

REACTION OF *MUSA* HYBRIDS TO *PRATYLENCHUS COFFEAEE* AND THEIR DIFFERENTIAL BIOCHEMICAL RESPONSES TO NEMATODE INFECTION

T. Damodaran^{1*}, N. Kumar², K. Poornima² and M. Kavino²

¹Division of Horticulture and Forestry, Central Agricultural Research Institute, Port Blair-744 101, India

²Department of Fruit Crops, Tamil Nadu Agricultural University, Coimbatore-641 001, India

Summary. Twenty-five hybrids, obtained by crossing six resistant diploids with commercial triploid bananas, were screened for their reaction to the root lesion nematode *Pratylenchus coffeae* in pots under glass-house conditions. Based on lesion indices of roots and rhizomes, the hybrids H-02-34, H-02-07, H-02-08, H-02-21, H-02-25, H-02-31 and NPH-02-01 were rated tolerant to the nematode. Among the tolerant hybrids H-02-34 and NPH-02-01 (AAB) appeared the most promising. The changes in root content of peroxidase (PO), polyphenol oxidase (PPO), and phenylalanine ammonia lyase (PAL) and lignin were also investigated. Relatively higher enzyme activity was observed in tolerant hybrids than in susceptible ones. An isozyme analysis of PPO revealed the induction of specific isoforms in the leaves of tolerant hybrids inoculated with the nematode.

Key words: Banana, enzyme activity, *Musa* sp., tolerance.

Banana production in India is severely threatened by many species of nematodes, including lesion producing nematodes such as *Radopholus similis* Cobb and *Pratylenchus coffeae* (Zimmermann) Goodey (Sarah *et al.*, 1992; Rajendran *et al.*, 1980) that are considered of economic importance. Sundararaju (1996) reported that *R. similis* caused severe root rotting, resulting in about 25-35% reduction in yield. Thus, the foremost aim in banana and plantain improvement is to enhance the quantitative and qualitative traits besides developing hybrids with increased resistance/tolerance to major nematode pests (Kumar and Soorianatha Sundaram, 2002). Previous screenings carried out at Tamil Nadu Agricultural University, India, resulted in the identification of potential nematode tolerant diploid bananas such as Anaikomban (AA), Matti (AA), Namarai (AA) and Pisang Lilin (AA) (Sathiamoorthy and Balamohan, 1993). These hybrids were thereafter crossed with commercial triploids to develop primary tetraploids/secondary triploids with good horticultural traits, including resistance to nematodes (Damodaran, 2003). Root histology of banana showed that resistant cultivars possess greater numbers of pre-formed phenolic cells in the root cortex with higher biochemical contents (Fogain, 1996; Collingborn *et al.*, 2000). The presence of a thick lignified and/or a suberized layer in the endodermis also restricts nematode penetration in the roots of banana clone Yangambi Km5 (Valette *et al.*, 1997).

Therefore, a screening of these new banana hybrids was conducted to assess their responses to *P. coffeae* and

to confirm the resistance/tolerance by assessing the content of peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), lignin and isozymes of polyphenol oxidase in the roots under pot conditions.

MATERIALS AND METHODS

Assessment of nematode response

The suckers were obtained from nematode-free banana hybrids and parents and tested in pots (60 × 45 cm) filled with a pot mixture (red earth, FYM and topsoil in the ratio of 1:1:2, respectively) sterilized by 4% formaldehyde. The plants were fertilized with Hoagland's No. 2 nutrient solution fortnightly. The population of the lesion nematode *P. coffeae* was maintained in 5 kg pots containing sterilized pot mixture and planted with the susceptible banana cultivar Robusta. Extraction and counting of the nematodes from the roots of the affected plants were done as per methods of Carlier *et al.* (2002). Five thousand nematode specimens were inoculated in the rhizosphere of the hybrids by the soil injection method (Sarah *et al.*, 1992). All hybrids screened for resistance were allowed to grow for 90 days in a glass-house at 25-27 °C. Then the length of five selected functional roots was reduced to 10 cm and the roots were sliced lengthwise. Scoring for migratory endoparasitic nematodes was carried out on one half of each of the five roots by assessing the percentage of root cortex showing necrosis. The maximum root necrosis score per root half was 20 per cent, giving a maximum root necrosis of 100 per cent for the five root halves added together. Necrosis of the individual roots was recorded as RN1 to RN5 and the sum was the total root necrosis of the sample (TOT RN).

