Indian J. Hort. 70(3), September 2013: 350-356

Screening of *Musa* hybrids for resistance to *Pratylenchus coffeae*

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ABSTRACT

Twenty four elite banana hybrids, six diploids (AA and AB), five triploids (AAA and AAB), ten tetraploids (AABB) and three pentaploids (AAABB) were screened under pot culture conditions for their reaction to lesion nematode, *Pratylenchus coffeae*. Among them the banana hybrids, H 516 and H 531 were rated resistant, while, H 511, H 534, H 537, H 571, H 572 and H 589 were rated tolerant to *P. coffeae*. The remaining accessions were rated as susceptible. Peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase enzyme activities and total phenol contents in roots were higher in H 531 and H 516 than other hybrids.

Key words: Banana, hybrids, resistance, *Pratylenchus coffeae*.

INTRODUCTION

Banana (*Musa* spp.) is one of the most important fruit crops grown in India and ranks second in area and production. Nematodes are a serious constraint of banana production world-wide among which the lesion nematode-*Pratylenchus coffeae* Goodey is the most important causing an yield loss to an extent of 44% in India (Sundararaju and Kumar, 14). It is a migratory endoparasitic nematode of root and corm tissues, which invade any potion of the root length and feeds on the cytoplasm of cortex cells, collapsing cell walls and causing cavities and tunnels. 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 (Sundararaju and Kumar, 14). 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 banana for nematode resistance is probably the best way of controlling this problem while keeping the environment safe. The objective of this study was to screen banana hybrids against *Pratylenchus coffeae* derived from crossings of potential diploids and hybrids developed at TNAU (resistant male parent) with commercial triploids (susceptible female parent) under pot culture conditions.

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MATERIALS AND METHODS

Pot culture experiments were conducted in the glass house (25±3°C) to evaluate the resistance of banana hybrids to *Pratylenchus coffeae*. Twenty four elite banana hybrids were drawn from the breeding programme of the Department of Fruit Crops, TNAU. The susceptible check cultivar was Rasthali (AAB), while the resistant reference cultivar used Pisang Lilin (AA). Before planting, the suckers were peeled to remove soil and superficial lesions and disinfected using hot water treatment (55°C for 20 min.). The experiment design was completely randomized design replicated three times with each replicate consisting of four suckers. The suckers were planted in pots (30 x 20 x 18 cm size) filled with sterilized pot mixture (red soil: sand: FYM in the ratio of 2: 1: 1 v/v). *Pratylenchus coffeae* was inoculated on 45 days of planting. Isolate of *P. coffeae* was originally collected from Thondamuthur village of Coimbatore district, Tamil Nadu on cv. Nendran and maintained monoxenically on carrot discs at 27°C (O'Bannon and Taylor, 10). *Pratylenchus coffeae* was extracted from the cultures, quantified, concentrated and resuspended in sterile deionized water (500 nematodes/ ml). Twenty ml of the nematode suspension containing 10,0000 nematodes (mixture of juveniles and adults) were added to the base of the each plant and covered with sand. Same set of replicated banana hybrids were also maintained as uninoculated check. The plants were fertilized with Hoagland's No. 2 nutrient solution fortnightly. The plants were allowed to grow for 90 days after the nematode inoculation date under glasshouse at 25±3°C. The plants were uprooted 90 days after inoculation and observations on root fresh weight, number of roots, root length and girth were recorded. Nematode population in 250 cc

sub-samples and in 5 g of root sub-samples were assessed. For extraction of nematodes from roots, the roots were macerated 3 times for 10 sec (separated by 5 sec intervals). The suspension was poured through 300 and 40 µm sieves and rinsed with tap water. The nematodes were collected from 40 µm sieve with 200 ml distilled water and the nematodes were counted using a stereomicroscope. Nematodes from soil were extracted by Cobb's sieving and modified Baermann's funnel method (Southey, 12).

