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Biochemical and Molecular Changes in Banana Plants Induced by *Pratylenchus coffeae* and *Meloidogyne incognita*

P. SUNDARARAJU AND K. PANDI SUBA

Crop Protection Laboratory, National Research Centre for Banana (ICAR) Thogamalai Road, Thayanur (Post), Tiruchirapalli – 620 102, Tamil Nadu.

ABSTRACT: A pot culture experiment was conducted to understand the biochemical and molecular changes associated with resistance reaction of banana against root-lesion and root-knot nematodes on five varieties of banana viz., Nendran (AAB), Robusta (AAA), Pisang Jari Buaya (AA), Karthobiumtham (ABB) and *Musa balbisiana* (BBB). The highest protein concentration and increased peroxidase activity was observed in cvs.Nendran and Robusta whereas minimum was observed in cvs.*Musa balbisiana,* Karthobiumtham and Pisang Jari Buaya. The activity of polyphenol oxidase was reduced in infected plants of cvs.Nendran and Robusta. But, the activity increased in cvs.Pisang Jari Buaya and *Musa balbisiana.* The phenylalanine ammonia lyase (PAL) activity was significantly lower in cvs.Nendran and Robusta compared to other three varieties. The phenolic accumulation was increased by 56% in cv.Nendran after nematode infection whereas there was only 2% increase in Karthobiumtham. The molecular analysis indicated a higher rate of mRNA synthesis soon after nematode infection.

Key words: Banana varieties, *Pratylenchus coffeae*, *Meloidogyne incognita*, biochemical alterations, resistance/susceptible.

The root-lesion (*Pratylenchus coffeae)* and root-knot nematodes (*Meloidogyne incognita)* are considered to be an important nematode pests of banana causing extensive crop damage (Reddy *et al.*, 1989; Jonathan & Rajendran, 2000 and Sundararaju & Cannayane, 2003). One of the most economical and effective ways to control plant parasitic nematodes is exploiting resistant / tolerant cultivars of banana. Resistance is an incompatible reaction of plants towards nematode infestation. Narayana & Reddy (1980) correlated the levels of phenols in roots with resistance in tomato cultivars against root-knot nematode *M.incognita*. Hence, the present investigation was undertaken to study the biochemical and molecular changes in nematode resistant and susceptible cultivars of banana infested with *P.coffeae* and *M.incognita*.

MATERIALS AND METHODS

Healthy suckers of five varieties viz., Nendran (AAB), Robusta (AAA), Pisang Jari Buaya (AA), Karthobiumtham (ABB) and *Musa balbisiana* (BBB) were planted in earthen pots containing 5 kg sterile soil (red soil, sand and FYM in 2:1:1 ratio). Three sets of each variety with 3 replications were maintained. One set of plants were kept as healthy ones and the other two sets were meant for nematode inoculations. Three months after planting, root-knot and root-lesion nematodes @2J_2/ g of soil were inoculated separately to both the sets kept under green house conditions. The root-lesion nematode, *P. coffeae* required for inoculation was extracted from the banana roots collected from the NRCB research farm. The root-knot nematode, *M. incognita* was collected from the egg masses of infected plant material and incubated for hatching. The other set of plants were maintained as check. After inoculation, the pots were arranged in a randomized manner at a place of even light in a greenhouse with a temperature range of 27-34^{o}C and watered daily with boiled and cooled water.

Three months after nematode inoculations, plants with entire root system were removed from the pots and growth characters were recorded. Root samples collected from both healthy and nematode inoculated plants were washed thoroughly in order to remove adhering debris and soil particles. Later the samples were stored at 4°C for biochemical analysis. Estimation of protein by Lowry's method (Lowry *et al.* 1976), peroxidase assay by Hammerschmaid & Smigocki (1982), Phenylalanine ammonia lyase assay by the method of Ross (1992), polyphenol oxidase and estimation of phenol by Spies method (Spies, 1955), were performed. The data were statistically analyzed (Gomez & Gomez, 1994) and treatment means were compared by Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

The results revealed significant difference in protein concentration between healthy and nematode inoculated plants. The highest protein concentration was recorded in cvs.Nendran and Robusta, and the lowest in *Musa balbisiana* and Pisang Jari Buaya(Table 1)**.** Peroxidase activity increased by 64 and 36% (Table-2) in cvs.Nendran and Robusta respectively. Quantitative increases in peroxidase activities were conspicuous throughout the period of observation in both the nematodes inoculated plants of both the varieties. The present study indicated the higher PO activity in susceptible cv.Nendran whereas the minimum in cv.Karthobiumtham. This was in agreement with the earlier findings of Veech & Endo (1970) who observed a higher activity of PO in the syncytia found in soybean roots infected with root-knot nematode. There was a narrow decrease of Polyphenol

Table 1. Concentration of Protein content in banana roots as influenced by *Pratylenchus coffeae* and *Meloidogyne incognita*

Varieties	Quantity (mg / g root)	
	P.coffeae	*M.incognita*
Nendran - Healthy	0.019a	0.018ab
Nendran - Inoculated	0.020a	0.020a
Robusta - Healthy	0.010a	0.010de
Robusta - Inoculated	0.018a	0.017b
Pisang Jari Buaya - Healthy	0.017a	0.017b
Pisang Jari Buaya - Inoculated	0.015a	0.015bc
Musa balbisiana - Healthy	0.010a	0.010e
Musa balbisiana - Inoculated	0.009a	0.009e
Karthobiumtham – Healthy	0.015a	0.013cd
Karthobiumtham - Inoculated	0.013a	0.017b
CD (P=0.05)	0.001	0.031
CV (%)	6.94	12.16

