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Endophytic fungi improve management of the burrowing nematode in banana (*Musa* spp.) through enhanced expression of defence-related genes

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Summary – The root-burrowing nematode, *Radopholus similis*, is reputedly the most damaging nematode pest of banana and responsible for major production losses. In this study, the endophytic potential of 13 fungal isolates was assessed for the management of *R. similis* in East African Highland bananas ('Ng'ombe'). All isolates successfully colonised tissue-cultured banana roots, with isolates from *Trichoderma*, *Fusarium* and *Hypocrea* producing the highest ($\geqslant$49.1%) and *Beauveria* isolates the lowest ($\leqslant$14.4%) colonisation. The fungal endophytes *T. asperellum* (ICIPE 700) and *H. lixii* (ICIPE 697) were the most effective in reducing *R. similis* densities (>81%) relative to the non-inoculated control. However, the combined inoculation of ICIPE 700 and ICIPE 697 led to greater suppression of *R. similis* (>21%) relative to individual inoculation. Suppression of *R. similis* following inoculation of banana roots with ICIPE 700 and/or ICIPE 697 was associated with the significant upregulation of the defence-related gene *PR-1*, the cell signalling gene *calmodulin* Ca^{2+} and the cell-wall-strengthening gene *β-1,3-glucan synthase*. This study demonstrates the potential for nematode management in bananas with fungal endophytes, especially using the isolates ICIPE 700 and ICIPE 697 when combined.

Keywords – biological control, endophyte, gene expression, microbial antagonists, *Radopholus similis*, tissue culture.

Plant-parasitic nematodes are a major threat to banana (*Musa* spp.) production in the tropics and subtropics. The East African Highland banana (EAHB), commonly grown in the Great Lakes region, is a key staple food and cash crop. However, it is highly susceptible to plant-parasitic nematodes. Nematode infection of banana leads to reduced bunch weight (Talwana *et al*., 2003), extends the crop cycle duration and can substantially affect yield (Fogain, 2000). Globally, *Radopholus similis* is viewed as the most devastating nematode parasite of banana (Sikora *et al*., 2018), but it occurs in a complex with other species, often including *Helicotylenchus multicinctus*, *Meloidogyne* spp. and *Pratylenchus* spp. among others (Speijer *et al*., 2001; Wang & Hooks, 2009; Coyne & Kidane, 2018; Nyang'au *et al*., 2021).

Chemical nematicides such as carbofuran and methyl bromide have been used for the management of banana nematodes (Bujulu *et al*., 1983). However, these have been linked to multiple health and environmental hazards (Satar *et al*., 2005; Gallegos-Avila *et al*., 2010), hence

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their use is discouraged or banned (WHO, 2010). Clean, healthy planting materials are key to the management of plant-parasitic nematodes in banana. Three techniques exist for the generation of healthy planting materials: *i*) paring banana suckers and dipping in boiling (100°C) water for 30 s; *ii*) micropropagation; and *iii*) tissue culture (TC) technique. Although the use of clean planting materials helps to reduce the initial nematode infection and retards population density build-up over crop cycles, the banana plants remain prone to in-field infection.

Beneficial fungal endophytes colonise host plants without inducing any penalty to the host or pathogenic symptoms, existing in a harmonious association with their plant hosts. Various studies have demonstrated the beneficial effects of the endophyte-banana plant associations in improving host resistance to both biotic and abiotic constraints, including plant-parasitic nematodes (Athman *et al*., 2007; Paparu *et al*., 2009; Waweru *et al*., 2013, 2014), and fungal and bacterial diseases (Thangavelu & Gopi, 2015; Cheng *et al*., 2020) among others.

The use of TC banana plants has numerous benefits, such as providing clean, healthy planting material that is free of pests and diseases. The shoot tip or TC culture technique (Vuylsteke, 1998), however, also eliminates any beneficial microbes that would otherwise naturally occur and confer protection against pests and diseases (Pereira *et al*., 1999). Inoculation of beneficial microbes into TC banana plants at the weaning stage can facilitate their re-establishment into the plant (Paparu *et al*., 2006; Kisaakye *et al*., 2022). Identifying the most useful endophytes, however, requires a thorough screening of potential, naturally occurring microbial isolates to assess their performance against key target pest(s) and to confirm the non-phytotoxicity of the potential beneficial microbial isolate(s) to the banana plant. The current study was therefore conducted to assess the endophytic potential of selected local isolates of fungal strains for the management of *R. similis* infection of banana in East Africa. The most appropriate spore concentration for effective management of *R. similis* infection was determined, with a focus on single and combined inoculation of the selected candidate isolates, and the mode of action of the candidate isolates assessed, with a bias on the expression profiles of selected defence-related genes in banana following inoculation with fungal isolate(s) and infection with (or without) *R. similis*.

Materials and methods

Fungal isolates

Thirteen fungal isolates: *Beauveria bassiana* (8), *Trichoderma asperellum* (2), *Trichoderma atroviride* (1), *Fusarium proliferatum* (1) and *Hypocrea lixii* (1), were screened for their endophytic potential against *R. similis* infection in banana (*Musa* spp.). Fungal cultures were sourced from culture repositories of the International Centre of Insect Physiology and Ecology (*icipe*) and RealIPM (Table 1). The identities of the selected fungal isolates were previously determined based on morphological features, as described by Goettel *et al*. (2000) and Humber (2012), and molecular tools based on the conserved internal transcribed spacer (ITS) regions of the respective fungal isolate DNA.

Table 1. Identity of selected fungal endophytic isolates used in the study.

Fungal species	Isolate	Year of isolation	Isolation host/source	Locality (Country) of isolation	Repository
Beauveria bassiana	ICIPE 273	2006	Soil	Mbita (Kenya)	*icipe*, Kenya
	ICIPE 284	2005	Soil	Mauritius	*icipe*, Kenya
	ICIPE 609	2008	Soil	Meru (Kenya)	*icipe*, Kenya
	ICIPE 621	2008	Soil	Kericho (Kenya)	*icipe*, Kenya
	ICIPE 622	2008	Soil	Kericho (Kenya)	*icipe*, Kenya
	ICIPE 644	2007	Soil	Mauritius	*icipe*, Kenya
	ICIPE 660	2008	Soil	Kemokock (Kenya)	*icipe*, Kenya
	SD 229-Bb01	2008	Soil	Thika (Kenya)	RealIPM, Kenya
Trichoderma asperellum	ICIPE 700	2009	Maize (roots & stalk)	Kenya	*icipe*, Kenya
	SD 228-TRC 900	2005	Tomato (roots)	Thika (Kenya)	RealIPM, Kenya
Trichoderma atroviride	ICIPE 710	2014	Onion	Kenya	*icipe*, Kenya
Fusarium proliferatum	ICIPE 712	2014	Onion	Kenya	*icipe*, Kenya
Hypocrea lixii	ICIPE 697	2014	Maize (roots & stalk)	Kenya	*icipe*, Kenya

The fungal isolates had been preserved on potato dextrose agar (PDA) media blocks in a sterile solution of 10% (v/v) glycerol in 2 ml Eppendorf tubes at −80°C. Fungal spores were revived by culturing on sterile PDA medium (39 g l^{-1} distilled water) in sterile 90 mm plastic Petri dishes and maintained in the incubator at 25 ± 2°C for 3-4 weeks until sporulation. For each isolate, a fungal spore suspension was prepared by washing each Petri dish with 10 ml of sterile distilled water and the spores scraped off using a sterile metal spatula. Suspensions of individual isolates were separately collected into sterile 30 ml universal bottles containing 3 mm diam. glass beads to form the stock suspensions. Spore viability for each respective fungal isolate was determined as described by Inglis *et al*. (2012).