The extent of nematode damage to roots and corms was assessed following the technical guidelines prescribed by INIBAP (Pinochet, 11). Roots were collected from plants were divided into dead roots and functional roots. Five functional primary roots at least 10 cm long were selected at random form each genotype in each replication. The lengths of the five selected functional roots were all reduced to 10 cm and the roots sliced lengthwise. The percentage of root lesions was assessed in one half of each of the five roots. The maximum root lesion index given per root half was 20, giving a maximum root lesion index of 100 (per cent) for all five together.

Corm damage assessment was done after thoroughly shaking off all soil and washing the corms with water. The outward half of the corm was assessed for damage after trimming the roots off. The number of roots showing black-purple lesions around their bases on the selected outward half of the corm was counted. The numbers of small lesions (SL, lesions smaller in diameter than the root bases) and large lesions (LL, lesions of equal or large diameter than the root bases) were counted and scoring was given as: 0 – no lesions; 1 – one small lesion; 2 – several small lesions; 3 – one large lesion; 4 – several large lesions. The oriental scale of plant response to lesion-forming nematodes used earlier by Pinochet (11) was followed to the hybrids as tolerant, susceptible or resistant as described below;

Plant response	Root lesion index (%)	Corm grade
Immune	0	0
Resistant	‹ 10	‹ 1
Tolerant	10 - 20	1 - 2
Susceptible	20 - 40	2 - 4
Highly susceptible	› 40	› 4

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, 13). 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*. (8) and the PPO activity was assessed using the modified method of Mayer *et al*. (9).

RESULTS AND DISCUSSION

Based on root lesion index and corm grade the hybrids were assessed for their levels of resistance against *P. coffeae*. The hybrids, H 508, H 548 and H 556 recorded higher root lesion index scale of 4, while H 516 and H 531 recorded the lower scale of 1 (Table 1). The hybrids H 508, H 548 and H 556 registered the highest corm grade of 5, whereas H 511, H 516, H 531, H 572 and H 589 registered the lowest grade of 1. The infestation of level of *P. coffeae* was also significantly different among the hybrids. The root population was found to be the lowest in hybrid H 516 (121/ 5 g root) and the highest in H 548 (416 / 5 g root). The soil population also varied among the hybrids and hybrid H 589 recorded the lowest of 73 and highest of 265 in H 548 and H 573. Total final nematode population was the lowest in H 572 (6,325/pot) and the highest of 15,731/pot in H 548. Nematode population buildup both in roots and soil had given more comprehensive picture of the reaction of various hybrids. Among the hybrids, *viz*., H 516, H 531, H 571 and H 572 recorded lower nematode population.

Significant variation was observed for the root characters of the hybrids, *viz.,* number of roots, root length, root girth and root weight among the hybrids (Table 2). H 531 recorded the minimum reduction of number of roots (3.93%) due to *P. coffeae* and it was higher (34.83%) in H 563. The reduction in root length was lesser in the hybrid H 516 and higher in H 508. The hybrid H 589 registered the highest root weight of 205.47 g under inoculated and 220.50 g under un-inoculated control. The per cent difference was the lowest in H 516 (2.63%) and the highest of 30.37 per cent in H 508.

Among them the banana hybrids, H 516 and H 531 were rated resistant, while, H 511, H 534, H 537, H 571, H 572 and H 589 were rated tolerant to *P. coffeae*. The remaining accessions were rated susceptible. Resistance/tolerance to nematodes can be clearly

Table 1. Root and corm damage assessment in banana hybrids under pot culture studies infected by *Pratylenchus coffeae* at 90 DAI.

