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Research Article

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Defence Responses of Cucurbitaceous Rootstocks and Bitter Gourd Scions against Root Knot Nematode *Meloidogyne Incognita* Kofoid and White

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Abstract

Pot culture experiments were conducted under *in vitro* to understand the biochemical changes takes place during nematode attack and to identify resistant rootstocks for grafting with bitter gourd scions. Results revealed that, three genotypes *viz.,*Kumatikai *(Citrulus colocynthis),* African horned cucumber *(Cucumis metuliferus)* and pumpkin *(Cucurbita moschata)* encompass the lowest value for number of galls per gram root, egg masses per gram root and numbers of females per gram of root and showed resistant reaction followed by two rootstocks *viz.,*Sponge gourd *(Luffa cylindrica)* and mithipakal (*Momordica charantia* var. *muricata)* bare minimum values and are moderately resistant. These resistant reaction yet again confirmed by assessment of defence enzymes *viz.,*phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenoloxidase (PPO), Acid phosphatase and biochemical components *viz.,* total phenol and ortho dihydroxy phenol (OD phenol). The present study indicates that rapid augmentation of PAL, PO, PPO, acid phosphatase, total phenol and OD phenols activities were noticed in resistant rootstocks *viz., C. colocynthis, C. metuliferus* and *C. moschata* and started to increase at 24 hours after inoculation, reached maximum at 96 hours after inoculation and thereafter declined. Similarly, native poly acralamide gel electrophoresis (PAGE) analysis revealed that one to six isoforms of defence enzymes were observed in aforementioned resistant rootstocks whereas fewer isoforms with less intensity were noticed in susceptible rootstocks and bitter gourd scions. These results suggest that the aforementioned resistant rootstocks are potential source for managing root knot nematode menace in bitter gourd by grafting.

Keywords

Meloidogyne incognita; Resistance; Rootstocks; Bitter gourd; Defence enzymes; Grafting

Introduction

Bitter gourd (*Momordica charantia*) is one of the important cucurbitaceous vegetables predominantly grown in South–East Asia. In India, the crop is cultivated over an area of 80,990 ha with an annual production of 8, 30,450 tonnes and the productivity of 10.25 t ha^{-1} during the year 2015-16 [1]. Depending upon the location, it has been known by different names such as 'Karela' in Hindi, Gujarathi and Punjabi, 'Karala' in Marathi, 'Beet Karela' in Assamese, 'Kakara kaya' in Telugu, 'Hagala kayi' in Kannada, 'Kayppayka' in Malayalam and 'Pavakai' in Tamil. The bitter gourd fruits are rich source of ascorbic acid, iron and vitamin C and also have antidiabetic properties due to the presence of a hypoglycaemic principle called "Cheratin". The intense bitter gourd cultivation is threatened by various soil borne diseases and root-knot nematode in tropics and sub-tropics. The three species *viz., Meloidogyne incognita*, *Meloidogyne javanica* and *Meloidogyne arenaria* endorsed major yield loss in cucurbits [2]. Among these three species, *Meloidogyne incognita* stands out which causes yield loss up to 38 to 48.2% in bitter gourd [3] results in significant loss in quality and quantity [4,5]. Root-knot nematodes are obligate parasites, feeds the cytoplasm of living plant cells leads to formation of abnormal, knotty growths on the roots, called galls can grow to be one inch or more, which affect nutrients uptake and translocation of materials to the above ground plant parts. As a result, infested plants become stunted.

In addition to the direct losses, root-knot nematodes also invite other secondary plant pathogens such as *Fusarium*, results in increased crop damage [6]. A range of strategy have been attempted to control root knot nematode which includes, use of nematicides, organic amendments and resistant cultivars. However, indiscriminate use of chemical nematicides becomes a major threat to environment and human health [7] besides high cost. The use of resistant varieties or cultivation of non-host crops against root knot nematode is economical and effective but it requires time and facilities to develop resistant varieties. Therefore, among the non chemical methods available, the use of resistant cucurbitaceous plants as a rootstock is considered as one of the most effective and sustainable method without harmful to the environment [8]. In short-term, the use of resistant rootstocks would be viable approach, as practiced in other crops [9,10,11]. Nevertheless, this practice would have greater applicability in cucurbits, because of the high commercial value-added.

In this context, the present study was carried out to study the morphological characters and biochemical changes taking place during the early stages of the disease process after initiation of infection, with reference to phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenoloxidase (PPO) and Acid phosphatase and biochemical components *viz.,* Total phenol and ortho dihydroxy phenol (OD phenol) using standard techniques.

Materials and Methods

Pot culture experiments and laboratory analysis was carried out from 2013 to 2014 at the Department of Nematology glasshouse and Micro analytical Lab, Department of Vegetable Crops, Tamil Nadu Agricultural University, Coimbatore, India (11^0 N latitude, 77^0 E longitude and an altitude of 426.26 m above mean sea level) to study the early biochemical changes in the rootstocks *viz.,* 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),* kumatikai *(Citrulus colocynthis)* and African horned cucumber

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(Cucumis metuliferus) and two scions of bitter gourd *viz.,* Palee F_1 and CO 1in response to *M. incognita* attack.

