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Phenylpropanoid enzymes, phenolic polymers and metabolites as chemical defenses to infection of *Pratylenchus coffeae* in roots of resistant and susceptible bananas (*Musa* spp.)

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Activity differences of the first (phenylalanine ammonia lyase, PAL) and the last (cinnamyl alcohol dehydrogenase, CAD) enzymes of phenylpropanoid pathway in the roots of resistant (Yangambi Km5 and Anaikomban) and susceptible (Nendran and Robusta) banana cultivars caused by root lesion nematode, *Pratylenchus coffeae*, were investigated. Also, the accumulation of phenolics and deposition of lignin polymers in cell walls in relation to resistance of the banana cultivars to the nematode were analyzed. Compared to the susceptible cultivars, the resistant cultivars had constitutively significantly higher PAL activity and total soluble and cell wall-bound phenolics than in susceptible cultivars. The resistant cultivars responded strongly to the infection of the nematode by induction of several-time higher PAL and CAD enzymes activities, soluble and wall-bound phenolics and enrichment of lignin polymers in cell wall and these biochemical parameters reached maximum at 7th day postinoculation. In addition, profiles of phenolic acid metabolites in roots of Yangambi Km5 and Nendran were analyzed by HPLC to ascertain the underlying biochemical mechanism of bananas resistance to the nematode. Identification and quantification of soluble and cell wall-bound phenolic acids showed six metabolites and only quantitative, no qualitative, differences occurred between the resistant and susceptible cvs. and between constitutive and induced contents. A very prominent increase of *p*-coumaric, ferulic and sinapic acids, which are precursors of monolignols of lignin, in resistant cv. was found. These constitutive and induced biochemical alterations are definitely the chemical defenses of resistant cvs. to the nematode infection.

Keywords: Cinnamyl alcohol dehydrogenase, *p*-Coumaric acid, Ferulic acid, Lignin, Phenylalanine ammonia lyase, Root lesion nematode, Yangambi Km5

The involvement of widely distributed and naturally occurring phenolics in plant resistance against pathogens attack is a well established phenomenon[1,2]. All phenolic moieties are generated through the biosynthetic route of phenylpropanoid pathway by a series of hydroxylation, methylation and dehydration reactions from cinnamic acid, which is formed from phenylalanine by the action of phenylalanine ammonia lyase (PAL), the branch point enzyme between primary shikimate pathway and secondary phenylpropanoid pathway[3,4]. Phenolic acids in plant cells are usually conjugated to sugars, other cell wall carbohydrates and organic acids and the complex cell wall polymer of lignin is formed from these phenylpropanoids and esters[5,6]. Lignin, composed of aromatic phenolic metabolites, is actively synthesized and deposited in the cell wall during the infection process as a chemical mechanism of resistance to the penetration of the pathogen[7]. Some phenolic molecules called phytoanticipins occur constitutively and act as preformed inhibitors of pathogens and others called phytoalexins, which are induced to form in response to pathogen infection.

The phytoanticipins is part of nonhost resistance mechanism and formation of phytoalexins is considered as part of an active defense response[8]. Cinnamyl alcohol dehydrogenase (CAD) is a key enzyme in the lignification pathway as it catalyses the final step in the synthesis of monolignols, thereby converting the cinnamaldehydes to the corresponding alcohols and is considered as an indicator of lignin biosynthesis[9]. CAD is regulated much like other well-studied enzymes of phenylpropanoid metabolism in response to pathogen infection and other stresses[10] and pathogen elicitors[11].

The relationship between phenolic metabolites and nematode infection in plants has been the subject of

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research for several years. On the host-pathogen interaction between the banana and nematodes, phenolic and proanthocyanidin compounds and lignifications of cell walls have been considered as resistance factors. Higher number of preformed phenolic cells and cells with lignified walls[12] and accumulation of phenols[13,14] were observed in roots of *Radopholus similes*-infected resistant banana cultivars. Induction of phenalenone-type phytoalexins[15,16] and higher accumulation of proanthocyanidins and phenolic acids[14,17] in roots were also observed and proposed as the biochemical responses of the resistant *Musa* species to *R. similis* infection. With respect to interaction between bananas and root-lesion nematode, *Pratylenchus coffeae*, phenols content and phenol-oxidizing enzymes have been positively correlated with resistance of banana cultivars to the nematode[18,19].

