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Full Length Research Paper

Response of cucurbitaceous rootstocks and bitter gourd scions to root knot nematode *Meloidogyne incognita* Kofoid and White

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Root knot nematodes are responsible for severe crop loss in bitter gourd to the tune of 38-48.2%. Pot culture experiments were conducted under glasshouse conditions to study the effect of root knot nematode, *Meloidogyne incognita* Kofoid and White in ten cucurbitaceous species and to identify resistant rootstocks 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. Results revealed that, three genotypes *viz.,*Kumatikai*(Citruluscolocynthis),* African horned cucumber *(Cucumismetuliferus)* and pumpkin *(Cucurbita moschata)* showed resistant reaction followed by two rootstocks *viz.,*Sponge gourd *(Luffa cylindrica)* and mithipakal (*Momordicacharantia* var. *muricata)* which are moderately resistant. The biochemical activities *viz*., Polyphenol oxidase (PPO), peroxidase (PO) and phenylalanine ammonia lyase (PAL) showed superiority in the aforementioned species. These rootstocks are further used for grafting with bitter gourd scions.

Keywords: Root knot nematode, *Meloidogyne incognita,* resistance, bitter gourd, Biochemical parameters, Polyphenol oxidase, Peroxidase, Phenylalanine ammonia lyase.

INTRODUCTION

Bitter gourd (*Momordica charantia* L.) is one of the important cucurbitaceous vegetables grown in India. 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). The crop is cultivated over an area of 80990 ha in India with an annual production of 830450 tonnes and the productivity of 10.25 t ha^{-1}(Anon., 2015-16). Due to limited availability of arable land and high market demand for bitter gourd, they are cultivated intensively in larger areas. The main problem with bitter gourd production is root knot nematode. Root knotnematode *(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 which penetrates the feeder roots and feed on the vascular tissue. A number of wild and cultivated cucurbit species are resistant to soil borne diseases and 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 onto 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 cucurbitaceous rootstocks to pathogenic potential of root knot nematode *M. incognita* and to identify resistant rootstocks for grafting with bitter gourd scions.

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MATERIALS AND METHODS

The pot culture experiment was carried out from 2013 to 2014 at the Department of Nematology glasshouse, Tamil Nadu Agricultural University, Coimbatore, India (11^0 N latitude, 77^0 E longitudes and an altitude of 426.26 m above mean sea level). The rootstocks *viz.,* mithipakal (*Momordicacharantia* 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 *(Cucumis metuliferus)*and two bitter gourd scions *viz.,* Palee F_1 and CO1 were used for screening. Second stage juveniles of *M. incognita* were inoculated at $2J_2$ per cc soil into the rhizosphere of 21 days old seedlings. Number of galls per gram of root, No. of egg masses/g of root and females per gram of root were determined at the end of the experiment.

Assay for defence related enzymes

Biochemical analysis

The biochemical constituents *viz.,*Polyphenol oxidase (PPO), peroxidase (PO) and phenylalanine ammonia lyase (PAL) were estimated in cucurbitaceous rootstocks and bitter gourd scions. 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 (-80º C) until used for biochemical analysis. Samples obtained from different time interval was homogenized in chilled pestle and mortar with 2 mL of ice cold 0.1 M sodium phosphate buffer (pH 7.0, at 4ºC). 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.

Poly phenol oxidase (PPO) activity

PPO activity was determined as per the procedure given by Mayer et al. (1965). The PPO activity was expressed as change in OD minute^{-1} g^{-1} of protein.

Peroxidase (PO) activity

Peroxidase activity was assayed by using the method of Srivastava (1987). The enzyme activity was expressed as change in ODmin^{-1}g^{-1}of protein (Hammerschmidt et al., 1982).

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 nmoltranscinnamic acid minute^{-1} g^{-1} tissue (Dikerson et al., 1984).

RESULTS AND DISCUSSION

All the rootstocks and scions developed characteristic galls caused by *M.incognita*. Significant differences were noticed among the rootstocks for number of galls per gram root, number of egg masses per gram root and number of females per gram of root (Table 1). Kumatikai *(Citrulus colocynthis)* (3.11, 4.20 and 8.93)followed by African horned cucumber (*Cucumis metuliferus)* (4.20, 1.54 and 3.74) and pumpkin *(Cucurbita moschata)* (8.93, 3.74, 5.56) recorded the lowest number of galls, number of egg masses and number of females per gram of root respectively and showed resistant reaction (Table 1).Siguenza *et al*. (2005) reported similar results in African horned cucumber (*Cucumis metuliferus)* and pumpkin *(Cucurbita moschata)*. Sponge gourd *(Luffa cylindrica)* followed by mithipakal (*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 F_1 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 masses 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.