Samples of root bearing galls along with their rhizospheric soils were collected from farmer's field in bitter gourd growing areas of Coimbatore district, Tamil Nadu using standard sampling method and brought to the laboratory for isolation [12,13]. The isolated *M. incognita* was identified microscopically by examining the perineal pattern of females and later confirmed from the Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. Highly susceptible tomato cultivar PKM-1 was used for developing pure culture of *M. incognita*. 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 pot and maintained for further studies.

Sixty days of inoculation, tomato plants were removed from pots and 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.

Seeds of the aforementioned cucurbitaceous rootstocks and bitter gourd scions were surface sterilized with 0.1% $HgCl_2$ solution and washed three times with sterile water. After storage under moisture for two days they were sown in protrays filled with sterilized coco pit. The two weeks seedlings were transplanted into earthen pots having 2.5 kg sterilized pot mixture [Red soil: Sand: Farmyard manure (1:1:1 ratio)]. A week after transplanting freshly hatched second stage juveniles (J_2) were inoculated at $2J_2$ per cc soil into the rhizosphere at a depth of 3 cm and replicated four times. Regular watering was done until harvest.

Seedlings were uprooted carefully 60 at days after inoculation with minimum root disturbance and washed with tap water to remove the adhering soil particles. From the fresh root sample, number of galls, number egg masses and number females per gram of root were counted under stereoscopic microscope after staining with acid fuchsin lacto phenol. The nematode population in soil were assessed by using Cobb's sieving and decanting method followed by modified Baermann funnel technique [12,13]. The final nematode population (Pf) was calculated as total number of nematodes extracted from both roots and soils. The root knot index was calculated as per Heald et al. [14].

Assay for defence related enzymes

The defence enzymes such as phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenoloxidase (PPO), acid phosphatase and biochemical components *viz.,* Total phenol and Ortho dihydroxy phenol (OD phenol) was measured in aforementioned cucurbitaceous rootstocks and bitter gourd scions.

Sample collection

Recently matured physically active roots of five randomly selected plants after inoculation were taken for biochemical analysis. Root of aforementioned species were collected at 0, 24,48,72,96 and 120 hours after nematode inoculation, washed in running tap water and stored in deep freezer (-80°C) until used for biochemical analysis.

Enzyme extraction

Samples obtained from different hours interval was homogenized in chilled pestle and mortar with 2 mL of ice cold 0.1 M sodium phosphate buffer (pH 7.0, at 4°C). The homogenate was centrifuged at 16000 rpm at 4^0C for 15 minutes in a refrigerated centrifuge and the supernatant was used as enzyme source.

Peroxidase (PO) activity

Peroxidase activity was assayed by using the method of Srivastava [15]. The enzyme activity was expressed as change in $OD min^{-1} g^{-1}$ of protein [16].

Poly phenol oxidase (PPO) activity

PPO activity was determined as in Mayer et al. [17]. The PPO activity was expressed as change in OD $minute^{-1}$ g^{-1} of protein.

Phenylalanine ammonia lyase (PAL) activity

PAL activity was determined as the rate of conversion of L-phenylalanine to transcinnamic acid at 290 nm and enzyme activity was expressed as nmol transcinnamic acid $minute^{-1}$ g^{-1} tissue [18].

Acid phosphatase activity

Acid phosphatase activity was assayed by using the method of Srivastava [15]. The released p-nitro phenol was monitored at 405 nm and expressed as *m*moles of p-nitro phenol released $minute^{-1}$ mg^{-1} protein.

Total phenol

Folinciocalteau reagent method was followed for estimation of total phenols at 660 nm and expressed as mg g^{-1} [19].

Orthodihydroxy phenol (OD Phenol)

Arnow's method was followed for the estimation of orthodihydroxy phenol at 515 nm and expressed as mg g^{-1} [20].

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

One gram of powdered root sample was extracted with one ml of 0.1 M sodium phosphate buffer (pH 7.0) under 4°C. The homogenate was centrifuged for 20 min. at 10000 rpm and the supernatant was used for the SDS-PAGE. Hundred microgram of protein from different cucurbitaceous rootstocks and bitter gourd scions was taken and mixed with 10 µl of sample buffer in a microfuge tube, boiled for 4 min and incubated at 4°C for 30 min. Then the samples containing equal amount of proteins were loaded into the wells of polyacrylamide gels (Sigma-Aldrich Techware system, Sigma, USA). The medium range molecular weight markers (Bangalore Genei, India) were used and electrophoresis was carried out at constant voltage of 75 volts for 2 h. The gels were stained with 0.2 % Coomassie brilliant blue (R250) solution. Based on the R*f* value of each protein band stained, the molecular weight was calculated.