The endoparasitic migratory root lesion nematode, *P. coffeae*, is a serious biotic threat to banana cultivation in India and crop loss by this nematode is over 40%[20]. The symptom, reddish-brown lesions in the root epidermis and cortical parenchymatic tissues, induced by *P. coffeae* infection are similar to those caused by *R. similis*[21,22] and moreover, the biochemical responses of bananas to both the nematodes are also similar[23]. Screening of banana clones to the reaction of nematodes infection revealed presence of a certain level of resistance/tolerance to the parasitism of *P. coffeae*. The banana cultivars Yangambi Km5 and Anaikomban are considered as resistant sources[24,25] while cultivars Nendran and Robusta are categorized as susceptible sources[18,26] to the nematode. Enhancement of cell wall lignifications and phenol containing cells as physical and chemical defense barriers against the parasitism in *R. similis* are reported. Similar investigation on host-pathogen interaction between bananas and *P. coffeae* is limited to correlating the activities of stress-related, phenol-oxidizing enzymes and total phenols contents with the resistance of the banana cultivars. The present experiments aimed to probe and elucidate the enzymes of phenolics synthesis and phenolic polymer and metabolites in the roots of banana cultivars with respect to resistance against the root lesion nematode. Differential changes in enzymes activities, lignin and phenolic metabolites synthesis after parasitism of the nematodes are found and these could definitely be the contributory factors of resistance of the banana cultivars to the nematode.

Materials and Methods

Plant materials and nematode inoculation— Uniformly sized (0.9-1.2 kg) suckers of the resistant banana cultivars *viz*., Yangambi Km5 (*Musa*, AAA) and Anaikomban (*Musa*, AA)[24,25] and susceptible cultivars *viz*., Nendran (*Musa*, AAB) and Robusta (*Musa*, AAA)[18,26] were planted in individual cement pots of 75 liter capacity filled with sterilized soil. Plants were grown with normal watering, recommended dose of fertilizers and need-based plant protection measures for three months. Nematode inoculum was mixture of female adults and infective juveniles of a virulent strain of *P. coffeae* isolated from banana roots and the inoculation suspension was adjusted for 250 nematodes/mL. The nematode was inoculated on roots of 2-months-old sucker-derived plants according to the method of Niere *et al*[27]. Briefly, white young tender fast-growing roots of 5-8 cm length were inserted into plastic cup of 250 mL volume half-filled with soil and the inoculum was directly applied on the roots and closed with soil. Each root was inoculated with 1000 nematodes (4 mL of suspension) and four roots/plant and six plants/cultivar were inoculated. For analysis, roots were sampled from uninoculated (control) plants to ascertain constitutive levels of enzymes and biochemicals and at 4, 7, 10, 15 and 30 days postinoculation from the inoculated plants.

Enzymes extraction and activity assays— *Phenylalanine ammonia lyase (PAL)*: Root pieces excised from plants were quickly washed and immersed in liquid nitrogen. Enzyme source (the supernatant) was obtained by pulverizing one g of root tissue in liquid nitrogen, homogenizing in 2 mL of 0.3 mM sodium borate buffer, *p*H 8.8, with 1 mM EDTA, 1 mM dithiothreitol, 5% insoluble polyvinylpolypyrrolidone (Sigma Chemical Co., St. Louis, USA) and spinning at 12,000 *g* for 20 min[28]. The activity of PAL was determined spectrophotometrically by measuring the amount of *trans*-cinnamic acid produced from L-phenylalanine. Enzyme source (1 mL) was mixed with 2 mL of the reaction mixture containing 0.3 mM borate buffer, *p*H 8.8 and 0.03 mM L-phenylalanine. The absorbance was read at 290 nm after 1 h incubation at 35 ^{o}C against the blank containing D-phenylalanine[29].

Cinnamyl alcohol dehydrogenase: The buffer system was 0.1 M Tris-HCl, *p*H 7.5 with 15 mM β-mercaptoethanol, 10% (v/v) ethylene glycol and 5% insoluble polyvinylpolypyrrolidone[28] and assay of

CAD was according to Wyrambik and Grisebach[30] and the activity was measured following the oxidation of hydroxycinnamyl alcohol at 30 °C. Assays with coniferyl alcohol as the substrate were monitored by following the formation of coniferaldehyde at 400 nm and with sinapyl and cinnamyl alcohols at 340 nm following the formation of NADPH. The protein content of the supernatants was determined by Bradford method with BSA as standard[31]. The activity of the enzymes, both PAL and CAD, is expressed as picokatals/mg protein.