Tissue culture banana plants

EAHB 'Ng'ombe' (genomic group, EA-AAA) was used in this study. Tissue culture plants at the deflasking stage were sourced from a commercial tissue culture laboratory (Stokman Rozen Kenya). The plants were generated using the shoot-tip culture method of Vuylsteke (1998). Upon deflasking, the plant roots were washed free of rooting media with distilled water and planted in 66 multi-cell plug plastic propagation trays (L × W × H = 50 × 28 × 4.3 cm) containing a steam-sterilised potting mixture of forest soil and composted cow manure (2:1, v/v). Forest soil was sourced from a local landscaping company (Afroscape).

Nematode culture

A *R. similis* nematode population used in this study was isolated from infected roots of dessert banana ('Cavendish') in Homa Bay County, Kenya. Nematodes were extracted from roots using a modified Baermann method (Coyne *et al*., 2018; Hallmann & Subbotin, 2018); adult females were then used to establish monoxenic nematode cultures on carrot discs (Coyne *et al*., 2014). The *R. similis* population identity was confirmed based on morphological features as described by Luc (1987) and Loof (1991). The nematodes were sub-cultured onto fresh carrot discs once every 6-8 weeks and maintained in a temperature-controlled incubator (27 ± 0.5°C) until required for use.

Screening of fungal isolates against *Radopholus similis* infection in banana

To screen fungal isolates for their endophytic potential against *R. similis*, approximately 50 ml of spore concentration 1.0 × 10^7 spores ml^{-1} was prepared for each individual fungal isolate; this formed the 'working spore suspension', which was used for drenching the banana plants in the 66 multi-cell plug plastic propagation trays. Each plantlet was individually inoculated with the fungal isolates by drenching each seedling plug with the spore suspension at approximately 10% (v/v) of the volume of the potting mixture; this was equivalent to approximately 4 ml of spore suspension per plant. Estimation of the inoculation volume was based on the maximum volume the potting mixture in the seedling plug could absorb without leaking. For each plant, the spore suspension was carefully applied to the potting mixture from above, using a pipette, to soak gradually into and completely drench the potting mixture.

Drenching with the fungal suspension was performed on two occasions: *i*) at the deflasking stage, on the day of establishment in the seedling trays; and *ii*) at 4 weeks post-deflasking. Control plants were drenched with an equivalent volume of sterile distilled water. Plants were maintained in a humidity chamber (relative humidity >80%, temperature 25 ± 3°C) for 5 weeks post-deflasking, then transferred singly into 2 l plastic pots filled with the same steam-sterilised potting mixture used for maintaining the TC banana plantlets. The plants were maintained in the screenhouse and watered regularly. Endophyte-inoculated and control plants were each infected with 1000 *R. similis* (juveniles and adult females) at 4 weeks after transfer into the 2 l pots. Infection of banana plants with *R. similis* was performed using the method described by Speijer & De Waele (1997). Plants were maintained in the screenhouse at 25 ± 3°C and a natural photoperiod of 12:12 h (light:dark). The treatments were arranged in a completely randomised block design and the experiment was terminated 8 weeks after infection with *R. similis*. Each treatment was replicated six times and the experiment repeated once, except for fungal isolate ICIPE 712, which was not assessed in Experiment 2 due to poor sporulation of the isolate.

At experiment termination, each plant was gently removed from the pots and the roots rinsed free of adhering soil using tap water. The roots were cut from the banana corm using a kitchen knife, and plant pseudostem height, number of functional leaves, shoot fresh weight and root fresh weight growth parameters recorded.

To assess fungal root colonisation, three roots from each plant were randomly removed and colonisation determined as described by Kisaakye *et al*. (2022). The remaining roots were chopped into *ca* 0.5 cm pieces, thoroughly mixed and nematodes extracted from a 5 g root sub-sample using the modified Baermann method for 48 h (Coyne *et al*., 2018; Hallmann & Subbotin, 2018). Nematode suspensions were reduced to 10 ml and nematode densities estimated from 3 × 2 ml aliquots using a compound microscope (×20 magnification) (Leica Microsystems) and a 2 ml De Grisse counting dish (De Grisse, 1963). The nematode reproduction factor (RF) was estimated by dividing the final (P_f) by the initial (P_i) nematode density of each banana plant.

Determination of fungal isolate treatment and spore concentration for optimal efficacy against *Radopholus similis*

Two fungal isolates were selected and used to establish the optimal level of spore concentration for management of *R. similis* infection in banana when applied individually and in combination. The isolates were selected based on two criteria: *i*) high level of suppression to *R. similis* infection of banana; and *ii*) high level of root colonisation without hampering plant growth. Based on these criteria, two isolates, ICIPE 697 and ICIPE 700, were selected for spore concentration and isolate combination studies.

Efficacy of the selected fungal isolates was assessed at different spore concentrations, individually and in combination. Each fungal isolate and the combination were applied at four spore concentrations: 1.0×10^5, 1.0×10^6, 1.0×10^7 and 1.0×10^8 spores ml^{-1}, prepared from the stock suspensions. Inocula of the individual isolate treatments contained spores of a single isolate. Inocula of the combined isolate treatments contained half the spores of each isolate but with a total spore concentration equal to that of the individual isolate treatments at the respective level of spore concentrations.

Inoculation of the banana plantlets with the fungal inocula and subsequent infection with *R. similis* were performed as described previously. Each treatment was replicated 12 times, arranged in a completely randomised block design, and the experiment conducted twice. The plants were maintained in the screenhouse and watered regularly for 8 weeks after infection with *R. similis*. At experiment termination, nematodes were extracted and quantified as described above.

Treatment of banana plants for gene expression analysis

To establish the expression profile of selected defence-related genes in endophyte inoculated and/or *R. similis*-infected banana plants, TC banana plantlets 'Ng'ombe' were planted in seedling trays and separately inoculated with a spore suspension (1.0×10^7 spores ml^{-1}) of the fungal isolates ICIPE 697, ICIPE 700 individually and in combination at deflasking and 4 weeks post-deflasking stages, as described earlier. Control plants were inoculated with distilled water. The plants were transferred into 2 l plastic pots at 5 weeks post-deflasking. Each of the four treatments, ICIPE 697, ICIPE 700, ICIPE 697 + ICIPE 700 and untreated control, comprised a total of 72 plants. For each treatment, 18 plants were individually inoculated with 1000 *R. similis* (juveniles and adult females) 1 week post-transfer into the 2 l pots (Fig. 1) and the plants maintained in the screenhouse and watered regularly for a further 2 weeks.