Native PAGE Analysis of PO and PPO isozymes

To study the expression pattern of different PO and PPO isozymes in wild and cultivated cucurbitaceous rootstocks and bitter gourd scions, Native polyacrylamide gel electrophoresis (Native PAGE) was carried out [15,21]. After staking, the gel was washed with distilled water and photographed.

Statistical analysis

The data were statistically analyzed using the IRRISTAT version 92 developed by the International Rice Research Institute Biometrics Unit, the Philippines [22]. Data were subjected to analysis of variance (ANOVA) at two significant levels (P<0.05 and P<0.01). The means were compared by Duncan's Multiple Range Test (DMRT).

Results

In the present study, there were significant variations were noticed among the cucurbitaceous rootstocks and two bitter gourd scions for number of galls, number of egg masses and number of RKN females per gram of root against *M. Incognita* (Table 1). The lowest number of galls and egg masses per gram of root was observed in *C. colocynthis* (3.11 and 1.42) followed by *C. metuliferus* (4.20 and 1.54) while highest values were recorded by *C. Pepo* (161.80 and 50.80). The number of RKN females was also high in *C. Pepo* (100.90) followed by *C. ficifolia* (92.20) and the lowest count for the same trait was noticed in *C. colocynthis* (2.34) followed by *C. metuliferus* (2.56) respectively. The rootstock *C. colocynthis* recorded lowest nematode population per 200 cc of soil (40.43) followed by *C. metuliferus* (45.13) and *C. moschata* (48.86) respectively. However *C. Pepo* recorded the highest value (237.53) for the same trait.

Root knot index was used to evaluate the reaction (resistance or susceptible) of the plants against *M. Incognita* (Table 1). The results of this study revealed that *C. colocynthis, C. metuliferus* and *C. moschata* rootstocks found to be 'resistant' (R) with the root knot index of 2, whereas *M. charantia* var. *muricata* and *L. cylindrica* were identified as 'moderately resistant' (MR) which have the root knot index of 3. The rootstock *L. acutangula* comes under the reaction category of 'susceptible' (RKI 4) and rest of the rootstocks and bitter gourd scions were highly susceptible with the root knot index of 5.

The root knot nematode resistance identified by conventional screening were confirmed through calorimetric estimation of resistant enzymes and biochemical components *viz.,* phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenoloxidase (PPO), acid phosphatise, total phenol and orthodihydroxy phenol (OD Phenol) in the aforementioned rootstocks and bitter gourd scions at different hours after inoculation.

The results depicted in Figure 1 shows that significant increases in PAL activity was observed in *C. colocynthis* (15.48 nmol of trans cinnmic acid min^{-1} g^{-1} of root) followed by *C. metuliferus* (15.29 nmol of trans cinnmic acid min^{-1} g^{-1} of root) against *M. incognita.* The increased activity of this enzyme was observed up to 96 hours after inoculation and thereafter declined with decreasing rate. The enzyme activity remained constant in susceptible rootstocks and bitter gourd scions. Similarly enhanced PO and PPO activities were also observed in *C. colocynthis* followed by *C. metuliferus* and *C. moschata* rootstocks against root knot nematode (Figure 2 and Table 2). Similar trend of increasing activity was noticed for acid phosphatase activity. The estimation of enzyme activity in the roots of cucurbitaceous rootstocks and bitter gourd scions showed a gradual increase up to 96 hours and slightly declined thereafter. The highest acid phosphatase activity was recorded by *C. colocynthis* (118.55 mmoles p-nitrophenol min^{-1} mg^{-1} protein) followed by *Cucurbita moschata* (114.51*m* moles p-nitrophenol min^{-1} mg^{-1} protein) whereas the lowest activity (45.65 *m*moles p-nitrophenol $min^{-1}mg^{-1}$ protein) was observed in CO 1 bitter gourd scion (Figure 3).

Estimation of total phenols from cucurbitaceous rootstocks and bitter gourd scions inoculated with *M. incognita* under *in vitro* clearly showed the increased activity of this enzyme in response to *M. incognita*. Among the cucurbitaceous rootstocks and bitter gourd scions, increased activity of total phenol content was observed in *C. colocynthis* (17.11 mg g^{-1} of root) followed by *C. metuliferus* (16.20 mg g^{-1} of root). Very little induction of total phenol content was observed in susceptible rootstocks and bitter gourd scions (Table 3). Roots of cucurbitaceous rootstocks and bitter gourd scions were analyzed for ortho-dihydroxy phenol (OD phenol) content. The result revealed that there was a gradual increase in OD phenol content after inoculation of *M. incognita.* The increased level of OD phenols were noticed from 24 hours up to 96 hours after inoculation and thereafter declined. Among the cucurbitaceous rootstocks, *Citrullus colocynthis* recorded the highest OD phenol content of 13.16 mg g^{-1} of root followed by *Cucumis metuliferus* (12.99 mg g^{-1} of root) (Table 4).

