Int.J.Curr.Microbiol.App.Sci (2017) *6*(4): 1668-1684

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 4 (2017) pp. 1668-1684**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.604.202

Induction of Resistant to *Radopholus similis* and Defence Related Mechanism in Susceptible and Resistance Banana Hybrids Infected with *Radopholus similis*

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Keywords

Radopholus similis, Defence related mechanism, Polyphenol oxidase.

Article Info

Accepted:
15 March 2017
Available Online:
10 April 2017

A B S T R A C T

The burrowing nematode, *Radopholus similis* is considered to be the most destructive nematode associated with banana growth worldwide. Cultural and chemical management alone cannot guarantee full control. An alternative is to develop new banana hybrids with resistance to burrowing nematode. The twenty four new synthetic banana hybrids were screened under artificially inoculated pot condition for their reaction and resistance mechanism against *Radopholus similis*. Five hybrids namely, H 912, H 914, H 916, H 926, H 943 were rated resistance to *Radopholus similis* based on the nematode multiplication, lowest root lesion and corm lesion index. The nematodes inoculated resistant hybrid H 916 had higher total phenols (567.21 µg/g) and orthodihydroxy phenols (2.77 µg/g) activities than the susceptible cultivars. The enzymes changes in root content of peroxidase (2.78-3.32 abs/min/g), polyphenol oxidase (0.086-0.113 abs/min/g) and phenylalanine ammonia lyase (23.28-30.12 nmol/min/ml) of the hybrids in defense mechanism in response to nematode invasion indicated higher activities in resistance plants *viz-a-viz* susceptible ones.

Introduction

Bananas and plantains (*Musa* spp.) are the second largest fruit crop produced and exported in the world and ranking third in terms of total production (Dochez *et al.*, 2006). Plant parasitic nematodes are one of the major biotic stresses affecting banana production. Among them, the burrowing nematode, *Radopholus simili* is found in all major banana growing regions of the world (Hölscher *et al.*, 2014). This migratory endoparasitic nematode causes root and corm tissue cavities that evolve to form necrotic lesions that affect the ability of the plant to uptake water and nutrients, resulting in the reduced development of banana bunches, reduced fruit yield, toppling and also paving way to pathogenic microorganisms (Aravind *et al.*, 2010 and López-Lima *et al.*, 2013).

Crop losses by nematodes to banana are estimated to be very high, with an average annual yield loss of about 20 per cent worldwide (Seenivasan *et al.*, 2013). In addition, these parasites also interact with

other disease causing organisms of fungus *Fusarium oxysporum f. sp. Cubense* produce wilt disease complexes (Begum *et al.,* 2012). Chemical control is widely used to manage this nematode, but this has become highly unsustainable due to high costs, deteriorating soil health, ground water contamination, hampering non target organisms, residue in fruits and general environmental issues (López-Lima *et al.,* 2013). Therefore, host plant resistance has been recognized as one of the most economic, effective and environmentally-friendly measures for controlling the *Radopholus similis* nematodes.

Breeding of banana varieties with resistance to nematodes are considered a more sustainable management option, equally accessible for subsistence banana growers as well as commercial producers. The use of resistant cultivars is considered one of the most effective and environmental-friendly alternatives as nematode reproduction is reduced, no toxic residues in fruits, stabilizes the yield and blends with cultural control (Olowe, 2007; Moon *et al.,* 2010). A resistant plant restricts or prevents the nematode's reproduction by activating defense mechanisms which may limit penetration of second-stage juveniles, inhibit of feeding site and prevents the reproduction of the adult female (Rodrigo *et al.,* 2013). The identification and use of resistant or tolerant varieties can be a viable means of minimizing loss caused by nematodes (Gharabadiyan *et al.,* 2012). The genetic component of host management involves the identification and utilization of selected sources of resistance in the breeding programs for development of nematode resistant cultivars (Hussain *et al.,* 2014). Resistance can be considered as the ability of the plant to suppress development of pest and pathogens, whereas tolerance is the ability of the plant to grow well despite infection by pathogens (Nithya Devi *et al.,* 2007).

The resistance and susceptibility attributes of several crops to different insect-pests and pathogens has been assessed with the presence of secondary plant metabolites mainly phenols (Vandana Sukhla *et al.,* 2014; Pathipati and Yasur, 2010). The phenolic accumulation was increased by 56% in banana cv. Nendran after nematode infection whereas there was only 2% increase in cv. Karthobiumtham (Sundararaju and Pandi Suba, 2006). The biochemical contents like total phenols and lignin of the banana hybrids in defence mechanism in response to nematode invasion indicated higher activities in resistance genotypes *viz-a-viz* susceptible ones (Kavitha *et al.,* 2008). Polyphenol oxidase, phenylalanine ammonia lyase enzymes activities and total phenols contents in roots were higher in nematode resistance banana hybrids than in the susceptible hybrids (Das *et al.,* 2013, 2014).