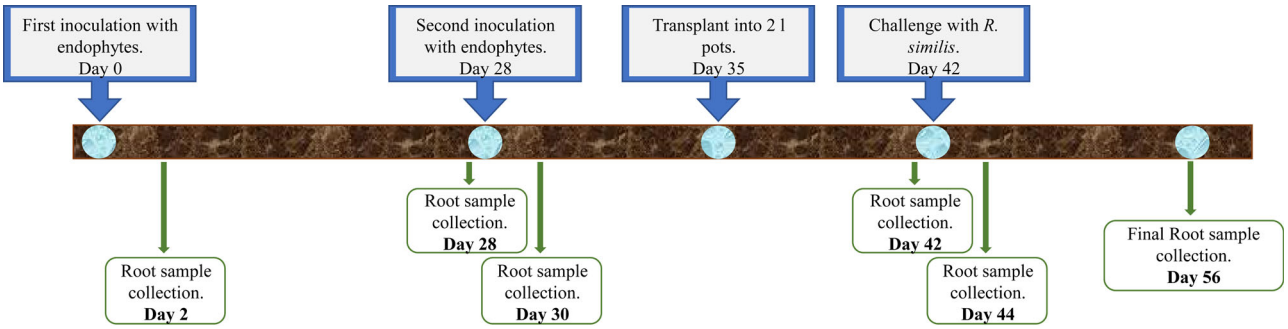

Fig. 1. Timeline for inoculation of banana roots of 'Ng'ombe' (EA-AAA) with fungal endophytes and infection with *Radopholus similis*, and root sample collection for qRT-PCR analysis.

Collection of root samples for gene expression analysis

Treatments were sub-sampled and banana roots collected for assessment of the expression levels of the selected defence-related genes. Sample collection was performed at six time points (Fig. 1): *i*) Day 2: at 2 days after the first endophyte inoculation; *ii*) Day 28: at 4 weeks after the first endophyte inoculation but before second inoculation; *iii*) Day 30: at 2 days after the second endophyte inoculation; *iv*) Day 42: at 2 weeks after the second inoculation with fungal endophytes (1 week after transfer of plants into 2 l pots) and just before infection with *R. similis*; *v*) Day 44: at 2 days after infection with or without *R. similis*; and *vi*) Day 56: at 2 weeks after infection of plants with or without *R. similis*. At each sample collection time, the plant roots were gently washed under running tap water to ensure that they were free of any adhering soil, then rinsed with sterile distilled water. The roots were blotted dry with sterile tissue paper, immediately frozen in liquid nitrogen and stored at −80°C for further analysis. Each treatment consisted of three biological replicates, each consisting of roots from three plants pooled together at harvest.

RNA extraction and cDNA synthesis

Extraction of total RNA from banana roots was conducted using the Isolate II RNA Mini Kit (Meridian Bioscience) according to the manufacturer's instructions. The purity and concentration of extracted RNA were determined by measuring the absorbance using a Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific). Only the RNA extract with a $A_{260\,nm}/A_{280\,nm}$ ratio of approx. 2 was considered pure and was used for cDNA synthesis. The cDNA was synthesised using the SensiFAST™ cDNA Synthesis Kit (Meridian Bioscience) according to the manufacturer's instructions. The resultant cDNA products were assayed for genomic DNA contamination using PCR and the actin-specific primer set actinF (5′-ACCGAAGCCCCTCTTAAC-CC-3′) and actinR (5′-GTATGGCTGACACCATCACC-3′), and the PCR products separated by electrophoresis through a 1.5% (w/v) agarose gel (Meridian Bioscience) stained with 10 mg ml^{-1} ethidium bromide (Sigma-Aldrich). The pure cDNA products were stored at −80°C for use in downstream processes.

Real-time PCR primers

The expression level of three defence-related genes, pathogenesis-related gene (*PR-1*), *calmodulin-*Ca^{2+} and *β-1,3-glucan synthase* (Table 2), in roots of the EAHB 'Ng'ombe' (EA-AAA) was determined. *PR-1* was previously reported to be upregulated in the nematode-susceptible Cavendish banana 'Williams' (genomic group AAA) (Van den Berg *et al*., 2007). Similarly, all three target genes were previously found to be upregulated in the nematode-tolerant banana 'Kayinja' (genomic group ABB) (Paparu *et al*., 2007). The *PR-1* primer sequences were previously identified by Van den Berg *et al*. (2007), while primer sequences for *calmodulin-*Ca^{2+} and *β-1,3-glucan synthase* were previously identified by Paparu

Table 2. Oligonucleotide sequences used in the expression analysis of defence-related genes in roots of banana 'Ng'ombe' (EA-AAA) inoculated with fungal endophyte(s) and infected with or without 1000 *Radopholus similis*.

Target gene	Function	Primer sequence (5′ → 3′)	Primer melting temperature (°C)	Annealing temperature (°C) *	Amplicon size (bp)
Calmodulin-Ca^{2+}	Cell signalling (Defence)	GTAGACTGCGTACCGACAAG	60.5	62	150
		GTGGAGGAAACAAGAGGAAG	58.4		
β-1,3-glucan synthase	Cell-wall strengthening (Callose synthesis)	TGTAGACTGCGTACCGACA	57.3	63	163
		CCATGGGAAGGATAAGGA	53.9		
Pathogenesis-related (*PR-1*)	General defence	TCCGGCCTTATTTCACATTC	56.4	59	126
		GCCATCTTCATCATCTGCAA	56.4		
Musa 25S rRNA	Reference gene	ACATTGTCAGGTGGGGAGTT	58.4	59	106
		CCTTTTGTTCCACACGAGATT	57.4		

* Determined by gradient PCR.

et al. (2013). The endogenous control gene, *Musa* 25S rRNA, previously identified by Van den Berg *et al*. (2007), was used as a reference gene since its expression remains relatively constant. All primers were synthesised as balanced pairs by Macrogen Europe Laboratory. For each primer sequence, the optimal annealing temperature was determined using gradient PCR, run in the Mastercycler Nexus gradient thermal cycler (Eppendorf), using the following conditions: initial denaturation for 2 min at 95°C, followed by 40 cycles each consisting of 30 s denaturation at 95°C, 45 s annealing at a gradient temperature range of 54.4-64.9°C, and a final extension step of 10 min at 72°C. PCR products were separated in a 1.5% (w/v) agarose gel (Meridian Bioscience) containing 10 mg ml^{-1} ethidium bromide (Sigma-Aldrich). Gel bands were visualised and documented using KETA GL imaging system transilluminator (Wealtec).

Gene expression analysis using qRT-PCR

Quantitative real-time reverse transcriptase (qRT-PCR) was used to quantify the expression levels of the target genes. This was performed using the SensiFAST™ SYBR® Hi-ROX Kit (Meridian Bioscience) using the first strand cDNA samples as template. A 10 μl reaction mixture for PCR amplification contained 5 μl SensiFAST SYBR® Hi-ROX mix, 2 μl cDNA template, 0.5 μl of forward and reverse primer (400 nM) and 2 μl PCR grade water. The non-template control treatment contained PCR-grade water instead of cDNA template. The qRT-PCR assays were performed in a Stratagene Mx3005P qPCR instrument (Agilent Technologies). All reactions were performed in three technical replicates and three independent biological replicates. The PCR conditions were performed as follows: pre-incubation at 95°C for 10 min, followed by 40 cycles, each consisting of 30 s denaturation at 95°C, 20 s annealing at the respective optimised annealing temperature for each gene (Table 2) and a final extension of 20 s at 72°C.

Data analyses

All data analyses were performed using R (Version 4.0.5) statistical software (R Core Team, 2021). In the experiment to screen for the endophytic potential of fungal isolates against *R. similis* infection of banana, nematode density, nematode reproduction (RF) and plant height data were subjected to a two-way analysis of variance (ANOVA) to investigate the effects and interactions between experiment repeat and endophyte treatment. Prior to ANOVA, nematode density data were $\log_{10}$-transformed, while RF data were square root transformed to conform to the requirements of normality (Shapiro & Wilk, 1965) and homogeneity of variances (O'Neill & Mathews, 2002). No transformation was performed on plant height data. Other plant growth parameters (number of functional leaves, root fresh weight and shoot fresh weight) were fitted with generalised linear model (GLM) with Gaussian distribution to check the main effects of experiment repeat and endophyte treatment, and the interaction effect. Due to the binary nature of endophyte colonisation data, colonised *vs* non-colonised, the data were fitted using GLM with binomial distribution (Warton & Hui, 2011).