In addition to calorimetric estimation of resistant enzymes and biochemical components, the protein banding pattern was also studied in aforementioned rootstocks and bitter gourd scions after challenge inoculation with *M. incognita*. In all cucurbitaceous rootstocks and bitter gourd scions a common banding pattern in the range of 20.1 and 97.4 kDa was noticed (Plate 1). The results revealed that among the cucurbitaceous rootstocks *C. colocynthis* exhibited a range of protein pattern from 20.1 kDa to 67 kDa whereas *C. moschata* exhibited a range of protein banding pattern from 20.1 kDa to 66 kDa. A difference in number and intensity of isoforms was observed among all cucurbitaceous rootstocks and bitter gourd scions after challenge inoculation with *M. Incognita* (Plates 2 and 3). Isoform analysis revealed that eight PO and seven PPO isoforms were observed respectively in the aforementioned species whereas one to two isoforms of PO & PPO were observed in the susceptible rootstocks and bitter gourd scions. The intensity of these enzymes was greater in *C. colocynthis* followed by *C. metuliferus.*

Discussion

Susceptibility of a plant to root-knot nematode depends on the ability of juveniles (J_2s) to penetrate the roots and cause the formation of giant cells which appears as galls on the roots [23]. The juveniles feed and moult twice before developing into the adult stage [24]. The outcome of this study pointed out that, significantly lowest number of galls was observed in the rootstocks *viz., C. colocynthis, C. metuliferus* and *C. moschata* as compared to *C. pepo* followed by *C. ficifolia* which recorded highest value for the same under *in vitro.* Nugent and Dukes [25] observed that the variety C 701 of *Cucumis metuliferus* was highly resistant to *M. incognita.* Formation of less number of 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 [26]. This was mainly due to non-cooperative action of host tissue or cells with *M. incognita*. The greater number of egg masses per gram of root system and egg-laying females was observed in *C. pepo* followed by *C. ficifolia* and minimum number of egg masses and egg-laying females was noticed in the rootstocks *viz., C. colocynthis* followed by *C. metuliferus* and *C. moschata* respectively. Khanzada et al. [27] reported that the number of root galls, number of egg masses and number of egg laying females per gram of root, was significantly increased in tomato variety Roma v.f. followed by Roma Holland as compared to Gola France and Sunehra and that was decreased in tomato variety Anmol. Sherly [28] and Dhivya [29] observed that number of females, root-galls and egg masses were decreased in *Solanum torvum* rootstock inoculated with

Table 1: Reaction of cucurbitaceous species to *Meloidogyne incognita.*

Rootstock	No. of galls / g of roots	No. of egg masses/g of root	No. of RKN females/ g of root	Nematode population in soil (200 cc)	Root knot index (RKI)	Degree of resistance
Mithipakal (*Momordicacharantia* var. *muricata)*	45.80	6.06	30.30	135.06	3	MR
Fig leaf gourd *(Cucurbita ficifolia)*	129.58	43.20	92.20	215.06	5	HS
Pumpkin *(Cucurbita moschata)*	8.93	3.74	5.56	48.86	2	R
Zucchini squash *(Cucurbita pepo)*	161.80	50.80	100.90	237.53	5	HS
Sponge gourd *(Luffa cylindrica)*	26.36	4.90	12.90	102.16	2	MR
Ridge gourd *(Luffa acutangula)*	74.20	25.26	42.23	183.66	4	S
Bottle gourd *(Lagenariasiceraria)*	86.40	35.60	46.70	228.46	5	HS
Ash gourd *(Benincasahispida)*	98.40	39.06	55.10	223.86	5	HS
Kumati kai *(Citruluscolocynthis)*	3.11	1.42	2.34	40.43	2	R
African horned cucumber *(Cucumismetuliferus)*	4.20	1.54	2.56	45.13	2	R
Scions						
PaleeF_1	76.60	22.66	33.93	178.80	5	HS
CO 1	78.30	26.76	38.20	198.96	5	HS
SEd	1.81	1.02	1.09			
CD (P=0.05)	3.75	2.10	2.26			

***Inoculation level 2J_2/g of soil; ** R- Resistant; MR- Moderately resistant; S- Susceptible; HS- Highly susceptible**

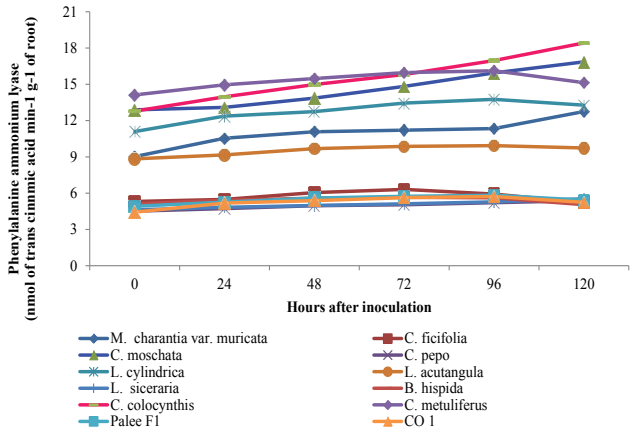


Figure 1: Phenylalanine ammonia lyase activity in cucurbitaceous rootstocks and bitter gourd scions roots inoculated with *M. incognita.*