Phenolics extraction and analysis—Two fractions *viz*., total methanol-soluble phenolics including non-conjugated, glycoside-bound and ester-bound phenolics and cell wall-bound (ester-bound phenolics incorporated in the cell wall) were prepared from the roots of bananas[32]. Root tissues (1 g) were separately ground in liquid nitrogen, homogenized and extracted with 2 mL of 50% (v/v) aqueous methanol by sonication for 20 min. The precipitates, after centrifugation at 3,000 *g* for 10 min and collection of supernatant, were reextracted with 80% methanol twice and the pooled supernatants of methanolic extracts containing total soluble phenolics was evaporated to dryness under reduced pressure and the residue was redissolved in 0.5 mL of aqueous methanol. The release of non-conjugated and glycoside-bound phenolics was achieved by acidification and hydrolysis of total methanol soluble phenolics fraction with 50 µL of concentrated HCl and ester-bound phenolics by alkali hydrolysis with 200 µL 2 M NaOH followed by 80 µL 1 M HCl. The total phenolics were extracted with 2 mL anhydrous diethyl ether[33] and evaporated to dryness and redissolved in standard volume of 50% methanol. The final residues, after extraction of total methanol-soluble phenolics, consisting of methanol-insoluble cell wall materials of root tissues were used for the extraction of wall-bound phenolics according to Campbell and Ellis[34]. The alcohol-insoluble cell wall residues were dried at 70 °C overnight and known quantity of materials (1 mL for 10 mg) were subjected to alkali hydrolysis using 2 N NaOH for 12 h. The supernatants were collected following centrifugation at 7,000 *g* for 10 min. The resulting pellets were treated twice with 6 N HCl (*p*H 1-2) for acid hydrolysis and the supernatants were collected by centrifugation and the residue pellets were saved for lignin analysis. From the combined supernatants, cell wall-bound phenolics were extracted with 2 mL anhydrous diethyl ether, evaporated to dryness under reduced pressure and the residue was redissolved in standard volume of 50% aqueous methanol. Quantification of total methanol-soluble phenolics and cell wall-bound phenolics were done with 1 N Folin-Ciocalteau reagent and phenolics contents are expressed as µg/g of fresh root weight.

Lignin analysis—After extraction of wall-bound phenolics, the residues of root tissues were washed with water and methanol twice separately, dried at 37 °C, weighed and used for determination of lignin contents by derivatization with thioglycolic acid[35]. Briefly, 25 mg of alcohol insoluble cell wall materials were treated with 1 mL of 2 M HCl and 0.2 mL of thioglycolic acid for 4 h at 95 °C. After removal of supernatant by centrifugation at 12,000 *g* for 10 min at room temperature and washing the pellets thrice with water, the pellets were treated with 1 mL of 0.5 M NaOH by vigorous shaking overnight. Following centrifugation, the supernatant was collected and pellets were washed with 0.5 mL of 0.5 M NaOH and the supernatant was saved. The combined supernatant of alkali extracts was acidified with 0.3 mL conc. HCl and was allowed to precipitate lignothioglycolic acid at 4 °C for 4 h. The brown colour pellets obtained after centrifugation was dried in vacuum and dissolved in 50 mL of 0.5 M NaOH. Absorbance of samples was read at 280 nm and lignin content as lignothioglycolic acid was calculated as µg lignin/mg cell wall dry weight using a calibration curve made with alkali lignin (Sigma-Aldrich, USA).

Phenolic metabolites analysis—Detection and quantification of phenolic metabolites were done in roots of control *i.e*., 0 day and at 7th day postinoculation of Yangambi Km5 and Nendran cvs. with six replications. The free and cell wall-bound phenolic metabolites extracted from roots were dissolved in 50% (v/v) methanol and mobile phases were filtered through a 0.22 µm filters (Millipore, Bedford, MA, USA) and analyzed by HPLC (Waters, Milford, MA, USA) consisting of a binary pump (Waters 515), an autosampler (Waters 717) and a PDA detector (Waters 2998). A Waters Atlantis® dC_{18} 0.5 µm column (250 × 4.6 mm^2) coupled to a guard column C_{18} ODS (4 × 3.0 mm^2) at 30 °C was used for analysis. A mixture of methanol and 1 mM trifluoroacetic acid (32:68 v/v) was employed as mobile solvents with a flow rate of 1 mL/min. Chromatograms were collected and data were analyzed using Empower2 software (Waters).