In the experiment to establish the ideal endophytic isolate combination and fungal spore concentration for optimal efficacy against *R. similis* infection in banana, nematode density data were subjected to a three-way ANOVA to establish the main effects of experiment repeat, fungal endophyte treatment and fungal spore concentration, and the interaction effects. Nematode density data were square root-transformed prior to ANOVA to conform to the requirements for normality of data and homogeneity of variances. RF data were fitted to GLM with Gaussian distribution to establish the main effects of experiment repeat, fungal endophyte treatment and fungal spore concentration, and the interaction effects. Apart from shoot fresh weight data, which were square root-transformed and fitted to a three-way ANOVA, data on other plant growth parameters (number of functional leaves, plant height and root fresh weight) were fitted to GLM with Gaussian distribution to check the main effects of experiment repeat, endophyte treatment and fungal spore concentration, and the interaction effects. Significance of the GLM models was established using an analysis of deviance (Wald chi-square test) followed by computation of least-square means using the 'emmeans' package (Lenth *et al*., 2021), and group mean separation performed by Tukey's (Honest Significance Difference: HSD) multiple comparisons using the 'cld' function of the 'multcomp' package (Hothorn *et al*., 2021).

The RT-PCR expression data were normalised using the endogenous control gene, *Musa* 25s rRNA using the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001; Schmittgen & Livak, 2008) and calibrated using the non-inoculated control at each time point to generate fold change (relative gene expression) at each time point. Gene fold change data were fitted to GLM with Gaussian distribution to check the main effects of fungal endophyte treatment

and *R. similis* infection, and the interaction effects on the relative expression of the target genes in endophyte-inoculated and/or *R. similis*-infected plants at each respective time point. Significance of the GLM models was established using an analysis of deviance (Wald chi-square test) followed by comparison of group means using the 'glht' function of the 'multcomp' package (Hothorn *et al*., 2021), separation of group means was performed by Tukey's (HSD) multiple comparisons.

Results

Screening fungal isolates against *Radopholus similis* infection of banana

Fungal colonisation of plant roots

There was no significant effect of experiment repeat on root colonisation by the fungal endophytes ($\chi^2 = 0.05$, $P = 0.82$). By contrast, root colonisation was significantly influenced by the fungal isolates ($\chi^2 = 1100.5$, $P < 0.001$). However, there was no interaction effect of experiment repeat and endophyte treatment ($\chi^2 = 3.37$, $P = 0.99$). Consequently, data were pooled across experiment repeats prior to further analysis. Colonisation of banana roots by each of the *Trichoderma* spp. isolates (ICIPE 700, SD 228 TRC 900 and ICIPE 710), the *H. lixii* isolate (ICIPE 697) and the *F. proliferatum* isolate (ICIPE 712) was significantly higher than root colonisation by the *B. bassiana* isolates (Fig. 2). No fungal colonisation was observed in the non-treated control plants.

Nematode density and reproduction

While the nematode density (pooled across treatments) in Experiment 1 (4013 *R. similis* (100 g root)$^{-1}$) was significantly higher than Experiment 2 (2164 *R. similis* (100 g root)$^{-1}$) ($F = 70.65$, $P < 0.001$), and there was a significant effect of endophyte treatment on nematode density ($F = 24.69$, $P < 0.001$), there was no interaction of experiment repeat and endophyte treatment ($F = 1.41$, $P = 0.17$). Consequently, nematode density data were pooled across experiment repeats prior to further analysis. The fungal isolates ICIPE 700, ICIPE 697, SD 228 TRC 900 and ICIPE 710 significantly supressed nematode densities by 86, 83, 76 and 62% relative to non-treated control, respectively (Table 3).

Generally, there was an overall suppression of RF, which differed significantly between the two experiment repeats ($F = 18.98$, $P < 0.001$). Reproduction of nematodes in Experiment 1 (RF = 0.52) was significantly lower than in Experiment 2 (RF = 0.76). Similarly, there was a significant effect of fungal endophyte treatment on RF ($F = 9.21$, $P < 0.001$). However, there was no interaction of experiment repeat and endophyte treatment ($F = 1.62$, $P = 0.09$). Consequently, RF data were pooled across experiment repeats prior to further analysis. The fungal isolates ICIPE 700, ICIPE 697, SD 228 TRC 900, ICIPE 710 and ICIPE 712 significantly suppressed RF by $\geqslant$65% relative to untreated control (Table 3).

Plant growth

There was a significant effect of experiment repeat on plant height ($F = 191.8$, $P < 0.001$), shoot fresh

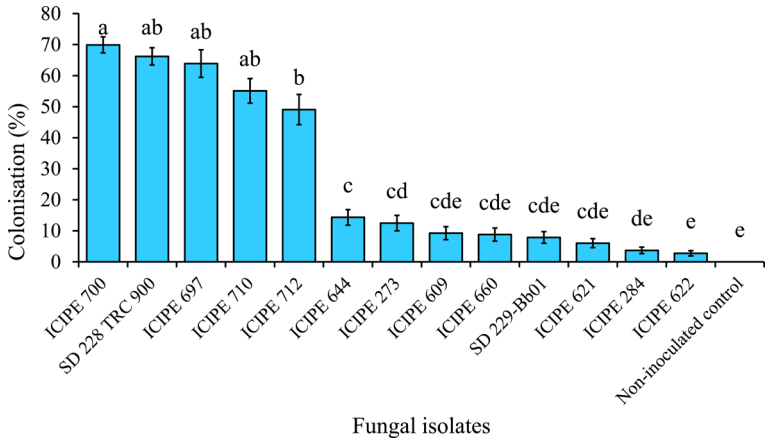

Fig. 2. Colonisation of banana root tissue of 'Ng'ombe' (EA-AAA) by endophytic fungal isolates at 8 weeks post-inoculation. Bars followed by the same letter(s) indicate no significant difference. Means separated by Tukey's (HSD) test at $P < 0.05$. Data pooled across two experiments.

weight ($\chi^2 = 246.5$, $P < 0.001$) and root fresh weight ($\chi^2 = 249.2$, $P < 0.001$) (Table 4). These three growth parameters were significantly higher in Experiment 2 than Experiment 1. There was an overall effect of endophyte treatment ($F = 2.00$, $P = 0.02$) and an interaction of experiment repeat and endophyte treatment ($F = 2.62$, $P = 0.004$) on plant height. In Experiment 2, the *Beauveria* isolate ICIPE 664 significantly improved plant height relative to non-treated control; however, this was not observed in Experiment 1 (Table 4). Similarly, there was an overall effect of endophyte treatment ($\chi^2 = 20.61$, $P = 0.01$) and an interaction effect of experiment repeat and endophyte treatment ($\chi^2 = 30.15$, $P = 0.003$) on shoot fresh weight (Table 4). There was an overall effect of endophyte treatment on root fresh weight ($\chi^2 = 24.44$, $P = 0.02$), but there was no interaction effect of experiment repeat and endophyte treatment ($\chi^2 = 16.10$, $P = 0.19$) (Table 4). By contrast, there was no effect of experiment repeat on the number of functional leaves of the banana plants ($\chi^2 = 0.88$, $P = 0.35$). However, there was an overall significant effect of endophyte treatment ($\chi^2 = 28.25$, $P = 0.008$) but no interaction effect ($\chi^2 = 1.17$, $P = 0.13$).

