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Screening of cucurbitaceous rootstocks against root knot nematode *Meloidogyne incognita* Kofoid and White

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ABSTRACT : Root knot nematodes are responsible for severe crop loss in cucurbits to the tune of 547.5 million. Pot culture experiments were conducted under glasshouse conditions to study the effect of root knot nematode, *Meloidogyne incognita* Kofoid and White in twelve wild and cultivated cucurbitaceous species and to identify resistant root stocks for grafting in bitter gourd. Second stage juveniles of *M. incognita* were inoculated at $2J_2$ per cc soil into the rhizosphere of 21 days old seedlings and changes in the growth rate *viz.,* shoot length, shoot fresh and dry weight, root length, root fresh and dry weight were recorded after sixty days after inoculation. Number of galls per 10 gram of root, egg mass and females per gram of root, soil nematode population per 200 cc soil, final nematode population and root gall indices were also determined at the end of the experiment. Soil and root population of nematodes were assessed by standard methods and growth parameters were worked out. Among ten rootstocks screened against *M. incognita*, three genotypes *viz.,* Kumati kai (*Citrulus colocynthus*), african horned cucumber (*Cucumis metuliferus*) and pumpkin (*Cucurbita moschata*) showed resistant reaction followed by two rootstocks *viz.,* Sponge gourd (*Luffa cylindrica*) and mithi pakal (*Momordica charantia* var. *muricata*) which were moderately resistant. These rootstocks were further used for grafting with bitter gourd scion.

KEY WORDS : Root knot nematode, *Meloidogyne incognita,* Resistance, Bitter gourd grafting

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Bitter gourd (*Momordica charantia* L.) is one of the important cucurbitaceous vegetables grown in our country. Among the cucurbits, bitter gourd is considered a prized vegetable because of its high nutritive value especially ascorbic acid and iron (Behera, 2004). Among the cultivated cucurbits, bitter gourd has been identified as one of the potent vegetables for export by Agricultural Processed Food Products and Export Development Authority (APEDA). Due to limited availability of arable land and high market demand for cucurbits (especially bitter gourd), they are cultivated intensively in larger areas. The main problem with bitter gourd production is Fusarium wilt, root knot nematode and low temperature during winter (Lin *et al*., 1998).

Root knot nematode (*Meloidogyne* spp.) are one of the most important group of plant parasitic nematodes attacking nearly every crop grown and have been reported to cause an annual loss of Rs. 547.5 million in cucurbits (Jain *et al.,* 2007). It is a sedentary endoparasite nematode which penetrates the feeder roots and feed on the vascular tissue. Berkeley (1855) was the first to report infestation by the root knot nematode, *Meloidogyne* spp. on cucumber in England. Root knot nematodes are worldwide in distribution (Sasser, 1977) and more than 3000 plants including cucurbits have been recorded as hosts (Krishnaveni and Subramanian (2005). *M. incognita* Kofoid and White is an obligate parasite which is highly destructive to crop in nature (Izuogu *et al*., 2010). Their worldwide distribution, extensive host ranges and interaction with other plant pathogens in disease complexes rank them among the major plant pathogens in the world. The major symptoms associated with nematode attack is the presence of root galls resulting in poor root growth, a decline in quality and yield of the crop and reduced stress resistance, leading to total crop loss (Chandra *et al.*, 2010 ; Izuogu *et al*., 2010). Additional losses to the grower are caused by inability of the damaged roots to utilize water and fertilizers effectively. The degree of damage done depends upon the population density of the nematode, taxa present, susceptibility of the crop and environmental condition such as fertility, moisture and presence of other pathogenic organisms, which may interact with nematodes.