Identification and quantification of phenolic metabolites of the samples was performed by comparing retention times (R_t) with those of authentic standards and quantification of each metabolite was achieved based on peak area of samples with that of standard using Empower2 software.

Statistical analysis—Data on the induction of enzymes activities and accumulation of phenolics and lignin and phenolic metabolites were analyzed using Proc Glm by SAS 9.2 software.

Results

Typical reddish-brown symptoms of *P. coffeae* infection started appearing from 4th day and reached maximum dark necrosis by 6th and 7th days after inoculation of the nematode. Necrotic lesions on the roots were more on the susceptible banana cvs., Nendran and Robusta, and less visible on the resistant cvs., Yangambi Km5 and Anaikomban.

Phenylpropanoid enzymes activities—The constitutive activity levels of PAL showed significant variation between the resistant (Anaikomban and Yangambi Km5) and susceptible (Nendran and Robusta) banana cvs. with higher levels in roots of resistant cvs. (mean activity of 26.5 picokatals/mg protein) than in the susceptible cvs. (14 picokatals/mg protein) (Fig. 1a). The temporal activity of PAL showed increment from 4th day reaching the maximum at 7th day with 163 picokatals/mg protein in resistant cvs., which was 6.2-time higher than the constitutive levels and 5.2-time greater than the susceptible cvs. The enzyme activity at 7th day in susceptible cvs. was only 2.2-time compared to the constitutive levels. The activity decreased from 10th day, however, the activity levels at 30th day were greater than constitutive levels and the difference in activity between resistant and susceptible cvs. was 4-time at 30th day.

Similar to PAL, the activity levels of CAD increased markedly from 4th day postinoculation in resistant cvs. reaching highest at 7th day (360 picokatals/mg protein), which was 18-time higher than constitutive level (20 picokatals/mg protein) and 7.3-time (49 picokatals/mg protein) greater than in the susceptible cvs. (Fig. 1b). The CAD activity in susceptible cvs. at 7th day was only 3.3-time from the constitutive levels. Started decreasing from 10th day postinoculation, the difference in activity was 7.6-time at 30th day between resistant and susceptible cvs.

Phenolic acids induction levels—Significant difference in the constitutive contents of total soluble phenolics between the resistant (56 µg/g fr. wt.) and susceptible (45.3 µg/g fr. wt.) cvs. was found (Fig. 2a). Prominent accumulation of total phenolics was observed at 7th day postinoculation in resistant cvs. with 245 µg/g fr. wt., which was 4.4-time higher than the corresponding control (56 µg/g fr. wt.) and 3.1-time (78 µg/g fr. wt.) greater than the susceptible cvs. The accumulation of soluble phenolics in susceptible cvs. was only 1.7-time of the constitutive levels. At 30th day, resistant cvs. had 2.6-time higher soluble phenolic levels than the susceptible cvs.

Significant difference existed in the constitutive contents of cell wall-bound phenolics between the resistant (19.4 µg/g fr. wt.) and susceptible (15.1 µg/g fr. wt.) cvs. (Fig. 2b). The accumulation of wall-bound phenolics at 7th day in resistant cvs. was 4.2-time (82 µg/g fr. wt.) higher than the corresponding control (19.4 µg/g fr. wt.) and 3-time (27.5 µg/g fr. wt.) greater than the susceptible cvs. In susceptible cvs., the infection induced only a moderate increase (1.8-time) in wall-bound phenolics at 7th day. From 10th day, the wall-bound phenolics contents reached plateau values and slightly increased up to 30th day with a 2.6-time difference existing between the resistant and susceptible cvs.

