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Variations in Biochemical Constituents for Tolerance to Nematode and *Fusarium* wilt Complex in Select Banana Hybrids

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Banana is one of the most important fruit crops cultivated worldwide and its cultivation is hampered by infestation by nematode and *Fusarium* wilt complex. A pot culture experiment was conducted to study the change in biochemical constituents due to the inoculation of nematodes followed by *Fusarium* pathogen in select banana hybrids. The results indicated that the increase in production of defense-related compounds and enzymes such as total phenols, lignin, PO, PPO and PAL in the root tissues of inoculated plants. Among the four select banana hybrids, NPH-02-01 recorded high total phenol content (586.92 μg^{-1}) and H 531 recorded high lignin content (1.58 %). Similarly the hybrid NPH-02-01 registered the higher activity of PO (3.5 $abs.min^{-1}.g^{-1}$), PPO (0.23 $abs.min^{-1}.g^{-1}$) and PAL (14.83 $abs.min^{-1}.g^{-1}$) when compared to other hybrids. The percentage increase of all the constituents in the hybrid NPH-02-01 was found to be comparatively higher with uninoculated plants and check cultivars.

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1. INTRODUCTION

Banana is the most valuable fruit crop with high nutritive value and is cultivated in 135 countries of the world. India is the leading producer of bananas accounting for 20.1 % of global production in an area of 0.80 million hectares with the production and productivity of 29.7 million tonnes and 34 MT/ ha respectively [1]. India, China,the Philippines, Ecuador, Indonesia and Brazil are the major producers of bananas in the world. Although, banana produces maximum yield under favourable conditions, its cultivation is highly limited by various biotic (fungi, viruses, bacteria and nematodes) and abiotic (drought and salinity) stresses that may lead to heavy economic loss. Nematodes and *Fusarium* wilt are the most destructive biotic factors that cause threat to most of the banana cultivars all over the world. Crop losses by nematodes in banana were estimated to be with an average annual yield loss of about 20 % worldwide [2]. *Fusarium* wilt disease caused by race 1 strain of *Foc* resulted in yield reduction up to 50-70% [3]. It has been proven that the combination of plant-parasitic nematodes disease increases the severity of disease caused by fungal infections [4,5]. Plant-parasitic nematodes are often referred to as primary pathogens since they have the potential to cause injury on their own. *Fusarium* spp. may fall into the second group, *i.e.,* secondary pathogen and may or may not be capable of producing disease on their own, as is the case with wilt field resistant cultivars [6]. Chemical management of the nematode is commonly used, but it has been highly unsustainable because of high costs, soil health deterioration and contamination of groundwater, hampering nontarget organisms, residue in fruit and general environmental problems [7]. The most effective way to manage *Fusarium* wilt is the use of resistant cultivars [8]. The fungal pathogen can enter plant roots by directly penetrating root epidermal cells, causing cell membrane damage and eliciting the formation of antioxidants such as superoxide dismutase (SOD), catalase (CAT) and peroxide (POS) [9]. The mechanism of resistance in plants is due to the higher activity of defense-related compounds such as total phenols, lignin, flavonoids, phytoalexins and other proteins are involved in the activation of plant defense-related genes [2]. These enzymes would break down the fungal cell wall and prevent pathogen growth and development in plant cells [10]. The activity of peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) was shown to be higher in resistant and tolerant bananas cultivars compared to susceptible cultivars, [11]. Therefore, host plant tolerance or resistance has been recognized as one of the most economic, effective and eco-friendly measures for controlling *Fusarium* wilt and nematode infestation in banana cultivation. Hence programs for improving bananas had targeted the development of hybrids with resistance or tolerance to *Fusarium oxysporum* f. sp. *cubense* and nematodes. In the present study, four select banana hybrids developed through conventional breeding at TNAU were assessed for the biochemical basis of tolerance to *Fusarium* wilt and nematode complex.