Chemical pest control is expensive, not always effective and can even harm the environment. The causal organisms may also develop resistance to a particular chemical or they may emerge as new races which may be resistant to chemical control measures. Use of resistant genotypes or varieties is one of the promising way of managing the nematodes which is safe and environment friendly method. Grafting of pest-susceptible vegetables on pest-resistant rootstocks is gaining popularity in the management of soil-borne pests. A number of wild and cultivated cucurbit species are resistant to soil borne diseases like root knot nematode, but attempt to incorporate this resistance is not been successful. One method to circumvent this problem is to graft the susceptible scion on to resistant rootstocks. But, very scanty reports exist on the screening of cucurbitaceous species against root knot nematode. Hence, the present study aims at evaluation of the reaction of ten wild and cultivated cucurbitaceous rootstocks to pathogenic potential of root knot nematode *M. incognita* and to identify resistant rootstocks against root knot nematode for grafting in bitter gourd.

RESEARCH METHODS

A pot culture experiment was conducted at the Department of Nematology glasshouse from December 2012 to February 2013 to evaluate the reaction of ten wild and cultivated cucurbitaceous rootstocks and bitter gourd scions to pathogenic potential of root knot nematode (*Meloidogyne incognita)* and to identify the resistant rootstocks to bitter gourd grafting. The rootstocks *viz.*, local cultivars and varieties of mithipakal (*Momordica charantia* var. *muricata),* fig leaf gourd *(Cucurbita ficifolia),* pumpkin *(Cucurbita moschata),* zucchini squash *(Cucurbita pepo),* sponge gourd *(Luffa cylindrica),* ridge gourd *(Luffa acutangula),* bottle gourd *(Lagenaria siceraria*), ash gourd *(Benincasa hispida),* kumati kai *(Citrulus colocynthus)* and african horned cucumber *(Cucumis metuliferus)* and two scions Palee F_1 and CO 1 of bitter gourd were used for screening.

Nematode culture:

Samples of root bearing galls were collected from farmer's field in tomato growing areas of Coimbatore district using standard sampling method and brought to the laboratory. Pure culture of root knot nematode, *M. incognita* was maintained at the laboratory. The identification of the species was confirmed by using taxonomic keys and by using the posterior cuticular pattern. Highly susceptible tomato cultivar PKM-1 was used for developing pure culture of root knot nematode. Plants of tomato (PKM-1) were raised in the pots filled with steam sterilized loamy soil mixed with fine river sand. The potted plants were inoculated with J_2 stage of *M. incognita* @ 2-3 per pots and maintained for further studies.

Experimental procedure:

Seeds of mithi pakal (*Momordica charantia* var. *muricata),* fig leaf gourd *(Cucurbita ficifolia),* pumpkin *(Cucurbita moschata),* zucchini squash *(Cucurbita pepo),* sponge gourd *(Luffa cylindrical),* ridge gourd *(Luffa acutangula),* bottle gourd *(Lagenaria siceraria*), ash gourd *(Benincasa hispida),* kumati kai *(Citrulus colocynthus)* and african horned cucumber *(Cucumis metuliferus)* and two scions Palee F1 and CO 1 were sown singly in protrays contains coco pit.

Pot mixture (Red soil: sand: farm yard manure at 2:1:1 v/v, respectively) were autoclaved at 15 kg/m^2 pressure at 121^0 C for 30 minutes. Fifteen days old seedlings of aforementioned species were transplanted in to earthen pots holding 2.5 kg of sterilized pot mixture.

Inoculation:

Inoculation of nematode was done as per the method suggested by Sasser *et al*. (1957). Egg masses were separated from the roots of tomato plants grown in pots infested with pure culture of root knot nematode one day prior to inoculation. Infested roots from pure culture were cut into small pieces of about 2cm long and placed in sodium hypochlorite (NaOCl) 0.5% solution. The container was shaken for about three minutes to dissolve the gelatinous matrix and freeing the eggs from the egg mass. The egg masses were kept on tissue paper on a wire mesh in a Petri plate containing water at a level touching the tissue paper and incubated for 24 hours at 25-28^0 C. The nematode suspension was collected from the plates and the second stage juveniles were counted in a counting dish under a stereoscopic microscope. The inoculum concentration was adjusted to a known number by addition of water. The eggs were kept in Petridishes and frequently aerated by an aerator to enable hatching. The nematode inoculum (J_2) was placed at 2 cm depth near to the rhizosphere of 21 days old seedlings at a depth of 3 cm 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 7 days after planting. About three replications were maintained for each cultivar. Regular watering of plants was done until termination. Pots were arranged in a completely randomized block design. The plants were allowed to grow 60 days in glasshouse and terminated. The experiment was terminated, 60 days after inoculation of nematode. The plants were carefully uprooted and the roots were washed under running tap water to remove the adhering soil particles. The growth parameters *viz.*, shoot length, shoot fresh weight as well as root fresh weight were recorded. Dry weights were determined after drying the plants in a hot air oven at 60^0C for 72 h.