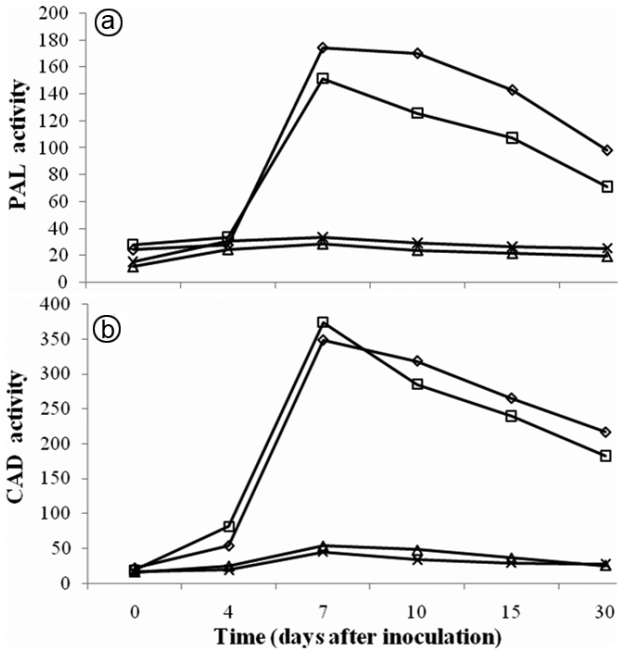

Fig. 1—Induction of phenylalanine ammonia lyase **(a)** and cinnamyl alcohol dehydrogenase **(b)** activity in resistant (◊-Anaikomban and □-Yangambi Km5) and susceptible (×-Nendran and Δ-Robusta) banana cvs. roots in response to *P. coffeae* infection. The enzyme activity was assayed from proteins extracts of roots and expressed as picokatals/mg protein. Values are mean±SE(n=6).

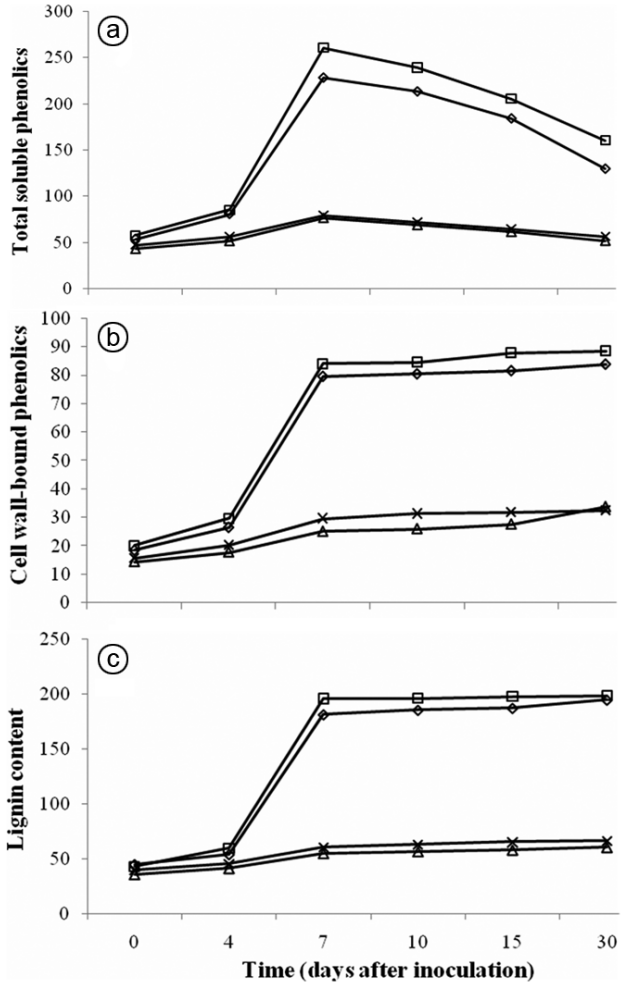

Fig. 2—Accumulation of total soluble phenolics **(a)**; cell wall-bound phenolics **(b)** and lignin **(c)** in resistant (◊-Anaikomban and □-Yangambi Km5) and susceptible (×-Nendran and Δ-Robusta) banana cvs. roots in response to *P. coffeae* infection. Phenolics content were estimated with the Folin- Ciocalteau reagent and expressed as µg/g root fresh weight. Values are mean±SE(n=6).

Lignin accumulation—No significant difference in the constitutive contents of lignin between the resistant and susceptible cvs. was found and the lignin contents (Fig. 2c) correlated well with the accumulation levels of both total soluble and wall-bound phenolics (Fig. 2a and b). The increase of lignin deposition in resistant cvs. was rapid reaching the maximum at 7th day with 188 µg/mg root cell wall dry wt., which was 4.3-time higher than the corresponding control (44 µg/mg root cell wall dry wt.) and 3.3-time (57 µg/mg root cell wall dry wt.) greater than the susceptible cvs. The susceptible cvs. had only 1.5-time higher lignin contents at 7th day. The lignin contents were stabilized and slightly increased between 10th and 30th day in both resistant and susceptible cvs. and the difference was 3.1-time between the resistant and susceptible cvs. at 30th day.