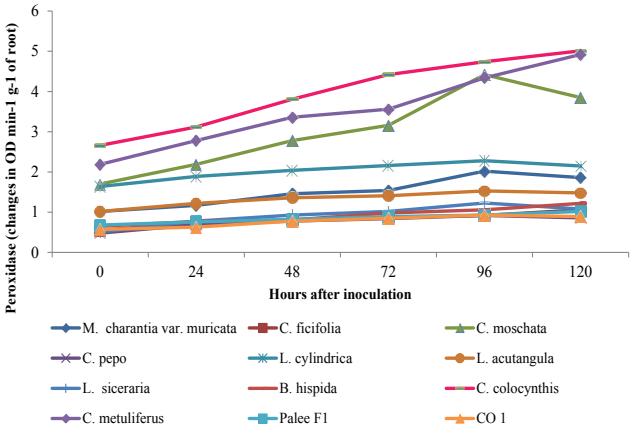


Figure 2: Peroxidase activity in cucurbitaceous rootstocks and bitter gourd scions roots inoculated with *M. Incognita.*

Table 2: Polyphenol oxidase in cucurbitaceous rootstocks and bitter gourd scions inoculated with *M. incognita.*

Rootstocks	Polyphenol oxidase (changes in OD min^{-1} g^{-1} of root)						
	Hours after inoculation						
	0	24	48	72	96	120	Mean
Mithipakal (*Momordica charantia* var. *muricata)*	1.56	1.98	2.51	3.34	3.65	3.2	2.70
Fig leaf gourd *(Cucurbita ficifolia)*	0.58	0.67	0.81	0.91	1.24	1.08	0.88
Pumpkin *(Cucurbita moschata)*	2.05	2.32	2.93	3.17	3.63	3.28	2.98
Zucchini squash *(Cucurbita pepo)*	0.41	0.63	0.82	0.93	1.04	0.89	0.78
Sponge gourd *(Luffa cylindrica)*	1.65	1.81	2.18	2.55	2.93	2.26	2.23
Ridge gourd *(Luffa acutangula)*	0.98	1.2	1.34	1.45	1.62	1.48	1.34
Bottle gourd *(Lagenaria siceraria)*	0.51	0.67	0.82	0.94	1.14	1.09	0.86
Ash gourd *(Benincasa hispida)*	0.63	0.78	0.85	0.98	1.23	1.15	0.93
Kumatikai *(Citrullus colocynthis)*	2.46	2.93	3.28	3.85	3.94	3.76	3.37
African horned cucumber *(Cucumis metuliferus)*	2.24	2.69	2.93	4.28	3.79	3.28	3.20
Scions							
Palee F_1	0.71	0.86	0.94	1.12	1.34	1.2	1.02
CO 1	0.62	0.77	0.83	0.94	1.12	0.9	0.86