* Corresponding author: Email: damhort2002@yahoo.com; kumarhort@yahoo.com

Table I. Orientative scale to assess the reaction of bananas to root lesion nematodes according to Pinochet (1988).

Plant response	Lesion index	
	Roots (%)	Rhizome
Immune	0	0
Resistant	< 10	< 1
Tolerant	10-20	1-2
Susceptible	20-40	2-4
Highly susceptible	> 40	>4

The corm was cleaned thoroughly with water. The number of root bases (RB) on the selected outward half of the corm was counted and scored for nematode related damage, which appears as blackish-purple lesions around the root bases and was scored as: 0 = no lesions; 1 = one small lesion, 2 = several small lesions, 3 = one large lesion, and 4 = several large lesions.

To estimate the population of the nematodes, the roots of the inoculated plants were washed free of soil, dried by wrapping in absorbent tissue and cut into pieces of 1 cm and weighed. Then, a sub-sample of 15 g of roots per replicate was put into 100 ml of distilled water in a kitchen blender and macerated 3 times for 10 sec and sieved through 250-106-40 μm sieves. The nematodes from the 40- μm sieve were collected in a beaker and made to a standard volume of 200 ml. The suspension was agitated with a pipette and 6 ml taken in a counting dish and nematodes counted under a stereomicroscope (Carrier *et al.*, 2002).

Assessment of biochemical changes

The content of the enzymes peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL), and the content of phenols and lignin 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 Ciocalteu reagent and measuring absorption at 660 nm in a spectrophotometer, and is expressed as mg/g root (Spies, 1955). The lignin content in roots was gravimetrically estimated following the method of Chesson (1978), using a mixture of 5 ml of concentrated H_2SO_4 and 50 ml of HCl for 16 hr at 25 °C in a shaker, and expressed as a percentage by weight.

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 homogenate was centrifuged for 20 min at 8000 g. 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 Hammer-

schmidt *et al.* (1982). The reaction mixture (3 ml) consisted of 0.25% v/v guaiacol in 10 mM potassium phosphate buffer (pH 6.9) containing 10 mM hydrogen peroxide.

The PPO activity was assessed using the modified method of Mayer *et al.* (1965), using 0.1 M phosphate buffer (pH 6.5) and 0.01 N catechol along with enzyme extract.

The PAL assay was conducted according to the method described by Ross and Sederoff (1992), using 500 μl of borate buffer and 600 μl of 12 mM L-phenylalanine along with 400 μl enzyme extract as reaction mixture.

Isozyme analysis of the enzyme PO. 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 homogenate was centrifuged for 20 min at 8000 g. The supernatant was used as crude enzyme extract for PO. For native anionic polyacrylamide gel electrophoresis, resolving gels of 8% acrylamide concentration and stacking zones of 4% acrylamide concentration were prepared. For estimation of PO, the gels were incubated in darkness in a solution containing 100 mg benzidine powder (dissolved in 0.5 ml of acetone) in 50 ml of acetate buffer for a period of 30 minutes after electrophoresis. Drops of 30% H_2O_2 were added with constant shaking till the bands appeared (Sindhu *et al.*, 1984).

Statistical design and analysis

Each hybrid was replicated three times according to a completely randomized design with each replicate consisting of four suckers. For enzyme assay, four samples from the roots of each hybrid were used for the analyses.

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

Assessment of nematode resistance under pot conditions

The proportion of root bases with lesions caused by *P. coffeae* varied from 8.7 to 70% among the hybrids and from 0.01 to 48.1% among the parents (Table II). Variation was also observed in the corm lesion index among hybrids (1.5 to 4) and parents (0 to 4). There was a considerable variation of the nematode population among the genotypes. The population level of the hybrids scored as tolerant ranged from 6,510 to 10,110 nematode specimens per 15 g of roots, and from 10,000 to 12,800 among the hybrids scored as susceptible. Among the hybrids, the smallest population (6,510) was recorded in H-02-34 and the largest in H-02-29 (12,800). The tolerant hybrids identified from the parents may provide unique genes for tolerance in combination with more acceptable agronomic characters. Based on this concept of tolerance, the hybrids H-02-34 and NPH-02-01 were scored as tolerant. These hybrids

had fewer nematode specimens per 15 g of roots than the susceptible cultivars and also registered the lowest root and corm lesion indices.