Table 3. *Radopholus similis* densities (100 g root)$^{-1}$ and reproduction factor (RF) in banana plants 'Ng'ombe' at 13 weeks post-inoculation with fungal endophytes and 8 weeks post-infection with 1000 *R. similis*.

Fungal isolate	*R. similis* (100 g root)$^{-1}$	RF
Control	4699 ± 498 ab	1.1 ± 0.1 a
ICIPE 273	3143 ± 288 abc	0.7 ± 0.2 abc
ICIPE 284	5923 ± 621 a	1.1 ± 0.2 a
ICIPE 609	2914 ± 665 bcd	0.8 ± 0.2 ab
ICIPE 621	3600 ± 466 abc	0.8 ± 0.1 abc
ICIPE 622	4803 ± 849 abc	0.7 ± 0.2 a-d
ICIPE 644	2532 ± 452 bcd	0.5 ± 0.1 a-e
ICIPE 660	4376 ± 554 abc	1.0 ± 0.1 a
SD 229-Bb01	4559 ± 851 abc	0.7 ± 0.1 a-d
ICIPE 700	649 ± 119 e	0.1 ± 0.0 e
SD 228 TRC 900	1116 ± 160 de	0.3 ± 0.1 cde
ICIPE 710	1802 ± 262 cd	0.4 ± 0.1 b-e
ICIPE 712	2476 ± 252 bcd	0.3 ± 0.1 b-e
ICIPE 697	798 ± 111 e	0.2 ± 0.0 de

Values represent means ± standard error. For each parameter, means followed by the same lower-case letter(s) indicate no significant difference between fungal isolate(s). Means were separated by Tukey's (HSD) test at $P < 0.05$. Data from two experiment repeats were pooled for analysis.

Dose-response study

Nematode infection

In the experiment to assess the effect of single and combined inoculation of TC banana plants with the fungal endophytes *H. lixii* (ICIPE 697) and *T. asperellum* (ICIPE 700) and the ideal spore concentration for optimal management of *R. similis* infection in the banana, there was a significant effect of experiment repeat ($F = 100.92$, $P < 0.001$), endophyte treatment ($F = 9.82$, $P < 0.001$) and fungal spore concentration ($F = 57.01$, $P < 0.001$) on *R. similis* densities. However, the interaction effects of experiment repeat, fungal endophyte and fungal spore concentration were not significant ($F \leqslant 0.43$, $P \geqslant 0.35$). Thus, nematode data were pooled across experiment repeat but split between endophyte treatment prior to further analysis.

Overall, the mean nematode density (pooled across treatments) in Experiment 2 (3565 *R. similis* (100 g root)$^{-1}$) was significantly higher than in Experiment 1 (2,215 *R. similis* (100 g root)$^{-1}$). Similarly, the combined inoculation of ICIPE 697 and ICIPE 700 led to a significantly higher suppression (50.4%) of *R. similis* densities relative to non-treated control, compared to ICIPE 697 (34.8%) and ICIPE 700 (35.6%). In all the three endophyte treatments (ICIPE 697, ICIPE 700 and ICIPE 697 + ICIPE 700), *R. similis* densities were inversely proportional to fungal spore concentration, attaining the lowest nematode density at a spore concentration of 1.0×10^7 spores ml^{-1}. However, a further increment in spore concentration beyond 1.0×10^7 spores ml^{-1} lessened the protective role of the fungal endophytes against *R. similis* infection of banana (Fig. 3).

Nematode RF was not significantly different between experiment repeat ($\chi^2 = 192.15$, $P < 0.001$), nor was there a significant interaction between experiment repeat, endophyte treatment and fungal spore concentration ($\chi^2 \leqslant 10.22$, $P \geqslant 0.11$). Thus, nematode RF data were pooled across experiments prior to further analysis.

While there was a general suppression of nematode reproduction, RF values (pooled across experiment repeat and fungal spore concentration) for ICIPE 697 (0.51), ICIPE 700 (0.58) and ICIPE 697 + ICIPE 700 (0.43) were significantly lower than for the non-treated control (0.85) ($\chi^2 = 28.83$, $P < 0.001$). Similarly, a substantial reduction in RF was evident as the fungal spore concentration increased ($\chi^2 = 50.39$, $P < 0.001$), reaching the lowest RF at 1.0×10^7 spores ml^{-1}. A further increment in spore concentration from 1.0×10^7 to 1.0×10^8 spores ml^{-1} did not yield additional suppression in nematode

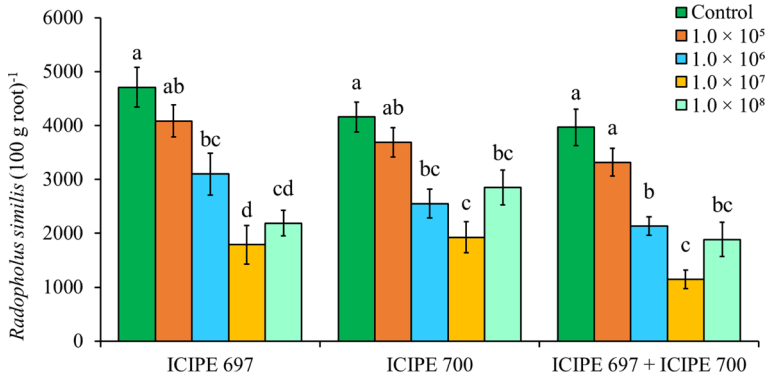

Fig. 3. *Radopholus similis* densities (100 g root)$^{-1}$ in banana 'Ng'ombe' (EA-AAA) inoculated with endophytes *Hypocrea lixii* (ICIPE 697) and *Trichoderma asperellum* (ICIPE 700) individually and in combination at spore concentrations 1.0×10^5, 1.0×10^6, 1.0×10^7 and 1.0×10^8 spores ml^{-1}. Control: non-treated control. Within each endophyte treatment, bars followed by same letter(s) are not significantly different by Tukey's (HSD) test at $P < 0.05$. Data pooled across two experiments.

reproduction; indeed, a slight increase in nematode reproduction was observed with increase in spore concentration (Fig. 4).

Plant growth

All plant growth parameters (plant height, shoot fresh weight, root fresh weight and number of functional leaves) were significantly higher in Experiment 2 than in Experiment 1 ($F = 6.08$ or $\chi^2 \geqslant 4.17$, $P \leqslant 0.04$). However, plant growth parameters were not significantly influenced by endophyte treatment ($F = 0.25$ or $\chi^2 \leqslant 1.33$, $P \geqslant 0.22$) or fungal spore concentration ($F = 0.2$ or $\chi^2 \leqslant 7.51$, $P \geqslant 0.11$), nor was there a significant interaction between experiment repeat, endophyte treatment and

Table 4. Growth of banana plants 'Ng'ombe' (EA-AAA) at 13 weeks post-inoculation with fungal endophytes and 8 weeks post-infection with 1000 *Radopholus similis*.