2. MATERIALS AND METHODS

The present study was carried out at the College orchard, Tamil Nadu Agricultural University, Coimbatore involving four select banana hybrids (CO2, H 531, H 96/7 and NPH-02-01) with checks (Pisang Lilin, Karpooravalli, Poovan and Grand Naine). The *Fusarium* wilt pathogen *Fusarium oxysporum* f.sp.*cubense* (*Foc*) was isolated from infected Karpooravalli cultivar by standard protocol given by Rangaswami [12]. The pathogen was confirmed through sequencing of Internal Transcribed Spacer region 1 and 4 (ITS) [13] and the sequence was submitted in Genebank. Second stage infective juveniles of nematode complex consisting of *Radopholus similis, Pratylenchus coffeae* and *Helicotylenchus multicinctus* were isolated from nematode infected banana rhizosphere soil and confirmed based on morphological observations.

2.1 Establishment of Pot Culture Experiment for Screening of Banana Hybrids against Fungal Nematode Complex

Healthy, disease-free and uniform sized (750 g) suckers of select banana hybrids and checks were collected from the germplasm block in the College orchard. The suckers were pared and planted in poly bags (30cm × 30cm) containing 7 kg of the sterilised potting mixture (1:1:1 red earth: sand: FYM).The experiment was laid out in

completely randomized block design with 8 treatments, replicated thrice, and comprising three plants per replication. A separate set of uninoculated plants was treated as a control.

2.2 Nematode Complex and *Fusarium* Pathogen Inoculation

1000 numbers of nematodes comprising of *viz., Radopholus similis, Pratylenchus coffeae* and *Helicotylenchus multicinctus* were inoculated per bag after 30 days of planting and conidial suspension of *Foc* at the rate of 10^6 conidial per ml through the sand-maize medium was inoculated 15 days after the inoculation of nematodes to the root zone of the banana plants. The root samples were collected from the control and inoculated plants on 150^{th} day after inoculation and analyzed for total phenol, lignin, PO, PPO and PAL activity.

2.3 Total Phenol Content

Folin – Ciocalteau reagent method was used to quantify total phenols. 20 % of Na_2CO_3 and Folin-Ciocalteau reagent (1:1) were added to diluted extracts after reduction to 3ml from 10ml concentration. The intensity of the blue color was measured at 660nm against a reagent blank. The standard curve was used to calculate the total phenol concentration in the samples, which was expressed as mg catechol equivalent of phenol/g sample [14].

2.4 Lignin Content

The content of lignin in banana root tissue was determined using the Chesson method [15]. One gram of the banana leaf or root tissue was treated in a combination of 5 ml of conc. H_2SO_4 and 50 ml of HCl for 16 hr at 25^o C in a shaker. After that, the mixture was transferred to a flask containing 450ml of distilled water. The contents of the flask were filtered through a Geena G3 glass filter after boiling for 20 minutes. The acid residue was rinsed with distilled water to neutralize it, then dried at 105°C and weighed. The results were expressed as a percentage of lignin content in the tissues on a basis of dry weight.

2.5 Enzyme Activity (PO, PPO and PAL)

The root sample of each genotype and the corresponding control replicates were taken and homogenized at the rate of 1g per 5ml of 0.1 M with 5ml of 0.1 M phosphate buffer with a pH of 6.5. The homogeneous product was centrifuged at 10,000 rpm for 20 minutes at 4 ^oC. For the extraction of polyphenyl ammonium lyase enzyme (PAL), 0.2 M borate buffer with a pH of 8.7 was used. The supernatant was used as the extract for estimation of enzyme activity and it was expressed as fresh weight unit min^{-1} g^{-1}. The activity of peroxidase (PO) enzyme was analyzed by the method suggested by Wuyts and coworkers [16] and the change in optical density of the reaction mixture was measured at 470 nm per 15 seconds for 3 minutes. Polyphenol oxidase (PPO) activity was measured using a modified method proposed by Gertzen and Escobar [17] and the absorbance was measured at 495 nm at 30 seconds intervals for 3 minutes. The activity of the polyphenyl ammonium lyase enzyme (PAL) was analyzed spectrophotometrically at 420 nm [18].

2.6 Statistical Analysis

The experimental data were satistically analysed using the techniques suggested by Panse and Sukhatme [19]. Data analysis was performed using OP STAT and critical difference was worked out at 5% level of significance.

