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IDENTIFICATION OF RESISTANCE AND BIOCHEMICAL CHANGES AGAINST *RADOPHOLUS SIMILIS* IN BANANA HYBRIDS UNDER POT CULTURE CONDITIONS

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ABSTRACT
Plant parasitic nematodes *Radophous similis* recognised as a significant factor limiting banana production worldwide. Identification of banana hybrids with resistance to nematodes may be the best option for sustainable nematode management. The present investigation was carried out to screen the new synthetic banana hybrids resistance *to Radophous similis*. The host response of 28 banana hybrids, parental bananas with reference cultivars were tested in pots under greenhouse conditions. Two hundred burrowing nematodes, reared in monoxenic carrot-disk culture, were used as inoculum for each container at 45 days after planting. The result revealed that six banana hybrids *viz*., H-11-08, H-11-21, H-11-23, H-11-25, H-11-36, H-11-69, H-11-70, H-11-71 and H-11-76 were found to have resistance and nine hybrids were found to be tolerant to the *Radopholus similis* and the remaining were rated as susceptible. An estimation of the biochemical activity of phenols, lignin, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase, revealed that resistance parents and hybrids had higher activities than susceptible ones. The hybrid H-11-25 registered maximum phenol content (582.62μg/g of roots), lignin (0.96%), peroxidase (3.84abs/min/g) and phenylalanine ammonia lyase activity (34.67 nmol/min/ml). The resistant and moderately resistant hybrids of banana could be further used in breeding programmes as well as be recognized as potential cultivars of commerce.

INTRODUCTION

Banana and plantain (*Musa* spp.) are second largest fruit crops produced and exported in the world and one of the world's major food crops, ranking third in terms of total production. Sustainable *Musa* production is threatened by several biotic and abiotic constraints, among which migratory endoparasitic burrowing nematode *Radopholus similis* is most widespread and damaging nematode throughout world, causing severe yield losses from crop grown for local consumption to desert banana for export (Hölscher *et al*., 2014).

This migratory endoparasitic nematode causes root and corm tissue cavities that evolve to form necrotic lesions that affect the ability of the plant to uptake water and nutrients, resulting in the reduced development of banana bunches, reduced fruit yield and toppling and also paving way to pathogenic microorganisms (Aravind *et al*., 2010 and López-Lima *et al*., 2013). Crop losses by nematodes to banana are estimated to be very high, with an average annual yield loss of 20 per cent worldwide (Seenivasan *et al*., 2013). In addition, these parasites also interact with other disease causing organisms to produce disease complexes (Begum *et al*., 2012).

Plant-parasitic nematode control has been based on chemical soil fumigants and nematicides (Candido *et al*., 2008). The alternative nematodes control measures such as fallow, crop rotation, paring and hot water treatment of the corms and the planting of in vitro micro-propagated plantlets (Queneherve, 2009; Coyne *et al*., 2010 and Vagelas and Gowen, 2012). Extensive application of chemical nematicides is the normal control practice in commercial banana growers. However, several problems have been associated with the use of these pesticides, including the contamination of soil, plants, groundwater, health risks to animals, farmers, and consumers (López-Lima *et al*., 2013). Therefore, host-plant resistance has been recognized as one of the most economic, effective and environmentally-friendly measures for controlling the *Radopholus similis* nematodes. Growing resistant cultivars of crops in crop rotations is economical for the farmers for reducing nematode populations gradually in the infested fields.

Banana breeding for nematode resistance is probably the best way of controlling *Radopholus similis* and keeping the environment safe. Host plant resistance to nematodes might be effective against only a single pathotype (Starr *et al*., 2002 and Buddenhagen, 2009), which might have implications for the durability of the resistance. This resistance may not be durable if the target nematode species has a high level of genetic variability. The variability in reproductive fitness and virulence among *R. similis* populations can influence the results of screening experiments for resistance (Uma *et al*., 2011).

In *Musa*, worldwide known and confirmed sources of resistance to *R. similis* are Pisang Jari Buaya and Yangambi km5 (Dochez *et al.,* 2012) to the major banana nematodes species. Identification and use of suitable sources of nematode resistance in *Musa* breeding are considered highly appropriate for the long lasting reduction of nematode problems in smallholder production systems. Development of hybrids with resistance against nematodes is key criteria in breeding programmes.

Knowing the mechanism of resistance to pests and diseases in resistance accessions is of great importance because it enables the breeder to select for a desired feature for the breeding programme (Nithya Devi *et al*., 2007). In response to restraining banana production, efforts aimed at the genetic improvement of *Musa* have gained renewed interest to generate resistance cultivars. None of the commercially grown clones have resistance to *R. similis*. It is an objective of many banana improvement programmes to incorporate sources of resistance or tolerance to this major nematode pest. The objective of the present study was to evaluation of banana hybrids for resistance to *Radopholous similis* and resistance mechanism. Therefore, a screening of these new synthetic banana hybrids was conducted to assess their responses to *R. similis* and confirm the resistance/tolerance by assessing corm and root damage and also nematode multiplication rate in roots.