Hybrid	Parent	Genome	Root population (5 g)	Soil population (250 cc)	Total population	Root lesion index	Corm grade	Reaction status
H 504	H-03-09 × PL	AAABB	305	216	11,140	3	2	S
H 508	ANK × PL	AA	345	220	11,042	4	5	HS
H 511	H-02-34 × Ykm-5	AABB	131	102	7,751	2	1	T
H 515	Mano × ANK	AAA	290	215	11,560	3	2	S
H 516	ANK × PL	AA	121	91	6,913	1	1	R
H 529	H-03-16 × ANK	AABB	290	218	13,697	3	2	S
H 530	H-03-13 (OP)	AABB	265	195	12,630	3	2	S
H 531	Poovan × PL	AAB	124	96	7,301	1	1	R
H 532	H-201 × Mano	AAB	365	245	12,658	3	2	S
H 534	H-03- 13 × Rose	AAB	133	122	7,850	2	2	T
H 537	(H-201 × PK) × Rose	AABB	127	120	7,648	2	2	T
H 540	(H-201 × PK) × Rose	AAABB	332	195	14,570	3	3	S
H 542	H-02-34 × ANK	AABB	345	225	13,253	3	3	S
H 547	H-02-23 (OP)	AABB	321	218	12,203	3	3	S
H 548	H-02-23 (OP)	AABB	416	265	15,731	4	5	HS
H 556	H-04-06 × Ykm-5	AABB	370	221	13,344	4	5	HS
H 563	H-201 × PL	AB	280	233	10,873	3	3	S
H 564	H-201 × PL	AB	319	231	10,900	3	3	S
H 571	H-04-05 × Ykm-5	AABB	123	88	6,332	2	2	T
H 572	H-03-35 (OP)	AAB	125	93	6,325	2	1	T
H 573	H-03-12 × Rose	AAABB	360	265	13,895	3	2	S
H 576	H-201 (OP)	AB	340	229	12,545	3	3	S
H 579	Mano × Rose	AA	325	232	14,268	3	3	S
H 589	H-03-19 (OP)	AABB	126	73	7,002	2	1	T
Reference cultivar								
Pising Lilin		AA	116	71	5,611	1	1	R
Rasthali		AAB	445	282	13,756	5	4	HS
$CD_{0.05}$			31.835	21.843	1261.88			

R = Resistant; T = Tolerant; S = Susceptible; HS = Highly susceptible

established by studying the damage caused to the root and corms of the sucker. The INIBAP method largely encompasses the ability of the genotype to resist nematode infection based on root and corm 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 (Gowen, 7). As the nematode population directly inflicts damage to the root system by causing lesions, assessment of root and corm damage becomes important. Pot screening showed that the resistant hybrids, *viz.*, H 516 and H 531 and tolerant hybrids, *viz.*, H 511, H 534, H 537, H 571, H 572 and H 589, had lesser number of nematodes in roots and soil resulting in minimum root lesion index and corm.

The resistant hybrids H 516, H 531 and the tolerant hybrids H 571 and H 572 registered lesser population than the susceptible hybrids. In some of the tolerant hybrids, the nematode population was more but the growth of the plant was not affected by the number. This could be because; the entry of the nematodes

Table 2. Effect of *Pratylenchus coffeae* on root characters of banana hybrids on 90th DAI under pot culture.