3. RESULTS

In plants, different kinds of defense-related compounds and enzymatic changes will be activated due to any stress and the level of biochemical constituents synthesized as a part of the defense mechanism might be responsible for conferring the resistance or tolerance against stress. In the present study, changes in biochemical constituents in the hybrids and checks inoculated with nematode wilt pathogen complex when compared to the control were assessed on 150 DAI and the data are presented. Total phenol and lignin, peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) activities varied significantly among inoculated plants when compared to control. In the present study, phenol production increased irrespective of the hybrids and checks in the inoculated set when compared to the respective control. Total phenol content in nematode wilt infected roots varied greatly, ranging from 175.08 to 586.92 µg g^{-1}. NPH-02-01 registered the highest total phenol content (586.92 µg g^{-1}) among the hybrids, followed by H 531 (563.24 µg g^{-1}). NPH-02-01 showed the highest percentage change in total phenols compared to other hybrids and control (69.22 %), whereas CO 2

recorded the lowest total phenol content (256.22 µg g^{-1}) with a percentage increase of 14.71%. In check varieties, Pisang Lilin registered the highest total phenol content (470.42 µg g^{-1}) as compared with other checks (Table 1). Increased phenol synthesis, followed by increased PPO activity, would have contributed in the conversion of phenols into polymers like lignin [20]. Among the hybrids, lignin content ranged from 0.97 % (CO 2) to 1.52 % (NPH-02-01) and in the check varieties the range was 0.78 to 1.58 %. Pisang Lilin had the highest lignin content rise of 60.82 % among the checks. The lowest lignin was recorded in Karpooravalli (15.97 %) (Table 1). The lignin content in the roots of banana hybrids indicated a positive response in nematode wilt pathogen inoculated plants compared to control.

The defense-related enzymes significantly confer tolerance/resistance against plant pathogens during the initiation of the pathogenesis process. The increased production of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase was observed in nematode and fusarium inoculated hybrids and checks when compared to control. The peroxidase activity varied considerably among the hybrids and checks. NPH-02-01 registered the highest percentage change in infected roots (38.66 %) compared to the uninfected control. The highest activity of peroxidase was recorded in NPH-02-01 (3.5 abs min^{-1} g^{-1}) followed by H 531 (2.89 abs $min^{-1}g^{-1}$). Among the checks, Pisang Lilin recorded the highest peroxidase activity and the least was recorded in Karpooravalli (Table 2). Similar to peroxidase, the hybrid, NPH-02-01 also recorded the highest polyphenol oxidase activity (0.23 abs min^{-1} g^{-1}) and phenylalanine ammonia lyase activity (14.06 abs min^{-1} g^{-1}), whereas the lowest activity of PPO and PAL activity (0.10 abs min^{-1} g^{-1} and 14.06 abs $min^{-1}g^{-1}$ respectively) was observed in CO 2. Among the checks, Pisang Lilin registered the highest PPO activity (0.205 abs $min^{-1}g^{-1}$) and the lowest activity was recorded in Karpooravalli (0.088 abs min^{-1} g^{-1}). Pisang Lilin registered the highest percentage increase in PAL activity (38.39 percent) among the checks, whereas Karpooravalli registered the lowest (9.58 %) (Table 2).

4. DISCUSSION

Infestation of banana plantations by nematode and *Fusarium* wilt complex hampers the growth and development leading to heavy economic loss and thereby disturbing the banana production system to a larger extent. Though chemical, biological and cultural practices are available [21], effective management can be achieved by evolving tolerant/resistant varieties to nematode wilt complex through breeding approaches. In the present study select banana hybrids along with checks were screened against nematode and *Fusarium* wilt pathogen to understand the level of change in biochemical constituents related to resistance mechanism *viz*., phenol, lignin, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase among the hybrids and checks. The results of the pot culture experiment revealed that among the select banana hybrids, NPH-02-01 was observed to accumulate a higher amount of total phenol (586.92 µ/g), lignin content (1.52 %) followed by the hybrid H 531.Earlier workers reported that the amount of total phenols was found to be lower in susceptible cultivars compared to tolerant and

Table 1. Total phenol and lignin content in select banana hybrids and checks inoculated with nematode and *Fusarium* pathogen