The hybrid H-02-34, tolerant to *P. coffeae* (Table II), also had a good bunch weight of 13.5 kg and produced a good number of functional roots and root bases with

Table II. Reaction level of banana hybrids and their parents to *Pratylenchus coffeae* in pots.

Hybrid	Parents	Genome	Corm lesion index	Root lesion index	Nematodes in 15g roots	Reaction level	Bunch weight (kg)
H-02-06	H 201 × AN	AB	4.00	87.50	12000	HS	4.50
H-02-07	H 201 × AN	AB	2.00	20.50	9890	T	5.00
H-02-08	H 201 × EV	AAB	1.50	15.00	9000	T	10.00
H-02-09	H 201 × PL	AB	3.00	26.50	11000	S	6.40
H-02-10	H 201 × H 110	AB	2.00	22.50	10200	S	6.75
H-02-11	H 201 × H 110	AB	2.50	22.50	10000	S	3.60
H-02-15	H 201 OP*	AB	3.00	29.00	11500	S	5.37
H-02-17	KV × PL	AABB	2.50	36.00	12000	HS	9.25
H-02-18	KV × PL	AABB	3.50	46.50	12500	HS	8.65
H-02-19	KV × EV	AABB	2.50	21.50	10900	S	8.50
H-02-20	KV × Red	AABB	4.00	47.50	11800	HS	8.85
H-02-21	KV × Red	AABB	2.50	19.50	9250	T	12.53
H-02-23	KV × Red	AABB	3.50	39.00	11950	HS	7.50
H-02-25	KV × Red	AABB	2.50	15.50	10000	T	6.57
H-02-26	KV × Red	AABB	4.00	31.50	12350	HS	9.25
H-02-27	KV × Red	AABB	3.50	29.50	11000	S	10.00
H-02-28	KV × Red	AABB	4.00	41.00	11950	HS	7.83
H-02-29	KV × Red	AABB	3.00	43.50	12800	HS	9.67
H-02-30	KV × Red	AABB	3.50	54.50	12315	HS	12.00
H-02-31	KV × Red	AABB	2.50	17.50	10110	T	10.75
H-02-32	KV × Red	AABB	2.50	24.50	11120	S	7.65
H-02-34	KV × Red	AABB	1.50	6.50	6510	T	13.50
H-02-36	KV × Rob	AABB	3.50	27.00	10110	S	9.50
NPH-02-01	H201 × AN	AAB	1.50	14.50	8350	T	13.00
NPH-02-02	H201 × AM	AB	2.50	26.50	11000	S	5.25
Parents							
H201		AB	0.00	4.50	7000	R	2.10
Anaikomban (AN)		AA	1.00	11.00	10650	T	5.60
Ambalakadali (AM)		AA	1.00	19.00	10800	T	9.25
Erachi Vazhai (EV)		AA	1.00	17.50	11000	T	4.27
Pisang Lilin (PL)		AA	0.50	15.50	10900	T	3.55
H110		AB	2.00	29.00	11250	S	2.82
Karpooravalli (KV)		ABB	2.00	17.00	10000	T	12.90
Red Banana (Red)		AAA	3.00	38.00	12250	HS	7.97
Robusta (Rob)		AAA	4.00	54.00	12650	HS	21.17
	CD (0.05%)		1.010	4.26	45.383		2.14
	SEd		0.496	0.29	22.321		1.05

Each figure is the average of three replicates.

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

*OP = Open pollinated.

few root lesions. Gowen (1993) reported that some tetraploids bred in Jamaica may develop larger root systems and show a lower level of susceptibility. Earlier screening had also indicated the resistance of hybrid H-02-34 to *Radopholus similis* (Krishnamoorthy, 2002), so it would be a good potential female parent in breeding for tolerance to nematodes in banana.