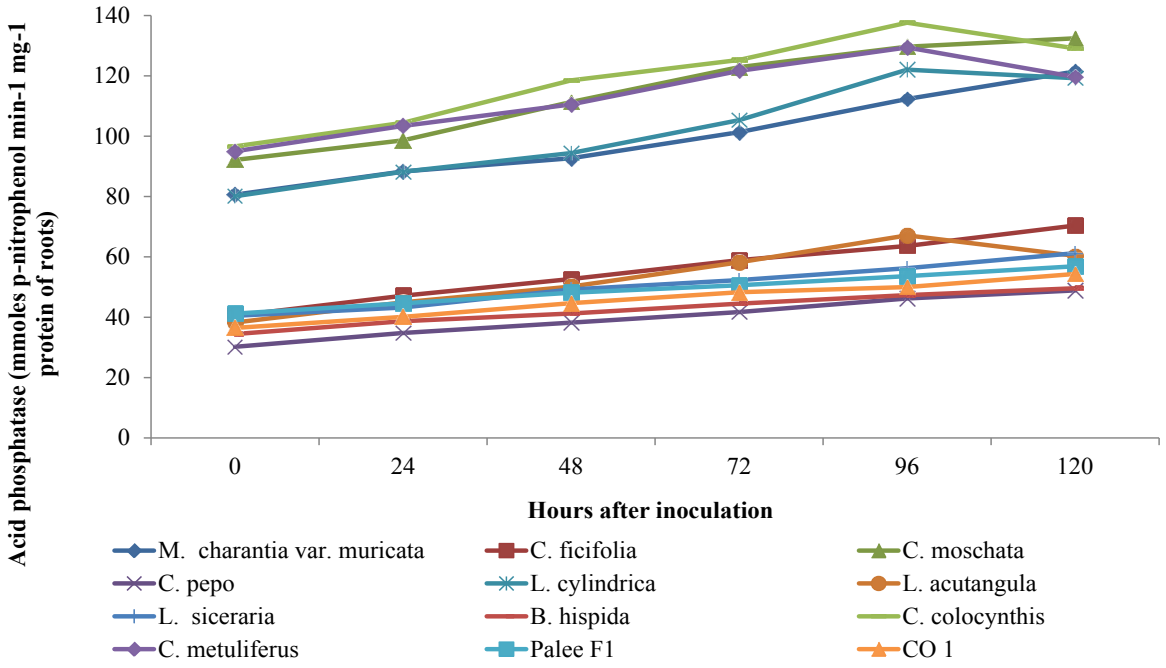


Figure 3: Acid phosphatase activity in cucurbitaceous rootstocks and bitter gourd scions roots inoculated with *M. incognita*.

Table 3: Total phenol in cucurbitaceous rootstocks and bitter gourd scions inoculated with *M. incognita*.

Rootstocks	Total phenol (mg g^{-1} of root)						
	Hours after inoculation						
	0	24	48	72	96	120	Mean
Mithipakal (*Momordica charantia* var. *muricata*)	7.24	8.98	10.34	11.31	12.74	12.09	10.45
Fig leaf gourd (*Cucurbita ficifolia*)	4.75	5.89	7.34	9.18	10.22	9.94	7.88
Pumpkin (*Cucurbita moschata*)	11.91	12.44	15.68	16.23	17.1	16.98	15.05
Zucchini squash (*Cucurbita pepo*)	4.26	5.43	7.58	8.98	9.68	9.22	7.52
Sponge gourd (*Luffa cylindrica*)	9.23	11.7	13.31	14.43	15.98	15.31	13.32
Ridge gourd (*Luffa acutangula*)	5.83	6.67	8.36	9.31	9.98	9.26	8.23
Bottle gourd (*Lagenaria siceraria*)	4.81	5.38	7.89	9.42	10.63	10.04	8.02
Ash gourd (*Benincasa hispida*)	4.48	5.38	7.89	9.23	10.27	9.96	7.86
Kumatikai (*Citrullus colocynthis*)	12.82	14.8	17.76	18.82	19.48	19.03	17.11
African horned cucumber (*Cucumis metuliferus*)	11.82	14.24	16.93	17.34	18.93	17.98	16.20
Scion							
Palee F_1	5.38	6.46	8.01	9.73	10.42	9.85	8.30
CO 1	4.63	5.48	7.74	9.28	9.98	9.15	7.71

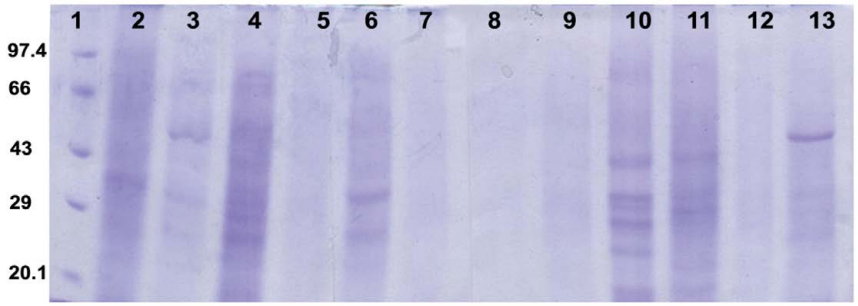
M. incognita and designated as resistant to the same. In contrast to this finding Fassuliotis [30] in USA demonstrated that *Cucurbita ficifolia* exhibited resistance reaction against *M. incognita*.

Nematode population build up both in roots and soil will provide more comprehensive reaction of the plants against them. The lowest nematode population per 200 cc of soil was recorded in *C. colocynthis* followed by *C. metuliferus* and *C. moschata*. However the *C. pepo* recorded the highest population per 200 cc soil followed by *C. ficifolia*. In *Cucumis* species, the potent chemicals, cucurbitacins (accumulated both seeds and roots) are believed to be responsible for nematode suppression, [31]. 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 [32] and Khan et al. [33].

The resistance or susceptibility of the plants to *M. incognita* was assessed by root knot index (RKI) score. Number of galls produced per gram of root was used as an index to work out root knot index (RKI) score. The results of this study revealed that *C. colocynthis, C. metuliferus* and pumpkin *C. moschata* rootstocks were identified as 'resistant' (R), whereas *M. charantia* var. *Muricata* and *L. cylindrica* exhibited 'moderately resistant' (MR) reaction. Punithaveni et al. [34] reported that among the seven cucurbitaceous species *C. colocynthis*, and *C. metuliferus* gave root knot index of 2 with resistant reaction at 45 days after challenge inoculation of M. incongita. Similar trends of results also reported by Darban et al. [35]; Pathan et al. [36] and Khanzada et al. [27] while artificially inoculating different tomato varieties with *M. incognita* under pot and field conditions.

