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Plant Pathology

Reaction of Banana Hybrids (Phase-II) for Resistance to *Meloidogyne incognita*

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Abstract

Plant parasitic nematodes are one of the major biotic stresses affecting banana production. Breeding works carried out at the Department of Fruit crops, Tamil Nadu Agricultural University (TNAU), India. The potential diploids and hybrids developed were crossed with commercial triploids to develop primary tetraploids and improved diploids. The susceptible check cultivar used was Rasthali (AAB), while the resistant reference cultivar used Pisang Lilin (AA). Banana suckers of uniform size and weight were collected, pared and planted in earthen part containing 5 kg sterilized pot mixture. Egg masses of *M. incognita* were picked from roots, allowed to hatch in a beaker of distilled water and the hatched juveniles (J2) were inoculated in the rhizosphere of the hybrids by soil injection method @ 5,000 nematodes / pot. Same set of replicated banana hybrids were also maintained as uninoculated check. The reactions of nineteen new synthetic banana phase II hybrids to *Meloidogyne incognita* was studied under field conditions as well as in controlled inoculation tests in pots. Hybrid H 531 (Poovan x Pisang Lilin) was found to be resistant and six hybrids, H-02-34, H-03-05, H-03-13, H-04-12, H-04-24 and NPH-02-01 were found to be toterant to the root-knot nematode, *Meloidogyne incognita* while the remaining were rated as susceptible and highly susceptible ones. Total phenols and PO, PPO, PAL and enzymatic activity of the hybrids in defense mechanism in response to nematode invasion indicated higher activities in resistant genotypes compare to susceptible ones. Hybrid H 531 had the maximum biochemical content and enzyme activity among the hybrids included in this study. The resistant and tolerant hybrids had enhanced contents of total phenol, PO, PPO and PAL.

Highlights

- Evaluation of 19 parthenocarpic *Musa* hybrids led to identification of a new banana hybrid 'H 531' with high yield potential as well as increased resistant to *Meloidogyne incognita*.
- The promising hybrid H 531' had high total phenol content and PO, PPO and PAL enzyme activities as resistant mechanism.

Keywords: Screening, banana, hybrids, resistance, *Meloidogyne incognita*

Nematodes are a serious constraint of banana production world-wide among which the root knot nematode, *Meloidogyne incognita* is the most important causing a yield loss to an extent of 31 % in India (Jonathan *et al*., 2011). It is a sedentary endoparasitic nematode which penetrates the root growing tips and feed the vascular region. Typical multinucleate giant cells are formed and found root gall and deposition of egg mass in root cortex in gelatinous matrix very close to the epidermis and reduced root system. The destruction of root and corm tissues reduces water and mineral uptake which results in a reduction of plant growth and development followed by toppling of flowering plants or reduction of bunch weight. Management of this nematode relies mainly on the repeated use of chemical nematicides which maintain yields 50% greater than in untreated plantations (Seenivasan *et al*., 2013). However, the use of chemical nematicides has many drawbacks among which are the potential residue in fruits, ground water contamination, effect on non target organisms and toxicity to applicators. This necessitates efforts to find alternative methods of nematode control in banana.

Breeding hybrid bananas with nematode resistance is an alternate strategy for controlling this pest simultaneously ensuring environmental safety. Most of the widely grown banana and plantain cultivars are susceptible to root knot nematodes. Pinochet (1988) evaluated 15 banana cultivars and accessions against *M. incognita* and found that all of them were susceptible to root knot nematode, although different degrees of susceptibility were detected. Breeding works carried out at Tamil Nadu Agricultural University (TNAU), India, resulted in development of 19 elite banana hybrids of one diploids (AB), four triploids (AAB) and fourteen tetraploids (AABB). The objective of this study was to screen these banana hybrids developed at Tamil Nadu Agricultural University against *M. incognita* under field and pot culture conditions.