The hybrids H-02-07, H-02-08, H-02-21, H-02-25, H-02-31 and NPH-02-01 showed tolerance to the nematodes. Hybrid NPH-02-01 showed the highest level of tolerance with the lowest lesion index (14.5%). This combination of characters may be due to incorporation of a resistance gene from the parent H201 and a tolerance gene from the cultivar Anaikomban (Table II).

Resistant \times tolerant crosses produced both tolerant and susceptible hybrids, suggesting that resistance/tolerance to nematodes is under polygenic control. 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 nematode.

The hybrids NPH-02-01 (13 kg of bunch weight) and H-02-08 (10 kg of bunch weight) had the lowest susceptibility indices, achieving scores of tolerant (Table II). The tolerance in these hybrids may be attributed to additive effect of the triploid genomes (AAB), which had inherited the entire genome of AB from the resistant diploid H 201 and the tolerant 'A' genome from Anaikomban or Erachi Vazhai, respectively.

Role of biochemical markers in imparting tolerance to nematodes

Among the various enzymes, peroxidase (PO) is considered as one of the important defence related enzymes. The enzyme activity of PO (Table III) varied considerably and ranged from 8.4 to 16.45 $\Delta A_{470} \text{ min}^{-1} \text{ g}^{-1} \text{ fw}$ in tolerant hybrids, with the exception of H-02-31 (3.85 $\Delta A_{470} \text{ min}^{-1} \text{ g}^{-1} \text{ fw}$), and from 2.79 to 9.52 $\Delta A_{470} \text{ min}^{-1} \text{ g}^{-1} \text{ fw}$ among susceptible hybrids. Within the tolerant hybrids, NPH-02-01 and H-02-34 registered higher peroxidase activity than their parents. Enzyme assay studies showed that time-course pattern of peroxidase accumulation was different in resistant and susceptible pearl millet seedlings (Shivakumar *et al.*, 2003). The role of peroxidases has been cited in a number of reports on defence mechanisms against invading pathogens, such as the hypersensitive response (Levine *et al.*, 1994). Polyphenol oxidase (PPO) activity varied from 0.36 to 1.10 $\Delta A_{495} \text{ min}^{-1} \text{ g}^{-1} \text{ fw}$ among tolerant hybrids and from 0.11 to 0.62 $\Delta A_{495} \text{ min}^{-1} \text{ g}^{-1} \text{ fw}$ among susceptible hybrids. 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). Phenylalanine ammonia lyase (PAL) activity ranged from 0.00636 to 0.1235 $\text{nmol min}^{-1} \text{ g}^{-1}$ in tolerant hybrids and from 0.00122 to 0.00425 $\text{nmol min}^{-1} \text{ g}^{-1}$ in highly susceptible hybrids. The hybrids NPH-02-01, H-02-34 H-02-31, H-02-25 and H-02-21 showed

higher PAL activity than their respective parents. Earlier reports, from our laboratory, on the involvement of phenylalanine ammonia lyase in banana/nematode interactions, recorded increased activity in highly tolerant varieties and decreased enzyme activity in highly susceptible varieties (Krishnamoorthy, 2002).

Besides phytoalexins, most plants synthesize toxic compounds such as phenols and lignin as part of normal development, and they are called phytoanticipins (Van Eten *et al.*, 1995). There were significant differences between the susceptible hybrids and the tolerant hybrids. Phenol content ranged from 490.6 $\mu\text{g g}^{-1}$ (H-02-25) to 722.37 $\mu\text{g g}^{-1}$ (H-02-34) among tolerant hybrids and from 213.91 $\mu\text{g g}^{-1}$ (H-02-20) to 385.27 $\mu\text{g g}^{-1}$ (H-02-17) among highly susceptible hybrids. Earlier studies of phenolic activity showed higher accumulation in resistant and lower in susceptible banana varieties (Krishnamoorthy, 2002). The accumulation of phenol may be due to the excess production of hydrogen peroxide by increased respiration (Farkas and Kirilay, 1962). However, in our study significant differences in phenol content were observed only between the highly susceptible hybrids and the most tolerant ones (H-02-21, H-02-34, H-02-25, NPH-02-01). The increased synthesis of phenols, followed by their oxidation by the higher PPO activity, would have helped to increase conversion of them into polymers such as lignin. The lignin content varied from 0.35 to 1.42% in tolerant hybrids but from 0.29 to 0.41% in highly susceptible hybrids. Tolerant plants, when subjected to biotic stress, showed elevated levels of free phenolics and contained more lignin (Kavino *et al.*, 2007). An increase in the number of lignified cells in tolerant cultivars compared to susceptible cultivars of banana had already been reported (Fogain, 1996), and their role in tolerance mechanisms has also been reported (Sariah *et al.*, 1999; Reuveni and Karchi, 1987).