Root infestation by the nematode was assessed by counting number of root galls in each plant and root gall index was assessed on a scale of 1-5, where 1 = 0 % galled roots (no galls/ egg mass) ; 2 = 1-25% galled roots (1-10 galls) ; 3 = 26-50 % galled roots (11-30 galls) ; 4 = 51-75 galled roots (31-100) ; 5 = 76-100 % galled roots (> 100 galls) by Heald *et al.* (1989). Soils from the pots were thoroughly mixed and eggs and J_2s in the egg masses in roots and soil population was assessed from 200 g of rhizosphere soils by the Cobb's sieving and decanting method followed by modified Baermann funnel technique (Cobb,1918; Schindler,1961). The final population (Pf) was calculated as total number of nematode extracted from roots and soil after 60 days. The reproduction factor (Rf) of the nematode in the different plants were calculated as the ratio of the final nematode population to initial nematode population (Rf =Pf / Pi). Five gram of root randomly taken from each plant was stained using acid fuchsin-lactophenol and number of females/ juveniles were counted. The data from the experiments were analyzed statistically following Panse and Sukhatme (1967).

RESEARCH FINDINGS AND DISCUSSION

The results obtained from the present investigation are summarized below :

Plant growth parameters:

Observation on plant growth parameters *viz.*, length, fresh weight and dry weight of shoots as well as roots revealed significant differences among the rootstocks (Table 1). Among the ten rootstocks, pumpkin *(Cucurbita moschata)*, kumati kai *(Citrulus colocynthus)* and african horned cucumber (*Cucumis metuliferus)* showed resistant reaction. Sponge gourd *(Luffa cylindrica)* and mithi pakal (*Momordica charantia* var. *muricata)* were found to be moderately resistant. Remaining rootstocks were highly susceptible to root knot nematode except ridge gourd *(Luffa acutangala)*. The highest shoot length was recorded in pumpkin *(Cucurbita moschata)* followed by kumati kai *(Citrulus colocynthus)* and african horned cucumber (*Cucumis metuliferus)* and the lowest vine length was recorded by zucchini squash followed by ash gourd and fig leaf gourd. Reduction in shoot length is mainly due to increased population of root knot nematode. An increase in shoot length was mainly due to resistant response of the plants and possibly by adaption of the plants in response to the stress induced by root knot nematode. The highest shoot fresh weight and dry weight were recorded in pumpkin followed by african horned cucumber (*Cucumis metuliferus)* and kumati kai *(Citrulus colocynthus)* and zucchini squash *(Cucurbita pepo)* followed by fig leaf gourd rootstocks were recorded the lowest value for the same characters. Highest shoot fresh and dry weight was mainly due to higher photosynthetic ability and assimilation of dry matter. This agrees with the earlier findings by Izuogu *et al.* (2010) in fluted pumpkin. Lowest shoot fresh and dry weight was mainly due to lower photosynthetic ability of leaves and less accumulation of dry matter. This result agrees with the works of Jain (2007).