Materials and Methods

Nineteen elite banana hybrids, one diploids (AB), four triploids (AAB) and fourteen tetraploids (AABB) were drawn from the breeding programme (Fig 1.) of the Department of Fruit crops, Tamil Nadu Agricultural Uiversity and screened against *M. incognita*. The susceptible check cultivar used was Rasthali (AAB), while the resistant reference cultivar used Pisang Lilin (AA).

Assessment of nematode response in pots (Fig2.)

Banana suckers of uniform size and weight were collected, pared and planted in earthen part containing 5 kg sterilized pot mixture (red soil : sand : farm yard manure 2:1:1 v/v respectively). The genomes were labelled and arranged in completely randomized block design, replicated thrice maintaining six plant in each replication. *Meloidogyne incognita* population used in this study was isolated from banana cv. Nendran at Thondamuthur village, Coimbatore district, Tamil Nadu and pure cultures were maintained on tomato Cv. Co 1. Egg masses of *M. incognita* were picked from roots, allowed to hatch in a beaker of distilled water and the hatched juveniles (J2) were inoculated in the rhizosphere of the hybrids by soil injection method @ 5,000 nematodes / pot. Same set of replicated banana hybrids were also maintained as uninoculated check. The plants were allowed to grow for 90 days in glass house at 25-27 ^{o}C and terminated. On termination the plants were uprooted and observations on total number of roots, nematode infected roots and root gall index recorded. Root gall index was assessed on a scale of 0–5, where 0 = 0% galled roots; 1 = <10%, 2 = 10 25 %, 3 = 25 50%, 4 = 50 75% and 5 = >75% galled roots (Seenivasan *et al*., 2012). Soil from pots were thoroughly mixed and J_2 population density was assessed from 250 g of sub-samples by Cobb's decanting and sieving technique followed by modified Baermann's funnel technique (Southey, 1986). Five gram of root randomly taken from each plant was stained using acid fuchsin-lactophenol and number of females/juveniles were counted.

The content of the enzymes peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) and the content of phenols in the roots were determined for each replicate after three months, just before root samples were scored for nematode damage. The total phenols in the roots was estimated using Folin Ciocalteau reagent and measuring absorption at 660 nm in a spectrophotometer, and is expressed as mg/g root (Spies, 1955). For enzyme extraction, one gram of root sample per replicate was homogenized with 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) at 4°C. The supernatant was used as crude enzyme extract for assaying peroxidase and polyphenol oxidase. Enzyme extracted in borate buffer was used for estimation of phenyl alanine ammonia lyase. The PO activity was assessed according to Hammerschmidt *et al*. (1982) and the PPO activity was assessed using the modified method of Mayer *et al*. (1965).

Assessment of nematode response in field

The field experiment was laid out in a randomized block design with three replicate blocks in a field naturally infested with > 1 *M. incognita*/ cm^3 of soil. Pits of 0.45 m^3 size were dug out at 1.8 m x 1.8 m spacing after thoroughly ploughing and leveling the field. Suckers of 19 hybrids and two reference cultivars were planted in the pits with one sucker per pit. Five plants of each accession were planted next to one another in each replicate block. Standard cultural practices were followed, but no nematicide was applied.

Nematode population in soil and roots was assessed at harvest. Roots were collected from a standard size excavation of 20 x 20 x 20 cm extending outward from the corm of the plant, a sub sample of 5 g was taken and stained using acid fuchsin-lactophenol and number of females/juveniles were counted. Soil from standard excavation size 20 x 20 x 20 cm was collected and a sub sample of 200 g was processed by Cobb's sieving and modified Baermann funnel method (Southey, 1986). The bunch weight per plant was also recorded. The data from the experiments were analyzed statistically following (Panse and Sukhatme, 1989).