Isozyme analysis

The induction pattern of PO in banana hybrids inoculated with *Pratylenchus coffeae* revealed five isoforms, PO1, PO2, PO3, PO4 and PO5 (Fig. 1). The isoform

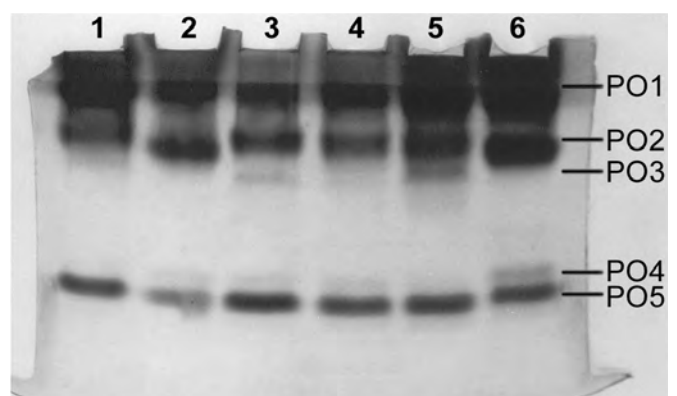


Fig. 1. Peroxidase activity in roots of banana hybrids and parents inoculated with *Pratylenchus coffeae* in pots. 1: Red Banana; 2: H-02-25; 3: NPH-0201; 4: H-02-26; 5: H-02-34; 6: H-02-15.

PO3 was expressed only in tolerant hybrids H-02-34 and NPH-02-01. These results suggest the possible involvement of acidic isoforms of PO in the host response, as reported by Miyazawa *et al.* (1998).

In conclusion, the overall evaluation of the 25 hybrids led to the identification of the hybrids H-02-34 and NPH-02-01 as having high yield potential and tolerance to nematodes. NPH-02-01 is a triploid (AAB) hybrid almost

Table III. Enzyme activities, phenol content and lignin percent in the roots of phase II banana of hybrids and parents inoculated with *P. coffeae* in pots.

Hybrid	Reaction level	Peroxidase ($\Delta A_{470} \text{ min}^{-1} \text{ g}^{-1} \text{ fw}$)	Polyphenol oxidase ($\Delta A_{495} \text{ min}^{-1} \text{ g}^{-1} \text{ fw}$)	PAL ($\text{nmol min}^{-1} \text{ g}^{-1}$)	Total Phenols ($\mu\text{g g}^{-1}$)	Lignin (%)
H-02-19	S	8.79	0.59	0.00645	583.70	0.90
H-02-36	S	4.02	0.41	0.00645	421.88	0.80
NPH-02-02	S	6.48	0.54	0.00730	510.50	0.84
H-02-09	S	6.44	0.58	0.00547	520.50	0.97
H-02-10	S	7.59	0.62	0.00747	607.65	1.05
H-02-11	S	9.52	0.55	0.00788	619.07	1.13
H-02-15	S	5.25	0.22	0.00312	457.00	0.55
H-02-32	S	4.22	0.46	0.00525	414.36	0.53
H-02-27	S	3.15	0.21	0.00490	347.58	0.35
H-02-06	HS	2.79	0.11	0.00122	211.00	0.29
H-02-30	HS	4.11	0.34	0.00144	267.23	0.31
H-02-17	HS	4.88	0.31	0.00300	385.27	0.60
H-02-18	HS	3.84	0.26	0.00250	292.45	0.33
H-02-20	HS	4.14	0.28	0.00334	213.91	0.33
H-02-26	HS	5.05	0.25	0.00425	363.85	0.39
H-02-23	HS	3.85	0.21	0.00227	275.54	0.33
H-02-28	HS	3.00	0.35	0.00344	312.87	0.37
H-02-29	HS	3.45	0.31	0.00280	365.15	0.41
H-02-07	T	8.40	0.36	0.00757	533.00	0.73
H-02-08	T	9.59	0.75	0.00855	644.91	1.24
H-02-21	T	12.70	0.61	0.00636	555.60	1.42
H-02-34	T	13.05	0.86	0.01160	722.37	0.35
H-02-25	T	11.65	0.85	0.00800	490.65	0.63
NPH-02-01	T	16.45	1.10	0.01235	656.74	1.27
H-02-31	T	3.85	0.64	0.00912	686.00	0.97
Parents						
H201	R	6.23	0.87	0.01135	605.00	1.32
Anaikomban	T	6.23	0.72	0.01020	636.50	1.30
Ambalakadali	T	6.00	0.86	0.00957	606.50	1.20
Erachi Vazhai	T	8.38	0.57	0.00818	738.00	1.24
Pisang Lilin	T	12.7	0.97	0.01227	745.50	1.59
H110	S	3.37	0.45	0.00338	313.50	0.85
Karpooravalli	T	4.41	0.30	0.00345	440.00	1.02
Red Banana	HS	3.55	0.22	0.00206	139.50	0.53
Robusta	HS	4.05	0.26	0.00211	229.50	0.61
CD (0.05%)		0.448	0.036	0.0006	4.412	0.122
SEd		0.220	0.018	0.0003	8.975	0.060