Phenol content had increased upto 50% at 30 days after sowing, which protected the plant from the pest by imparting high level of resistance (Taggar *et al.,* 2014). Moreover, high levels of two major oxidizing enzyme of plants such as poly phenol oxidase and peroxidase impart induced resistance to insect herbivores and pathogens (Rachana *et al.,* 2015; Bandi and Siva subramanian, 2012; He *et al.,* 2011). Similarly, polyphenol oxidase and peroxidase activities were higher in resistant genotypes compared to susceptible genotypes of *Capsicum annum* infested by *Bemisia tabaci* (Latournerie-Moreno *et al.,* 2015). Higher activity of peroxidise and polyphenol oxidase in faba bean was strongly associated with its resistant character against aphid *Aphis craccivora* (Soffan *et al.,* 2014). Induction of polyphenol oxidase activity in potato leaves resulted due to aphid *Myzus persicae* infestation led to enhancement of resistance in potato against the pest (Xiao-Lin *et al.,* 2013). Plants when attacked by *R. similis* nematodes show selective changes in

their metabolism due to host pathogen interaction, inducing an immune response of host the parasite. Hence, the present investigation was undertaken to screen the banana hybrids for resistance against *Radopholus similis* nematode which can be further used as resistance source.

Materials and Methods

Twenty four new synthetic banana hybrids and their parents were derived from hybrid maintained block in TNAU Orchard, were studied at Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, during 2011-2013. Healthy sword banana suckers of uniform size and weight (750 g) were collected, pared, treated in hot water (50-55°C) for 10 minutes and planted, in plastic bag (30 x 45 cm) containing twenty kilograms of pot mixture (red soil: sand: FYM at 2:1:1 respectively) sterilized with 4% formaldehyde. The individual pots were labeled with name of the hybrid. The experiment was conducted in a glass house in potted plants, which were artificially inoculated with nematodes. Four weeks after planting, 10 plants of each hybrid were inoculated with nematodes while another set of 10 plants were kept as nematode-free control. The experiment was laid out in completely randomized block design. The hybrids were evaluated along with the reference cultivar *viz.*, Yangambi km5 as the resistance cultivars and Grand Naine as the susceptible reference cultivar.

Culturing and extraction of nematodes and Inoculation of nematodes in pots

Healthy banana corms of cv. Robusta were selected and pared with a knife before planting to remove the outer layer of adhering roots and tissues to eliminate nematode infection. Sucker planted at the rate of one per pot, filled with autoclaved pot mixture consisting of sand, red soil and FYM mixed in equal proportions. Roots infested with *R. similis* were collected from infested field of banana, washed in water, cut into small bits and processed in a warring blender. The nematodes were extracted and the nematode suspension was then poured into the rhizosphere of the plants after the emergence of roots. Banana hybrids maintained in the pots were inoculated with infective juveniles of burrowing nematode, *R. similis* at 45 days after planting @ 1,000 nematodes /pot, respectively. The nematodes were extracted by the modified baermann funnel technique (Schindler, 1961). The nematode suspension was then poured in the holes made around the rhizosphere of the plants after the emergence of roots *i.e*. at 45 after planting (Nithya Devi *et al.,* 2007). After inoculation the soil was lightly watered. Biochemical estimation was done in roots at 90th day after inoculation.

Observations in pot culture

The nematode population in roots, root lesion index, corm grade and biochemical activity in roots were assessed 90 days after inoculation of nematode. 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 1cm and weighed. Then, a sub sample of 15 g of roots per replicate was put into 100 ml 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 was taken in a counting dish to count nematodes under a stereomicroscope (Carlier *et al.,* 2003).

The extent of nematode damage to roots and corms was assessed following the technical guidelines prescribed by INIBAP (Pinochet,

1988). Plants were removed from the pots and the soil washed from the roots and corm with tap water. Roots were collected from plants were divided into dead and functional roots. The percentage root necrosis was estimated for five randomly selected functional primary roots. Five primary root segments of 10 cm were cut lengthwise and the percentage of visible necrotic cortical tissue of five root halves was determined. Each root half could have a maximum percentage root necrosis of 20%, adding up to 100% for the five root halves together (Speijer and Gold, 1996; Speijer and De Waele, 1997). Corm damage assessment was done after thoroughly shaking off all soil and washing the corms with water. The number of root showing black –purple lesions around their bases on the selected 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 (Pinochet, 1988) was followed to the hybrids as resistance, tolerant or susceptible as described in table 1.