Results and Discussion

Significant differences were noticed among the hybrids for root population, soil population and total final population inoculated with *M. incognita* **(**Table 1). The lowest root population of 102 nematodes per 5 g of root was recorded in hybrids H 531 (Fig 3.), which was similar to reference cultivar Pisang Lilin (62) and the highest in H-03-06 (261). The hybrid H 531 recorded the lowest population of 106 nematodes per 200cc of soil and the highest of 275 by the hybrid H-03-16. Total nematode population also significantly varied among the hybrids. The hybrid H 531 registered the minimum of 6,917 nematodes, and the maximum of 14,766 nematodes by the hybrid H-03-06, which was found to be a highly susceptible. Nematode population buildup both in roots and soil had given more comprehensive representation of the reaction of various hybrids. Among the hybrids *viz*,

Table 1: Response of Banana hybrids to *Meloidogyne incognita* under pot culture

Hybrids	Soil population (200cc)	Root population (5g)	Total population	Totalno. of functional roots	No. of galled roots	% of galled roots	RGI	Levels of resistance
H-02-19	236	260	14,273	32.33	17.67	54.66	3	S
H-02-23	181	236	14,123	25.67	21.33	83.09	5	S
H-02-26	255	217	14,612	31.70	19.33	60.98	3	S
H-02-34	132	105	7,387	33.20	11.00	33.13	2	T
H-03-05	123	107	9,060	35.40	4.37	12.34	2	T
H-03-06	262	261	14,766	28.33	23.00	81.19	5	HS
H-03-13	138	129	8,159	34.20	5.60	16.37	2	T
H-03-16	275	228	14,002	29.43	24.00	81.55	4	S
H-03-17	108	114	9,516	36.50	23.00	63.01	4	HS
H-03-19	219	227	12,360	32.97	28.00	84.93	5	HS
H-04-05	222	223	13,410	41.75	25.97	62.20	5	S
H-04-06	201	189	10,964	38.20	27.07	70.86	5	S
H-04-10	217	225	13,761	27.30	21.30	78.02	5	S
H-04-12	118	148	11,423	40.50	5.60	13.83	2	T
H-04-21	199	223	11,598	32.25	24.50	75.97	5	S
H-04-24	148	110	9,601	43.50	17.30	39.77	2	T
NPH-02-01	109	112	9,557	39.20	7.00	17.86	2	T
H-510	114	110	7,125	36.30	21.25	58.54	3	S
H-531	106	102	6,917	41.25	4.60	11.15	1	R
Reference								
Pisang Lilin	339	368	16,453	34.20	2.50	7.43	1	R
Rasthali	104	62	4,983	27.57	16.92	61.29	5	HS
SEd	11.77	12.43	636.651	1.835	0.878	2.723		
CD(.05 %)	23.51	24.83	1285.116	3.666	1.754	5.440		
CD(.01%)	31.25	33.00	1717.841	4.872	2.331	7.231		

RGI- Root Galling Index; R - resistant; T- tolerant; S-susceptible; HS - Highly susceptible

H-02-34, H-03-05, H-03-13, H-03-17, H-04-12, H-04-24 and NPH-02-01 had recorded lesser nematode population. Significant differences were observed on the percentage of galled roots from various banana hybrids. The galling percent ranged from 11.1 (H 531) to 84.93 (H-03-19) per cent. The hybrid H 531 had the minimum roots galling index of 1 and the highest root galling index scale of 5 was noticed in the hybrids H-02-23, H-03-06, H-03-19, H-04-05, H-04-06, H-04-10, H-04-21 and also in Rasthali. However, the hybrids H-02-34, H-03-05, H-03-13, H-04-12, H-04-24 and NPH-02-01 recorded the lowest root galling index scale of 2. Based on the nematode population, functional roots and root gall index the hybrids, H 531 rated as resistant and H-02-34, H-03-05, H-03-13, H-04-12, H-04-24 and NPH-02-01 rated as tolerant to *M. incognita*. However, the rest of the hybrids except H-03-17, H-03-06 and H-03-19 were susceptible. The hybrids H-03-17, H-03-06 and H-03-19 were found to be highly susceptible among the hybrids evolved.