MATERIALS AND METHODS

The study was carried out at Department of Fruit Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. The twenty eight elite new synthetic banana hybrids were developed and parents' cultivars drawn from the main field maintained at Department of Fruit Crops, Tamil Nadu Agricultural University, Coimbatore was evaluated banana hybrids under greenhouse condition against *Radopholus similis* during 2012-2013. The experiments were conducted in a greenhouse in potted plants which were inoculated artificially. The experiment was laid out in complete randomized block design and replicated thrice. The hybrids were evaluated along with the reference cultivars *viz*., Yangambi km5 (AAA) as the resistance cultivar and Grand Naine (AAA) as the susceptible reference cultivar. The screening was done based on the root and corm damage assessment as followed in INIBAP technical guidelines 7 (Carlier *et al*., 2003).

Preparation of plant materials

Suckers of uniform size were pared immediately after detaching from the mother plants, and planted in pots filled with sterilized pot mixture. The soil was watered upto field capacity. Forty five days after planting, 10 plants of each hybrid were inoculated with *Radopholus similis* nematodes (1 nematode per gram of soil), while another set of 10 plants were kept as nematode free control. Nematode inoculum was obtained from cultures maintained on carrot discs, according to the technique described by Carlier *et al*. (2003). Nematode suspension obtained from the above method, containing infective juveniles of *Radopholus similis*, were inoculated into the pots, forty five days after planting, @ 1 N /g of soil, by making three deep holes around each sucker. Another set of pots was maintained as uninoculated control.

Observations in pot culture

Observations on nematode population in soil and root were made on 90th day after inoculation. Nematode population in soil was assessed using Cobb's sieving and decanting techniques followed by modified Baermann funnel technique (Southey, 1986). Nematode population in roots was determined by the method of Carlier *et al*. (2003). The extents of nematode damage of root lesion index and corm grade were assessed technical guidelines prescribed by INIBAP (Pinochet, 1988). The content of the biochemical phenols, lignin and orthohydroxy phenols and content of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in the root were determined for each replicate after 90 days, just before root samples were scored for nematode damage. The total phenol 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) and Ortho-dihydric phenol by Arnow's method (Arnow, 1937). The lignin content of banana roots was gravimetrically estimated methods of Chesson (1978). 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 4ºC. 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 peroxidase activity was assessed according to Hammerschmidt *et al*. (1982) and polyphenol oxidase activity was assessed using the modified method of Mayer *et al*. (1965). Data were subjected to analysis of variance using the SPSS 11.5 statistical package and means compared by the LSD at P = 0.05.

RESULTS AND DISCUSSION

Radopholus similis population densities, root and corm necrosis

The reactions of the banana hybrids and parents and reference cultivars to *Radopholus similis* are given in table 1. *Radopholus similis* population densities at 90 days after inoculation varied considerably by new hybrids and reference cultivars under greenhouse condition. The lowest reproductive factor was observed on the resistant reference cultivars Yangambi km 5 (92.00). Nematode population from the hybrids root sample recorded a high count of 451.00 nematodes per five grams of root in the hybrid H-11-22. H-11-08 recorded the lowest population of nematodes (97.00) with the highest mean densities recovered from susceptible check Grand Naine (432.00) for *R. similis*. Grand Naine supported generated reproduction of *R. similis* than the other genotype tested in the green house. The lower rate of multiplication might be due to lower rate of invasion of juveniles into the resistant cultivars and subsequent suppression of nematode development resulting into low a fecundity rate (Mukhtar *et al.,* 2013).

Resistant / tolerant hybrids produce thick and more number of healthy roots to overcome the nematode infection. In the present study, wherever the hybrid exhibiting resistances to nematodes are posses Pisang Lilin as one of the parents. This

Table 1: Population buildup of *R. similis* and assessment of root and corm damage caused by *R. similis* in banana hybrids and parents

