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Research Application Summary

Differential gene expression in nematode-susceptible and -tolerant East African highland bananas following inoculation with non-pathogenic *Fusarium oxysporum* endophytes

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Abstract

Endophytic non-pathogenic *Fusarium oxysporum* isolates have been shown to reduce *Radopholus similis* populations through induced resistance. We used cDNA-AFLPs to identify genes induced in banana plants susceptible (cv Nabusa, AAA-EA) and tolerant (cv Kayinja, ABB) to the nematode following colonization by endophytic isolates Emb2.4o and V5w2. Nematode challenge of plants was carried out 30 days after endophyte inoculation and roots harvested for RNA extraction 3 days later. Fifty up-regulated genes were assigned putative identities. Of interest to the current study was the up-regulation of genes involved in signal transduction, cell wall strengthening, the jasmonic acid pathway and transport of defence molecules. The expression profiles of differentially expressed fragments with similarity to *ABC transporter, coronatine insensitive 1 (COI1), lipoxygenase (LOX)* and *â 1,3-glucan synthase* genes were confirmed using quantitative real-time PCR. Challenge of endophyte-inoculated plants with *R. similis* resulted in further up-regulation of the activities of *â-1,3-glucan synthase* and *COI1* in the susceptible cv Nabusa, and that of *COI1* and *LOX* in the tolerant cv Kayinja. Our results confirm induced resistance as a mode of action for *F. oxysporum* endophyte control of *R. similis* in banana. This investigation represents the first report of the isolation and identification of genes involved in the interaction between endophytic *F. oxysporum* and banana.

Keywords: cDNA-AFLPs; expression profile; quantitative real-time PCR; *Radopholus similis*

Résumé

Les isolants endophytes non pathogènes de *Fusarium oxysporum* ont montré une réduction des populations *Radopholus similis* à travers la résistance induite. Nous avons utilisé l'ADNc-AFLPs

pour identifier les gènes induits dans les bananiers sensibles (cv Nabusa, AAA-EA) et tolérant (cv Kayinja, ABB) au nématode suite à la colonisation par les endophytes isolants Emb2.4o et V5w2. Le défi de nématode des plantes a été effectué 30 jours après l'inoculation d'endophyte et de racines récoltées pour l'extraction de l'ARN 3 jours plus tard. Cinquante gènes régulés à la hausse ont été affectés d'identités putatives. L'intérêt pour l'étude actuelle a été la régulation positive des gènes impliqués dans la transduction du signal, le renforcement de la paroi cellulaire, la voie de l'acide jasmonique et le transport de molécules de défense. Les profils d'expression des fragments se sont différemment exprimés, présentant une similitude avec des *transporteurs ABC, coronatine insensibles 1 (COI1), la lipoxygénase (LOX)* et les gènes d'un *1,3-glucane synthase* ont été confirmés par PCR quantitative en temps réel. Le défi des plantes inoculées avec endophytes R. similis a entraîné en outre une régulation des activités de A-1 ,3-glucane synthase et COI1 dans le cultivar sensible Nabusa, et celle de *COI1* et *LOX* dans le cv tolérante Kayinja. Nos résultats confirment la résistance induite par un mode d'action pour le contrôle de *F. oxysporum endophyte* de *R. similis* chez le bananier. Cette enquête constitue le premier rapport de l'isolement et l'identification de gènes impliqués dans l'interaction entre *F. oxysporum endophytes* et de la banane.

Mots-clés: AFLP-ADNc; profil d'expression; PCR quantitative en temps réel; *Radopholus similes*

Background

Fungal endophytes are organisms which, at some stage in their life cycle, colonize living plant tissues without causing any visible symptoms (Petrini, 1991). They have been isolated from almost every plant species studied, including banana (Schuster *et al*., 1995). The root burrowing nematode (*Radopholus similis* (Cobb) Thorne) is a major pest of banana in Uganda (Gold *et al*., 1994). The potential of using non-pathogenic *F. oxysporum* endophytes as antagonists against *R. similis* is considerable. This is because the endophytes occur within the plant where the destructive stages of the two pests are found. Similarly, the use of microbial antagonists that occur within the plant might offer a better control option than others because they are less exposed to environmental influences (Sikora, 1997). These endophytes have been shown to kill *R. similis* juveniles *in vitro* (Schuster *et al*., 1995), were successfully introduced into tissue culture plants (Schuster *et al*., 1995), where they are reported to reduce *R. similis* populations in *in vivo* pot trial. Recently, Athman (2006)

demonstrated induced resistance as the most likely mode of action of *F. oxysporum* endophytes against *R. similis*.

Literature Summary

Fungal endophytes trigger defence reactions within intact cell walls during penetration that lead to their reinforcement (Benhamou and Garand, 2001). This assumption is deduced from the limited ingress of endophytic fungi into the cortex and vascular bundle of plants (Paparu *et al*., 2006). Plant defence responses induced at the point of infection can also spread systemically throughout the plant and protect parts that have not been inoculated (Duijff *et al*., 1998; Athman, 2006). Biochemical changes that are associated with induced resistance include the accumulation of secondary metabolites such as phytoalexins (Kuc and Rush, 1985; Athman, 2006), production of PR proteins such as chitinases and â-1,3-glucanases (Duijff *et al*., 1998; Benhamou and Garand, 2001) and an increased activity of enzymes involved in the phenylpropanoid pathway (lignin synthesis).

Study Description

cDNA-AFLP analysis was used to investigate differential gene expression in EAHB cultivars highly susceptible (Nabusa, AAA-EA) and tolerant (Kayinja, ABB) to *R. similis* following inoculation with endophytic *F. oxysporum* and the nematode. Differentially expressed transcript derived fragments (TDFs) were isolated, re-amplified and sequenced using ABI Bigdye terminator chemistry on ABI3100 instruments with the universal M13-pUC vector primers at Macrogen Corp. (Rockville, USA). The expression profiles of up-regulated genes were studied using quantitative real-time reverse transcription (qRT)-PCR on a LightCycler version 1.2 instrument (Roche Diagnostic).

Research Application

We observed the up-regulation of defense-related genes such as *Glycolate oxidase, Coronatine insensitive 1* (*COI1*), *Cathepsin B-like protease, Calmodulin-ca2+* and *Lipoxygenase*; and cell

Table 1. Defense-related genes up-regulated in roots and rhizomes of susceptible (Nabusa, AAA-EA) and tolerant (Kayinja, ABB) East African Highland banana following *Fusarium oxysporum* endophyte inoculation and *Radopholus similis* challenge.

Genbankaccession	Putative ID	E-value	Isolate	Function
GR972475	Glycolate oxidase	9e-28	Emb2.4o	Oxidative burst
GR972511	COI1	3e-36	Emb2.4o/V5w2	Jasmonate response
GR972513	calmodulin-Ca2+	2e-23	Emb2.4o/V5w2	Defence
GR972516	Lipoxygenase	3e-14	Emb2.4o/V5w2	Jasmonate response
GR972507	â 1,3-glucan synthase	1e-23	Emb2.4o/V5w2	Callose synthesis
GR972476	Cellulose synthase	5e-12	Emb2.4o	Cell wall appositions

wall biosynthesis genes such as *Cellulase synthase* and *β-1,3-glucan synthase* (Table 1).

Expression profiling of isolated genes revealed up-regulation of *COI 1, β-1,3-glucan synthase and LOX* in endophyte-inoculated and nematode-challenged banana plants of both the susceptible and tolerant cultivars.

The genes identified in the current study can be used as markers to screen potential endophytic candidates for bio-enhancement of East African Highland bananas.

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