Resistance / tolerance to nematodes can be clearly established by studying the damage caused to the root. The INIBAP method largely encompasses the ability of the genotype to resist nematode infection based on root damage assessment besides its ability to tolerate more population of nematodes. The nematode though can live in the soils, it cannot enter into the roots of resistant hybrids and multiply at a faster rate (Kumar and Kumar, 2009). As the nematode population directly inflicts damage to the root system by causing galls, assessment of root damage becomes important (Das *et al*., 2011). Root number and per cent galled roots are considered as critical in the assessment of nematode damage, because, the rate of root destruction is not directly related to the population density in the root system as a whole, but to the number of individual colonies on the roots (Das *et al*., 2010). Healthy and sturdy root are the sign of resistant exhibited by the genotypes. In the present study, significant differences were observed in the ability of the hybrids and parents to produce more number of functional roots. Resistant/tolerant hybrids produced thick and more number of healthy roots to overcome the nematode infection. According to (Hartman *et al*., 2010) good root development potential favours resistance. All the resistant/tolerant hybrids reported in this study were also found to possess better root characters than the susceptible hybrids which are in confirmation with the findings of Janarthani *et al.* (2002). In the present study, wherever the hybrids exhibiting resistance to nematodes are posses Pisang Lilin (AA) as one of the parents. This showed a clear indication of that Pisang Lilin (AA) might be the contributing the trait for the resistant to *M. incognita*.

The enzyme activities and total phenol contents were estimated in roots and found to vary significantly different among the hybrids (Table 2). The hybrid H 531 recorded the highest peroxidase activity of 2.83 abs/min/g under control and 3.30 abs/min/g by H 531 under inoculated condition. However, the hybrid H-04-10 recorded the lowest peroxidase activity of 0.51 abs/min/g under control and 0.54 abs/min/g under inoculated condition. The minimum per cent increase for peroxidase activity of 5.41 per cent was found in the hybrid H-04-21 and the maximum of 37.16 per cent in H-02-34. The highest polyphenol oxidase activity of 0.110 and 0.150 abs/min/g under control and inoculated condition was found in H 531 whereas the lowest in H-03-16 (0.012 abs/min/g in control and 0.013 abs / min/g under inoculated). The minimum per cent increase of 5.71 per cent was found in H-03-06 and the maximum of 36.36 by the hybrid H 531. Among the hybrids, H 531 registered the highest phenylalanine ammonia lyase (PAL) content of 16.25 nmol/min/ml in control and 19.89 nmol/min/ml under inoculated condition while, H-03-16 registered the lowest content of 6.02 and 6.60 nmol/min/ml under control and inoculated condition respectively. The maximum per cent increase in PAL was recorded by H 531 (22.40 per cent), while the minimum by H-03-19 (7.04 per cent). The hybrid H 531 registered the highest total phenol content of 419.48 µg/g in control and 491.23 µg/g under inoculated condition, while H-03-16 registered the lowest total phenol content of 134.17 and 142.75 µg/g under control and inoculated condition respectively. The per cent increase of total phenol content was the maximum in H 531 (17.80per cent) and the minimum in H-02-26 (4.0 per cent).

Enzyme activity is one of the important tools to confirm the resistance to nematodes. When a pathogen infects the host tissue, a small number of specific genes are induced to produce mRNA's that permit synthesis of similar number of specific proteins (Seenivasan and Murugan, 2011). Many of these proteins are enzymes such as phenylalanine ammonia lyase, polyphenol oxidase, peroxidase and b-1-3 glucanase (Seenivasan, 2011). These are involved in the synthesis of low molecular weight substances such as phytoalexins, phenols and lignin, which are inhibitory to the invading pathogens (Seenivasan, 2011). Hence, estimation of these biochemical markers, which provide mechanism for resistance to pathogens, is highly essential.

Table 2: Enzyme activity and phenol content of hybrids inoculated with *Meloidogyne incognita* under pot culture.