S.No.	Hybrids	Parentage	Nematodes population (No/5 g roots)	DE	Roots OK	DE%	Total RN%	Corm Grade	Root lesion index	Reaction status
01.	H-11-01	ANK x PL	376	8	24	33.33	17	3	3	S
02.	H-11-02	ANK x PL	319	7	23	30.43	14	2	2	T
03.	H-11-03	ANK x PL	190	3	26	11.54	4	1	2	T
04.	H-11-06	ANK x PL	282	5	28	17.86	9	2	2	T
05.	H-11-07	ANK x PL	362	14	46	30.43	10	3	3	S
06.	H-11-08	AMB x cv.Rose	97	4	50	8.00	7	1	1	R
07.	H-11-12	AMB x PL	267	4	21	19.05	9	1	2	T
08.	H-11-18	EV x PL	376	5	26	19.23	17	2	2	T
09.	H-11-21	Tongat x PL	207	3	40	7.50	2	1	1	R
10.	H-11-22	Tongat x PL	451	11	36	30.56	53	4	5	HS
11.	H-11-23	Tongat x PL	124	4	41	9.76	10	1	1	R
12.	H-11-24	Tongat x YKM5	136	5	29	17.24	12	1	2	T
13.	H-11-25	Tongat x YKM5	113	3	38	7.89	8	1	1	R
14.	H-11-36	H 911 x YKM5	106	4	38	10.53	6	1	1	R
15.	H-11-37	H 911 x YKM5	294	6	29	20.69	11	2	2	T
16.	H-11-41	H 940 x YKM5	174	6	23	26.09	23	3	3	S
17.	H-11-49	ANK x cv.Rose	276	4	24	16.67	21	2	2	T
18.	H-11-50	ANK x cv.Rose	362	10	29	34.48	23	3	3	S
19.	H-11-51	ANK x cv.Rose	321	7	18	38.89	30	4	4	HS
20.	H-11-52	ANK x cv.Rose	315	14	30	46.67	37	4	4	HS
21.	H-11-65	AMB x H572	321	8	47	17.02	7	1	2	T
22.	H-11-69	H 201 x FHIA-1	135	2	26	7.69	14	1	1	R
23.	H-11-70	H 201 x FHIA-1	107	2	31	6.45	8	1	1	R
24.	H-11-71	H 201 x cv.Rose	141	3	34	8.82	10	1	1	R
25.	H-11-74	H-02-34 x cv.Rose	315	7	18	38.89	29	3	3	S
26.	H-11-75	H-02-34 x cv.Rose	367	8	18	44.44	31	4	4	HS
27.	H-11-76	H-02-34 x cv.Rose	286	2	29	6.90	8	1	1	R
28.	H-11-78	H-02-34 x cv.Rose	278	6	27	22.22	25	2	2	T
Parents										
01.	Anaikomban		112	2	23	8.70	10	1	1	R
02.	Ambalakadali		154	5	27	18.52	13	2	2	T
03.	Erachivazhai		295	7	29	24.14	16	2	2	T
04.	Pisang Lilin		102	2	28	7.14	8	1	1	R
05.	cv.Rose		272	3	38	7.89	9	1	1	R
06.	Tongat		126	3	31	9.68	10	1	1	R
07.	H 911		287	4	21	19.05	13	2	2	T
08.	H 940		345	8	19	42.11	24	3	3	S
09.	H 201		266	3	37	8.11	14	1	1	R
10.	H 572		158	6	24	25.00	14	2	3	T
11.	FHIA-1		325	4	33	12.12	11	2	2	T
12.	H-02-34		147	6	39	15.38	12	2	2	T
	Reference cultivars									
01	YKM5		92	3	41	7.32	7	1	1	R
02.	Grand Naine		432	8	18	44.44	44	4	5	HS

YKM5 – Resistance to *R. similis;* Grand Naine – Susceptible to *R. similis;* ANK-Anaikomban; AMB-ambalakadali; PL-Pisang Lilin; YKM5- Yangambi km5; EV- Erachivazhai DE- Dead root; OK-Functional root; DE% - Dead root percentage; R- Resistant; T- Tolerant; S- Susceptible; HS- Highly susceptible

showed a clear indication of that Pisang Lilin might be the contributing the trait for the resistance to *R. similis*. Plants show a variety of responses when they attempt to resist the attack by pathogens. When these responses are successful and prevent or inhibit nematode growth, the plant is considered to have complete or fully functional resistance (Nithya Devi *et al*., 2007).

Inbuilt host plant resistance is the most promising strategies the management of parasitic nematodes. In breeding for resistance the basic requirement is the availability of an efficient screening technique, which should clearly distinguish resistant genotypes from susceptible ones. The root number and percent dead roots are considered as critical in the assessment of nematode damage as Gaidashova *et al*. (2008) reported that 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. At 90 day after inoculation, hybrids H-11-08, H-11-21, H-11-23, H-11-25, H-11-69, H-11-70 and H-11-76 had significantly less dead roots compared to other hybrids. The reference resistance variety, Yangambi km5 had less dead root (7.32) compared to susceptible check variety Grand Nine (44.40%). Among the hybrids, there were more dead roots in H-11-52 and less dead roots in H-11-70.

In the current study greater percentage root necrosis was associated with reduction of number of roots in inoculated

Table 2: Total phenols, OD phenols and lignin content in the roots of banana hybrids and parents inoculated with *R. similis*.

