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Research Article

Screening of cucurbitaceous rootstocks and cucumber scions for root knot nematode resistance (*Meloidogyne incognita* Kofoid and White)

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Abstract
A study was carried out under glasshouse condition at the Department of Nematology, Tamil Nadu Agricultural University, Coimbatore during 2012-2013 to identify resistant rootstocks of cucurbitaceous species for grafting of cucumber against root knot nematode, *Meloidogyne incognita*. Seven wild and cultivated cucurbitaceous rootstocks and two cucumber scions (variety and hybrid) were used for screening studies against the root knot nematode. Forty fifth day after inoculation, the plants were evaluated for shoot length, root length, shoot fresh and dry weight as well as root fresh and dry weight, number of galls per 10 gram of root, egg mass and females per gram of root, root knot index, soil nematode population per 200 cc of soil and reproduction factor. The lowest number of galls and egg masses were observed in *Citrullus colocynthis* followed by *Cucumis metuliferus* which exhibited resistant reaction with root knot index (RKI) of 2. The other rootstocks *viz*., *Cucurbita ficifolia, Cucurbita moschata, C. maxima and Luffa cylindrica* were found to be moderately resistant to root knot nematode with root knot index of 3. The two cucumber scions (Green Long variety and NS 408 hybrid) were observed to be highly susceptible to *M. incognita* with RKI of 5.

Key words
Cucumber, root knot nematode, screening, cucurbitaceous rootstocks

Introduction

Cucumber (*Cucumis sativus* L.) (2n=2x=14) is an important and commercially popular cucurbitaceous vegetable crop which holds a much desired position in the vegetable market. When vegetable crops production taken under greenhouses, the incidence soil borne diseases and nematodes cause most of the damage due to mono cropping and intensive cropping. Cucumber crop suffers from several infections by serious fungal diseases and nematodes resulting in severe loss of yield and quality. Root knot nematode, *Meloidogyne incognita* and *Fusarium* wilt, *Fusarium oxysporum* f. sp. *cucumerinum* are the most serious soil borne diseases in cucumber rhizosphere (Sharma *et al.*, 1995).

Meloidogyne spp. is considered to be the most important parasites of cucumber which cause yellow foliage, unthrifty growth, reduced fruit size, poor yield, heavy root galling, root decay and reduced root system. *M. incognita* race 3 is predominant in Tamil Nadu. Krishnaveni and Subramanian (2005) estimated yield loss due to M. *incognita* race 3 on cucumber as 69.24 per cent. In addition, these parasites also interact with other disease causing organisms to produce disease complexes and break down of resistance against other pathogens and reduce plant tolerance to environmental stress.

The management of nematodes has been done by using resistant plants, crop rotation, cultural practices and chemical nematicides. Chemical control is highly expensive and hazardous to ground water, environment, animal and human health (Kacjan Marsic and Osvald, 2004). Although there has been much progress in selecting and breeding for root knot nematode resistance varieties/ hybrids in many other important horticultural crops, limited works has been made in cucumber and attempts to produce viable interspecific hybrids between cucumber and several related resistant wild *Cucumis spp.* also have failed. Successful gene exchange between *C. sativus* and related wild species is difficult using conventional hybridization techniques, since *C. sativus* has a different chromosome number than other related wild *Cucumis* spp. Screening methods have been standardized for evaluation of resistance against root knot nematodes and several diseases (Fassuliotis, 1979).

For these reasons, looking for alternative safety approaches and methods such as antagonistic plants and grafting onto selected resistant rootstocks is needed. Grafting of cucumber onto resistant rootstocks seems to be effective against *Meloidogyne incognita* (Abd-El Wanis *et al*., 2013). Eventhough cucumber is not easily crossable with other wild *Cucumis* sp., the interspecific hybrids of other cultivated and wild species of curcurbitaceous family *viz*., *Cucubita maxima* X *Cucurbita moschata* can be used as resistant rootstock for successful cucumber cultivation. Under heavy population of root knot nematode, grafting might be an economically feasible pest control measure to maintain a profitable production giving that the risk of economic crop losses due to root knot nematode. Generally, breeding new cultivars that are resistant to plant parasitic nematodes requires much time and investment.