Means followed by a common letter are not significantly different at 5% by DMRT

Oxidase in nematode inoculated plants of cvs.Nendran and Robusta followed by slightly reduced enzyme activity in infected cv.Karthobiumtham and the increased activity in nematode inoculated plants of cvs.Pisang Jari Buaya and *Musa balbisiana.*

Phenylalanine Ammonia Lyase (PAL) activity was estimated using 1mM L-phenylalanine as a substrate. The results of the study showed significantly lower activity of PAL in nematode inoculated plants of susceptible cultivars, while in the resistant varieties, the PAL activity increased in both healthy and nematode inoculated plants of cv.Karthobiumtham. The highest quantity of phenol was accumulated in cv.Nendran infected plant (218.74 µg/g root), followed by cv.Robusta (161.16 µg/g root). The lowest amount was observed in Pisang Jari Buaya (45.00 µg/g root) of healthy plants (Table-3). 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. The ratio between PPO & phenol was observed to be lower in resistant cvs. Karthobiumtham and *M. balbisiana* and much higher in susceptible cvs. Nendran and Robusta (Table 3). It was in agreement with Giebel (1974) who explained the function of polyphenols in contributing resistance.

The accumulation of phenols varied depending on the nematode infection. There was higher phenolic accumulation in susceptible varieties after nematode infection. In Nendran, 56% increase in phenolic content was observed. The lowest phenolic accumulation was noted in cv.Karthobiumtham. Even though the phenolic accumulation was very low in resistant plant, but PPO activity was high. It might have resulted in oxidation of polyphenols and accumulation of monophenols which are responsible for resistant reaction. More amounts of polyphenols accumulated in cv.Nendran resulted in susceptible reaction. The molecular analysis revealed a higher rate of mRNA synthesis soon after nematode infection. Raja & Dasgupta (1986) reported that initiation of symptom expression in nematode induced plant diseases was triggered by *de novo* synthesis of new species of mRNA, along with rapid alteration of plant metabolism at gene transcriptional level after nematode infection.

Table 2. Changes in activity of Peroxidase, Polyphenol oxidose (PPO) and phenylalanine ammonia lyase (PAL) in banana roots as influenced by *Pratylenchus coffeae* and *Meloidogyne incognita*

Varieties	Enzyme activity (Units / g of root)					
	Peroxidase		PPO		PAL	
	P. coffeae	*M. incognita*	*P. coffeae*	*M. incognita*	*P. coffeae*	*M. incognita*
Nendran – Healthy	70.45f	67.75d	12.43e	10.96d	51.20g	51.50f
Nendran - Inoculated	185.21a	189.09a	7.32i	6.63e	47.89i	48.50g
Robusta – Healthy	95.42e	93.68c	25.28a	24.54a	65.24b	67.16b
Robusta - Inoculated	150.22b	147.19b	9.11g	7.98e	60.45d	65.60bc
Pisang Jari Buaya - Healthy	20.45j	18.26e	8.42h	7.71e	60.76cd	65.16c
Pisang Jari Buaya - Inoculated	24.84i	21.65e	12.11f	11.25d	67.35a	70.50a
Musa balbisiana – Healthy	108.64d	102.71c	19.27c	19.23b	54.60f	56.60e
Musa balbisiana - Inoculated	112.36c	104.66c	22.98b	23.16a	55.80e	57.60e
Karthobiumtham – Healthy	36.21g	32.41e	17.20d	16.23c	50.00h	51.00f
Karthobiumtham - Inoculated with nematodes	29.54h	28.55e	12.12f	11.88d	61.15c	63.30d
CD (P=0.05)	0.095	15.84	0.014	2.35	0.55	1.79
CV (%)	0.067	11.53	0.585	9.9	0.56	1.76

Means followed by a common letter are not significantly different at 5% by DMRT

Table 3. Accumulation of Phenols and ratio of polyphenol oxidase (PPO) and phenol in banana roots as influenced by *Pratylenchus coffeae* and *Meloidogyne incognita*

Varieties	Quantity of phenol (Units / g of root))		Ratio (PPO:Phenols) (Units / g of root))	
	P. coffeae	*M. incognita*	*P.coffeae*	*M. incognita*
Nendran - Healthy	94.816c	96.15c	1:8	*1:9*
Nendran - Inoculated	212.453a	218.74a	1:31	1:33
Robusta – Healthy	69.143f	68.17f	1:2.6	1:30
Robusta - Inoculated	156.940b	161.16b	1:18	1:20
Pisang Jari Buaya - Healthy	43.210j	45.00j	1:5	1:6
Pisang Jari Buaya - Inoculated	56.666g	58.80g	1:4.5	1:4.5
Musa balbisiana – Healthy	88.310d	87.003e	1:4.5	1:3.2
Musa balbisiana – Inoculated	76.256e	74.98d	1:3.3	1:3
Karthobiumtham – Healthy	46.726i	48.47i	1:2.7	1:3
Karthobiumtham - Inoculated	50.450h	49.68h	1:4	1:4.1
CD (P=0.05)	2.809	0.42		
CV (%) 1.830 0.27				

Means followed by a common letter are not significantly different at 5% by DMRT

The results of the present study indicated the cultivars, Pisang Jari Buaya, *Musa balbisiana* and Karthobiumtham to be resistant to both nematodes whereas cvs.Nendran and Robusta are known to be susceptible to both the nematodes.

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