Each figure is the average of three replicates.

akin to Pome group in terms of bunch traits. This hybrid needs to be tested in the field in areas where the nematodes are endemic to assess its yield potential. The female and male fertility of this hybrid offers further scope for improvement. Of the other hybrids, H-02-34 is a tetraploid AABB with female fertility and H-03-08 is a triploid that can be backcrossed with tolerant diploids, such as Anaikomban and Pisang Lilin, to produce secondary triploids.

LITERATURE CITED

- Carlier J., De Waele D. and Escalant J.V., 2002. Global evaluation of *Musa* germplasm for resistance to *Fusarium* wilt and *Mycosphaerella* leaf spot diseases and nematodes, INIBAP Technical guidelines (Vézina A. and Picq C., eds). INIBAP, Montpellier, France, pp. 68.
- Chesson A., 1978. The maceration of line flax under anaerobic condition. *Journal of Applied Bacteriology*, 45: 219-230.
- Collingborn F.M.B., Gowen S.R. and Mueller-Harvey I., 2000. Investigations on the biochemical basis for nematode resistance in roots of three *Musa* cultivars in response to *Radopholus similis* infection. *Journal of Agriculture, Food and Chemistry*, 48: 5297-5301.
- Damodaran T., 2003. Breeding of banana (*Musa* spp.) for resistance to *Fusarium* wilt and nematodes. Ph.D. Thesis. Tamil Nadu Agricultural University, Coimbatore, India, pp. 65-68.
- Farkas G.L. and Kirlyay Z., 1962. Role of phenolic compounds in the physiology of plant disease resistance. *Phytopathology*, 44: 105-150.
- Fogain R., 1996. Evaluation of *Musa* spp. for susceptibility to nematodes and study of resistance mechanisms. *Acta Horticulturae*, 540: 215-224.
- Gowen S.R., 1993. Possible approaches for developing nematode resistance in bananas and plantains. Pp. 123-128. *In: Breeding banana and plantain for resistance to diseases and pests* (Gany J., ed.). INIBAP, Montpellier, France.
- Hammerschmidt R., Nuckles E.M. and Kuc J., 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiological Plant Pathology*, 20: 73-82.
- Kavino M., Harish S., Kumar N., Saravanakumar D., Damodaran T., Soorianathasundaram K. and Samiyappan R., 2007. Rhizosphere and endophytic bacteria for induction of systemic resistance of banana plantlets against bunchy top virus. *Soil Biology and Biochemistry*, 39: 1087-1098.
- Krishnamoorthy V., 2002. Breeding for resistance to sigatoka leaf spot and nematodes in banana (*Musa* spp.). Ph.D. thesis. Tamil Nadu Agricultural University, Coimbatore, India, pp. 238.
- Kumar N. and Soorianatha Sundaram K., 2002. Progress and current strategies in banana improvement in Tamil Nadu (India). Global Conference on Banana and Plantain, 28-31, October 2002, Bangalore, India, pp. 12-14
- Levine A., Tenhaken R., Dixon R. and Lamb C., 1994. H₂O₂ from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell*, 79: 583-593.
- Mayer A.M., Haul E. and Shaul R.B., 1965. Assay of catechol oxidase, a critical comparison of methods. *Phytochemistry*, 5: 783-789.
- Miyazawa J., Kawabata T. and Ogasawara N., 1998. Induction of an acidic isozyme of peroxidase and acquired resistance to wilt disease in response to treatment of tomato roots with 2-furoic acid, 4-hydroxybenzoic hydrazide or salicylic hydrazide. *Physiology and Molecular Plant Pathology*, 58: 115-125.