In present study, the seven cucurbitaceous rootstocks and two cucumber scion plants were screened against the incidence of root knot nematode under pot culture with artificial inoculation of nematodes under glasshouse condition. Further, the resistant rootstocks could be used for grafting with susceptible scions.

Material and method

A pot culture experiment was conducted to study the relative performance of different cucurbitaceous species on incidence of root knot nematode under glasshouse condition at the Department of Nematology, TNAU, Coimbatore during 2012-2013**.** The experiment was laid out in a completely randomized block design with three replications. The seven cucurbitaceous rootstocks *viz*., Fig leaf gourd (*Cucurbita ficifolia*), Pumpkin (*Cucurbita moschata*), Winter squash (*Cucurbita maxima*), Bottle gourd (*Lagenaria scireraria*), Sponge gourd (*Luffa cylindrica*), African horned cucumber (*Cucumis metuliferus*) and Colocynth (*Citrullus colocynthis*) and two cucumber scions NS 408 (hybrid) and Green Long (variety) were used in present study. Seedlings were raised in protrays and then 15 days old seedlings were transplanted into pots containing five kilogram of sterilized pot mixture (Red soil: Sand: FYM in 2:2:1 ratio) for artificial inoculation.

The root galls were collected from root knot nematode infested cucumber plant and confirmed under stereoscopic microscope after thorough washing with tap water and staining with acid fuchsin lactophenol. Highly susceptible cucumber and tomato plants were used for developing pure culture of root knot nematode

*i. Inoculation:*The method of Sasser *et al*. (1957) was followed for inoculation of root knot nematodes. Infected roots from pure culture were cut into small pieces of about 2 cm and placed in 0.5 per cent sodium hypochlorite (NaOCl) solution. The container was shaken for about 3 minutes to dissolve the gelatinous matrix and freeing the eggs from the egg mass and incubated for 48 hours under room temperature. The eggs were kept in beakers and frequently aerated with the use of aerator to enable hatching. The nematode concentration was adjusted to a known number by addition of water for inoculation. The nematode inoculation (J_2) was done at 2 cm depth near the rhizosphere and covered with sterile sand. Each pot was inoculated with J_2 of *M. incognita* at the rate of two juveniles (J_2) / g of soil on fifteenth days after planting.

ii. Assessment of nematode population: Seedlings were uprooted carefully 45 days after inoculation with minimum root disturbance and washed with tap water to remove the adhering soil particles. Plant growth parameters *viz.*, shoot length (cm), root length (cm), shoot fresh and dry weight (g) as well as root fresh and dry weight (g) were recorded. Dry weight was determined after drying the plants in a hot air oven at 60 ˚C for 72 hours. From the fresh root sample, number of galls per 10 gram of root, number egg masses and number females per gram of root were counted under stereoscopic microscope after staining with acid fuchsin lacto phenol. The population of *M. incognita* in soil were assessed by using Cobb's decanting and sieving method followed by modified Baermann funnel technique (Cobb, 1918 and Schindler, 1961). The final nematode population (Pf) was calculated as total number of nematodes extracted from both roots and soils. The reproduction factor (Rf) of the nematode in the different treatments were obtainted by dividing the final population densities by the initial population densities (Rf= Pf/Pi). The data from the experiments were analyzed statistically following the method suggested by Panse and Sukatme (1989).

*iii. Assessment of nematode resistance (Root knot index):*The degree of resistance is indicated by the root knot index and it was done as per Heald *et al*. (1989).

Percentage of roots with galls	Root knot index	Reaction
0	1	Highly Resistant (HR)
1-25	2	Resistant (R)
26-50	3	Moderately Resistant(MR)
51-75	4	Susceptible (S)
76-100	5	Highly Susceptible (HS)

Result and discussion

Management of nematode populations using resistant cultivars is considered an important strategy. In this present study, reactions of seven cucurbitaceous rootstocks and two cucumber scions against *M. incognita* were assessed on the basis of gall produced on the roots and rate of nematode build up. Significant variations were noticed among seven cucurbitaceous species and two cucumber cultivars in their response to the nematode.