Hybrid	No. of roots			Root length (cm)			Root girth (cm)			Root weight (g)		
	C	I	%	C	I	%	C	I	%	C	I	%
H 504	32.00	22.50	29.69	34.20	27.05	20.91	1.50	1.22	18.67	120.50	90.40	24.98
H 508	30.40	20.30	33.22	30.25	20.20	33.22	1.45	1.15	20.68	115.35	80.32	30.37
H 511	51.75	48.50	6.28	60.50	56.40	6.78	2.10	2.00	4.78	210.60	198.54	5.73
H 515	32.50	24.20	25.54	30.00	22.05	26.67	1.15	0.90	21.73	136.70	106.65	21.98
H 516	40.00	37.75	5.63	46.45	44.30	4.63	1.75	1.65	5.71	195.25	190.12	2.63
H 529	36.25	27.20	24.97	40.00	30.00	25.00	1.55	1.20	22.58	185.30	145.26	21.73
H 530	37.50	30.40	18.93	41.30	31.50	23.73	1.40	1.10	21.43	170.60	135.48	20.59
H 531	52.20	50.15	3.93	52.60	49.50	5.89	1.90	1.78	6.32	204.80	197.64	3.50
H 532	33.35	25.25	24.29	31.40	23.30	25.80	1.10	0.80	27.27	121.55	89.50	26.37
H 534	42.60	39.40	7.51	48.25	44.20	8.39	1.80	1.65	8.33	190.50	180.47	5.27
H 537	48.00	45.00	6.25	49.00	45.00	8.16	1.95	1.80	7.69	196.00	183.60	6.63
H 540	40.28	33.10	17.83	43.60	33.40	23.39	1.50	1.15	23.33	180.70	150.55	16.69
H 542	37.10	27.00	27.22	33.25	26.20	21.20	1.33	1.05	21.05	145.65	110.56	24.09
H 547	38.30	30.00	21.67	30.40	22.00	27.63	1.50	1.25	16.67	135.50	105.20	22.36
H 548	32.80	24.30	25.91	31.20	21.00	32.69	1.45	1.10	24.14	142.60	109.45	23.25
H 556	34.25	25.00	27.01	37.35	30.20	19.14	1.35	1.05	22.22	140.90	105.67	25.00
H 563	24.55	16.00	34.83	26.45	18.30	30.81	1.20	0.90	25.00	115.25	90.16	21.77
H 564	25.15	17.00	32.41	27.00	20.00	25.93	1.15	0.85	26.09	110.50	80.34	27.29
H 571	45.25	42.20	6.74	50.50	47.41	6.12	1.95	1.85	5.13	182.50	168.00	7.95
H 572	46.75	42.70	8.67	45.25	48.20	6.52	1.75	1.60	8.57	171.55	160.49	6.45
H 573	28.85	20.80	27.90	36.30	26.25	27.69	1.42	1.15	19.01	126.30	105.15	16.75
H 576	27.70	20.60	25.63	20.72	15.55	24.95	1.35	1.00	25.93	122.40	100.30	18.05
H 579	30.95	21.90	29.24	31.81	23.60	25.81	1.20	0.95	20.83	165.50	130.28	21.28
H 589	53.50	49.45	7.57	62.22	57.10	8.23	2.00	1.85	7.50	220.50	205.47	6.82
Reference cultivar												
Pisang Lilin	40.60	38.55	5.05	36.54	34.40	5.69	1.50	1.45	3.33	170.50	165.37	3.01
Rasthali	32.25	18.25	45.20	46.84	32.72	30.15	1.45	1.05	27.58	110.40	75.35	31.75
$CD_{0.05}$	4.340	3.686	2.589	4.605	3.950	.463	0.174	0.151	2.094	18.248	15.859	2.083

DAI = Days after inoculation; C = Control; I = Inoculated; % = per cent difference over control

and their reproduction in those hybrids did not affect the crop growth. This is in line with the findings of Das *et al*. (3). All the resistant/ tolerant hybrids showed better root characters than the susceptible hybrids. Binks and Gowen (2) also found higher root weight with primary roots in resistant cultivars compared to susceptible cultivars. Resistant/ tolerant hybrids produced thick and more number of healthy roots to overcome the nematode infection. According to Fogain (5) good root development potential favours resistance. It was interesting to note that a few hybrids claimed to be resistant under field conditions were found to be tolerant under pot culture. This is due to more bombardment of nematodes in the root zone for forceful infection (Dosselaere *et al*., 4).