Among the wild and cultivated cucurbit species used,

Table 1 : Effect of *Meloidogyne incognita* and reaction of cucurbit rootstocks and scions on plant growth parameters 60 days after inoculation

Rootstocks	Shoot / vine length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
Mithi pakal (*Momordica charantia* var. *muricata)*	99.03	12.20	1.27	11.60	0.84	0.06
Fig leaf gourd *(Cucurbita ficifolia)*	60.60	8.43	0.85	4.32	0.34	0.03
Pumpkin *(Cucurbita moschata)*	160.53	22.43	3.73	15.70	1.07	0.16
Zucchini squash *(Cucurbita pepo)*	36.93	6.26	0.64	2.23	0.61	0.07
Sponge gourd *(Luffa cylindrica)*	126.46	16.63	1.46	12.36	0.90	0.14
Ridge gourd *(Luffa acutangula)*	95.43	12.56	0.75	8.73	0.81	0.07
Bottle gourd *(Lagenaria siceraria)*	56.76	10.33	0.92	4.36	0.28	0.05
Ash gourd *(Benincasa hispida)*	50.40	14.06	1.05	2.36	0.22	0.02
Kumati kai *(Citrulus colocynthus)*	144.73	17.46	1.11	13.60	0.91	0.14
African horned cucumber *(Cucumis metuliferus)*	138.86	18.76	1.40	16.46	1.81	0.21
Scions						
Palee F1	84.20	10.56	1.05	9.40	1.02	0.09
CO 1	78.23	8.20	0.56	8.36	0.98	0.07
S.E.±	2.19	1.00	0.12	0.64	0.15	0.02
CD (P=0.05)	4.53	2.07	0.24	1.33	0.31	0.04

*Inoculation level 5000 J_2/pot

highest root length (16.46), root fresh (1.81) and dry weight (0.21), was observed in african horned cucumber followed by pumpkin (15.70 cm, 1.07 g and 0.16 g, respectively). The cultivars that showed resistance reaction occasionally exhibited significant reduction in fresh as well as dry weight of shoot and root. Significant reduction in length, fresh and dry weight of shoot as well as root was recorded in almost all the susceptible cultivar. Stunted shoot and root growth as well as deformed root system were accompanied by heavy infestation. Deformed root system developed by root knot nematode infestation limits water and nutrient translocation from infested roots to above ground plant tissues subsequently leading to plant growth impairment. These results coincide with the findings obtained by Mahapatra *et al.* (1999) in pointed gourd and Krishnaveni and Subramanian (2002) in cucumber were more than 1000 J_2 of *M. incognita* race 3 as initial inoculums level caused significant reduction in shoot length, root length and shoot weight. The cultivars which greatly supported nematode pathogenesis (susceptible) exhibited greater reduction in various plant growth parameters considered. Progressive decrease in plant growth with nematode infestation was also reported by Khan *et al.* (2006) and Chandra *et al.* (2010).

Reaction of wild and cultivated cucurbitaceous species to *Meloidogyne incognita* :

All the rootstocks and scions developed characteristic galls caused by *M. incognita*. Significant differences were noticed among the rootstocks for number of galls per 5 gram root, number of egg masses per gram root and number of females per gram of root (Table 2). Kumati kai *(Citrulus colocynthus)* followed by african horned cucumber (*Cucumis metuliferus)* and pumpkin *(Cucurbita moschata)* recorded the lowest number of galls, number of egg masses per gram root and number of females per gram of root, respectively and showed resistant reaction. Siguenza *et al.* (2005) reported similar results in african horned cucumber (*Cucumis metuliferus)* and pumpkin *(Cucurbita moschata)*. Sponge gourd *(Luffa cylindrica)* followed by mithi pakal (*Momordica charantia* var. *muricata)* showed moderately resistant reaction and ridge gourd showed susceptible reaction. The four rootstocks *viz.*, zucchini squash *(Cucurbita pepo)*, fig leaf gourd *(Cucurbita ficifolia)*, bottle gourd *(Lagenaria siceraria)* and ash gourd *(Benincasa hispida)* and scions (Palee F1 and CO 1) exhibited highly susceptible reaction as rhizosphere of these cultivars favoured maximum population build up in terms of number of galls, number of egg mass per gram root and number of females per gram of root and maximum root damage due to gall formation. Similar results were reported by Chandra *et al*. (2010) in bitter gourd. In contrast to this finding Fassuliotis (1970) in USA demonstrated that fig leaf gourd *(Cucurbita ficifolia)* had exhibited resistance to root knot nematode.

