

Reaction of *Musa* hybrids to *Fusarium* wilt and *Radopholus similis*, burrowing nematode complex

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ABSTRACT

Forty three banana hybrids developed by crossing *Fusarium* and nematode resistant male parents, viz., Pisang Lilin, Anaikomban, Pisang Jari Buaya, Ambalakadali, Rose, H-56, H-201 and Yangambi KM5 with commercial triploid bananas, viz., Karpooravalli, Poovan, Hill banana, Manoranjitham and Rasthali. The resultant hybrids were screened for their reaction to FOC (Race 1) alone and in combination with *Radopholus similis* in pots under glasshouse conditions. When the hybrids were inoculated with FOC alone, H 511, H 516, H 531, H 534, H 537, H 571, H-02-34, H-03-05, H-03-13, H-03-17, H-04-12 and NPH-02-01 were found resistant with a wilt score 1.0. When FOC was inoculated along with *R. similis*, the hybrids H 516 and H 531 recorded a root lesion index of 3.0, a wilt score of 1.0 and rated as resistant to both fungus and nematode. The hybrids H 511, H 534, H 537, H 571, H-02-34, H-03-05, H-03-13, H-03-17, H-04-12 and NPH-02-01 were found to be resistant to FOC and tolerant to *R. similis*. The percent reduction of plant height, plant girth, number of leaves/plant and number of roots/plant after combined inoculation was the lowest in H 531. Polyphenol oxidase, phenylalanine ammonia lyase enzyme activities and total phenols contents in root were higher in H 531 than in the other hybrids. This screening trial indicated that the new banana hybrid H 531 has good combined resistance against FOC and nematodes.

Key words: Banana, *Fusarium*, *Radopholus similis*, pathogen, complex.

INTRODUCTION

Radopholus similis, is amongst the most important nematodes associated with banana and plantain (Seenivasan *et al.*, 13). Banana nematodes attack root and corm tissues causing damage that can result in lengthening of the vegetative growth cycle, production of small bunches, shortened life of the production unit and toppling of the plants. Yield loss in south India alone due to FOC was estimated to 2-90% (Thangavelu *et al.*, 16). Infestation of *R. similis* makes the banana plants highly susceptible to the attack of FOC and it acts as predisposing agent for the entry of FOC and both pathogens cause destructive disease complex in banana (Poornema *et al.*, 9). In commercial banana production, nematicides and fungicides are often used to maintain the productivity all over the world. But the continuous use of nematicides and fungicides has been increasingly affecting the environment and causing various health hazards to both human and animals. Breeding hybrid bananas with nematode and *Fusarium* wilt resistance is an alternate strategy of controlling these pest and disease simultaneously ensuring environmental safety.

The current study was framed to develop resistant hybrids by hybridization of crossing *Fusarium* and nematode resistant male parents. The resistance of newly developed hybrids to *R. similis* and FOC was tested along with a study of their biochemical constituents.

MATERIALS AND METHODS

The present study was undertaken at Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. Unopened anthers, just prior to dehiscence, were collected from the inflorescence and pollen smeared over the surface of receptive stigma of female flowers for production of new hybrids (Rowe and Richardson, 10). Hybridization was attempted by crossing *Fusarium* and nematode resistant male parents. The new *Musa* hybrids obtained were assessed for ploidy level using stomatal density and flow cytometry analysis (Damodaran *et al.*, 2).

The suckers of the hybrids were screened against FOC alone and in combination with *R. similes* in pots (30 × 20 × 18 cm) filled with 4% formaldehyde sterilized pot mixture (red soil: sand: FYM in the ratio of 2:1:1 v/v). Each hybrid were treated by FOC alone and in combination with *R. similis* replicated three times according to completely randomized design

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with each replicate consisting of four suckers. For FOC alone treatment, pot mixture soil was inoculated with FOC at 10 µl of conidial suspension per pot 15 days after planting. For combination treatment, *R. similis* (multiplied by carrot disc culture technique) and *Fusarium* fungus (maintained in sand/maize medium containing 12×10^3 colony forming units/g) were inoculated in the rhizosphere of the plants at 5,000 nematodes/pot and 10 µl of conidial suspension respectively on 15 day after planting. Pots were kept under glasshouse conditions ($30 \pm 2^\circ\text{C}$). After three months, the suckers were drugged out from each of pots and resistance for nematode was assessed on the scale of root lesion index per cent based on the INIBAP guidelines and the wilt score were assessed based on discoloration of corms index (Speijer and De Waele, 14).