Assessment of biochemical changes

The content of the biochemical phenols, lignin and orthohydroxy phenols and content of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in the root were determined for each replicate after 90 days, just before root samples were scored for nematode damage. The total phenol 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, 1955) and Ortho-dihydric phenol by Arnow's method (Arnow, 1937). The lignin content of banana roots was gravimetrically estimated methods of Chesson (1978). 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^{o}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 peroxidase activity was assessed according to Hammerschmidt *et al.*, (1982) and polyphenol oxidase activity was assessed using the modified method of Mayer *et al.*, (1965). Data were subjected to analysis of variance using the SPSS 20 statistical package and means compared by the LSD at P= 0.05.

Results and Discussion

Nematode population densities and root necrosis index were the most discriminate in this study on showing the difference in host reaction by banana hybrids to burrowing nematodes. Significant differences were observed among the hybrids for root population of *R. similis* at 90^{th} days after nematodes inoculation (Table 2). In all experiments, the mean average number of nematodes in roots of *R. similis* was low on the resistant reference cultivar Yangambi km5 (104 nematodes/5 g of roots) and high on the susceptible reference cultivar Grand Naine (417 nematodes/5 g of roots). The final nematode population of *R. similis* on H 943 (103 nematodes/5 g of roots) and H 912 (105 nematodes/5 g of roots) were not significantly different from Yangambi km5 and significantly lower than Grand Naine. Hybrids, H 914, H 926 and H 916 showed a partially resistant host response. On these hybrids, the average nematode population of *R. similis* ranged from 122 to 137 and the final nematode population was significantly higher than on Yangambi km5 and lower than Grand Naine, while H 922 recorded highest population of 484 nematodes/5 g of roots. The hybrids H 903, H 904, H 906, H 911, H 913, H 915, H 923, H 939 and H 952 were considered tolerant with the Nematodes

population ranges from 167 to 344. Some of the tolerant plant had higher nematode population but the growth of plant was not affected. This could be because, these plants allowed entry of the nematodes and their reproduction, but did not support further nematode growth. Similar results were also observed by Ramesh kumar *et al.,* (2012) and Das *et al.,* (2014).

Based on the intensity of lesions on roots and corm, they were assessed for their level of resistance (Table 2). Functional root numbers and dead roots percentage are considered as assessment of nematode damage in banana. Number of dead roots ranged from 3 for resistance check cultivar Yangambi km5 and 14 for susceptible check cultivar Grand Naine. Functional roots for resistance check cultivar 40 and susceptible check cultivar 34. The maximum number of functional roots were recorded in the H 916 (50 roots) followed by H 926 (47 roots) and least were found in H 912 (31). Percentage of dead root was 7% for Yangambi km5 and 41.18% for Grand Naine. For the resistant, bananas hybrids the percentage of dead root ranged from 2 to 5%. Among hybrids the dead root per cent was lowest in H 912 (6.45 per cent) and the highest in H 922 (38.46 per cent).

The damages caused by *R.similis* on the banana root system of resistance cultivars were always lower than on susceptible cultivars, from 10.5-19% to 48-56% (Dochez *et al.,* 2006). Root necrosis was a useful parameters in rating host resistance or susceptible to *R. similis* (Table 2). The percent necrosis of roots ranged from 8.00 to 40.00. Total root necrosis per cent was the minimum in H 912 and H 926 (8 per cent) and the maximum of 40.00 per cent in H 922. The root lesion index ranged from 1 to 5 and corm grade ranged from 1 to 4 among the hybrid. The minimum root lesion index of 1 was recorded by H 912, H 914, H 916, H 926 and H 943 and maximum root lesion index of 5 was registered by hybrid H 922 and H 925. The minimum corm grade of 1 was recorded by H 912, H 914, H 916, H 926 and H 943. In reference cultivar Yangambi km5 was recorded minimum root lesion index (1) and corm grade (1) while Grand Naine recorded maximum root lesion index (5) and corm grade (4). Good root development with healthy roots and corm favours resistance. Among the hybrids, 5 exhibited resistance, 10 exhibited tolerance, 5 were moderately susceptible and 4 were highly susceptible to nematode infestation. However, the use of dead roots percentage, nematode density and number of large lesions appears effective and an efficient approach to identify nematode resistant genotypes than the root necrosis index (Hartman *et al.,* 2010).