Nematode population build up both in roots and soil had given more comprehensive representation of reaction of rootstocks and scions. The lowest nematode population per 200 cc of soil (Table 3) was recorded in kumati kai *(Citrulus colocynthus)* followed by african horned cucumber *(Cucumis metuliferus)* and pumpkin (*Cucurbita moschata)*. However, the zucchini squash recorded the highest population per 200 cc soil followed by fig leaf gourd. Final / total nematode population also significantly varied among the rootstocks and scions. Kumati kai *(Citrulus colocynthus)* registered the lowest nematode population followed by african horned cucumber *(Cucumis metuliferus)* and pumpkin

Table 2 : Reaction of wild and cultivated cucurbitaceous species to *Meloidogyne incognita*

Rootstock	No. of galls / 10g of roots	No. of egg masses/g of root	No. of RKN females/ g of root
Mithi pakal (*Momordica charantia* var. *muricata)*	45.80	6.06	30.30
Fig leaf gourd *(Cucurbita ficifolia)*	129.58	43.20	92.20
Pumpkin *(Cucurbita moschata)*	8.93	3.74	5.56
Zucchini squash *(Cucurbita pepo)*	161.80	50.80	100.90
Sponge gourd *(Luffa cylindrica)*	26.36	4.90	12.90
Ridge gourd *(Luffa acutangula)*	74.20	25.26	42.23
Bottle gourd *(Lagenaria siceraria)*	86.40	35.60	46.70
Ash gourd *(Benincasa hispida)*	98.40	39.06	55.10
Kumati kai *(Citrulus colocynthus)*	3.11	1.42	2.34
African horned cucumber *(Cucumis metuliferus)*	4.20	1.54	2.56
Scions			
Palee F1	76.60	22.66	33.93
CO 1	78.30	26.76	38.20
SEd	1.81	1.02	1.09
CD (P=0.05)	3.75	2.10	2.26

*Inoculation level 5000 J_2/pot

(*Cucurbita moschata)* whereas zucchini squash *(Cucurbita pepo)* registered the highest value for the same trait and final nematode population was higher in all susceptible rootstocks.

The suitability of the host for plant parasitic nematodes expressed as the ability of the nematode to multiply in the plant and is measured by reproduction factor (Rf) which is the ratio of number of nematodes recovered at the end of the experiment (Pf) to the number of nematode units used to inoculate the plants (Pi) (Liebanas and Castillo, 2004). A wide range of variation was recorded in reproduction factor (Rf) which ranged from 0.83 (kumati kai - *Citrulus colocynthus)* to 3.72 (Zucchini squash-*Cucurbita pepo)* followed by CO 1 scion (2.99).On the basis of nematode reproduction, kumati kai *(Citrulus colocynthus)*, african horned cucumber *(Cucumis metuliferus)* and pumpkin *(Cucurbita moschata)* were categorized as resistant to root knot nematode. Significantly lower Rf value exhibited by these rootstocks in this study are a further indication of high level of resistance present in this genotypes than susceptible rootstocks. This result corresponds with reports by other authors (Di Vito *et al*., 1991; Charchar *et al*., 2003) in terms of *M. incognita* race 1 and *M. javanica* on tomato. In greenhouse study, control watermelon cultivars had higher Rf values and root gall numbers in inter generic grafts Pofu *et al.* (2011) and other water melon studies (Theies *et al*., 2011). The Rf values lower than one on *Cucumis* rootstocks and the absence of noticeable root galls indicated their resistance to *Meloidogyne* species as described in plant parasitic nematodes and plant growth was not affected.

Nugent and Dukes (1997) observed that the variety C 701 of african horned cucumber *(Cucumis metuliferus)* was highly resistant to *M. incognita.* Formation of fewer galls in these 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 (Sobczak *et al*., 2005). Biochemical mechanism of invasion supports this mechanism which occur due to noncooperative action of host tissue or cells. The chemical inhibitors in the host tissue counteract or neutralize the gaint cell inducing effect of salivary secretions of the nematode (Barrons, 1939). In *Cucumis* species, the potent chemicals, cucurbitacins, which are believed to be responsible for nematode suppression, accumulate to the greatest extent in both seeds and roots (Jeffrey, 1978). The rootstocks and scions susceptible to nematode infection supporting the highest population and galling compared to resistant rootstocks. The present study agrees with Liebanas and Castillo (2004) and Khan *et al.* (2006).