The content of the enzymes peroxidase (POX), 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 and FOC damage. The total phenols in the roots were estimated using Folin-Ciocalteu reagent and measuring absorption at 660 nm (Spies, 15). 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 POX activity was assessed according to Hammerschmidt *et al.* (6) and the PPO activity was assessed using the modified method of Mayer *et al.* (7).

RESULTS AND DISCUSSION

A total of 7,550 crosses were performed which resulted in the production of 2,026 seeds, of which 122 germinated. Forty three parthenocarpic hybrids were selected for further evaluation (Table 1). Poor fertility and poor viability of seeds is common in hybridization. This may be due to the presence of structural hybridity and chromosomal aberrations (Dodds, 4). Analysis of hybrids for inheritance revealed that resistant diploid, triploid and tetraploid hybrids had either Pisang Lilin or Anaikomban as one of the parents. However, hybrid H-03-13 exhibited resistance despite the fact that both parents used were susceptible. Recovery of resistance genes from a (susceptible x susceptible) combination may be due to the transgressive segregation of the hybrids because of the heterozygous nature of the parents used in crossing (Ortiz and Vuylsteke, 8). When the hybrids were inoculated with FOC alone, H 511, H

516, H 531, H 534, H 537, H 571, H-02-34, H-03-05, H-03-13, H-03-17, H-04-12 and NPH-02-01 were found resistant with a wilt score 1.0. When FOC was inoculated along with *R. similis*, the hybrids H 516 and H 531 recorded a root lesion index of 3.0, a wilt score of 1.0 and rated as resistant to both fungus and nematode. The hybrids H 511, H 534, H 537, H 571, H-02-34, H-03-05, H-03-13, H-03-17, H-04-12 and NPH-02-01 were found to be resistant to FOC and tolerant to *R. similis*. The lesser lesion index in H 516 and H 531 may be due to less nematode population due to lesser multiplication rate as suggested by Das *et al.* (3). Since, the less entry points existed in these hybrids made simultaneously resistance to FOC.

Peroxidase activity in susceptible hybrids was nearly two to three times less than that of resistant hybrids under control (Table 2). Compared to control, all the plants exhibited higher peroxidase activity when inoculated with *Fusarium*. In case of combined inoculation also, a similar trend was observed. The maximum increase in peroxidase activity was recorded in H 531 in both the treatments (49.10% under *Fusarium* and 52.70% under *Fusarium* + nematodes inoculated) and lowest increase in peroxidase activity was recorded in H-02-19 in both the treatments (3.13% under *Fusarium* and 7.03% under *Fusarium* + nematodes inoculated). The resistance hybrids showed at least two to three times higher polyphenol oxidase activity than the susceptible hybrids. The hybrid H 531 recorded the maximum polyphenol oxidase activity in *Fusarium* (324.74 abs/min/g) and *Fusarium* + nematode (386.60 abs/min/g) conditions. However, the minimum was noticed in H-03-16. Under *Fusarium* and *Fusarium* + nematodes inoculated treatments increase in phenylalanine ammonia lyase activity was observed. The hybrids H 531 registered the maximum of 43.96 per cent for *Fusarium* inoculated while, the hybrid H-03-13 registered the maximum of 50.07 per cent for *Fusarium* + nematodes treated.

For total phenols, H 531 registered the maximum per cent increase of 34.25 over control under *Fusarium* inoculated, whereas, 36.70 per cent by the hybrid H 572 under *Fusarium* + nematode inoculated. Enzyme activity is one of the important tools to confirm the resistance to root pathogens. 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 (Seenivasan, 11). Many of these proteins are enzymes such as phenylalanine ammonia lyase, polyphenol oxidase, peroxidase and β -1-3 glucanase. 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, 11).

Table 1. Genome characteristics and reaction of banana hybrids to FOC and *Radopholus similis*.