The lower percentage of root lesion index and corm lesion index in the resistance hybrids might be due to lower nematode population, less multiplication rate in soil and roots. Similar finding were earlier reported by Das *et al.,* (2010). The resistance banana cultivars had lower density of nematodes and least root damage. They may be considered as partially resistance to *Radopholus similis* (Gaidashova *et al.,* 2008). The resistance cultivar Gros Michel had lower root infestation reflect a lower nematode carrying capacity, probably linked to its lower sensitivity related to secondary metabolism (Quenneherve *et al.,* 2009).

The resistance hybrids exhibited its roots higher phenolic content and lignified cells also confirmed by histological studies. Carlier *et al.,* (2002) reported that assessment of root and corm damage will give a better understanding of resistance or tolerance of the cultivars under both field and glass house conditions. Banana varieties Gros Michel and Culcatta 4 were much lower root necrosis, percentage of dead roots and population

densities which were considered partially resistance to *R. similis* nematode (Speijer and Ssango, 1999).

Several physiological processes in the host are stimulated due to the activation of certain enzymes. Resistance to nematode is often governed by the intrinsic capability of the cells to deter the movement and feeding or interrupt the nematode reproduction by way of synthesis of certain chemical substances. Many of these proteins are enzymes such as phenylalanine ammonia lyase, polyphenol oxidase, peroxidase and b-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 nematodes (Seenivasan *et al.,* 2012).

Phenolic compounds are known to play a major role in the defense mechanism of plants against various external infectious agents. The total phenol content of the banana hybrids was estimated in roots and the results showed that there was a significant difference among the hybrids, treatments and their interaction (Table 3). In nematodes uninoculated hybrids (control), phenol registered the highest activity in H 926 (368.20µg/g) while lowest was observed in H 940 (166.34µg/g). Among the hybrids screened, the nematode inoculated plants recorded the maximum phenol content of 567.21 µg/g root in the hybrid H 916. The lowest phenol content (124.76 µg/g) was recorded in H 940 which was 7.24 per cent over control. Maximum increase in activity was observed in H 916 which showed a 69.44 per cent increase over control. In the present study, total phenol estimated in roots of banana hybrids showed that these compounds were higher in inoculated and uninoculated resistance hybrids *viz*., H 912, H 914, H 916, H 923 and H 946 than susceptible hybrids. Similar findings were observed by Fogain and Gowen (1996; Damodharan (2007), Kavitha *et al.,* (2008), Karunakaran (2010) Das *et al.,* (2014) and Ranchana *et al.,* (2016). The plant infected by nematodes and the accumulation of phenolic compounds in the plants (Selim *et al.,* 2014). Many of these compounds, especially oxidized forms, are toxic to repelling the juveniles or by adversely affecting the development of juveniles nematode and act as mechanical barriers to nematodes enter into the plant.

Increase in phenol content and enzyme activities were negatively related with the degree of infestation. The accumulation of phenol may be due to the excess production of hydrogen peroxide by increased respiration (Farkas and Kraly, 1962) or due to the activation of hexose monophosphate shunt pathway, acetate pathway and release of bound phenols by hydrolytic enzymes (Goodman *et al.,* 1967; Seenivasan, 2011). Fogain 1996 and Valette *et al.,* (1997) found that higher amount of phenolics in the resistance banana cultivar of Yangambi km5. Most of the nematode resistance plant is found in hypersensitive type of response that involves change in enzyme activity, phenol metabolism and deposition of the newly synthesized material in cell walls and regulation of free redical O_2 (Ganguly and Dasgupta, 1980; Zacheo *et al.,* 1995). Similar results were also observed by Das *et al.,* (2011) for nematode resistance in banana.

Irrespective of the hybrids screened, the nematodes inoculated plants registered higher Ortho – dihydroxy phenol content in roots compare to uninocualed hybrids (Table 3). The highest Ortho – dihydroxy phenol content was observed in the hybrids, H 916 (2.77 µg/g) which showed an increase of 48.92 per cent over the control. The OD phenol was found to be lower (0.98 µg/g) in H 922 which showed an increase of 13.95 per cent over control. Studies on the changes in the Orthodihydroxy phenol clearly indicated that

there was an enhancement of activities of this biochemical in the resistance hybrids.

Phenol and OD-phenol content had increased upto 50% at 30 days after sowing, which protected the plant from the pest by imparting high level of resistance (Taggar *et al.,* 2014). The increased levels of orthodihydroxy phenols might have resulted as a means of defensive reaction to nematode infestation since orthodihydroxy phenols are known to be reactive and upon oxidation yield quinones which are still more toxic to invading organisms (Indu Rani *et al.,* 2008). The per cent mean accumulation of orthodihydroxy phenol content in genotypes between before pathogen initiation stage to peak stage of infection was more in resistant genotypes (55.80%), than moderately resistant (46.27%) and susceptible (37.36%) genotypes (Sowmya, 2011).