- Pinochet J., 1988. A method for screening bananas and plantains to lesion forming nematodes. Pp. 62-65. *In: Nematodes and borer weevil in bananas: Present status of research and outlook*. INIBAP Montpellier, France.
- Rajendran G., Bhakthavatsalu C.M., Madhava Rao V.N. and Abdul Khader J.B.M.Md., 1980. Studies on the distribution of nematodes of banana in Tamil Nadu. Proceedings of the National Seminar on Banana Production Technology. Tamil Nadu Agricultural University, Coimbatore, India, pp. 220.
- Reuveni R. and Karchi J.F., 1987. Peroxidase activity – A possible marker for resistance of melon against downy mildew (Abstract). *Phytopathology*, 77: 1724.
- Ross W.W. and Sederoff R.R., 1992. Phenylalanine ammonia lyase from loblolly pine; purification of the enzyme and isolation of complementary DNA clones. *Plant Physiology*, 98: 380-386.
- Rowe P.R. and Rosales F.E., 1996. Bananas and plantains. Pp. 167-211. *In: Fruit Breeding Vol. 1* (Janick J. and Moore J.N., eds). John Wiley & Sons Inc., New York, USA.
- Sarah J.L., Blavignac C., Sabatini C. and Boisse M., 1992. Une méthode de laboratoire pour le criblage varietal des bananiers vis-a-vis de la résistance aux nématodes. *Fruits* 47: 559-564.
- Sariah M., Lim C.L. and Tariq S.A., 1999. Use of biochemical marker as a measure of resistance towards *Fusarium* wilt of banana. Proceedings of the International Workshop on the banana *Fusarium* wilt disease, 18-20 October 1999, Genting, Malaysia, pp. 243-249.
- Sathiamoorthy S. and Balamohan T.N., 1993. Improvement of Banana. Pp. 285-287. *In: Advances in Horticulture, Vol. 1 - Fruit Crops, Part I* (Chadha K.L. and Pareek D.P., eds). Malhotra Publishing House, New Delhi, India.
- Shivakumar P.D., Geetha H.M. and Shetty H.S., 2003. Peroxidase activity and isozyme analysis of pearl millet seedlings and their implications in downy mildew disease resistance. *Plant Science*, 164: 85-93.
- Sindhu J.S., Ravi S. and Minocha J.L., 1984. Peroxidase isozyme patterns in primary trisomics of pearl millet. *Theoretical and Applied Genetics*, 68: 179-182.
- Spies J.R., 1955. *Methods in Enzymology* (Colowick S.P. and Kaplan W.O., eds). Academic Press, New York, USA, 467-468 pp.
- Sundararaju P., 1996. Nematode pests of banana and their management. Proceedings of Conference on Challenges in Banana Production and Utilization in the 21st Century, September, 24-25, Trichy, India, pp. 17-19.
- Valette C., Nicole M., Sara J.L., Boisseau M., Boher B., Fargette M. and Geiger J.P., 1997. Ultrastructure and cytochemistry of interactions between banana and the nematode *Radopholus similis*. *Fundamental and Applied Nematology*, 20: 65-77
- Van Etten H.D., Sandrock R.W., Wasmam C.C., Soby S.D., Mellusky K. and Wang P., 1995. Detoxification of phytoanticipins and phytoalexins by phytopathogenic fungi. *Canadian Journal of Botany*, 73(Suppl.): 518-S525.
- Vidhyasekaran P., 1988. *Physiology of disease resistance in plants*. Vol. I. CRC Press, Boca Raton, Florida, USA, 149 pp.