Among the rootstocks screened for the study significant difference was observed in plant growth parameters. The highest shoot length was observed in and *Cucurbita ficifolia* (253.80 cm) followed by *Cucumis metuliferus* (157.24 cm). The highest shoot fresh weight and dry weight was observed in

Cucurbita maxima (45.36 g and 5.03 g) followed by *Cucurbita moschata* (42.53 g and 4.88 g) and *C. ficifolia* (40.25 g and 4.16 g) respectively.

C. moschata recorded the highest root length (60.45 cm) followed by *C. maxima* (54.20 cm). The highest root fresh weight and dry weight was measured in the cases of the *C. maxima* (2.83 g and 0.45 g) and *C. moschata* (2.75 g and 0.32 g). The root weight of the *Cucumis sativus* scion plants *viz*., Green Long variety and NS 408 hybrid were served as a control and was found to be significantly lower in comparison with the root weight of other five rootstocks. But the resistant rootstock *C. metuliferus* recorded the lowest root fresh and dry weight (0.12 g and 0.01 g) compared to the control plants of *Cucumis sativus*. Similar results was obtained by Siguenza *et al.* (2005) that the shoot weight was noted to be higher but root weight, number of gall produced and final nematode populations were noted to be lower on *C. metuliferus* rootstocks compared to *C.moschata* and control melon plants.

Citrullus colocynthis recorded significantly reduced number of galls (4.69 galls/10 g of root) followed by *C. metuliferus* (5.24 galls/10 g of root) compared to other species and *Cucumis sativus* scions used in present study (Table 2). These two species also recorded the lowest number of egg masses per gram of root (1.94 and 1.98) and number of females per gram of root (3.85 and 4.81) respectively and showed resistant reaction. The *Cucumis sativus* scion NS 408 hybrid and Green Long variety were recorded the highest number of galls (83.25 & 76.35 galls/ 10 g of roots) more number egg masses (29.35 & 23.39 per gram of root) and more number of females (49.31 & 46.35 per gram of root) which showed highly susceptible reaction to incidence of root knot nematode. Sobczak *et al*. (2005) observed the formation of fewer galls in resistant rootstocks was probably due to failure of nematode juveniles to produce functional feeding site in the host after invasion and to develop subsequently as reproducing females.

The suitability of the host for plant parasitic nematode expressed as the ability of the nematode to multiply in the plant and is measured by reproduction factor (Rf) which is the ratio of the number of nematodes recovered at the end of the experiment (Pf) to the number of nematode units used to inoculate the plants (Pi). The RF values measure the reproductive potential of a nematode in a host, serving as an indicator for host status to the test nematode (Liebanas and Castillo, 2004). A wide range of variation was recorded in reproduction factor (Rf) which ranged from 0.61 (Colocynth) to 3.0 (cucumber hybrid NS 408) (Table 3). On the basis of nematode reproduction *Citrullus colocynthis* and *Cucumis metuliferus* were categorized as resistant to root knot nematode which recorded the reproduction factor of 0.59 and 0.68 respectively which might be due to the host was comparatively less suitable for reproduction and the presence of resistant mechanism that limits the reproduction of *M. incognita*. The resistance might be due to the some biochemical compounds present in the resistance species. In *Cucumis* species, the potent chemical cucurbitacins, which were believed to be responsible for nematode suppression, accumulated to the greatest extent in both seeds and roots (Pofu *et al.*, 2012). Tamilselvi (2014) reported that the root knot nematode resistance in *Citrullus colocynthis* and *Cucumis metuliferus* was due to presence of high amount of phenol content and peroxidase activity. Mondoki (2012) recorded the same result that *C. metuliferus* noted to be immune to *M. incognita*. Biochemical mechanism of invasion supports this mechanism which occurred due to non co-operative action of host tissue or cells. The chemical inhibitors in the host tissue counteract or neutralize the giant cell on inducing effect of salivary secretions of the nematode (Barons, 1939). The rootstocks and scions susceptible to nematode infection supporting the highest population and number of galls compared to resistant rootstocks. The results of present study was agreed with result obtained by Liebanas and Castillo (2004).