The infestation due to nematodes increased the lignin in all banana hybrids compared to uninoculated plants and the differences were significant. Among the hybrids screened, uninoculated hybrids (control) registered the highest lignin was registered in H 926 (1.12 per cent) while lowest was recorded in H 940 (0.51 per cent) (Table 3). In nematode inoculated hybrids, H 912 registered the highest lignin content of 1.93 per cent. The percentage increase of lignin activity over control was more in the hybrid H 912 (78.70%).

Lignin is one of the most abundant bio-polymers, which provides resistance to plants against *R. similis* and makes the cell wall more resistant to nematodes attack. The deposition of lignin around the vascular bundle has been implicated as a defense response in banana resistant cultivar to nematodes. Similar results were also observed by Kavitha *et al.,* (2008) and Karunakaran (2010) in banana.

A close relationship between lignification and disease resistance has been showed that resistant plants accumulated lignins more rapidly and/or exhibited enhanced lignin deposition as compared with susceptible plants (Yates *et al.,* 1997). Lignin and phenol are synthesized via phenyl propanoid pathways which impart resistance against nematode attack. The role of phytoalexins and other toxic compounds like phenols and lignin in resistance mechanism have been reported by earlier workers (Reuveni *et al.,* 1992 and Sariah *et al.,* 1999). An increase in the number of lignified cells in tolerant cultivars compared to susceptible cultivars of banana was noted by Fogain, (1996) and Kavino *et al.,* (2007).

Enzyme activity is one of the important tools to confirm the resistance to root pathogenic nematodes. When a nematode infects the host tissue, a small number of specific genes are induced to produce mRNA's that permit synthesis of similar number of specific proteins (Vidhyasekaran, 1993). 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 nematodes (Seenivasan *et al.,* 2012)

The infestation due to nematodes increased the peroxidase activity in all banana hybrids compared to uninoculated plants and the differences were significant (Table 4). The uninoculated hybrids, H 914 (2.47abs/min/g) registered the highest peroxidase content while the lowest was observed in H 941 (0.97abs/min/g). Among the inoculated hybrids, peroxidase activity of 3.32 abs/min/g fresh weight was highest in H 914 which showed 34.41 per cent increase over control conditions. The lowest activity of 1.16

abs/min/g fresh weight was recorded in H 922 with an increase of 10.48 per cent over control. Estimation of peroxidase activity in the current study elicits that all the resistant hybrids possessed higher peroxidase activity than the susceptible ones. A critical analysis of their activity in this study revealed that resistance hybrids *viz.*, H 912, H 914, H 916, H 923 and H 943 recoded highest peroxidase activity than the susceptible hybrids. The percentage increase was more in resistant hybrids as compared to susceptible banana hybrids. Similar finding was also reported by Das *et al.,* (2011 and 2014) and Tapamay Dhar *et al.,* (2016).

Peroxidase activity in nematodes infected roots of tomato were considered, there total peroxidase activity was twice in resistance plants as compared to susceptible (Zacheo *et al.,* 1993). Peroxidase makes cellular environment toxic and extremely unfavorable for pathogen by producing reactive species of oxygen and nitrogen (Passardi *et al.,* 2005; Gill and Tuteja, 2010; *Liu et al.,* 2010; Schaffer and Bronnikova, 2012). Peroxidase enzyme provide mechanism for resistance to pathogens, is highly essential Peroxidase enzymes also play a vital role in alleviating free radical toxicity in plant tissues (Fogain and Gowen, 1996; Valette *et al.*, 1997; Elsen *et al.,* 2002). Increased activity of peroxidase in tomato and phenylalanine ammonia lyase in brinjal was positively correlated with nematode resistance (Rajasekar *et al,* 1997; Sirohi and Dasgupta, 1993). Polyphenol oxidase and peroxidase, the enzymes involved in the oxidation of phenols to more toxic quinones, are known to increase in resistant plants (Yamamoto and Tani, 1978). Significant difference was noticed between the hybrids, treatments and the interaction with regard to polyphenol activity (Table 4). The hybrid H 923 expressed the maximum activity of 0.096 abs/min/g under control and 0.126 abs/min/g when inoculated while the minimum was in H 922 (0.032 abs/min/g) under control and (0.037abs/min/g) when inoculated. Increase in polyphenol activity was the highest in H 926 (41.56%) and the lowest in H 905 (9.30%) followed by H 901 (9.62%). Following inoculation of banana hybrids with the nematodes, the polyphenol activity increased in both the resistant and susceptible hybrids. However, the final enzyme concentration was the maximum in resistance hybrids like H 912, H 914, H 916, H 923 and H 943. Polyphenol activity was found to increase in all the hybrids following nematode inoculation. However, the final enzyme concentration was higher in the resistance hybrids. Increased peroxidase activity due to nematode infestation was reported by Fogain and Gowen (1996), Krishnamoorthy (2002); Wayts *et al.,* (2005); Karunagaran (2010); Anitha an Samyappan, (2012); Xiao-Lin *et al.,* (2013); Das *et al.,* (2014); Soffan *et al.,* (2014) and Latournerie-Moreno *et al.,* (2015).