Root knot index was used to evaluate the resistance or susceptibility of wild and cultivated cucurbit species against *M. incognita*. The results revealed that kumati kai *(Citrulus colocynthus)* african horned cucumber (*Cucumis metuliferus) and* pumpkin *(Cucurbita moschata)* rootstocks had the least count of gall index of 2, attaining a reaction category of 'resistant' (R), whereas mithi pakal (*Momordica charantia* var. *muricata)* and sponge gourd *(Luffa cylindrica)* exhibited higher number of galls and recorded a gall index of 3 and the corresponding reaction category of 'moderately resistant' (MR). The gall index of 4 was observed in ridge gourd *(Luffa acutangula)* which attained the reaction category of 'susceptible' (S) and other rootstocks and scions *viz*., fig leaf gourd *(Cucurbita ficifolia),* zucchini squash *(Cucurbita pepo)*, bottle gourd *(Lagenaria siceraria*), ash gourd *(Benincasa hispida),* Palee F1 and CO 1 recorded the gall index of 5 which is came under the category of 'highly susceptible'.

This study indicated that the preliminary evaluation of cucurbitaceous rootstocks exhibited significant differential

Table 3 : Reaction of cucurbitaceous rootstocks and scions to *Meloidogyne incognita* in pots glasshouse condition

Rootstocks	Soil population (200 cc)	Soil population (J2/kg soil) Initial population (Pi)	Final population (Pf)	Reproduction factor (Rf = Pf / Pi)	Gall index (GI)	Degree of resistance
Mithi pakal (*Momordica charantia* var. *muricata)*	135.06	5000	8007.09	1.60	3	MR
Fig leaf gourd *(Cucurbita ficifolia)*	215.06	5000	14670.40	2.93	5	HS
Pumpkin *(Cucurbita moschata)*	48.86	5000	4779.81	0.95	2	R
Zucchini squash *(Cucurbita pepo)*	237.53	5000	18623.21	3.72	5	HS
Sponge gourd *(Luffa cylindrical)*	102.16	5000	8140.30	1.62	2	MR
Ridge gourd *(Luffa acutangula)*	183.66	5000	11342.40	2.26	4	S
Bottle gourd *(Lagenaria siceraria*)	228.46	5000	13978.70	2.79	5	HS
Ash gourd *(Benincasa hispida)*	223.86	5000	12026.10	2.40	5	HS
Kumati kai *(Citrulus colocynthus)*	40.43	5000	4163.80	0.83	2	R
African horned cucumber *(Cucumis metuliferus)*	45.13	5000	4673.50	0.93	2	R
Scions						
Palee F1	178.80	5000	14021.40	2.80	5	HS
CO 1	198.96	5000	14978.20	2.99	5	HS

*Inoculation level 5000 J_2/pot

response to *M. incognita*. However, majority of the cultivars found susceptible to nematode. Three rootstocks kumati kai (*Citrulus colocynthus*), african horned cucumber *(Cucumis metuliferus)* and pumpkin (*Cucurbita moschata*) which exhibited resistant reaction and Sponge gourd (*Luffa cylindrica*) and mithi pakal (*Momordica charantia* var. *muricata*) showed moderately resistant reaction and can be used for grafting in bitter gourd which could be developed into a valuable crop management tool to reduce the deleterious effect of root knot nematodes on bitter gourd. Root knot nematode is the main problem in bitter gourd cultivation worldwide and is well known that grafting bitter gourd with interspecific species can provide best solution to soil borne diseases. These rootstocks are further used to assess the graft compatibility with bitter gourd scions. Once the compatibility between rootstocks and scions achieved, grafting is considered to part of an integrated approach to control soil borne diseases.

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