Hybrid	Parentage	Genome	BW (g)	Reaction to FOC		Reaction to FOC + <i>R. similis</i>		
				Wilt score	Status	Lesion index	Wilt score	Status
H 504	H-03-09 × PL	AAABB	4.50	2	S	40	3	S
H 508	ANK × PL	AA	2.50	3	S	47	3	S
H 511	H-02-34 × Ykm-5	AABB	9.50	1	R	8	2	S
H 515	Mano × ANK	AAA	6.00	2	S	35	3	S
H 516	ANK × PL	AA	7.50	1	R	3	1	R
H 529	H-03-16 × ANK	AABB	4.00	2	S	31	3	S
H 530	H-03-13 (OP)	AABB	8.00	2	S	34	3	S
H 531	Poovan × PL	AAB	12.50	1	R	3	1	R
H 532	H-201 × Mano	AAB	1.50	2	S	40	2	S
H 534	H-03-13 × Rose	AAB	8.50	1	R	10	2	S
H 537	(H-201 × P) × Rose	AABB	11.00	1	R	9	2	S
H 540	(H-201 × PK) × Rose	AAABB	7.00	2	S	32	2	S
H 542	H-02-34 × ANK	AABB	8.50	2	S	28	2	S
H 547	H-02-23 (OP)	AABB	5.00	3	S	30	3	S
H 548	H-02-23 (OP)	AABB	5.00	3	S	41	3	S
H 556	H-04-06 × Ykm-5	AABB	6.50	3	S	35	3	S
H 563	H-201 × PL	AB	1.50	3	S	30	3	S
H 564	H-201 × PL	AB	2.00	3	S	29	3	S
H 571	H-04-05 × Ykm-5	AABB	8.00	1	R	7	2	S
H 572	H-03-35 (OP)	AAB	7.00	2	S	9	2	S
H 573	H-03-12 × Rose	AAABB	6.50	2	S	39	3	S
H 576	H-201 (OP)	AB	1.50	2	S	31	3	S
H 579	Mano × Rose	AA	6.00	2	S	33	3	S
H 589	H-03-19 (OP)	AABB	15.00	2	S	11	2	S
H-02-19	KAR × RED	AABB	13.00	3	S	42	4	S
H-02-23	KAR × RED	AABB	14.50	3	S	47	4	S
H-02-26	KAR × RED	AABB	17.00	3	S	39	4	S
H-02-34	KAR × RED	AABB	12.50	1	R	17	2	S
H-03-05	Peykunnan (OP)	AABB	11.50	1	R	18	2	S
H-03-06	H-02-32 × PL	AB	9.00	3	S	22	3	S
H-03-13	Peykunnan × EV	AABB	15.50	1	R	12	2	S
H-03-16	Peykunnan × PL	AABB	9.50	1	R	47	2	S
H-03-17	Peykunnan × PL	AABB	12.50	3	S	8	3	S
H-03-19	Peykunnan × EV	AABB	17.50	3	S	31	3	S
H-04-05	H-02-32 × PL	AABB	5.50	3	S	31	3	S
H-04-06	H-02-32 × PL	AABB	18.50	2	S	30	3	S
H-04-10	Peykunnan (OP)	AAB	13.50	3	S	51	3	S
H-04-12	Pisang Sabax PL	AABB	22.50	1	R	8	2	S
H-04-21	H-02-10 × PL	AAB	8.00	3	S	32	2	S
H-04-24	Peykunnan (OP)	AABB	15.00	2	S	17	2	S
NPH-02-01	H 201 × ANK	AAB	17.50	1	R	7	2	S
H-510	Poovan (OP)	AABB	14.50	2	S	17	2	S
H-531	Poovan × PL	AAB	13.50	1	R	5	1	S

PL = Pisang Lilin; ANK = Anaikomban; Mano = Manoranjitham; EV = Erachi Vazhai; OP = Open pollinated; KAR = Karpooravalli; RED = Red banana; R = Resistance; S = Suscetible; BW = Bunch weight; FOC = *Fusarium oxysporum* f. sp. *cabense*

Table 2. Reaction of banana hybrids to FOC and *Radopholus similis*.