Peroxidase involved in defense mechanism considered the condensation of phenolic monomers derived from the phenylpropanoid pathway into insoluble polymers (Robb *et al.,* 1991).

Devarajan and Seenivasan (2002) observed that inoculation of nematodes increase the polyphenol oxidase activity in banana. It might have resulted in oxidation of polyphenols and accumulation of monophenols which are responsible for resistance reaction. 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 root tissues (Das *et al.,* 2010). Thus, the overall estimation analysis of these enzymes in resistant and susceptible hybrids indicates the role of these enzymes in conferring resistance to nematodes.

Table.1 Orientative scale to assess the reaction of banana root lesion nematodes according to Pinochet (1988)

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

Table.2 Nematode reproduction and percentage root necrosis on banana hybrids at 90 days after root inoculation with *Radopholus similis* under pot culture

S. No.	Hybrids	Parents	Nematodes population in roots	Roots			Total RN %	Root lesion index	Corm grade	Reaction status
				DR	OK	DR %				
1	H 901	Poovan × Rose	316	9	42	21.43	27	3	3	S
2	H 902	Poovan × Rose	312	12	45	26.67	35	3	3	S
3	H 903	Poovan × Rose	344	10	41	24.39	25	3	2	T
4	H 904	Poovan × Rose	268	7	44	15.91	15	3	2	T
5	H 905	Poovan × PL	413	12	42	28.57	31	4	4	S
6	H 906	Poovan × PL	167	8	44	18.18	17	2	2	T
7	H 911	H 516 × Rose	286	6	34	17.65	12	2	2	T
8	H 912	H 516 × Rose	105	2	31	6.45	8	1	1	R
9	H 913	Poovan × ABK	316	11	40	27.50	22	2	3	T
10	H 914	Poovan × ABK	122	5	46	10.87	10	1	1	R
11	H 915	Poovan × ABK	162	4	37	10.81	13	1	2	T
12	H 916	Poovan × EV	137	5	50	10.00	9	1	1	R
13	H 921	H 516 × YKM5	312	7	33	21.21	29	3	3	S
14	H 922	Poovan × ABK	484	15	39	38.46	40	5	4	HS
15	H 923	Poovan × H 516	294	6	42	14.29	15	2	2	T
16	H 924	H 201 × ANK	384	9	43	20.93	33	3	3	S
17	H 925	H 201 (OP)	445	13	36	36.11	39	5	4	HS
18	H 926	H 201 × ANK	133	5	47	10.64	8	1	1	R
19	H 934	Poovan × EV	395	7	36	19.44	22	4	2	T
20	H 939	H 201 × ANK	294	5	35	14.29	12	3	2	T
21	H 940	H 201 × H 516	371	11	32	34.38	28	4	4	S
22	H 941	H 201 × H 516	385	12	39	30.77	33	4	3	S
23	H 943	Rose × H 516	103	3	33	9.09	9	1	1	R
24	H 952	H 201 (OP)	296	5	33	15.15	14	2	2	T
Reference cultivars										
1	Yangambi km5		104	3	40	7.00	7	1	1	R
2	Grand Naine		417	14	34	41.18	43	5	4	HS

DR - Dead roots; OK - Functional roots; RN – Root necrosis; R - Resistant; T-Tolerant; S- Susceptible; HS – Highly susceptible; ANK - Anaikomban; EV - Erachivazhai; PL - PisangLilin; ABK - Ambalakadali; BC - Bareli Chinia; Rob - Robusta; OP - Open Pollinated; Parentage: H 516 (ANK × PL); H 201 (BC × PL) × Rob

Table.3 Total phenol content, OD phenol and lignin percent in the roots of banana hybrids inoculated with *R. similis* in pot