Polyphenol oxidase (PPO) oxidizes the colourless dihyroxy phenols to highly coloured orthoquinones and hence it is considered to play an important role in pest and disease resistance, particularly those affecting the tissues. Among the ten cucurbitaceous rootstocks, kumatikai *(Citrullus colocynthis)* recorded the highest polyphenol oxidase activity of 3.37 changes in OD min^{-1}g^{-1} of root followed by African horned cucumber *(Cucumis metuliferus)* (3.20 changes in OD min^{-1}g^{-1} of root). After inoculation, the polyphenol oxidase activity was increased upto 96 hours and there was a slight decrease in this activity (Table2). The role of PPO in *M. incognita* resistance had been well documented by Sherly (2010).

Among the various enzymes, peroxidase is considered as one of the important disease resistant enzyme due to its role in catalyzing the condensation process of phenolic compounds into lignin. Estimation of peroxidase activity in all the resistant rootstocks possessed higher peroxidase activity than the susceptible ones. In the present study, peroxidase activity in the cucurbitaceous rootstocks and bitter gourd scions showed an induction after the inoculation and it was increased upto 96 hours. After that there was a slight decrease in the activity of peroxidase. Among the cucurbitaceous rootstocks tested, kumatikai *(Citrullus colocynthis)* recorded the highest value of 3.96 changes in OD min^{-1}g^{-1} of root followed by African horned cucumber *(Cucumis metuliferus)* (3.52 changes in OD

Table 1. Reaction of cucurbitaceous rootstocks and bitter gourd scions to *M. incognita*.

Rootstocks	**No. of galls / g of roots**	**No. of egg masses/g of root**	**No. of RKN females/ g of root**
T_1- Mithipakal (*Momordica charantia* var. *muricata)*	45.80	6.06	30.30
T_2- Fig leaf gourd *(Cucurbita ficifolia)*	129.58	43.20	92.20
T_3-Pumpkin *(Cucurbita moschata)*	8.93	3.74	5.56
T_4-Zucchini squash *(Cucurbita pepo)*	161.80	50.80	100.90
T_5-Sponge gourd *(Luffa cylindrica)*	26.36	4.90	12.90
T_6-Ridge gourd *(Luffa acutangula)*	74.20	25.26	42.23
T_7-Bottle gourd *(Lagenaria siceraria)*	86.40	35.60	46.70
T_8-Ash gourd *(Benincasa hispida)*	98.40	39.06	55.10
T_9-Kumati kai *(Citrulus colocynthis)*	3.11	1.42	2.34
T_{10}-African horned cucumber *(Cucumis metuliferus)*	4.20	1.54	2.56
Scions			
T_{11}-Palee F_1	76.60	22.66	33.93
T_{12}-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.

Table 2. Polyphenol oxidase (changes in OD min^{-1} g^{-1} of root) in cucurbitaceous rootstocks and bitter gourd scions against *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	3.20	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
Kumati kai *(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

$min^{-1}g^{-1}$ of root) and Pumpkin *(Cucurbita moschata)* (3.01 changes in OD $min^{-1}g^{-1}$ of root) whereas zucchini squash *(Cucurbita pepo)* recorded the lowest peroxidase activity of 0.76 changes in OD $min^{-1}g^{-1}$ of root. Among the two bitter gourd scions, Palee F_1 and CO 1 recorded the lowest peroxidase activity of 0.85 changes in OD $min^{-1}g^{-1}$ of root and 0.77 changes in OD $min^{-1}g^{-1}$ of root respectively (Fig.1). These findings fall in line with the findings of Indurani*et al.* (2008) and Sundhariaya (2008) in tomato.