Soluble and cell wall-bound phenolic metabolites—HPLC analysis revealed six metabolites from both soluble and cell wall-bound fractions of resistant and susceptible cvs. (Fig. 3a and b) with retention times of protocatechuic acid (R_t=6.47), vanillic acid (R_t=10.13), caffeic acid (R_t=11.54), *p*-coumaric acid (R_t=12.69), ferulic acid (R_t=21.76) and sinapic acid (R_t=25.60). Nematode infection of resistant and susceptible cvs. induced a prominent and a moderate increase respectively in both soluble and wall-bound phenolic metabolites concentrations compared to the corresponding controls. In commensurate with total soluble and wall-bound phenolics, the induction of phenolic metabolites particularly in roots of resistant cvs. was quantitatively twice as that of the corresponding controls. With all six metabolites constitutively present, there was no qualitative change in the number of metabolites between the resistant and susceptible cvs. and between infected and uninfected control roots. However, the cell wall-bound phenolics fraction contained only a small amount of caffeic acid (Fig. 3b).

Quantification of individual phenolic metabolites showed that the induction of vanillic, *p*-coumaric, ferulic and sinapic acids from soluble phenolics fraction of nematode-infected resistant cv. were 3.70-, 2.86-, 2.97- and 3.19-time higher compared to the corresponding control (Table 1). The constitutional content of phenolic metabolites between resistant and susceptible cvs. was significantly different except for vanillic acid and the metabolites induction in susceptible cvs. roots was less than 2-time. From cell wall-bound phenolics fraction, the induction of protocatechuic, *p*-coumaric and sinapic acids were 3.3-, 2.9- and 4.2-time higher in inoculated roots of resistant cvs. compared to the corresponding controls. Significant constitutional contents of phenolic metabolites between resistant and susceptible cvs. was observed except for vanillic and caffeic acid in cell wall-bound fraction. The induction of metabolites in cell wall-bound fraction in susceptible cvs. infected roots was also less than 2-time compared to control. The content of caffeic acid in the cell wall was only 2-6% of total phenolic acids.

Discussion

The biochemical roles of phenylalanine ammonia lyase and cinnamyl alcohol dehydrogenase in defense

responses of plants during pathogens infection have been well documented. PAL is the first and key enzyme that controls the phenylpropanoid biosynthetic pathway through which phenolic compounds of lignin, flavonoids and hydroxycinnamyl conjugates are synthesized. PAL activity is an extremely sensitive indicator of stress conditions including pathogen infection as basically PAL is a stress-related enzyme[28]. CAD is the last enzyme involved in the synthesis of monolignols in the phenylpropanoid pathway and its activity is a direct indicator of lignin biosynthesis[6,36]. In the present experiments, the results indicate infection of nematode highly elevated the activities of PAL and CAD in the roots of nematode-resistant banana cvs. Anaikomban and Yangambi Km5 against a marginal increase of these enzymes activities in the roots of susceptible cvs. Nendran and Robusta. The highly elevated activities of these phenylpropanoid enzymes are the indicators of synthesis and supply of carbon skeletons for