S. No.	Hybrids	Reaction level	Total phenols (µg/g)			OD phenols (µg/g)			Lignin (%)		
			C	I	%	C	I	%	C	I	%
01.	H-11-01	S	235.26	265.10	12.68	1.76	2.62	48.86	0.74	0.80	8.11
02.	H-11-02	T	211.83	269.78	27.36	1.27	1.74	37.01	0.69	1.04	50.72
03.	H-11-03	T	252.14	354.30	40.52	1.82	2.41	32.42	0.82	1.19	45.12
04.	H-11-06	T	275.63	372.05	34.98	1.66	2.30	38.55	0.77	1.03	33.77
05.	H-11-07	S	270.89	318.57	17.60	1.95	2.46	26.15	0.68	0.76	11.76
06.	H-11-08	R	326.34	518.15	58.78	1.70	2.87	68.82	0.84	1.32	57.14
07.	H-11-12	T	257.00	332.46	29.36	1.58	2.64	67.09	0.71	0.83	16.90
08.	H-11-18	T	348.90	471.77	35.22	1.86	2.50	34.41	0.79	1.00	26.58
09.	H-11-21	R	321.75	498.26	54.86	3.28	4.84	47.56	0.87	1.46	67.82
10.	H-11-22	HS	192.36	214.30	11.41	1.35	1.69	25.19	0.64	0.76	18.75
11.	H-11-23	R	369.15	617.50	67.28	2.14	3.40	58.88	0.89	1.36	52.81
12.	H-11-24	T	242.88	342.30	40.93	1.36	2.21	62.50	0.78	1.13	44.87
13.	H-11-25	R	384.25	582.62	51.63	2.61	4.19	60.54	0.96	1.64	70.83
14.	H-11-36	R	294.00	406.26	38.18	2.83	3.78	33.57	0.92	1.40	52.17
15.	H-11-37	T	252.67	330.34	30.74	1.78	2.43	36.52	0.88	1.22	38.64
16.	H-11-41	S	276.85	328.40	18.62	1.54	2.37	53.90	0.75	0.80	6.67
17.	H-11-49	T	248.51	333.65	34.26	1.60	2.28	42.50	0.78	0.85	8.97
18.	H-11-50	S	190.24	223.88	17.68	1.23	1.71	39.02	0.73	0.78	6.85
19.	H-11-51	HS	215.40	234.56	8.90	1.16	1.64	41.38	0.68	0.74	8.82
20.	H-11-52	HS	168.05	192.70	14.67	1.34	1.58	17.91	0.60	0.62	3.33
21.	H-11-65	T	272.80	368.74	35.17	1.62	2.25	38.89	0.79	1.20	51.90
22.	H-11-69	R	318.56	484.53	52.10	1.78	2.60	46.07	0.92	1.34	45.65
23.	H-11-70	R	320.17	464.03	44.93	1.52	2.46	61.84	0.81	1.27	56.79
24.	H-11-71	R	362.74	587.54	61.97	2.37	3.90	64.56	0.88	1.40	59.09
25.	H-11-74	S	190.65	231.33	21.34	1.24	1.66	33.87	0.61	0.66	8.20
26.	H-11-75	HS	158.35	169.02	6.74	1.45	1.80	24.14	0.64	0.67	4.69
27.	H-11-76	R	314.81	464.82	47.65	3.12	4.36	39.74	0.84	1.38	64.29
28.	H-11-78	T	187.62	204.18	8.83	1.32	1.61	21.97	0.62	0.80	29.03
Parents											
01.	Anaikomban	R	246.25	372.70	51.35	2.47	3.09	25.10	0.82	1.24	51.22
02.	Ambalakadali	T	221.60	285.12	28.66	2.10	2.52	20.00	0.74	1.10	48.65
03.	Erachivazhai	T	194.14	226.75	16.80	1.37	1.85	35.04	0.65	0.93	43.08
04.	Pisang Lilin	R	327.02	517.47	58.24	2.53	3.67	45.06	0.92	1.68	82.61
05.	cv.Rose	R	278.56	405.18	45.46	1.85	3.12	68.65	0.85	1.37	61.18
06.	Tongat	R	251.35	335.82	33.61	2.48	3.44	38.71	0.87	1.32	51.72
07.	H 911	T	216.50	257.08	18.74	1.31	1.87	42.75	0.74	1.01	36.49
08.	H 940	S	142.24	155.51	9.33	1.19	1.51	26.89	0.52	0.55	5.77
09.	H 201	R	255.81	354.17	38.45	1.51	2.35	55.63	0.78	1.29	65.38
10.	H 572	T	242.45	309.20	27.53	1.40	2.01	43.57	0.81	1.00	23.46
11.	FHIA-1	T	276.96	375.71	35.65	1.66	2.65	59.64	0.87	1.21	39.08
12.	H-02-34	T	252.65	320.00	26.66	1.45	2.27	56.55	0.76	1.03	35.53
	Reference cultivars										
01.	YKM5	R	332.48	513.40	54.42	2.36	3.80	61.02	0.92	1.55	68.48
02.	Grand Naine	HS	175.15	203.62	16.25	1.14	1.55	35.96	0.61	0.67	9.84
		Sources	G	T	GT	G	T	GT	G	T	GT
		SEd	7.936	1.711	11.223	0.057	0.012	0.080	0.024	0.005	0.033
		CD (p=0.05)	15.664	3.378	22.151	0.112	0.024	0.158	0.047	0.010	0.066

OD Phenol-orthodihydroxy phenols; C = Control; I = Inoculated; % = per cent difference over control

pots. The percentage of root necrosis ranged 7 for resistance cultivar Yangambi km5 and 44 for susceptible cultivar, Grand Naine. The resistance hybrids had root necrosis ranged from 2 to 14 percentages. The lowest percentage of root necrosis recorded in H-11-21 (2%). This combination of characters may be due to incorporation of a resistance gene from resistance the parent Tongat and Pisang Lilin. Among the hybrids highest root necrosis recorded in H-11-22 (53.00%).

Based on root lesion index and corm grade the hybrids were assessed for their level of resistance against *R. similis* (Table 1). Banana hybrids H-11-03, H-11-08, H-11-12, H-11-21, H-11-23, H-11-24, H-11-25, H-11-36, H-11-65, H-11-69, H-11-70, H-11-71 and H-11-76 and parents *viz*., Anaikomban, cv.Rose, Pisang Lilin and Tongat and H 201 were considered resistance to the nematode as the root lesion index and corm grade rate was lowest grade of 1. The lower percentage of root necrosis and corm lesion index in the hybrids might be due to lower nematodes populations due to less multiplication rate (Hartman *et al*., 2010).