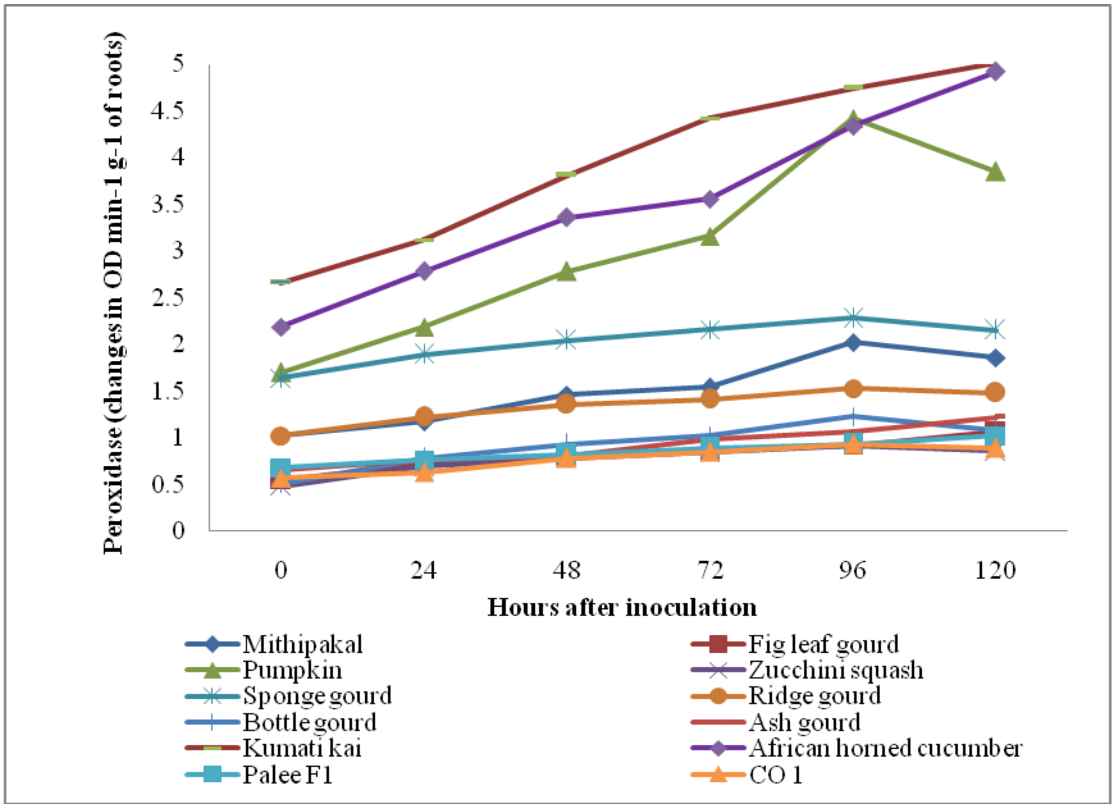

Fig.1. Peroxidase activity in cucurbitaceous rootstocks and bitter gourd scions roots against *M. incognita.*