Hybrids	Peroxidase(abs/min/g)			Polyphenol oxidase (abs/min/g)			PAL (nmol/min/ml)			Total phenol(µg/g)		
	C	I	%	C	I	%	C	I	%	C	I	%
H-02-19	1.27	1.36	7.09	0.052	0.057	9.62	7.17	8.01	11.72	256.75	270.36	5.30
H-02-23	1.22	1.31	7.38	0.061	0.065	6.56	6.58	7.28	10.64	269.34	281.72	4.60
H-02-26	1.32	1.39	5.30	0.056	0.060	7.14	9.14	10.03	9.74	242.25	251.95	4.00
H-02-34	1.83	2.51	37.16	0.082	0.097	18.29	13.27	15.92	19.97	296.26	348.58	17.66
H-03-05	1.82	2.05	12.64	0.086	0.098	13.95	13.75	16.10	17.09	327.19	379.24	15.91
H-03-06	1.17	1.24	5.98	0.035	0.037	5.71	7.63	8.51	11.53	218.71	237.59	8.63
H-03-13	2.16	2.41	11.57	0.084	0.096	14.29	11.99	13.60	13.43	289.56	327.18	12.99
H-03-16	0.98	1.06	8.16	0.012	0.013	8.33	6.02	6.60	8.73	134.17	142.75	6.39
H-03-17	1.63	1.85	13.50	0.071	0.085	19.72	12.15	14.59	20.08	348.97	387.65	11.08
H-03-19	1.46	1.58	8.22	0.057	0.061	7.02	8.52	9.12	7.04	246.52	264.64	7.35
H-04-05	1.35	1.44	6.67	0.054	0.058	7.41	6.07	7.01	16.45	283.30	303.51	7.13
H-04-06	1.38	1.46	5.80	0.047	0.051	8.51	8.40	9.81	16.79	284.37	308.69	8.55
H-04-10	0.51	0.54	5.88	0.064	0.071	10.94	8.57	9.50	10.85	221.51	236.27	6.66
H-04-12	2.26	2.55	12.83	0.087	0.110	26.44	12.05	14.21	17.93	314.69	343.43	9.13
H-04-21	1.48	1.56	5.41	0.056	0.060	7.14	9.29	10.90	17.33	299.28	327.46	9.42
H-04-24	2.02	2.49	23.27	0.095	0.121	27.37	11.99	13.71	14.35	253.31	293.74	15.96
NPH-02-01	1.85	2.12	14.59	0.098	0.118	20.41	11.99	13.71	14.35	347.52	433.26	24.67
H-510	1.77	1.92	8.47	0.079	0.093	17.72	11.84	13.97	17.99	261.49	288.77	10.43
H-531	2.83	3.302	16.68	0.110	0.150	36.36	16.25	19.89	22.40	419.48	491.23	17.10
References												
Pisang Lilin	2.84	3.29	15.61	0.121	0.162	33.88	15.58	18.71	20.08	363.65	419.56	15.37
Rasthali	0.52	0.56	6.49	0.041	0.044	7.31	5.64	6.03	6.91	120.53	127.60	5.87
SEd	0.094	0.109	0.767	0.004	0.005	0.583	0.678	0.777	0.799	15.445	17.281	0.641
CD(0.05%)	0.188	0.219	1.532	0.008	0.009	1.166	1.354	1.553	1.595	30.856	34.522	1.281
CD(0.01%)	0.251	0.291	2.037	0.011	0.012	1.550	1.799	2.064	2.121	41.012	45.885	1.703

DAI- Days after inoculation; C- Control; I- Inoculated; %- per cent difference over control PAL - Phenylalanine Ammonia Lyase

Among the various enzymes, peroxidase is considered as one of the important defense related enzymes due to its role in catalyzing the condensation of phenolic compounds into lignin. Estimation of peroxidase activity in the current study elicits that all the resistant genotypes possessed higher peroxidase activity than the susceptible ones. Moreover, the isozyme analysis of the inoculated hybrids indicated that the host explicit its resistance to the nematodes either by production of specifics isoforms in the form of either peroxidase or polyphenol oxidase. Enhanced peroxidase activity has been associated with hybrids resistant to nematodes (Das *et al*., 2011)