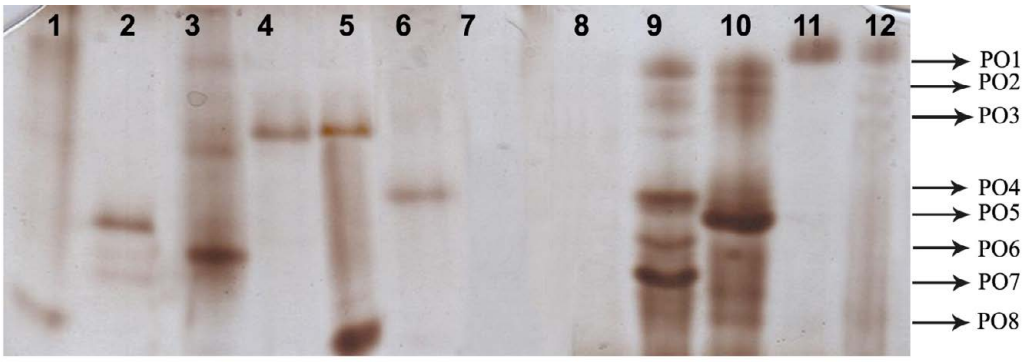
Table 4: Ortho dihydroxy phenol (OD phenol) in cucurbitaceous rootstocks and bitter gourd scions inoculated with *M. incognita.*

Rootstocks	Ortho dihydroxy phenol (mg g^{-1} of root)						
	Hours after inoculation						
	0	24	48	72	96	120	Mean
Mithipakal (*Momordica charantia* var. *muricata)*	7.72	8.42	9.06	9.61	10.14	10.97	9.32
Fig leaf gourd *(Cucurbita ficifolia)*	6.22	6.34	7.05	7.68	8.05	7.14	7.08
Pumpkin *(Cucurbita moschata)*	8.68	9.82	11.04	14.01	14.98	15.74	12.37
Zucchini squash *(Cucurbita pepo)*	2.93	3.18	3.73	4.01	4.38	4.52	3.79
Sponge gourd *(Luffa cylindrica)*	7.43	8.02	8.34	9.34	10.78	10.04	8.99
Ridge gourd *(Luffa acutangula)*	3.45	3.96	4.21	4.84	5.04	5.28	4.46
Bottle gourd *(Lagenaria siceraria)*	3.02	3.38	3.96	4.14	4.86	5.03	4.06
Ash gourd *(Benincasa hispida)*	2.08	3.34	3.65	4.18	5.08	4.82	3.85
Kumatikai *(Citrullus colocynthis)*	10.18	10.46	12.43	13.97	16.08	15.84	13.16
African horned cucumber *(Cucumis metuliferus)*	9.86	10.15	12.48	14.31	15.87	15.28	12.99
Scions							
Palee F_1	5.41	5.98	6.76	7.28	8.28	8.08	6.96
CO 1	5.02	5.64	6.36	6.87	7.15	7.93	6.49



Lane 1: Medium range molecular weight Marker	**Lane 2:** *M. charantia* var. *muricata*	**Lane 3:** *C. ficifolia*
Lane 4: *C. moschata*	**Lane 5:** *C. pepo*	**Lane 6:** *L. cylindrica*
Lane 7: *L. acutangula*	**Lane 8:** *L. siceraria*	**Lane 9:** *B. hispida*
Lane 10: *C. colocynthis*	**Lane 11:** *C. metuliferus*	**Lane 12:** Palee F_1
Lane 13: CO 1		

Plate 1: SDS-PAGE analysis of cucurbitaceous rootstocks and bitter gourd scions inoculated with *M. incognita.*



Lane 1: *M. charantia* var. *muricata*	**Lane 2:** *C. ficifolia*	**Lane 3:** *C. moschata*
Lane 4: *C. pepo*	**Lane 5:** *L. cylindrica*	**Lane 6:** *L. acutangula*
Lane 7: *L. siceraria*	**Lane 8:** *B. hispida*	**Lane 9:** *C. colocynthis*
Lane 10: *C. metuliferus*	**Lane 11:** Palee F_1	**Lane 12:** CO 1

Plate 2: Expression of PO isoforms in cucurbitaceous rootstocks and bitter gourd scions inoculated with *M. incognita.*