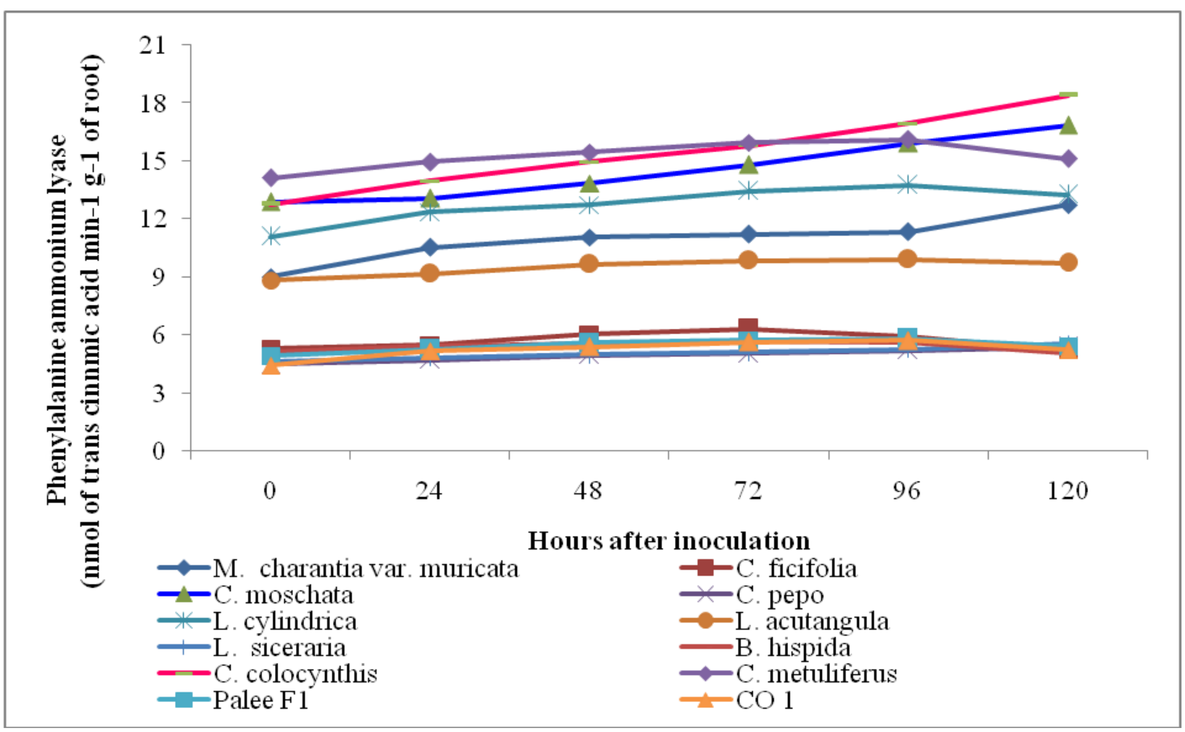

Fig.2. Phenylalanine ammonia lyase activity in cucurbitaceous rootstocks and bitter gourd scions roots against *M. incognita.*

Phenylalanine ammonia lyase (PAL) is one the important enzyme in the synthesis of phenolics, phytoalexin and lignin. Hence it is considered as the most important enzyme in disease resistance. Among the cucurbitaceous rootstocks, kumatikai *(Citrullus colocynthis)* recorded the highest PAL activity of 16.97nmol of trans cinnmic acid min^{-1} g^{-1} and followed by African horned cucumber *(Cucumis metuliferus)*(16.12nmol of trans cinnmic acid min^{-1} g^{-1}). The lowest activity was recorded in zucchini squash *(Cucurbita pepo)* (5.21nmol of transcinnmic acid min^{-1} g^{-1} of fresh root) (Fig. 2).The activity levels of phenylalanine ammonia-lyase and anionic peroxidase induced early resistance response to many other pathogens and also increased in resistant tomato cultivars after nematode inoculation (Rajasekar*et al.,* 1997).

CONCLUSION

This study indicated that the preliminary evaluation of cucurbitaceous rootstocks exhibited significant differential response to *M. incognita*. Three rootstocks kumatikai *(Citrulus colocynthis),* African horned cucumber *(Cucumismetuliferus)* 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 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.

REFERANCES

Anon., (2015-16). *www.indiastat.com*

Chandra P, Sao RS, Gautam K, Poddar, AN (2010). Initial population density and its effect on the pathogenic potential and population growth of root knot nematode *(Meloidogyne incognita)* in four species of Cucurbits. Asian J. Plant Pathol., 4: 1-15

Dikerson DP, Pascholati SF, Hagerman AE, Butler LG, Nicholson RL (1984). Phenyl alanine ammonia-lyase and hydroxycinnamate-CoA ligase in maize mesocotyls inoculated with *Helminthosporiummaidis*(or) *H. carbonum*. Physiol. Plant Path.25: 111-123.

Hammerschmidt R, Nuckless EM, Kue J (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Collectotrichumlagenarium*. Physiol. Plant Path., 20: 73-82.

Indurani C, Veeraragavathatham D, Sanjutha S (2008). Analysis on biochemical basis of root knot nematode *Meloidogyne incognita* resistance in tomato (*Lycopersiconesculentum*Mill.).Res. J. Agric. Bio. Sci., 4(6): 866-870.

Jain RK, Mathur KN, Singh RV (2007). Estimation of losses due to plant parasitic nematodes on different crops in India.Ind.J.Nematol., 37:219-221

Mayer AM, Harel E, Shaul RB (1965). Assay of catechol oxidase: A critical comparison of methods. Phytochem.5:783-789.

Rajasekar, S.P., A.K. Ganguly and S.C. Swain. 1997. Quantitative changes in superoxide dimutase, catalase and peroxidase with reference to resistance in tomato to *Meloidogyne incognita*. Indian Nematol., 27: 79-85.

Sherly J (2010).Studies on grafting of brinjal accessions (S*olanum melongena*L.) with wild s*olanum* rootstocks.Ph.D (Hort.) Thesis,Tamil Nadu Agricultural University, Coimbatore.

Siguenza C, Schochow M, Turini T, Ploeg A (2005). Use of *Cucumismetuliferus* as a rootstock for melon to manage *Meloidogyne incognita*.J. Neamtol., 37: 276-280.

Srivastava SK (1987). Peroxidase and polyphenol oxidase in *Brassica juncea* plants infected with *Macrophominaphaseolina* (Tassi.) Goid and their implication in disease resistance.J. Phytopath., 120: 249-254.

Sundhariaya P (2008). Stability analysis for combined resistance to leaf curl virus and root knot nematode *Meloidogyne incognita* race 3 in tomato (*Solanum lycopersicum* L.).Ph.D (Hort.) Thesis, Tamil Nadu Agricultural University, Coimbatore.