Banana hybrids/ Check cultivars	Genotype	Total phenol (µg g^{-1})			Lignin (%)		
		C	I	%	C	I	%
CO2	AB	223.35	256.22	14.71	0.81	0.97	19.75
NPH-02-01	AAB	346.82	586.92	69.22	0.92	1.52	65.75
H 531	AAB	420.07	563.24	34.08	1.26	1.58	25.45
H 96/7	ABB	238.28	275.45	15.59	1.18	1.37	16.35
Karpooravalli	ABB	253.71	284.34	12.07	0.67	0.78	15.97
Grand Naine	AAA	151.32	175.08	15.70	0.93	1.12	20.49
Pisang Lilin	AA	340.21	470.42	38.27	1.09	1.75	60.82
Poovan	AAB	276.53	317.61	14.85	0.94	1.18	25.61
C.D.		35.71	36.28		0.14	0.11	
SE(d)		16.7	16.97		0.06	0.05	

C- Control, I- Inoculated, %- Percent change over control

Table 2. Peroxidase (PO), Polyphenol oxidase (PPO) and phenyl ammonia lyase in select banana hybrids and check cultivars inoculated with nematode and *Fusarium* pathogen

Banana hybrids / check cultivars	Genotype	PO (abs $min^{-1}g^{-1}$)			PPO (abs $min^{-1}g^{-1}$)			PAL (abs $min^{-1}g^{-1}$)		
		C	I	%	C	I	%	C	I	%
CO-2	AB	1.95	2.36	23.15	0.07	0.10	49.25	11.89	14.06	18.22
NPH-02-01	AAB	1.96	3.50	38.66	0.10	0.23	134.70	10.44	14.83	42.08
H 531	AAB	2.22	2.89	23.07	0.11	0.17	54.54	15.61	19.66	25.94
H 96/7	ABB	1.95	2.34	28.01	0.08	0.12	40.47	14.30	16.61	16.18
Karpooravalli	ABB	1.72	2.01	14.96	0.06	0.09	46.67	11.96	13.11	9.58
Grand Naine	AAA	1.28	1.57	16.75	0.07	0.11	60.56	16.67	19.61	1.65
Pisang Lilin	AA	2.22	4.08	33.30	0.09	0.20	130.33	1.72	16.22	38.39
Poovan	AAB	1.50	1.82	17.53	0.05	0.09	57.14	15.33	17.28	12.67
C.D.		0.22	0.23		0.013	0.03		1.78	1.06	
SE(d)		0.10	0.11		0.006	0.01		0.83	0.49	

C- Control, I- Inoculated, %- Per cent change over control

resistant cultivars and it might be due to the early accumulation of phenolic compounds at the infection site that slows the pathogen's growth by early cell death and is a feature of defense responses [2, 22]. The higher levels of phenols result in vascular lignification and endodermal cell suberisation which may inhibit nematode and fungal pathogen xylem invasion and their proliferation in the vascular tissues. Increase in total phenol content after inoculation of nematode and wilt pathogen in combination is reported by earlier workers [2,4]. In a similar line, variations in level of infection of *Fusarium* wilt and nematode in banana cultivars *viz*., Terra Maranhão, 'BRS Pacovan Ken, BRS Vitória and BRS Platinade were reported by Rocha [5]. Reduction in infection severity of *Fusarium* pathogen inoculated plants was observed due to production of higher concentrations of total phenolics and lignin-thioglycolic acid derivatives in banana [23]. The resistance towards *Foc* in banana cultivar GCTCV-218 is due to the production of cell wall-associated phenols during the infection process of *Foc* [24]. The level of resistance in banana genotypes was evaluated based on production of defense-related enzyme activity, like peroxidase, polyphenol oxidase,1,3 glucanase, chitinase and phenolics [25]. There is significant increase in PO, PPO and PAL in the hybrid NPH-02-01(38.66 %, 134.70 % and 42.08) followed by the hybrid H 531 compared to uninoculated banana plants and check varieties. The upregulation of PO, PPO, and PAL in combination involves the addition of a barrier that prevents pathogens from establishing themselves in a resistant and tolerant host [26]. The increased activity of enzymes *viz*., peroxidase, polyphenol oxidase, phenylalanine ammonia lyase was observed in the resistant cultivars compared to the susceptible cultivars by several earlier workers [27, 28].

5. CONCLUSION

It is concluded from the present study that all the four select hybrids of banana were found to register an increase in the defense related compounds and enzymes when inoculated with nematodes followed by *fusarium* pathogen. The percentage in an increase of defense related compounds when compared to control was higher in NPH-02-01 and hence it exerts higher level of tolerance to nematode and *Fusarium* fungal complex.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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