S. No.	Hybrids	Total phenol (µg/g)			OD Phenol (µg/g)			Lignin (%)		
		C	I	%	C	I	%	C	I	%
1	H 901	271.26	341.55	25.91	1.64	2.04	24.39	0.76	0.84	10.53
2	H 902	194.51	224.60	15.47	1.18	1.40	18.64	0.87	0.98	12.64
3	H 903	152.19	181.00	18.93	1.17	1.34	14.53	0.53	0.64	20.75
4	H 904	265.50	369.40	39.13	1.60	2.19	36.88	1.05	1.70	61.90
5	H 905	160.88	176.58	9.76	1.08	1.19	10.19	0.56	0.65	16.07
6	H 906	247.21	338.24	36.82	1.64	2.29	39.63	1.04	1.62	55.77
7	H 911	234.26	289.51	23.58	1.26	1.52	20.63	0.83	1.24	49.40
8	H 912	318.60	514.65	61.53	1.76	2.58	46.59	1.08	1.93	78.70
9	H 913	183.02	223.28	22.00	1.20	1.42	18.33	0.74	1.02	37.84
10	H 914	276.59	395.00	42.81	1.54	2.08	35.06	1.03	1.70	65.05
11	H 915	290.44	426.16	46.73	1.74	2.23	28.16	0.94	1.50	59.57
12	H 916	334.76	567.21	69.44	1.86	2.77	48.92	1.07	1.82	70.09
13	H 921	205.54	248.50	20.90	1.68	1.98	17.86	0.78	1.13	44.87
14	H 922	133.92	154.22	15.16	0.86	0.98	13.95	0.46	0.51	10.87
15	H 923	271.23	366.30	35.05	1.44	1.89	31.25	0.84	1.36	61.90
16	H 924	143.50	171.62	19.60	1.54	1.82	18.18	0.87	1.02	17.24
17	H 925	160.82	187.00	16.28	1.15	1.41	22.61	0.73	0.91	24.66
18	H 926	368.20	548.65	49.01	1.87	2.67	42.78	1.12	1.93	72.32
19	H 934	288.00	382.49	32.81	1.35	1.76	30.37	0.94	1.23	30.85
20	H 939	214.67	276.85	28.97	1.26	1.58	25.40	0.76	1.09	43.42
21	H 940	116.34	124.76	7.24	1.30	1.47	13.08	0.51	0.60	17.65
22	H 941	146.13	177.14	21.22	1.22	1.45	18.85	0.94	1.08	14.89
23	H 943	274.91	379.00	37.86	1.68	2.33	38.69	0.98	1.66	69.39
24	H 952	220.47	262.73	19.17	1.26	1.60	26.98	0.81	1.17	44.44
Reference cultivars										
1	Yangambi	324.11	529.15	63.26	2.31	3.70	60.17	1.08	1.93	78.70
2	GrandNaine	142.65	168.43	18.07	0.94	1.10	17.02	0.83	0.94	13.25
		G	T	G x T	G	T	G x	G	T	G x T
	SEd	7.369	1.762	10.421	0.046	0.110	0.065	0.028	0.007	0.039
	CD(p=0.05)	14.573	3..484	20.609	0.091	0.022	0.129	0.054	0.013	0.077

C - Control; I - Inoculated; % - Per cent difference over control

Table.4 Peroxidase, polyphenol oxidase, phenyl alanine ammonia lyase activities in banana hybrids inoculated with *R. similis* in pot