Fungal isolate	Plant height (cm)		Fresh shoot weight (g)		Fresh root weight (g)*	NSL*
	Experiment 1	Experiment 2	Experiment 1	Experiment 2		
Control	14.5 ± 1.0 aB	17.7 ± 0.8 aA	38.7 ± 7.7 aB	76.1 ± 9.3 abA	26.5 ± 4.2 ab	7.9 ± 0.3 ab
ICIPE 273	15.1 ± 1.6 aA	17.3 ± 0.5 aA	41.1 ± 9.0 aB	63.6 ± 5.6 bA	22.7 ± 3.5 ab	7.2 ± 0.4 b
ICIPE 284	13.3 ± 1.2 aB	18.2 ± 0.5 abA	22.6 ± 5.0 aB	87.9 ± 6.9 abA	21.3 ± 4 ab	7.3 ± 0.3 ab
ICIPE 609	15.3 ± 0.6 aB	20.2 ± 0.8 abA	42.2 ± 6.4 aB	104.6 ± 9.3 aA	33.6 ± 4.3 a	8.2 ± 0.3 ab
ICIPE 621	15.2 ± 1.0 aA	17.5 ± 0.9 aA	36.5 ± 4.9 aB	62.7 ± 9.2 bA	26.6 ± 5.6 ab	7.4 ± 0.3 ab
ICIPE 622	12.8 ± 0.8 aB	18.1 ± 0.6 abA	26.5 ± 5.4 aB	66.8 ± 9.5 abA	20 ± 4.2 b	7.5 ± 0.3 ab
ICIPE 644	12.7 ± 0.7 aB	21.6 ± 1.0 bA	26.2 ± 1.0 aB	100.7 ± 14.8 abA	27.1 ± 5.7 ab	7.3 ± 0.4 ab
ICIPE 660	14.3 ± 0.5 aB	20.4 ± 0.5 abA	37.4 ± 4.2 aB	90.9 ± 2.5 abA	26.8 ± 3.8 ab	8.0 ± 0.4 ab
SD 229-Bb01	11.2 ± 0.6 aB	19.3 ± 0.8 abA	23.1 ± 3.7 aB	88.2 ± 8.6 abA	22.4 ± 4.5 ab	7.5 ± 0.2 ab
ICIPE 700	12.5 ± 0.9 aB	17.5 ± 0.4 aA	37.4 ± 6.0 aB	68.4 ± 3.6 abA	25.5 ± 2.9 ab	8.6 ± 0.3 a
SD 228 TRC 900	11.9 ± 1.2 aB	17.6 ± 0.7 aA	35.0 ± 8.2 aB	85.7 ± 2.0 abA	27 ± 4.6 ab	8.3 ± 0.3 ab
ICIPE 710	13.5 ± 1.4 aB	18.4 ± 0.9 abA	26.5 ± 8.0 aB	69.1 ± 9.2 abA	22.6 ± 4.8 ab	7.3 ± 0.2 ab
ICIPE 712	11.9 ± 1.4 a	**	26.4 ± 6.9 a	**	12.1 ± 3.6 b	7.2 ± 0.5 b
ICIPE 697	15.6 ± 1.6 aA	17.5 ± 0.8 aA	49.5 ± 12.3 aB	80.2 ± 6.4 abA	30 ± 4.4 ab	8.0 ± 0.3 ab

Values represent means ± standard error. For each growth parameter and within each experiment, means followed by the same lower-case letter(s) indicate no significant difference between fungal isolate(s). For each fungal isolate, means followed by same upper-case letter indicate no significant difference between Experiments 1 and 2. Means separated by Tukey's (HSD) test at $P < 0.05$.

* Data pooled across experiments.

** Not enough fungal spores were available to conduct Experiment 2 due to poor sporulation of the fungal isolate.

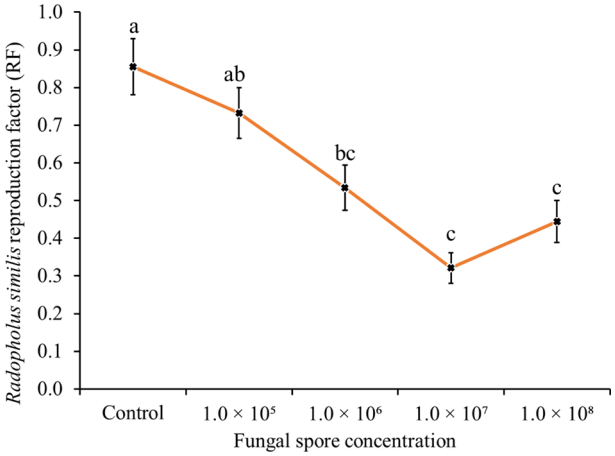

Fig. 4. *Radopholus similis* reproduction in banana plants cv. Ng'ombe (EA-AAA) inoculated with fungal endophytes *Hypocrea lixii* (ICIPE 697) and *Trichoderma asperellum* (ICIPE 700) (data pooled) at five spore concentrations: 1.0×10^5, 1.0×10^6, 1.0×10^7 and 1.0×10^8 spores ml^{-1}. Control: non-treated control. Fungal spore concentrations with the same letter(s) are not significantly different by Tukey's (HSD) test at $P < 0.05$. Data pooled across two experiments.

fungal spore concentration ($F = 1.66$ or $\chi^2 \leqslant 8.58$, $P \geqslant 0.10$).

Gene expression

The relative expression of the cell signalling gene, *calmodulin-Ca*$^{2+}$, was significantly upregulated in the roots of banana plants at day 2 ($\geqslant$7.36-fold) and day 28 ($\geqslant$1.34-fold) post initial inoculation of the plants with the fungal endophytes ICIPE 697 and ICIPE 700, applied individually or in combination ($\chi^2 \leqslant 38137$, $P < 0.001$), except for the individual application of ICIPE 697, which did not differ significantly from the non-inoculated plants at day 28 ($P = 0.23$) (Fig. 5A). Boosting of banana plants with fungal endophytes at day 28 led to a further significant upregulation of the *calmodulin-Ca*$^{2+}$ gene in all fungal-treated plants at day 30 ($\geqslant$9.3-fold; $\chi^2 = 634$, $P < 0.001$) and day 42 ($\geqslant$2.04-fold; $\chi^2 = 234$, $P < 0.001$). The expression profile of the *calmodulin-Ca*$^{2+}$ gene was maintained at significantly higher levels in the endophyte-inoculated plants than the non-inoculated plants at day 44 ($\chi^2 = 231$, $P < 0.001$) and 56 ($\chi^2 = 120$, $P < 0.001$), although this was at a relatively lower-fold difference compared to earlier time points (1.29-1.52-fold). However, the expression of the *calmodulin-Ca*$^{2+}$ gene in plants inoculated with a mixed inoculum of both endophytes did not differ from the non-inoculated plants at day 44 ($P = 0.79$) and 56 ($P = 0.16$). Conversely, when plants dually inoculated with the fungal isolates were infected with *R. similis* at day 42, the expression of this gene was maintained at a higher level in these plants ($\geqslant$1.45-fold; $P < 0.001$) relative to the endophyte-free control. Furthermore, infection of the non-inoculated plants with *R. similis* at day 42 led to a significant suppression of the *calmodulin-Ca*$^{2+}$ gene at day 44 (0.68-fold; $P < 0.001$) and day 56 (0.6-fold; $P < 0.01$), relative to the control plants.