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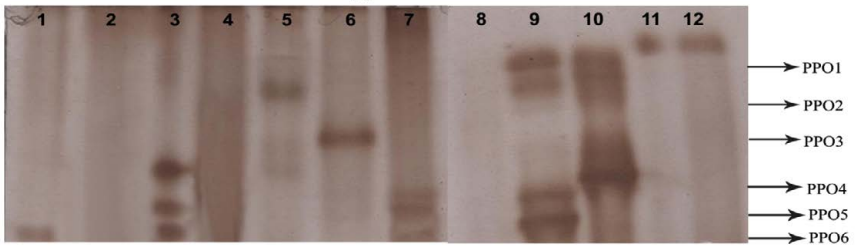

Lane 1: *M. charantia* var. *muricata*	**Lane 2:** *C. ficifolia*	**Lane 3:** *C. moschata*
Lane 4: *C. pepo*	**Lane 5:** *L. cylindrica*	**Lane 6:** *L. acutangula*
Lane 7: *L. siceraria*	**Lane 8:** *B. hispida*	**Lane 9:** *C. colocynthis*
Lane 10: *C. metuliferus*	**Lane 11:** Palee F_1	**Lane 12:** CO 1

Plate 3: Expression of PPO isoforms in cucurbitaceous rootstocks and bitter gourd scions inoculated with *M. incognita*.

Results on calorimetric assay revealed that significantly increased activities of phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenoloxidase (PPO), acid phosphatase, total phenol and orthodihydroxy phenol (OD Phenol) were observed among the cucurbitaceous rootstocks and bitter gourd scions after challenge inoculation of *M. incognita* under *in vitro*. Significant increases in PAL activity was observed in *C. colocynthis* followed by *C. metuliferus* and *C. moschata* against *M. incognita* and these rootstocks excelled for this trait**.** These enzymes activity gradually increased when the nematode infestation started and attained the peak at 96 hours after inoculation and then declined. The enzyme activity remained constant in susceptible rootstocks and bitter gourd scions. Significantly enhanced PO, PPO and acid phosphatase activities were observed in *C. colocynthis* followed by *C. metuliferus* and *C. moschata* rootstocks against *M. incognita*. The two bitter gourd scions (Palee F_1 and CO 1) showed a slow increase in PO, PPO and acid phosphatase activity from 24 hours after inoculation and thereafter showed a declining trend for the same trait. Similar results have been reported by Sherly [28] and Dhivya [29] in *Solanum torvum* rootstock against *M. incognita.*

Estimation of total phenols from cucurbitaceous rootstocks and bitter gourd scions inoculated with *M. incognita* under *in vitro* clearly showed enhanced enzyme activity in response to *M. incognita*. Among the cucurbitaceous rootstocks and bitter gourd scions, increased activity of total phenol content was observed in *C. colocynthis* followed by *C. metuliferus* and *C. moschata*. Very little induction of total phenol content was observed in susceptible rootstocks and bitter gourd scions. The result on estimation of OD phenol content revealed that there was a gradual increase in the OD phenol content up to 96 hours, after that there was a slight decline. There was a clear cut difference in OD phenol content have been noticed between rootstocks and scions. Among the cucurbitaceous rootstocks *C. colocynthis* recorded the highest OD phenol content followed by *C. metuliferus* and *C. moschata.* The accumulation of phenolic compounds in the nematode injured area and the activity of associated oxidative enzymes have been reported by Dhivya [29] and Punithaveni [34] against *M. incognita* in tomato and cucumber respectively.

The protein banding pattern was studied in the roots of cucurbitaceous rootstocks and bitter gourd scions after inoculation with *M. incognita*. The results revealed that nematode inoculation showed more induction of protein. Among the cucurbitaceous rootstocks *C. colocynthis* exhibited a range of protein pattern from 20.1 kDa to 67 kDa. A difference in number and intensity of PO and PPO isoforms was observed among all cucurbitaceous rootstocks and bitter gourd scions after challenge inoculation with *M. Incognita.* The intensity of these enzymes was greater in *C. colocynthis* followed by *C. metuliferus* and *C. moschata.* Arzoo et al. [37]; Manikandan and Raguchander [38]; Murthy et al. [39] reported similar result in tomato against *Fusarium oxysporum* and *Ralstonia solanacearum* by plant extract and *Pseudomonas fluorescens* respectively.

From this study it could be concluded that the preliminary evaluation of cucurbitaceous rootstocks exhibited significant differential response to *M. incognita*. However, majority of the cultivars found susceptible to *M. incognita*. Three rootstocks kumatikai *(Citrulus colocynthis),* African horned cucumber *(Cucumis metuliferus)* and pumpkin *(Cucurbita moschata)* which exhibited resistant reaction and sponge gourd *(Luffa cylindrica)* and mithipakal (*Momordica charantia* var. *muricata)* showed moderately resistant reaction and can be used for grafting with bitter gourd scions which could be developed into a valuable crop management tool to reduce the deleterious effect of root knot nematodes in bitter gourd cultivation. Grafting in bitter gourd with these resistant and moderately resistant rootstocks is said to be an alternative tool to achieve nutritional security in a sustainable way.

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