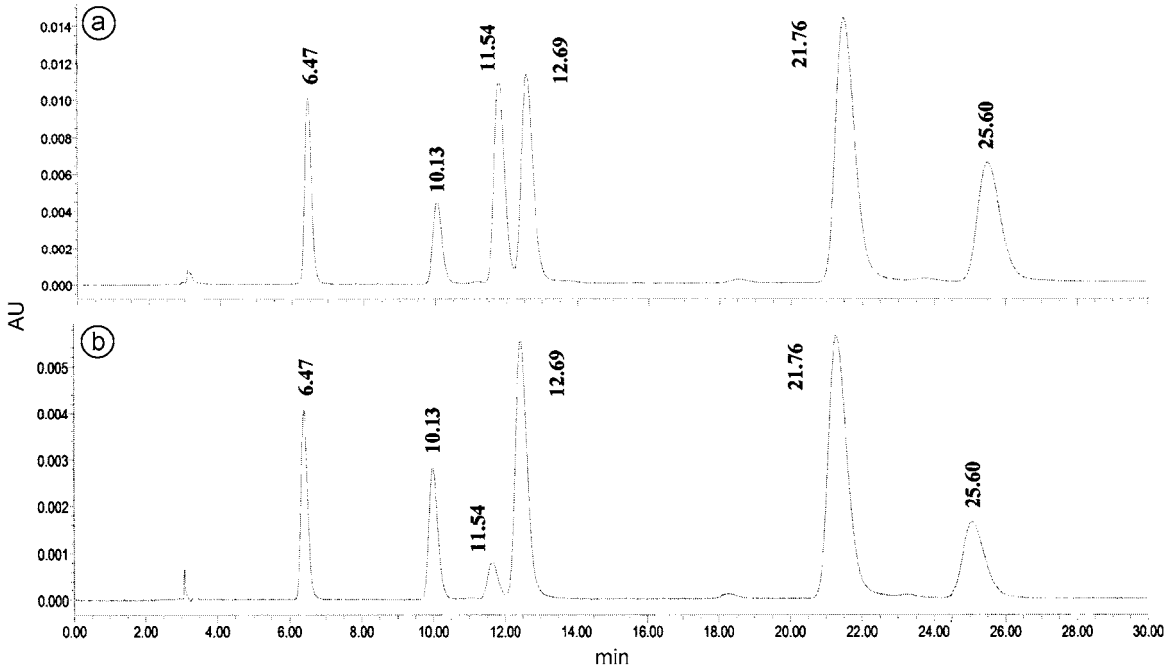

Fig. 3—HPLC chromatogram of total methanol soluble phenolic acids eluted from roots of resistant banana cv. Yangambi Km5 **(a)** and susceptible banana cv. Nendran **(b)** following infection with *P. coffeae*. Protocatechuic acid (R_t=6.47); Vanillic acid (R_t=10.13); Caffeic acid (R_t=11.54); *p*-Coumaric acid (R_t=12.69); Ferulic acid (R_t=21.76) and Sinapic acid (R_t=25.60). The extracts were made at 7 days postinoculation and control (preinoculation) and phenolic acids were separated by reverse phase HPLC on a C_{18} column using MeOH-1 mM aqueous Trifluoroacetic acid and eluents were monitored at 280 nm.

Table 1—Contents of individual phenolic acid metabolites (µg/g root fresh weight) in resistant (Yangambi Km5) and susceptible (Nendran) banana cvs. roots in response to *P. coffeae* infection

Phenolic metabolites	Total soluble acids				Cell wall-bound acids			
	Yangambi Km5 (control)	Yangambi Km5 (inoculated)	Nendran (control)	Nendran (inoculated)	Yangambi Km5 (control)	Yangambi Km5 (inoculated)	Nendran (control)	Nendran (inoculated)
Protocatechuic acid	9.90 C	26.22 A	7.37 D	12.25 B	4.91 C	16.32 A	3.43 D	6.47 B
Vanillic acid	7.43 C	27.53 A	7.26 C	11.40 B	5.72 C	14.33 A	5.24 C	8.01 B
Caffeic acid	15.83 C	41.32 A	8.58 D	16.62 B	1.22 C	1.74 A	1.12 C	1.61 A
p-Coumaric acid	12.44 C	35.53 A	10.39 D	14.25 B	7.14 C	21.04 A	5.85 D	8.74 B
Ferulic acid	16.54 C	49.21 A	14.98 D	22.04 B	10.26 C	26.49 A	8.76 D	16.07 B
Sinapic acid	14.83 C	47.37 A	9.74 D	17.37 B	5.22 C	21.81 A	4.72 D	6.71 B

The metabolites were extracted from the roots of control (preinoculation) and at 7 days postinoculation and quantified by HPLC. Values are the mean of 7 replications.

Comparisons were made separately among total methanol soluble metabolites and cell wall-bound metabolites and values in a row with different letters are significantly different by Tukey's test.

secondary products of phenolics, which are precursor of lignin. The observed increase in PAL and CAD activities in infected roots is presumably related to the lignification process as opined by Ascensao and Dubery[28]. Numerous experiments demonstrated similar results of metabolic changes of increased enzymes activities, particularly of PAL, resulting from *P. coffeae* and other nematodes infection in banana systems[18,37].