Inoculation of banana plants with the fungal endophytes ICIPE 697 and ICIPE 700 singly or in combination led to an up regulation of the *β-1,3-glucan synthase* gene at day 2 ($\geqslant$3.5-fold; $\chi^2 = 289.3$, $P < 0.001$) and day 28 ($\geqslant$3.9-fold; $\chi^2 = 205.9$, $P < 0.001$) relative to the non-inoculated control (Fig. 5B). Re-inoculation of banana plants with the fungal endophytes at day 28 further enhanced the expression levels of the *β-1,3-glucan synthase* gene at day 30 ($\geqslant$5.1-fold; $\chi^2 = 457.2$, $P < 0.001$) and day 42 ($\geqslant$5.6-fold; $\chi^2 = 215.6$, $P < 0.001$), relative to the endophyte-free control. Furthermore, the expression level of the *β-1,3-glucan synthase* gene was maintained significantly higher in endophyte inoculated plants at day 44 ($\geqslant$5.6-fold; $\chi^2 = 1350.7$, $P < 0.001$) and day 56 ($\geqslant$5.9-fold; $\chi^2 = 1769.5$, $P < 0.001$). Similarly, infection of endophyte inoculated plants with *R. similis* at day 42 yielded a significantly higher relative expression of the *β-1,3-glucan synthase* gene at day 44 ($\geqslant$6.0-fold; $P < 0.001$) and day 56 ($\geqslant$6.2-fold; $P < 0.001$), relative to the endophyte-free control. However, infection of endophyte-free plants with *R. similis* at day 42 yielded no difference in the expression levels of the *β-1,3-glucan synthase* gene at day 44 (1.0-fold; $P = 1$) and day 56 (1.0-fold; $P = 1$), relative to endophyte-free control.

The expression level of the pathogenesis-related gene (*PR-1*) was increased by >2.0-fold at day 2 ($\chi^2 = 122.2$, $P < 0.001$) and >1.8-fold at day 28 ($\chi^2 = 51.7$, $P < 0.01$) in endophyte-inoculated plants, relative to the non-inoculated control. Re-inoculation of banana plants with the fungal endophytes at day 28 maintained the expression levels of the *PR-1* gene significantly higher at day 30 ($\geqslant$2.6-fold; $\chi^2 = 132.4$, $P < 0.001$), day 42 ($\geqslant$2.4-fold; $\chi^2 = 68.0$, $P < 0.001$), day 44 ($\geqslant$3.5-fold; $\chi^2 = 233.5$, $P < 0.001$) and day 56 ($\geqslant$3.2-fold; $\chi^2 = 193.0$, $P < 0.001$), relative to the endophyte-free control. Similarly, infection of endophyte inoculated plants with *R. similis* at day 42 further maintained the expression level of the *PR-1* gene at a significantly high level at day 44 ($\geqslant$4.8-fold; $\chi^2 = 233.5$, $P < 0.001$) and day 56 ($\geqslant$6.9-fold; $\chi^2 =$

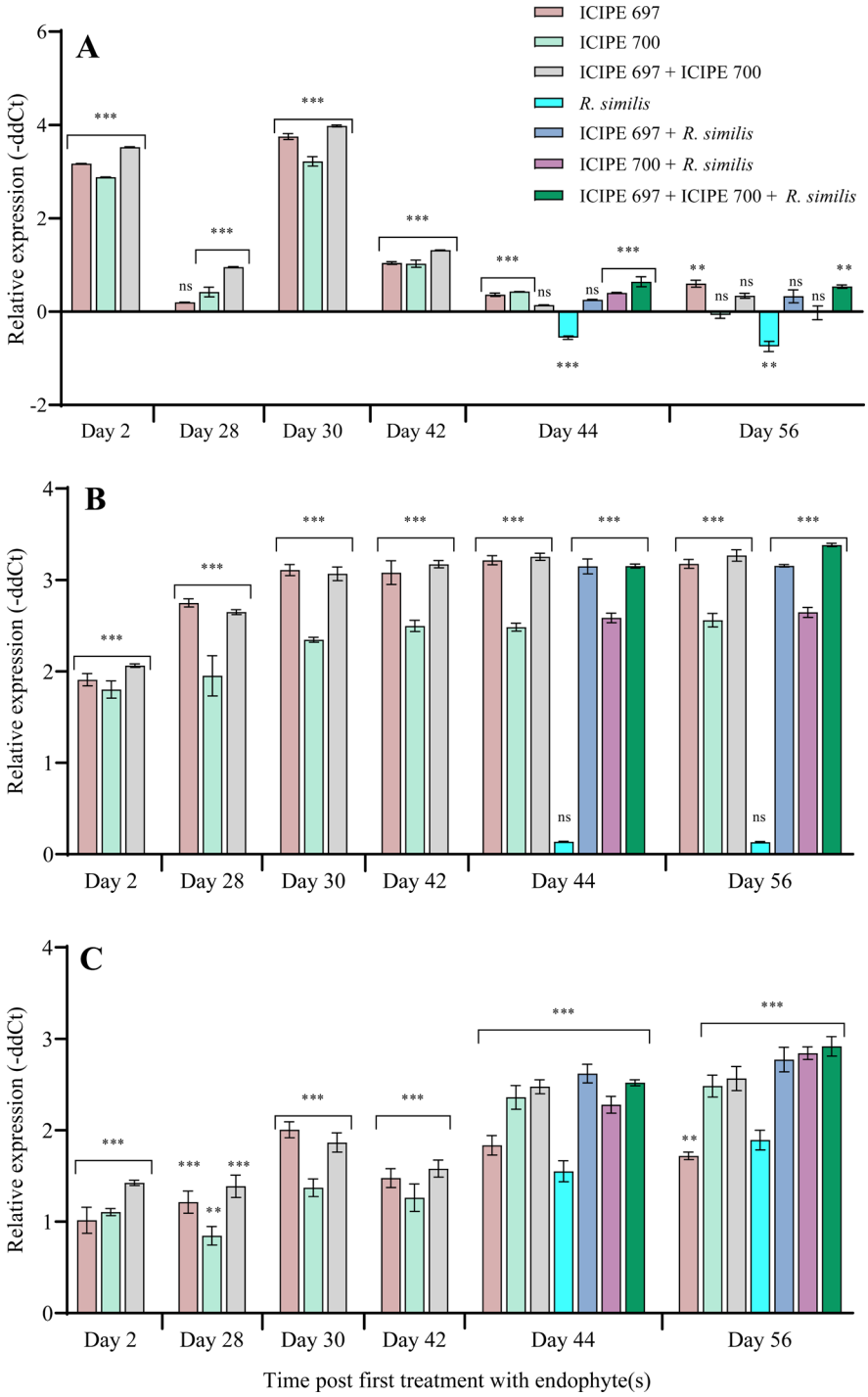

Fig. 5. Expression of defence-related genes *calmodulin-Ca*$^{2+}$ (A), *β-1,3-glucan synthase* (B) and *PR-1* (C) in roots of fungal endophyte inoculated banana plants 'Ng'ombe' (EA-AAA) infected with or without 1000 *Radopholus similis* relative to non-treated control plants. At each day, asterisks indicate statistical significance relative to non-treated control as shown by Tukey's (HSD) test (***$P < 0.0001$; **$P < 0.01$; ns: no significant difference).

193.1, $P < 0.001$). Furthermore, infection of endophyte-free plants with *R. similis* at day 42 significantly increased the expression of the *PR-1* gene at day 44 ($\geqslant$4.8-fold; $P < 0.001$) and day 56 ($\geqslant$6.9-fold; $P < 0.001$), relative to the untreated control (Fig. 5C).

Discussion

The current study demonstrates that the combined inoculation of *T. asperellum* isolate ICIPE 700 and *H. lixii* isolate ICIPE 697 at 1.0×10^7 spores ml^{-1} provides good suppression of *R. similis* infection in the EAHB 'Ng'ombe'. We also established that increasing the spore concentration beyond 1.0×10^7 spores ml^{-1} led to the reduced effectiveness of the fungal endophytes. We further revealed that the potential of these two fungal isolates to suppress *R. similis* infection is achieved through enhancement of the plant's defence mechanisms by upregulation of the defence-related gene *PR-1*, the cell signalling gene *calmodulin Ca2*$^+$ and the cell-wall-strengthening gene *β-1,3-glucan synthase*.