Among them the banana hybrids, H-11-08, H-11-21, H-11-23, H-11-25, H-11-36, H-11-69, H-11-70, H-11-71 and H-11-76 were rated resistant, while H-11-02, H-11-03, H-11-06, H-11-12, H-11-18, H-11-24, H-11-37, H-11-49, H-11-65 and H-11-78 were rated tolerant to *R. similis*. The hybrids, H-11-22, H-11-51, H-11-52 and H-11-75 were highly susceptible. These resistance hybrids could be used in breeding programmes for developing new hybrids as male parents. The remaining hybrids were rated susceptible. Resistant can be considered as the ability of the plant to suppress development of nematodes, whereas tolerance is the ability of the plant to grow well despite infection by nematodes (Gaidashova *et al*., 2008). Tolerant x resistance crosses produced resistant, tolerant and susceptible hybrids, suggesting that resistance/ tolerance to nematodes in under polygenic control.

Table 3: Enzyme activities in the roots of banana hybrids and their parents inoculated with *R. similis*

S.No.	Hybrids	Reaction level	Peroxidase(abs/min/g)			Polyphenol oxidase(abs/min/g)			PAL(nmol/min/ml)		
			C	I	%	C	I	%	C	I	%
01.	H-11-01	S	1.65	1.85	12.12	0.063	0.076	20.63	14.65	16.12	10.03
02.	H-11-02	T	1.42	1.90	33.80	0.045	0.051	13.33	12.16	13.94	14.64
03.	H-11-03	T	2.29	3.12	36.24	0.062	0.084	35.48	16.74	21.12	26.16
04.	H-11-06	T	1.54	1.97	27.92	0.085	0.112	31.76	13.15	16.00	21.67
05.	H-11-07	S	1.88	2.19	16.49	0.067	0.084	25.37	17.81	19.48	9.38
06.	H-11-08	R	2.16	3.32	53.70	0.128	0.182	42.19	22.43	28.70	27.95
07.	H-11-12	T	2.34	3.20	36.75	0.097	0.131	35.05	14.05	16.63	18.36
08.	H-11-18	T	1.61	2.14	32.92	0.069	0.091	31.88	15.76	19.92	26.40
09.	H-11-21	R	2.87	4.33	50.87	0.117	0.168	43.59	24.55	28.07	14.34
10.	H-11-22	HS	1.59	1.75	10.06	0.034	0.046	35.29	14.61	16.18	10.75
11.	H-11-23	R	2.53	3.74	47.83	0.121	0.157	29.75	18.38	24.53	33.46
12.	H-11-24	T	1.98	2.51	26.77	0.070	0.102	45.71	20.00	22.41	12.05
13.	H-11-25	R	2.69	3.84	42.75	0.112	0.160	42.86	25.14	34.67	37.91
14.	H-11-36	R	2.27	3.16	39.21	0.096	0.132	37.50	19.60	25.84	31.84
15.	H-11-37	T	1.89	2.43	28.57	0.065	0.077	18.46	14.92	17.58	17.83
16.	H-11-41	S	2.46	2.88	17.07	0.070	0.091	30.00	12.59	15.16	20.41
17.	H-11-49	T	2.13	2.66	24.88	0.065	0.090	38.46	16.64	19.12	14.90
18.	H-11-50	S	1.54	1.66	7.79	0.076	0.088	15.79	11.86	12.92	8.94
19.	H-11-51	HS	1.82	2.17	19.23	0.067	0.083	23.88	12.15	14.00	15.23
20.	H-11-52	HS	1.21	1.36	12.40	0.049	0.054	10.20	14.43	17.26	19.61
21.	H-11-65	T	1.82	2.44	34.07	0.067	0.091	35.82	19.00	23.53	23.84
22.	H-11-69	R	2.39	3.51	46.86	0.091	0.130	42.86	19.57	25.69	31.27
23.	H-11-70	R	2.18	3.11	42.66	0.087	0.114	31.03	23.75	27.25	14.74
24.	H-11-71	R	2.54	3.78	48.82	0.134	0.196	46.27	20.98	27.13	29.31
25.	H-11-74	S	1.12	1.19	6.25	0.047	0.053	12.77	13.47	14.86	10.32
26.	H-11-75	HS	1.33	1.57	18.05	0.032	0.040	25.00	12.08	13.50	11.75
27.	H-11-76	R	2.56	3.61	41.02	0.076	0.110	44.74	21.97	26.65	21.30
28.	H-11-78	T	1.35	1.60	18.52	0.058	0.071	22.41	13.45	14.89	10.71
Parents											
01.	Anaikomban	R	2.21	3.17	43.44	0.075	0.104	38.67	15.66	19.01	21.39
02.	Ambalakadali	T	2.10	2.62	24.76	0.082	0.114	39.02	12.48	14.74	18.11
03.	Erachivazhai	T	1.84	2.14	16.30	0.064	0.076	18.75	12.21	13.85	13.43
04.	Pisang Lilin	R	2.51	3.80	51.39	0.112	0.163	45.54	21.85	28.67	31.21
05.	cv.Rose	R	2.76	3.84	39.13	0.103	0.141	36.89	19.36	25.84	33.47
06.	Tongat	R	2.16	3.48	61.11	0.091	0.134	47.25	16.50	20.13	22.00
07.	H 911	T	1.93	2.30	19.17	0.061	0.077	26.23	14.96	17.15	14.64
08.	H 940	S	1.48	1.72	16.22	0.042	0.051	21.43	12.62	13.98	10.78
09.	H 201	R	2.23	3.28	47.09	0.102	0.148	45.10	15.04	18.65	24.00
10.	H 572	T	1.97	2.50	26.90	0.068	0.085	25.00	12.09	14.67	21.34
11.	FHIA-1	T	2.16	2.82	30.56	0.074	0.101	36.49	15.80	19.04	20.51
12.	H-02-34	T	2.17	2.57	18.43	0.092	0.117	27.17	14.55	17.12	17.66
Reference cultivars											
01.	YKM5	R	2.69	3.87	43.87	0.126	0.185	46.83	18.96	25.20	32.91
02.	Grand Naine	HS	1.46	1.72	17.81	0.054	0.068	25.93	12.04	13.80	14.62
	Sources		G	T	GT	G	T	GT	G	T	GT
	SEd		0.059	0.0127	0.0838	0.0024	0.0005	0.0034	0.4601	0.0992	0.6507
	CD(p=0.05)		0.1169	0.0252	0.1654	0.0048	0.0010	0.0068	0.908	0.1958	1.2845