S. No.	Hybrids	Peroxidase (abs/min/g)			Polyphenol oxidase (abs/min/g)			Phenyl alanine ammonia lyase (nmol/min/ml)		
		C	I	%	C	I	%	C	I	%
1	H 901	1.74	2.02	16.09	0.052	0.057	9.62	13.40	15.20	13.43
2	H 902	1.96	2.20	12.24	0.067	0.077	14.93	17.69	19.84	12.15
3	H 903	1.28	1.40	9.37	0.041	0.046	12.20	14.20	16.52	16.34
4	H 904	1.74	2.16	24.14	0.070	0.096	37.14	24.00	29.67	23.63
5	H 905	1.33	1.44	8.27	0.043	0.047	9.30	9.51	10.33	8.62
6	H 906	2.09	2.70	29.19	0.082	0.112	36.59	19.22	24.46	27.26
7	H 911	1.57	1.98	26.11	0.076	0.086	13.16	15.73	18.34	16.59
8	H 912	2.23	2.87	28.70	0.062	0.086	38.71	18.55	23.28	25.50
9	H 913	1.30	1.58	21.54	0.046	0.055	19.57	16.86	19.40	15.07
10	H 914	2.47	3.32	34.41	0.065	0.089	36.92	18.50	23.73	28.27
11	H 915	1.62	2.04	25.93	0.058	0.080	37.93	13.41	16.70	24.53
12	H 916	2.21	2.93	32.58	0.084	0.113	34.52	22.63	29.50	30.36
13	H 921	1.63	1.95	19.63	0.042	0.052	23.81	16.49	19.85	20.38
14	H 922	1.05	1.16	10.48	0.032	0.037	15.63	11.24	12.58	11.92
15	H 923	1.79	2.17	21.23	0.096	0.126	31.25	19.10	23.55	23.30
16	H 924	1.20	1.46	21.67	0.056	0.071	26.79	16.80	18.60	10.71
17	H 925	1.90	2.16	13.68	0.061	0.073	19.67	14.59	16.73	14.67
18	H 926	2.23	2.85	27.80	0.077	0.109	41.56	21.72	30.12	38.67
19	H 934	2.16	2.47	14.35	0.062	0.077	24.19	17.24	20.91	21.29
20	H 939	1.32	1.60	21.21	0.047	0.060	27.66	15.34	19.15	24.84
21	H 940	1.44	1.66	15.28	0.044	0.055	25.00	12.40	13.81	11.37
22	H 941	0.97	1.18	21.65	0.053	0.062	16.98	18.70	21.36	14.22
23	H 943	2.10	2.78	32.38	0.078	0.109	39.74	19.34	25.58	32.26
24	H 952	1.35	1.69	25.19	0.051	0.063	23.53	16.12	18.78	16.50
	Reference cultivars									
1	Yamgambi km5	2.31	3.05	32.03	0.092	0.143	55.43	22.48	30.31	34.83
2	Grand Naine	1.24	1.40	12.90	0.053	0.061	15.09	13.51	15.10	11.77
		G	T	G x T	G	T	G x T	G	T	G x T
	SEd	0.051	0.012	0.072	0.003	0.001	0.003	0.479	0.115	0.678
	CD (p=0.05)	0.100	0.024	0.142	0.005	0.001	0.007	0.948	0.227	1.340

C - Control; I - Inoculated; % - Per cent difference over control

The result of the study showed significantly lower activity of PAL in nematode inoculated plants of susceptible cultivars while in the resistance hybrids, the PAL activity increased in both healthy and nematode inoculated plants of hybrid (Table 4). Among the hybrids, H 904 registered the highest phenylalanine ammonialyase content of 24.00 nmol/min/ml in control and 29.67 nmol/min/ml under inoculated condition while H 905 registered lowest content of 9.51 nmol/min/ml and 10.33 nmol/min/ml under

control and inoculated condition respectively. The maximum per cent increase in PAL was recorded by H 926 (38.67 nmol/min/ml), while the lowest by H 905 (8.62 nmol/min/ml). Phenylalanine ammonia lyase is the most important enzyme in the synthesis of phenolics, phytolexin and lignin. Hence it is considered as the most important enzyme in nematode resistance.

The phenylalanine ammonia lyace activity in resistance diploid accessions of banana roots had higher activity after nematode inoculation compared to susceptible banana (Nithya Devi *et al.*, 2007) Das *et al.*, (2014). PAL is the first enzyme in phenylpropanoid metabolism involved in the production of phenolics and phytoalexins that prevent establishment of the pathogen (Mariutto *et al.*, 2011). This enzyme induces the formation of necrosis, which resulted in the localization of the juveniles and prevented their further movement. The maximum activity of glucanase, chitinase and PAL was found to be associated with resistant status of mungbean cultivars (Deepak and Choudhary, 2012). The overall evaluation of 24 banana hybrids led to identification of the hybrids H 912, H 914, H 916 H 926 and H 943 were resistant to *R. similis*. These studies indicate that higher content of phenols, lignin and PO, PPO and PAL might have produced toxic compound against nematodes. These resistance hybrids can be utilized in future breeding programme for developing nematode resistance hybrid. Indeed, the highest yielding hybrids evaluated in multi-location trials and could be released for cultivation.

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How to cite this article:

Sankar, C., K. Soorianathasundaram, N. Kumar, G. Karunakaran and Sivakumar, M. 2017. Induction of Resistant to *Radopholus similis* and Defence Related Mechanism in Susceptible and Resistance Banana Hybrids Infected with *Radopholus similis. Int.J.Curr.Microbiol.App.Sci.* 6(4): 1668-1684. doi: https://doi.org/10.20546/ijcmas.2017.604.202