Significant variation was observed among the hybrids for peroxidase, polyphenol oxidase, phenylalanine ammonia lyase activity and total phenol content (Table 3). The highest peroxidase activity of 2.26 abs/min/g in control and 2.75 abs/min/g in nematode inoculated plants was recorded by H 531 and H 589, respectively. However, the lowest of 1.12 abs/min./g in control and 1.22 abs/min./g in inoculated plants was recorded by H 563. Significance variation was found among hybrids and also inoculated and uninoculated treatments. The hybrid H 531 also

Table 3. Enzyme activity and phenol content of banana hybrids inoculated with *Pratylenchus coffeae* at 90th DAI under pot culture.

Hybrid	Peroxidase (abs/min/g)			Polyphenol oxidase (abs/min/g)			PAL (nmol/min/ml)			Total phenols (µg/g)		
	C	I	%	C	I	%	C	I	%	C	I	%
H 504	1.65	1.77	7.27	0.043	0.047	9.30	13.50	14.60	8.15	262.60	286.90	9.25
H 508	1.30	1.40	7.69	0.051	0.056	9.80	12.30	13.45	9.35	240.25	250.35	4.20
H 511	1.95	2.27	16.92	0.082	0.098	19.51	15.65	17.72	13.23	295.65	335.85	13.60
H 515	1.20	1.29	7.50	0.048	0.051	6.25	14.65	15.82	7.98	215.35	230.55	7.06
H 516	2.15	2.60	20.93	0.098	0.130	32.65	16.64	19.81	19.05	337.50	370.70	9.84
H 529	1.40	1.52	8.57	0.049	0.053	8.16	12.67	13.92	9.87	205.50	210.60	2.48
H 530	1.45	1.53	5.51	0.056	0.061	8.93	13.55	14.58	7.60	235.70	250.85	6.43
H 531	2.26	2.70	19.47	0.098	0.140	42.86	17.55	20.65	17.66	339.45	385.55	13.58
H 532	1.36	1.52	11.76	0.051	0.057	11.76	13.70	14.76	7.74	195.20	215.30	10.30
H 534	2.15	2.53	17.67	0.088	0.108	22.73	15.80	17.85	12.97	288.56	315.65	9.39
H 537	2.20	2.64	20.00	0.089	0.107	20.22	15.75	17.90	13.65	295.48	330.60	11.89
H 540	1.40	1.56	11.43	0.052	0.056	7.59	10.55	10.92	3.50	222.15	240.30	8.17
H 542	1.36	1.48	8.82	0.060	0.063	5.00	11.30	12.35	9.29	207.20	225.45	8.81
H 547	1.28	1.44	12.5	0.063	0.068	7.94	10.70	11.72	9.53	205.25	225.55	9.89
H 548	1.43	1.57	9.79	0.054	0.057	5.56	11.65	12.66	8.67	190.65	210.90	10.62
H 556	1.38	1.46	5.79	0.062	0.068	9.68	12.40	13.43	8.31	185.70	196.90	6.03
H 563	1.12	1.22	8.93	0.036	0.039	8.33	10.25	10.75	4.88	165.30	178.45	7.96
H 564	1.14	1.23	7.89	0.038	0.041	7.89	10.45	10.96	4.88	170.50	185.65	8.89
H 571	2.05	2.52	22.93	0.092	0.120	30.43	15.50	18.65	20.32	279.50	300.75	7.60
H 572	2.08	2.51	20.67	0.093	0.120	29.03	16.20	19.45	20.06	282.70	315.90	11.74
H 573	1.50	1.64	9.33	0.047	0.052	10.64	12.75	13.55	6.27	170.50	185.60	8.86
H 576	1.44	1.55	7.58	0.045	0.050	11.11	11.85	12.95	9.28	139.25	145.85	4.78
H 579	1.45	1.58	8.97	0.055	0.059	7.27	12.60	13.85	9.92	182.25	195.15	7.08
H 589	2.21	2.75	24.43	0.080	0.099	23.75	16.75	19.90	19.16	315.50	356.85	13.09
Reference cultivar												
Pisang Lilin	2.21	2.62	18.55	0.120	0.140	33.33	17.45	20.70	18.62	336.45	375.55	10.14
Rasthali	0.62	0.68	9.68	0.010	0.010	0.00	9.50	9.85	3.68	115.35	121.50	5.33
$CD_{0.05}$	0.187	0.213	1.596	0.009	0.028	2.103	1.546	1.743	1.377	27.126	29.912	1.030