C = Control; I = Inoculated; % = per cent difference over control; PAL = Phenylalanine Ammonia Lyase

Segregation for resistance/tolerance and susceptibility was expected because of the heterozygous nature of the parents. Rowe and Rosales (1996) indicated that one or more dominant alleles control genetic resistance to burrowing nematodes. It could be seen that use of both resistant parents resulted 9 resistant hybrids. Nine out of 28 crosses were of tolerant x resistant combination that resulted in resistant progenies. In the tolerant x tolerant combination only two hybrids progenies *viz*., H-11-65 and H-11-67 had resulted in resistance. These results again depicts that it is necessary to involve resistant parents in the breeding programme although occasionally resistant progeny may develop due to recombination of favorable genes in tolerant x tolerant combinations. Some of the cross combinations involving resistant x resistant parents *viz*., Anaikomban x cv.Rose (H-11-51 and H-11-52) and Anaikomban x Pisang Lilin (H-11-07), Tongat x Pisang Lilin(H-11-22) were susceptible to nematodes probably because of the heterozygous nature of parents for this trait.

Resistance within a plant species is often due to specific genes that can segregate within the species. By contrast, for non-host species or resistant cultivars, the nematode cannot reproduce on that species or group of plants due to a broader absence of host traits required for parasitism (Abad *et al*., 2009). An additional feature of nematode resistance is the effect on the development of nematode penetrate the epidermis and migrate through the root cortex to establish a feeding site in the vascular parenchyma typically associated with compatible nematode plant interactions on susceptible host plants. In many incompatible interactions on resistant host plants, nematode infection site is reduced or lacking, depending on the resistance mechanism (Roberts *et al*., 2008).

Biochemical and enzyme assays

Significant variation was observed among the hybrids of

inoculated treatments for total phenol, OD-phenol, lignin, peroxides, and polyphenol oxidase and phenylalanine ammonia lyase activity (Table. 2). Among the various biochemicals, total phenol is considered as one of the important defense related chemical. Table 2 shows a sequential development of total phenol activity in both the resistant and susceptible banana hybrids to *R. similis* infection.

The constitutive level of total phenol activity in roots was found to be higher in resistant hybrids than in susceptible one. In nematode-infected resistant roots, the activity of total phenol varied considerable and ranged from 406.26 to 617.50 µg/g. Among the hybrids, the highest total phenol content was registered in H-11-23 (617.50 µg/g root) followed by H-11-25 (582.62 µg/g root). Maximum percentage change of infected compared with the non-infected control was recorded in H-11-23 (67.28 %). The lowest phenol content (169.02µg/g) was recorded in H-11-75. In the *R. similis* resistance check cultivars, Yangambi km5 registered maximum total phenol activity compared with susceptible cultivar of Grand Naine. Most of the resistant hybrids records higher phenols content as that of the resistance reference cultivar. Similar finding were earlier reported in banana by Das *et al*. (2013).

Yangambi km5 infected with nematodes which induced and accumulation of more phenolics in roots (Karunakaran, 2010). Most of this resistance is found in hypersensitive type of responses that involve changes in enzyme activity, phenol metabolism, soluble antimicrobial, deterrent compounds or they may cross-link with callose, polysaccharides in the cell walls, and regulation of free radical O_2 in cell walls (Torres *et al*., 2012; Vaganan *et al*., 2014 and Wang *et al*., 2015).

The total phenolic substances in the roots of both healthy and resistant cultivars had higher compared to the susceptible cultivars (Nayak, 2015). The accumulation of phenol may be due to the excess production of hydrogen peroxide by increased respiration or due to the activation of hexose monophosphate (HMP) shunt pathway, acetate pathway and release of bound phenols by hydrolytic enzymes (Seenivasan, 2012). The resistance and susceptibility attributes of several crops to different insect-pests and pathogens has been explained by many researchers due to the presence of secondary plant metabolites mainly phenols (Pathipati and Yasur, 2010 and Vandana Sukhla *et al*., 2014).

