 1). Among the resistant accessions, the lowest number of root galls was 1, which was observed in accession EC0517140, whereas the highest was 5 in IC0253277. The susceptible tomato cv. Pusa Ruby developed numerous galls (GI=5) indicating viable and sufficient inoculum. Thus, the environmental conditions were conducive for critical evaluation of host status of cowpea accessions and experiments were conducted during the summer cowpea-growing season.

Nematode penetration, development and reproduction (egg masses) differed among the cowpea accessions (P >0.05) (Table 3). Penetration was lower in resistant cowpea accessions compared to the susceptible cowpea assessions (Table 3). In accession IC0253277, nematode penetration was

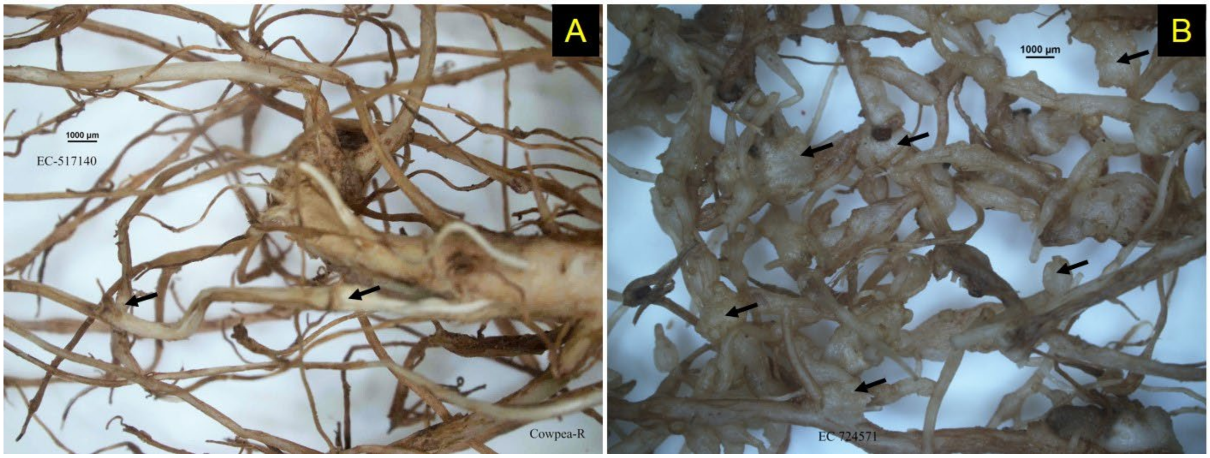

Fig. 1. Host reaction of cowpea accessions to *Meloidogyne incognita* infection. A: Resistant accession EC0517140 with few small galls. B: Susceptible accession EC0724571 showing numerous large-sized root galls. Arrows indicates the root galls induced by *M. incognita*.

Table 3. Nematode penetration into roots and egg-mass formation by *Meloidogyne incognita* in resistant and susceptible accessions of cowpea.

Cowpea accession	Nematode penetration (%) at DAI[x]				No. of egg masses per root after 45 DAI (mean±SD)[y]
	2	5	7	10	
EC0517140	2.02 a[z]	2.64 a	3.46 a	3.28 a	0.0±1a
EC0528391	3.12 bc	4.48 c	5.22 c	5.12 b	3±1 a
EC0723686	2.54 ab	3.66 bc	4.86 bc	5.06 b	1±1 a
EC0724523	2.04 a	2.88 a	3.60 a	3.34 a	1±1 a
EC0725122	2.82 b	3.62 ab	4.28 ab	4.92 b	1±1 a
IC0253277	3.70 c	4.92 c	5.32 c	4.70 b	2±1 a
EC0724571	6.88 d	13.9 d	17.02 d	20.04 c	173±10 b

[x]DAI: days after inoculation

[y]Means and standard deviations are of five replications.

[z]Means (n = 5) followed by different letters in the column are different according to Duncan Multiple Range Test ($P < 0.05$).

the highest (5%) at 7 DAI among the six resistant accessions. This difference increased substantially with passage of time after inoculation. Up to 7 and 10 days after inoculation, about 17 and 20% of the inoculum penetrated the roots of the susceptible accession (EC0724571), whereas only 3-5% of J2 penetrated the roots of resistant accessions (Table 3). The number of egg masses observed at 45 DAI in the susceptible accession EC0724571 was 173, comprising 20% of J2 inoculation. Very few egg masses were formed in any of the resistant cowpea accessions (Table 3). Egg-mass formation at 45 DAI was in the range of 1-3 per plant in resistant accessions. The lowest number of egg mass (>1 per plant) was observed on accession EC0517140 and the maximum (3) was recorded on accession EC0528391.

DISCUSSION

In the present study, 350 accessions of cowpea were screened for resistance to *M. incognita*. Six accessions were resistant, showing less than 10 root galls per root system (GI<2.0). These resistant cowpea accessions showed a varied reaction from each other in their response to *M. incognita* infestation. The differences in reaction might be due to genetic variability among the accessions (Adegbite *et al*., 2005; Adomako *et al*., 2013). Nematode penetration occurred in all 6 resistant accessions, but was lower when compared to a susceptible accession. Very few egg masses formed (0-3) in the resistant accessions, suggesting that the development or reproduction of nematodes was inhibited after penetration in the resistant plant roots.

Resistance to nematode infection can be either pre-infectional or post-infectional resistance. In pre-infectional resistance, nematodes are unable to enter the roots of the plant due to the presence of toxic chemicals or physical barriers whereas in post-infection resistance, nematodes are able to penetrate roots but fail to develop (Bendezu and Starr, 2003; Moon *et al*., 2010). It was suggested in cotton (Anwar and Mckenry, 2000) and pepper (Pegard and Brizzard, 2005; Moon *et al*., 2010) that the failure of J2 to penetrate roots of resistant accessions may be related to physical or chemical root barriers. The inhibition of nematode development or reproduction after penetration may be related to the inhibited formation and development of giant cells (Jones, 1981; Moon *et al*., 2010). Our study also showed less J2 penetration and reduced egg mass numbers as resistance responses, which differed in degree among the accessions.

Host resistance and susceptibility is governed genetically (Jacquet *et al*., 2005; Castagnone-Sereno, 2006). The differences in the resistance reaction to *M. incognita* in cowpea accessions may be due to differences in their genetics, which can be explained in terms of number of galls. Accession 'EC0724571' was highly susceptible as the maximum number of root galls and egg masses were observed on the roots wherein maximum number of J2 penetrated and completed their life cycles successfully. On the other hand, the resistant accessions allowed only a limited number of J2 of *M. incognita* to enter the roots, leading to less root galling. Roberts *et al*. (1998) reported that root galling is often, but not always, closely correlated to nematode reproduction. This may be because root galling is induced by chemicals released by the nematode (Trudgill, 1991) whereas nematode reproduction is influenced by the host plant (Giebel, 1982). In conclusion, the drastic reduction in nematode penetration into the roots, the reduced number of galls, and the lower numbers of egg masses suggest that resistance may be both pre-infectional and post-infectional in these cowpea accessions. These resistant accessions will be useful in cowpea plant breeding programs for crop improvement.

ACKOWLEDGMENTS

Authors are thankful to the Director, ICAR-NBPGR, New Delhi, for support and encouragement, and the Indian Council of Agricultural Research (ICAR), New Delhi, for financial support.

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Received:
Recibido: *3/I/2017*

Accepted for publication:
Aceptado para publicación: *5/II/2018*