Number of galls per 10 g of roots, Number of egg masses per gram of roots and Number of females per gram of root of *Cucurbita ficifolia* was six fold higher than the resistant species *viz*., *Citrullus colocynthis* and *Cucumis metuliferus* but soil nematode population (per 200 cc of soil) and Reproduction factor of *Cucurbita ficifolia* was just two fold higher than the *Citrullus colocynthis* and *Cucumis metuliferus.* This was due to the population regulation of nematode; naturally mortality of nematode will occur when the nematode population is very high.

The reproduction factor depends on the susceptibility of plants for nematode infection. Normally reproduction factor will be high in susceptible species. Therefore population of soil nematode will be increased with high number of galls, egg masses and females. Thus these parameters were directly associated with one another.

The resistance and susceptibility of wild and cultivated species to root knot nematode was assessed by root knot indexing (RKI). Index based on number of galls is also one of the methods of scoring resistance to root knot nematode. It was revealed from data that among the seven cucurbitaceous species *C. colocynthis*, and *C. metuliferus* gave root knot index of 2 with resistant reaction. Nugent and Dukes (1997) observed that the variety C 701 of *Cucumis metuliferus* was found to be highly resistant to *M. incognita*. Similar trends of results were also obtained by Tamilselvi *et al.* (2013).

Cucurbita ficifolia, Cucurbita moschata, Cucrbita maxima and Luffa cylindrica were found to be moderately resistant against root knot nematode incidence with root knot index of 3. Among the moderately resistant species *C. ficifolia* recorded the lower rate of nematode multiplication and suffered less damage than other species. The lower rate of multiplication might be due to lower rate of invasion of juveniles into the resistant cultivars and subsequent suppression of nematode development resulting into low a fecundity rate. *Lagenaria siceraria* showed susceptible reaction with root knot index of 4 (Table 3). Resistance within a plant species is often due to specific genes that can segregate within the species. By contrast, for non-host species or resistant cultivars, the nematode could not be reproduced on that species or group of plants due to a broader absence of host traits required for parasitism. To reproduce, the infective second-stage juveniles must be attracted to host roots, penetrate the epidermis and migrate through the root cortex to establish a feeding site in the vascular parenchyma that provides sufficient nutrition for development and egg production (Abad *et al.,* 2009). Resistance genes, in response to nematode infection, block or suppress one or more of several critical steps in nematode parasitism. The two cucumber scions NS 408 hybrid and Green Long variety showed highly susceptible reaction with root knot index of 5. Resistant reaction was observed in wild species due to the presence of nematode resistant gene. This finding was observed to be in line with the reports of Roberts and May (1986) who found that resistance due to the gene and these genes made the plant less attractive for attack by nematodes.

Developing interspecific hybrids between *C. sativus* and other resistant wild cucurbitaceous species such as *C. metuliferus* is difficult due to different chromosome numbers of wild species. Franken *et al.* (1988) reported development of hybrid between these two species was restricted by strong barriers in hybridization including pollen tube growth arrestment in the stylar region of the pistil or abortion of the hybrid embryo at the globular stage. Hence, these resistant species could be used as rootstock for grafting with other cucurbitaceous crop to produce root knot nematode resistant plants.

The species *Citrullus colocynthis* and *Cucumis metuliferus* were noted to be resistant to root knot nematode and *Cucurbita ficifolia, C. moschata, C. maxima* and *Luffa cylindrica* were moderately resistant to root knot nematode. Hence, these species were found to be promising materials to be used as rootstocks for grafting studies in cucumber. Thus, there is a potential for use of these cucurbitaceous species as rootstock in cucumber cultivated areas infested with the root knot nematode. Grafting of cultivated cucumber varieties / hybrids on related soil borne resistant wild species as rootstocks, will be a profitable alternative for the production of healthy, toxic free cucumber to the consumers. However, further studies are required concerning graft compatibility between root knot nematode resistant rootstocks with cucumber and also breeding of interspecific hybrid as rootstock needed in cucurbits with wild and cultivated species for production of resistant plants.