The Ortho-dihydrogen phenol (OD phenol) activity increased in both root from nematode-infected resistant and susceptible hybrids when compared to non-infected hybrids. The percentage increase in OD phenol activity in nematode-infected resistant hybrids compared with the non-infected control. The OD phenol content differed significantly between the genotypes, treatments and their interactions (Table 2). Among the hybrids, the highest OD phenol content was observed in H-11-21 (4.84µg/g) which showed an increase of 47.56 per cent over control. The OD phenol was found lower (1.58µg/g) in H-11-52 which showed an increase of 17.91 per cent over control. In case of parent's cultivar, Rose registered maximum percentage of OD phenol content (68.65) and Ambalakadali was registered minimum percentage of OD phenol content (20.00). In case reference cultivars Yangambi km5 recorded maximum increase activity of 61.02 per cent while minimum activity was recorded in Grand Naine 35.96 per cent.

Karunakaran (2010) found that biochemical contents like total phenols, OD phenols and lignin were highly active in resistant genotypes. Phenol and OD-phenol content had increased upto 50 per cent in plant, which protected the plant from the pest by imparting high level of resistance (Taggar *et al*., 2014).

The increased synthesis of phenols, followed by the higher PPO activity, would have helped to increase conversion of them into polymers such as lignin (Kavitha *et al*., 2008). Lignin content varied from 0.60 per cent (H-11-52) to 0.96 per cent (H-11-25) in nematodes uninoculated plants. In the nematode inoculated plants, the range of lignin was between 0.62 and 1.64 per cent. Among the parents, Pisang Lilin recorded the maximum per cent increase of lignin content as 82.61 per cent and H 940 recorded the minimum per cent lignin content of 5.77 per cent. In the reference cultivars, Yangambi km5 exhibited maximum increase in lignin content (1.55 per cent) and minimum increase in lignin content was recorded in Grand Naine (0.67 per cent). The resistance hybrid H-11-25 expressed the maximum activity of lignin activity as 0.96 per cent under control and 1.64 per cent under nematode inoculated plants. The lignin content in the roots of the banana hybrids indicated a positive response due to nematode infestation and was more as compared to uninoculated banana roots.

Lignin and phenol are synthesized *via* phenyl propanoid pathways which impart resistance against nematode attack. The role of phytoalexins and other toxic compounds like phenols and lignin (which are otherwise called phytoanticipins and are synthesized as a part of the normal plant development) in resistance mechanism have been reported by earlier workers (Reuveni *et al*., 1992 and Sariah *et al*., 1999). The tolerant plants, when subjected to biotic stress, showed elevated levels of free phenolics and contain lignin (Kavino *et al*., 2007). Lignin activity was found to increase in banana after nematode inoculation. However, the final lignin concentration was higher in the resistance plants (Damodaran, 2007; Kavitha, 2008; Karunakaran, 2010 and Das *et al*., 2011).

Among the various enzyme studies, peroxidase has been correlated with plant defense mechanism by catalyzing the condensation of phenolic compounds into lignin. The current model that involves peroxidase in defense mechanism considered the condensation of phenolic monomers derived from the phenylpropanids pathway into insoluble polymers (Robb *et al*., 1991and Lattanzio *et al*., 2006).

Changes in peroxidase activity in nine resistant, ten tolerant and nine susceptible banana hybrids were studied 90 days after *R. similis* infection (Table 2). The inoculation of banana plants with the nematodes, the peroxidase activity increased in both the resistance and susceptible genotypes. In nematode-infected resistant roots, enzyme activity increased compared to that in non-infected hybrids. Maximum percentage change of infected compared with the non-infected control recorded H-11-08 (53.70 per cent) in roots. The peroxidase activity differed significantly among the hybrids and respective checks. The highest polyphenol oxidase activity was highest (4.33 abs/min/g) in H-11-21 which was on par with H-11-23 (3.74 abs/min/g), H-11-25 (3.84 abs/min/g), H-11-76 (3.61 abs/min/

g). The susceptible check of Grand Naine registered lower peroxidase activity. However, the final enzyme concentration was the maximum in resistance hybrids like H-11-08, H-11-21, H-11-23, H-11-25, H-11-69, H-11-71 and H-11-76. Among the parents, Tongat recorded increased peroxidase activity content of 61.11 while minimum activity was recorded in H 940 (16.22 per cent). Resistance hybrids have shown highest level of peroxidase activity compared to susceptible. Similar result was found by (Das *et al*., 2013; Anitha and Samyappan, 2012; Latournerie- Moreno *et al*., 2015).

The role of peroxidase has been cited in a number of reports on defense mechanisms against invading pathogens, such as the hypersensitive response (Vaganan *et al*., 2014). Hypersensitive response leads to the production of reactive oxygen species such as singlet oxygen, superoxide radical, hydrogen peroxide, and the hydroxyl radical in the region surrounding the infection to limit the growth and spread of pathogens (Passos *et al*., 2013).