Hybrid	Peroxidase activity (abs/min/g)			Polyphenol oxidase (abs/min/g)			Phenylalanine ammonia lyase (nmol/min/ml)			Total phenols (µg/ g)		
	Uninoculated	FOC	FOC + <i>R. similis</i>	Uninoculated	FOC	FOC + <i>R. similis</i>	Uninoculated	FOC	FOC + <i>R. similis</i>	Uninoculated	FOC	FOC + <i>R. similis</i>
H 504	1.26	1.31	1.35	0.045	0.048	0.053	13.00	15.50	15.57	260.65	276.27	277.59
H 508	1.21	1.27	1.29	0.050	0.057	0.062	12.50	14.62	14.66	238.75	246.38	247.58
H 511	1.98	2.39	2.56	0.083	0.236	0.239	15.46	21.56	21.59	288.63	351.75	366.95
H 515	1.16	1.23	1.26	0.047	0.097	0.099	14.50	15.79	15.82	217.38	231.31	232.54
H 516	2.16	2.86	2.97	0.099	0.376	0.488	16.65	23.96	24.58	338.76	398.50	399.89
H 529	1.31	1.39	1.42	0.048	0.089	0.093	12.70	18.93	13.95	201.43	216.47	218.93
H 530	1.42	1.48	1.50	0.057	0.072	0.090	13.66	14.86	14.71	237.70	253.88	256.86
H 531	2.19	2.76	2.85	0.097	0.412	0.472	17.82	25.31	25.48	340.55	457.19	461.86
H 532	1.37	1.42	1.41	0.056	0.086	0.093	13.45	15.62	14.67	196.26	231.36	232.92
H 534	2.07	2.68	2.72	0.085	0.025	0.257	15.67	21.65	21.68	292.75	386.57	394.43
H 537	2.10	2.62	2.81	0.082	0.236	0.242	15.89	22.60	22.21	301.56	391.35	398.85
H 540	1.40	1.47	1.49	0.055	0.087	0.089	10.42	12.10	12.12	230.67	276.56	278.79
H 542	1.35	1.44	1.46	0.066	0.082	0.087	11.67	13.35	12.34	220.35	250.68	253.76
H 547	1.27	1.36	1.39	0.065	0.093	0.096	10.93	12.86	11.89	218.83	236.50	239.62
H 548	1.42	1.51	1.54	0.058	0.095	0.088	11.67	18.78	12.81	295.76	223.78	224.75
H 556	1.37	1.46	1.49	0.067	0.089	0.092	12.93	13.85	13.87	287.75	215.36	218.76
H 563	1.23	1.38	1.33	0.043	0.078	0.083	10.25	12.28	11.80	168.79	179.55	182.93
H 564	1.16	1.24	1.27	0.039	0.085	0.087	9.26	11.96	12.12	176.75	189.38	192.45
H 571	2.12	2.68	2.79	0.091	0.215	0.226	15.82	21.56	21.58	282.87	373.76	381.85
H 572	2.09	2.63	2.75	0.095	0.226	0.231	16.55	22.89	22.92	289.50	382.73	395.76
H 573	1.35	1.40	1.53	0.053	0.086	0.091	12.69	14.83	14.86	176.81	193.33	195.76
H 576	1.40	1.49	1.54	0.049	0.095	0.098	11.53	13.56	13.57	143.37	158.74	159.82
H 579	1.43	1.53	1.55	0.058	0.088	0.093	12.86	14.76	14.78	227.63	293.95	295.26
H 589	2.17	2.67	1.78	0.089	0.225	0.227	17.56	22.17	22.22	332.55	385.35	393.38
H-02-19	1.28	1.32	1.37	0.054	0.069	0.083	9.56	10.79	10.99	258.93	269.53	270.85
H-02-23	1.23	1.29	1.36	0.063	0.078	0.087	8.92	9.88	9.95	267.54	283.75	285.85
H-02-26	1.34	1.40	1.48	0.057	0.077	0.081	10.46	11.85	11.91	253.56	264.85	270.63