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Table 1. Reaction of wild and cultivated cucurbitaceous rootstocks and cucumber scions to M. incognita on growth parameters at 45 days after inoculation

Treatments	Mean shoot / vine length (cm)	Mean shoot fresh weight (g)	Mean shoot dry weight (g)	Mean root length (cm)	Mean root fresh weight (g)	Mean root dry weight (g)
Fig leaf gourd (Cucurbita ficifolia)	253.80	40.25	4.16	23.28	1.47	0.21
Pumpkin (Cucurbita moschata)	79.35	42.53	4.88	60.45	2.75	0.32
Winter squash (Cucurbita maxima)	61.33	45.36	5.03	54.20	2.83	0.45
African horned cucumber (Cucumis metuliferus)	157.24	20.12	1.60	17.25	0.12	0.01
Colocynth (Citrullus colocynthis)	79.48	8.56	0.81	23.85	1.19	0.20
Bottle gourd (Lagenaria siceraria)	87.56	32.10	1.70	35.54	1.83	0.28
Sponge gourd (Luffa cylindrica)	89.36	20.08	1.52	42.27	1.02	0.16
Cucumber (Green Long) (Cucumis sativus)	96.42	15.15	1.43	13.85	0.56	0.06
Cucumber (NS 408) (Cucumis sativus)	112.55	22.50	2.89	24.76	0.83	0.10
SEd	2.0600	0.4906	0.0504	0.5935	0.0270	0.0038
CD (P=0.05)	4.3280	1.0308	0.1058	1.2470	0.0568	0.0081

Inoculation level 10000 J_2 /pot

Table 2. Reaction of cucurbitaceous rootstocks and cucumber scions on incidence of root knot nematode (M. incognita) under pot culture

Treatment	No. of galls / 10 g of roots	No. of egg masses / g of roots	No. of females / g of root
Fig leaf gourd (Cucurbita ficifolia)	25.32	6..36	18.32
Pumpkin (Cucurbita moschata)	34.26	9.94	29.94
Winter squash (Cucurbita maxima)	31.43	9.61	28.50
African horned cucumber (Cucumis metuliferus)	5.24	1.94	3.85
Colocynth (Citrullus colocynthis)	4.69	1.98	4.81
Bottle gourd (Lagenaria siceraria)	69.55	19.14	43.63
Sponge gourd (Luffa cylindrica)	38.36	13.27	31.94
Cucumber (Cucumis sativus) Green Long	76.35	23.39	46.35
Cucumber (Cucumis sativus) NS 408	83.25	29.35	49.31
SEd	0.863	0.315	0.560
CD (P=0.05)	1.813	0.663	1.177

** (1-5 scale Index : 1= No galls; 2 = 1-10 galls; 3 = 11- 30galls ; 4 = 31-100 galls; 5 = more than 100galls)

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Table 3. Soil nematode population and root knot index of cucurbitaceous rootstocks and cucumber scions to root knot nematode (Meloidogyne incognita)

Rootstock/scion	Soil nematode population (per 200 cc of soil)	Nematode population		Reproduction factor (Rf=Pf/Pi)	Root Knot Nematode Index (RKI)**	Reaction
		Initial (Pi)	Final (Pf)			
Fig leaf gourd (Cucurbita ficifolia)	98.98	10000	9694.43	0.97	3	MR
Pumpkin (Cucurbita moschata)	137.50	10000	15056.72	1.51	3	MR
Winter squash (Cucurbita maxima)	118.78	10000	12054.29	1.20	3	MR
African horned cucumber (Cucumis metuliferus)	48.85	10000	6807.40	0.68	2	R
Colocynth (Citrullus colocynthis)	43.34	10000	5869.44	0.59	2	R
Bottle gourd (Lagenaria siceraria)	235.64	10000	24089.65	2.40	4	S
Sponge gourd (Luffa cylindrica)	197.27	10000	18045.84	1.80	3	MR
Cucumber (Cucumis sativus) Green Long variety	249.92	10000	28785.47	2.87	5	HS
Cucumber (Cucumis sativus) NS 408 hybris	268.73	10000	29958.79	3.00	5	HS

R - Resistant, MR - Moderately resistant, S - Susceptible, HS - Highly susceptible