Polyphenol oxidase (PPO) oxidizes the phenols to highly toxic quinones and hence is considered to play an important role in disease resistance, particularly those affecting the tissues (Abbattista and Matta, 1975). Thus, the overall analysis of estimation of these enzymes in resistant and susceptible hybrids indicated the role of these enzymes in conferring resistance to nematodes. A critical analysis of their activity within the hybrids reveals that the resistant hybrids viz., H 531 and the tolerant hybrids viz., H-02-34, H-03-05, H-03-13, H-04-12, H-04-24 and NPH-02-01 recorded higher peroxidase and poly phenol oxidase activity than the susceptible ones. Similar finding were earlier reported in banana by Das *et al*. (2010).

The data recorded under field conditions are in line with pot culture results. It was interesting to note that a few hybrids recorded least nematode population in roots under field conditions were found to posses relatively more population under pot culture. This is due to more bombardment of nematodes in the root zone for forceful infection (Dosselaere *et al*., 2003). In some of the tolerant hybrids such as H-03-19 and H-04-06, the nematode population was more but the growth of the plant was not affected by the nematodes. This could be because, the entry of the nematodes and their reproduction in those hybrids did not affect the crop growth. This is in line with the findings of Janarthani (2002). Among the hybrids, H-

Table 3: Population of *Meloidogyne incognita* in the fields of banana hybrids and parents under evaluation (200cm^3 of soil and 5g $^{-1}$ of roots).

Hybrids	Parents	Genome	At harvesting		RGI	Bunch weight (kg)
			Soil	Root		
H-02-19	KAR x RED	AABB	326	397	3	13.00
H-02-23	KAR x RED	AABB	326	382	5	14.50
H-02-26	KAR x RED	AABB	321	384	3	17.00
H-02-34	KAR x RED	AABB	291	204	2	12.50
H-03-05	Peykunnan (OP)	AABB	194	145	2	11.50
H-03-06	H-02-32 x PL	AB	207	235	5	9.00
H-03-13	Peykunnan x EV	AABB	190	147	2	15.50
H-03-16	Peykunnan x PL	AABB	324	340	4	9.50
H-03-17	Peykunnan x PL	AABB	249	163	4	12.50
H-03-19	Peykunnan x EV	AABB	343	429	5	17.50
H-04-05	H-02-32 x PL	AABB	258	262	5	5.50
H-04-06	H-02-32 x PL	AABB	360	296	5	18.50
H-04-10	Peykunnan (OP)	AAB	240	267	5	13.50
H-04-12	Pisang Saba x PL	AABB	223	128	2	22.50
H-04-21	H-02-10 x PL	AAB	263	268	5	8.00
H-04-24	Peykunnan (OP)	AABB	250	157	2	15.00
NPH-02-01	H 201 x ANK	AAB	221	129	2	17.50
H-510	Poovan (OP)	AABB	236	144	3	14.50
H-531	Poovan x PL	AAB	203	126	1	13.50
Pisang Lilin			162	82	1	3.75
Rasthali			296	370	5	9.50
SEd			4.41	6.10		0.320
CD(.05 %)			8.82	12.20		0.640
CD(.01%)			11.73	16.22		0.851

RGI- Root Galling Index

04-12 recorded the maximum bunch weight of 22.5 kg/ plant and may be attributed to heterotic vigour, which indicated that parents with wider genetic base as well as geographical diversity might result in better heterotic vigour in banana. The nematode resistant hybrid H 531(Fig3.) recorded relatively higher yield of 13.5 kg/plant. In conclusion, the overall evaluation of 19 parthenocarpic Musa hybrids led to identification of the hybrid H 531(Fig 3.) with high yield potential as well as increased resistant to *M. incognita*.

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