To identify microbial isolates with likely biological activity against a target pest, it is essential to conduct a preliminary screening of the candidate microbial isolates against the target pest. In our study, the potential of 13 fungal isolates to suppress *R. similis* infection and reproduction in banana TC plants 'Ng'ombe' endophytically was assessed. In both experiments, *B. bassiana* isolates displayed the lowest efficacy against *R. similis* infection in comparison to the *Trichoderma* spp. (ICIPE 700, ICIPE 710 and SD 228 TRC 900), *H. lixii* (ICIPE 697) and *F. proliferatum* (ICIPE 712) isolates. The potential for a fungal isolate to express its antagonistic efficacy endophytically against a target pathogen largely depends on its ability to establish a mutualistic endophytic association with its host plant, as this mutualistic association helps modify the host plant's physiological and biochemical processes and ultimately induce the host's defence mechanism against target pests and pathogens (Paparu *et al*., 2007; Waweru *et al*., 2014; Contreras-Cornejo *et al*., 2016; Khare *et al*., 2018; Kiarie *et al*., 2020; Li *et al*., 2021). Thus, failure of the *Beauveria* isolates to supress *R. similis* infection in our study could be attributed to the low levels of root colonisation ($\leqslant$14%) attained by the *Beauveria* isolates. On the other hand, all the *Trichoderma* spp. and the *H. lixii* (ICIPE 697) isolate(s) tested in our study supressed *R. similis* densities and reproduction. This suppression of nematode density and reproduction could be attributed to the relatively high level of root colonisation ($\geqslant$55%) exhibited by these isolates.

The potential for *Trichoderma* spp. to manage *R. similis* infection endophytically in banana has been studied previously (*e.g.*, zum Felde *et al*., 2005, 2006; Vargas *et al*., 2015). However, only limited information is available on the endophytic potential of *Hypocrea* spp. for nematode management in banana. In the experiment to screen the endophytic potential of the fungal isolates against *R. similis* infection of banana, we observed between 56-86% reduction in *R. similis* infection of banana upon drenching the banana roots with 10 ml of 1.0×10^7 spores ml^{-1} of either *Trichoderma* spp. or *H. lixii* isolate(s), with the *T. asperellum* isolate ICIPE 700 and the *H. lixii* isolate ICIPE 697 recording the highest level of nematode suppression at $\geqslant$85.8 and $\geqslant$81.2%, respectively. Similar to our findings, zum Felde *et al*. (2005) observed up to 80% reduction in *R. similis* infection of banana ('Grande Naine') in a pot experiment following inoculation of the banana plantlets with *Trichoderma* spp. isolates.

Fungal endophytes form mutualistic associations with their host plants. They exert their antagonistic activity through various means, which are expressed as modifications to host plant physiological and biochemical processes (Paparu *et al*., 2013; Khare *et al*., 2018; Plett & Martin, 2018; Li *et al*., 2021). Inoculation of the banana plants with ICIPE 700 and ICIPE 697 individually or in combination led to activation of the cell-wall-strengthening gene, *β-1,3-glucan synthase*, and the general defence pathogenesis-related gene, *PR-1*, irrespective of whether the plants were infected with (or without) *R. similis*. However, there was no upregulation of the *β-1,3-glucan synthase* gene when the endophyte-free banana plants were infected with *R. similis*, an indication that upregulation of this defence gene was dependent on inoculation of the plants with the fungal endophytes.

Callose, a polysaccharide of β-1,3-glucan, is a key component of plant cell wall and plays a vital role in cell-wall strengthening (Hayashi, 1989; Chang *et al*., 2021). The burrowing nematode, *R. similis*, is a migratory endoparasite. The nematode moves inter- and intracellularly, feeding on the cell cytoplasm, to which it gains access by piercing the plant cell wall with its stylet. Upon inoculation of banana plants with the fungal endophytes ICIPE 700 or ICIPE 697, we observed a reduction in the levels of *R. similis* infection. The observed suppression of *R. similis* infection could be attributed to deposition of extra callose in the plant cell walls following the activation and increased expression of the *β-1,3-glucan synthase*

gene in the endophyte-inoculated plants. The role of β-1,3-glucans and callose deposition in the suppression of *R. similis* infection of banana has been reported (Valette *et al*., 1997).

A persistent expression of the *PR-1* gene was observed in endophyte inoculated banana plants until 42 days post-inoculation (DPI), with a slight increment in the expression level at 44 and 56 DPI when the plants were infected with *R. similis*. Similar to our findings Paparu *et al*. (2007), reported an increase in the expression level of *PR-1* when the nematode-tolerant banana 'Kayinja' (genomic group ABB) was inoculated with the endophytic non-pathogenic *Fusarium oxysporum* isolate V5w2 and infected with (or without) *R. similis*, an indication that the *PR-1* gene can be upregulated in different banana cultivars, and by different genera (or isolates) of fungal endophytes.

In an experiment to compare root dip, rhizome injection and soil-solid substrate as methods for introducing the *B. bassiana* isolate G41 as an endophyte in banana, Akello *et al*. (2007) reported that the root dip method was the most appropriate method for maximum colonisation of banana roots by *B. bassiana*, achieving root colonisation levels $\geqslant$49%. Similarly, Akello *et al*. (2008, 2009) attained root colonisation levels of $\geqslant$40% when *B. bassiana* isolate G41 was introduced into banana plants using the root dip method. Thus, the low level of banana root colonisation by *B. bassiana* isolates reported in our study ($\leqslant$14%) could be further attributed to the method of application (soil drench) as opposed to the root dip method demonstrated in previous studies. Indeed, when comparing soil drench and root dip methods for introducing *B. bassiana* isolate G41 as an endophyte in roots of TC banana plants, Kato (2013) established that the level of root colonisation in dipped plants was double that of drenched plants.

Similar to our findings, fungal isolates belonging to the *Trichoderma* spp. group have been shown successively to colonise roots and corms of banana (*Musa* sp.) (Thangavelu & Gopi, 2015), and roots, stems and leaves of pineapple (*Ananas comosus*) (Kiriga *et al*., 2018), onion (*Allium cepa*) (Muvea *et al*., 2014), French bean (*Phaseolus vulgaris*) (Paradza *et al*., 2021), tomato (*Solanum lycopersicum*) (Paradza *et al*., 2021) and maize (*Zea mays*) (Batool *et al*., 2020; Kiarie *et al*., 2020), among other crops, with resultant antagonistic activity against specific plant pests and pathogens.

In our study, we demonstrated that inoculation of the EAHB 'Ng'ombe' with a combination of *T. asperellum* isolate ICIPE 700 and *H. lixii* isolate ICIPE 697 at 1.0×10^7 spores ml^{-1} is optimal for the suppression of *R. similis* infection, which is achieved through enhancement of the defence mechanisms of the banana plant. However, studies to elucidate other possible modes of action will provide a better understanding of the functioning of these isolates. Additionally, further studies to explore the potential of these two candidate isolates, *T. asperellum* isolate ICIPE 700 and *H. lixii* isolate ICIPE 697, in the management of other banana pests and diseases would help us to understand the full benefits of these candidate fungal isolates in the management of other biotic constraints in banana.

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