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

expressed the maximum activity of polyphenol oxidase as 0.098 abs/min/g under control and 0.140 abs/min/g under inoculated while, the minimum in H 563 (0.036 abs/min/g under control and 0.039 abs/min/g under inoculated). Per cent increase in polyphenol oxidase activity was the highest in H 531 (42.86) and the lowest in H 542 (5.00). The hybrid H 531 expressed the maximum activity of phenylalanine ammonia lyase activity as 17.55 nmol/min/ml under control and 20.65 nmol/min/ml under inoculated, while, the minimum in H 563 (10.25 nmol/min/ml under control and 10.75 nmol/min/ml under inoculated). The hybrid H 531 registered the highest total phenol content of 339.45 and 385.55 µg/g under control and inoculated respectively. However, the lowest phenol content of 139.25 µg/g under control and 145.85 µg/g under inoculated was recorded by the hybrid H 576. Percent increase in total phenol content over control was the maximum in H 511 (13.60) and the minimum in H 529 (2.48) than control among the hybrids.

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 mRNAs that permit synthesis of similar number of specific proteins. Many of these proteins are enzymes such as phenylalanine ammonia lyase, polyphenol oxidase, peroxidase and β-1-3 glucanase (Vidhyasekaran, 15). These are involved in the synthesis of low molecular weight substances such as phytoalexins, phenols and lignin, which are inhibitory to the invading pathogens (Vidhyasekaran, 15). Hence, estimation of these biochemical markers, which provide mechanism for resistance to pathogens, is highly essential. 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 pathogen either by production of specific isoforms in the form of either peroxidase or polyphenol oxidase. Enhanced peroxidase activity has been associated with hybrids resistant to nematodes (Das *et al.*, 3).

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, 1). 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 516 and H 531 and the tolerant hybrids, *viz.*, H 511, H 534, H 537, H 571, H 572 and H 589 recorded higher peroxidase and poly phenol oxidase activity than the susceptible ones. Out of all the hybrids, H 516, H 531, H 537 and H 589 had higher peroxidase and poly phenol oxidase activity along with higher yield and quality. Similar findings were earlier reported in banana by Das *et al.* (3). Vidhyasekaran (15) described the occurrence of many kinds of phenolics in plants. Among them, total phenols play a unique role in response to pathogen and nematode invasion. The significant increase in phenol content in the resistant hybrids H 516 and H 531 and the tolerant hybrids, *viz.*, H 511, H 534, H 537, H 571, H 572 and H 589 *vis-à-vis* in susceptible ones. 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 (Goodman *et al.*, 6). Similar results were also observed by Das *et al.* (3) for nematode resistance in banana. The hybrids H 516 and H 531 were found to be resistant and H 511, H 534, H 537, H 571, H 572, and H 589 were found to be tolerant to the lesion nematode, *Pratylenchus coffeae*, when screened under artificially inoculated conditions. Hybrids H 516 and H 531 had the maximum biochemical content and enzyme activity among the hybrids taken for this study. In conclusion, the overall evaluation of 24 parthenocarpic *Musa* hybrids led to identification of the hybrid H 531 and H 516 with increased resistance to *P. coffeae*. However, the performance of these hybrids for yield and quality parameters under field conditions is warranted to exploit them for further breeding programmes.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support of the Flemish office for Development Cooperation and Technical Assistance (VVOB), Belgium and the International Network for the Improvement of Banana and Plantain (INIBAP) obtained through NRC for Banana, Trichy.

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Received: July, 2011; Revised: July, 2013;
Accepted: August, 2013