Peroxidase (PO) oxidizes phenolics to quinones and generates H_2O_2. The latter not only is antimicrobial in itself, but it also releases highly reactive free radicals and in that way further increases the rate of polymerization of phenolic compounds into lignin like compounds. These substances are deposited in cell walls and papillae and inhibited further growth and development of pathogen (Ghosh, 2015). Peroxidase enzyme corresponding with provoked resistance and are concerned in numerous plant defense mechanisms like oxidative cross-linking of plant cell walls, lignin biosynthesis and production of vigorous oxygen species (Faize *et al.,* 2004). Peroxidase makes cellular environment toxic and extremely unfavorable for pathogen by producing reactive species of oxygen and nitrogen (Passardi *et al.,* 2005; Gill and Tuteja, 2010; Schaffer and Bronnikova, 2012).

Polyphenol oxidase oxidizes the phenols to highly toxic quinines and hence is considered to play an important role in pest resistance. The PPO activity is normally induced in plants infected by different parasites, particularly those affecting the vascular tissues (Soffan *et al*., 2014). The highest of polyphenol oxidase activity was recorded maximum in H-11-71 (0.196 abs/min/g fresh weight) while lowest activity was recorded in H-11-75 (0.040 abs/min/g fresh weight).Yangambi km5 recorded increased polyphenol oxidase activity of 0.185 nmol/min/ml while Grand Naine recorded minimum activity of 0.068 nmol/min/ml. A critical analysis of their activity in this study revealed that resistance hybrids H-11-08, H-11-21, H-11-23, H-11-25, H-11-69, H-11-71, H-11-76 recorded higher polyphenol oxidase activity than the susceptible ones. In the case of parents, the highest activity was recorded in Pisang Lilin (0.163abs/min/g fresh weight) which showed an increase of 45.54 per cent over control while H 940 recorded lowest activity of 21.43 per cent over the control. Out of all the hybrids, H-11-21, H-11-23 and H-11-25 had higher polyphenol oxidase activity along with higher yield.This may be due to the alteration of redox potential of the host leading to the abrupt rise in the activity of PPO (Vidhyasekaran, 1988). High levels of two major oxidizing enzyme of plants such as poly phenol oxidase and peroxidase impart induce resistance to insect herbivores and pathogens (Ranchana *et al*., 2015). Polyphenol oxidases catalyse the oxidation of ortho-oriented dihydroxy phenolic compounds, thereby generating quinones, which are highly reactive molecules that can either spontaneously polymerise or damage proteins, amino acids and nucleic acids via an alkylation reaction (Constabel and Barbehenn, 2008).

The important role of polyphenol oxidase is to oxidize polyphenols in the phenolic complex. Most phenols occur in plant tissues in bound form which contains both mono and polyphenols. Accumulation of mono phenols is an important criterion for resistance (Sundraraju and Pandi suba, 2006).

PPO activity is ubiquitous in higher plants, and functions attributed to the enzyme include phenol metabolism and a defense mechanism against nematodes (Das *et al.,* 2013). Several observations have identified a role for PPO in the polymerization of monolignols into olignols, precursor molecules of lignin. The possible involvement of PPO in defense in banana roots was suggested by the markedly increased levels of PPO activity after elicitation.

The PAL percentage increase was more in resistant hybrids as compared to susceptible banana hybrids. Among the hybrids, the maximum phenylalanine ammonia lyase activity of 34.67 nmol/min/ml was registered by H-11-25 under inoculated condition (Table 3). However, the lowest activity of 12.92 nmol/min/ml was recorded in H-11-50. Among the parents, per cent increase in PAL activity was the highest in cultivar Rose (33.47 %) and the lowest in H 940 (10.78 %). In case of reference cultivars maximum increased activity of 32.91 per cent Yangambi km5, whereas minimum increase activity in Grand Naine (14.62 per cent). The resistant check cultivar of Yangambi km5 registered maximum activity of 25.20 nmol/min/ml, whereas the susceptible cultivar of Grand Naine recorded minimum increase activity of 13.80 nmol/min/ml. Das *et al*. (2014) found that the activity of phenylalanine ammonia lyase were more in resistant and tolerant cultivars of banana in pot culture studies when compared to susceptible ones.

Increased activities of phenylalanine ammonia lyase and polyphenol oxidase were associated with reduced *R. similis* population in cultivar Yangambi km5 as compared to the susceptible cultivar 'Nabusa' (Paparu *et al*., 2008). The maximum activity of glucanase, chitinase and PAL was found to be associated with resistant status of mungbean cultivars (Koche and Choudhary, 2012). In susceptible plant, nematodes would have broken the defense barrier by producing certain offensive chemicals while in the case of resistance plant synthesized certain toxic compounds known as phytoalexins.

Generally these phytoalexins compounds are antimicrobial activity that are synthesized and accumulated in the cells. It has been well established that phenylalanine ammonia lyase is the prime enzyme involved in the phenyl propanoid pathway which impart resistance against nematode attack. The PAL and ascorbic acid oxidase enzymes might have increased the chlorogenic acid and ascorbic acid content in the plant tissues, which act as toxic compounds against nematodes (Ramesh kumar *et al.,* 2012).

Resistance mechanism in plant is due to increased activity of defense-related enzymes such as polyphenoloxidase, peroxidase, phenylalanine ammonia-lyase, and glucanase, and also increased production of antifungal compounds, such as

phenolic metabolism products (lignin), flavonoids, phytoalexins, pathogenesis-related proteins in plants and activation of some plant defense-related genes (Fauteux *et al.,* 2005; Datnoff *et al.,*2007; Van Bockhaven *et al.,* 2013).

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