The results on phenolics clearly demonstrate that infection of the nematode induced a rapid accumulation of both total soluble and cell wall-bound phenolics in the roots of resistant cvs. and in contrast, the roots of susceptible cvs. responded by slow accumulation of phenolics. Synthesis and accumulation of phenolic compounds at the site of pathogen infection is a typical characteristic of defense response of plants to pathogenesis, which causes rapid cell death slowing the development and penetration of pathogen[8,38]. Free phenolics and other intermediates generated through the general phenylpropanoid and lignin specific pathways are toxic and possess highly antipathogenic activities[39,40]. In plants, most of the phenolic compounds are present in conjugated esters form with a sugar molecule attachment in one or two hydroxyl groups of phenolics. From the point of nematode infection, the wall-bound ester conjugated phenolic acids are very important as the esterification of phenols to the cell wall materials is a common biochemical process of resistance and accumulation of polymerized phenols is also known to occur as a rapid response to pathogen infection[41,42]. The esterification of phenolic acids and cross-linking of such esters to form lignin polymers in the primary cell wall of the host along with polysaccharides has been suggested to act as chemical barrier and defense to pathogens and the phenols in the primary cell wall function as a template for further esterification and lignification of the cell wall[2,3]. In light of the findings of this study and results from previous investigations, it could therefore be opined that high activity of PAL coupled with high accumulation of phenolics in resistant cvs. constitute part of their resistance mechanism against *P. coffeae*. Previous investigations[12-14] by histochemical staining analysis found the increased phenolics enrichment detected in the roots cell walls of resistant cvs. including Yangambi Km5 in response to *R. similis* infection and these results are in complete endorsements of the findings of the present experiments.

The results seem to indicate that the resistant banana varieties responded actively to the nematode infection through strong synthesis and deposition of lignin. Lignin in the plant cell wall is a free radical polymer synthesized from *p*-coumaryl, coniferyl and sinapyl alcohols. Induced accumulation of lignin in infected root tissues is widely proved as one of several plant defense responses against pathogens including nematodes[17,32,43]. Lignified cell wall, by nature of its high resistance, is an effective physical and chemical barrier to nematode penetration and development limiting its access to cell wall polysaccharide[44-46]. Considering these facts, strong and rapid lignifications play a significant role in the elevated defense response of resistant cvs. to the nematode. Collingborn *et al*[17] previously reported slightly more lignin content in Yangambi Km5 roots due to *R. similis* infection. The stabilization and slight increase of wall-bound phenolics and lignin contents in the roots after 7th day postinoculation implies that the incorporated phenolics and polymer remain in the cell walls and continuation of physiological lignification process respectively despite decline of enzymes activities and soluble phenolics.

The remarkable increase of phenolic metabolites *p*-coumaric, ferulic and sinapic acids in total soluble phenolic fraction and *p*-coumaric and sinapic acids in wall-bound fraction from roots of resistant cvs. upon the nematode infection explains the activation of phenylpropanoid pathway through which these metabolites are synthesized leading to production of monolignols of *p*-coumaryl, coniferyl and sinapyl alcohols, which are chemical blocks of lignin[6]. It has been observed and proved by histochemical analysis that infection of burrowing nematode caused high deposition of lignin and phenolic metabolites including ferulic and sinapic acids in root tissues of resistant banana cvs.[13,14] Lignin and phenolic acids are constitutive compounds involved in the defense of plants as chemical barriers to the penetration of the nematode in roots of resistant cvs[13]. As reported in the case banana host and *R. similis* interactions[14], no qualitative differences in the phenylpropanoid phenolic acids profile was found between resistant and susceptible cvs. and also between uninoculated control and *P. coffeae* infected banana roots in the present experiments.

The results clearly demonstrate that phenylpropanoid enzymes, phenolic metabolites and phenolic polymer of lignin play a pronounced role in

the resistance of bananas to the nematode infection. The existence of constitutively significant higher levels of stress-related PAL activity and soluble and wall-bound phenolics in the roots of tested resistant banana cvs. and thrust in PAL and CAD activities and rapid synthesis and deposition of phenylpropanoids and lignin in the roots of resistant cvs. upon infection of the root lesion nematode are definitely metabolic initiatives and chemical defenses of bananas to provide resistance by biochemical means to the nematode infection.

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