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Hybrid	Peroxidase activity (abs/min/g)			Polyphenol oxidase (abs/min/g)			Phenylalanine ammonia lyase (nmol/min/ml)			Total phenols (µg/g)		
	Uninoculated	FOC	FOC + <i>R. similis</i>	Uninoculated	FOC	FOC + <i>R. similis</i>	Uninoculated	FOC	FOC + <i>R. similis</i>	Uninoculated	FOC	FOC + <i>R. similis</i>
H-02-34	1.84	2.58	2.67	0.084	0.138	0.267	15.47	19.57	19.85	295.76	352.17	365.27
H-03-05	1.85	2.69	2.75	0.097	0.149	0.285	15.82	19.68	20.94	329.28	384.56	375.84
H-03-06	1.16	1.35	1.39	0.039	0.041	0.044	9.52	10.39	10.55	215.42	228.67	236.28
H-03-13	2.10	2.76	2.87	0.088	0.112	0.113	14.56	18.76	21.85	288.76	328.19	332.25
H-03-16	0.96	1.21	1.28	0.022	0.023	0.024	9.76	10.77	10.83	138.26	142.29	149.28
H-03-17	1.66	2.27	2.38	0.076	0.249	0.358	14.53	19.83	20.95	401.20	478.56	483.76
H-03-19	1.42	1.58	1.76	0.061	0.078	0.085	9.74	10.89	10.93	250.49	258.75	263.35
H-04-05	1.46	1.60	1.65	0.058	0.068	0.073	8.55	9.83	9.84	275.50	287.74	289.93
H-04-06	1.39	1.53	1.62	0.067	0.079	0.085	9.72	10.86	10.92	268.35	282.53	287.79
H-04-10	0.59	0.74	0.86	0.049	0.066	0.068	9.83	10.95	10.98	256.79	264.37	265.82
H-04-12	2.13	2.53	2.65	0.086	0.238	0.241	14.67	18.97	20.22	310.68	354.62	357.35
H-04-21	1.45	1.66	1.78	0.050	0.069	0.078	10.52	11.78	11.89	276.36	289.45	293.75
H-04-24	2.11	2.53	2.67	0.122	0.243	0.335	15.50	20.85	21.93	258.65	324.16	327.55
NPH-02-01	1.87	2.16	2.28	0.119	0.218	0.323	14.96	18.80	19.92	337.49	393.77	395.79
H-510	1.78	2.28	2.32	0.099	0.139	0.255	14.52	18.72	19.84	263.44	283.56	289.78
H-531	2.22	3.31	3.39	0.142	0.478	0.479	16.72	21.82	22.89	338.75	426.85	434.94
Pisang Lilin	2.24	2.57	2.64	0.123	0.437	0.448	16.70	22.26	23.37	325.70	398.55	399.68
Rasthali	0.54	0.68	0.72	0.042	0.065	0.067	8.52	9.25	9.26	128.54	139.52	140.29
CD _{0.05}	0.231	0.261	0.264	0.021	0.024	0.067	3.086	4.189	4.421	41.555	49.803	50.072

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. Enhanced peroxidase activity has been associated with hybrids resistant to both *Fusarium* wilt (Damodaran *et al.*, 2) and nematodes (Seenivasan *et al.*, 12). Polyphenol oxidase (PPO) oxidises 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 *Fusarium* wilt and nematodes. A critical analysis of their activity within hybrids reveals that the FOC + nematode resistant hybrids, viz., H 516 and H 531 and the FOC alone resistant/tolerant hybrids, viz., H 511, H 534, H 537, H 571, H 572, H 589, H-02-34, H-03-05, H-03-13, H-03-17, H-04-12, H-04-24 and NPH-02-01 recorded higher peroxidase and poly phenol oxidase activity than the susceptible ones. Out of all the hybrids, H 516, H 531, H 537, H 589, H-03-05, H-04-12, H-04-24 and NPH-02-01 had higher peroxidase and polyphenol oxidase activity. Similar finding were earlier reported in banana by Das *et al.* (3). Total phenols play a unique role in response to pathogen and nematode invasion. The results of the present study revealed a significant increase in phenol content in hybrids, viz., H 516, H 531, H 511, H 537, H 571, H 572, H 589, H-02-34, H-03-05, H-03-13, H-03-17, H-04-12, H-04-24 and NPH-02-01 *vis-à-vis* in others. The accumulation of phenols may be due to the excess production of hydrogen peroxide by increased respiration (Seenivasan, 11) or due to the activation of hexose monophosphate (HMP) shunt pathway, acetate pathway and release of bound phenols by hydrolytic enzymes (Seenivasan *et al.*, 12). The overall evaluation of 43 parthenocarpic *Musa* hybrids led to identification of the hybrid H 531 with high yield potential as well as increased resistant to both FOC and *R. similis*.

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, Thiruchirappalli.

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Received: November, 2012; Revised: November, 2013;
Accepted: December, 2013.