

Defense-related gene expression in susceptible and tolerant bananas (*Musa* spp.) following inoculation with non-pathogenic *Fusarium oxysporum* endophytes and challenge with *Radopholus similis*

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Abstract

Radopholus similis is a major pest of East African highland cooking bananas (*Musa* spp.) in Uganda. Non-pathogenic *Fusarium oxysporum* endophytes, isolated from bananas in farmers' fields, have shown potential to reduce *R. similis* numbers in tissue culture banana. The mechanism through which endophytes confer resistance to nematodes has previously been demonstrated to involve induced resistance. In this study, the expression of eight defense-related genes in banana was investigated using quantitative real-time reverse transcription PCR. Plants of susceptible (cv. Nabusa, genomic group AAA-EA) and tolerant (cv. Kayinja, genomic group ABB) banana cultivars were inoculated with endophytic *F. oxysporum* strain V5w2. Gene expression levels were analysed following endophyte inoculation and nematode challenge. Endophyte colonization of roots of the tolerant cultivar induced transient expression of *POX* and suppressed expression of *PR-3*, *lectin*, *PAE*, *PAL* and *PIR7A*. *Catalase* and *PR-1* activities were up-regulated in the tolerant cultivar 33 days after endophyte colonization of roots, but their expressions were further up-regulated following nematode challenge. Apart from *POX* and *lectin*, the other genes analysed were not responsive to endophyte colonization or *R. similis* challenge in the susceptible cv. Nabusa. This is the first report of endophyte-induced defense-related gene expression in banana.

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1. Introduction

Fungal endophytes occur in all plants, and often infect their hosts without causing any observable disease symptoms [1,2]. Apart from grasses, in which they have been intensively studied, endophytes have been isolated from agricultural crops such as banana (*Musa* spp.) [3,4], rice (*Oryza sativa* L.) [5], cocoa (*Theobroma cacao* L.) [6] and barley (*Hordeum vulgare* L.) [7]. While in the plant,

endophytes may be mutualists, commensalists and even parasitic [8,9]. In a mutualistic association between an endophyte and its host, the latter provides nutrients and protection, and aids in transmission to the next generation of hosts for vertically transmitted endophytes. The endophyte in return, is believed to offer increased resistance or tolerance to biotic (pests and diseases) and abiotic (drought and salinity) stresses. The commensalists benefit from the host, but neither harms nor benefits their hosts. Parasites are detrimental to their hosts [10].

The use of pest management practices in Sub-Saharan Africa is severely hampered by economical and environmental constraints. For example, the use of synthetic pesticides is discouraged due to their persistence in soils and their negative effect on the environment and human

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health. These pesticides are also expensive, often unavailable and poorly understood by farmers [11]. For many crops, breeding for host resistance is slow due to the lack of knowledge of resistance mechanisms, resistance markers and genetics of resistance [12]. In banana, durable resistance against major pests such as the burrowing nematode *Radopholus similis* (Cobb) Thorne is yet to be successfully bred into economically important cultivars. Problems associated with the use of traditional pest management practices have led to many crop protectionists turning to control strategies that are naturally occurring and hence environmentally friendly. These include the use of microbial control agents such as entomopathogenic fungi, rhizobacteria and fungal endophytes. Fungal endophytes are particularly attractive for control of pests such as the burrowing nematode because they occur inside the plant where the destructive stages of this pest exist.

Fungal endophytes are beneficial to host plants through various mechanisms, including the production of secondary metabolites, which are used in direct antagonism against pests and diseases [13–17], changes in host physiology, which lead to increased plant growth [18,19], and induction of pest and disease resistance in plants [20–23]. Two types of induced resistance exist. Systemic acquired resistance (SAR) and induced systemic resistance (ISR) can be differentiated on the basis of signal molecules and genes up-regulated following resistance induction, but not their phenotype. SAR is dependent on the salicylic acid pathway and its onset is characterized by expression of genes that encode pathogenesis-related (PR) proteins such as β -1,3-glucanases (PR-1 family), endo-chitinases (PR-3 family) and thaumatin-like proteins (PR-5 family) [24–27]. SAR is widely known to be associated with pathogen attack or in response to the exogenous application of chemicals such as salicylic acid and benzothiadiazole. ISR, conversely, is reported due to root colonization by plant growth-promoting rhizobacteria and its onset is not characterized by accumulation of PR proteins [28–35].

Endophyte-induced defenses in plants are expressed through structural and biochemical mechanisms. Structural mechanisms include the reinforcement of plant cell walls by deposition of newly formed molecules of callose, lignin and phenolic compounds [22,36]. Other physical mechanisms of resistance include the occlusion of colonized vessels by gels, gums and tyloses [37,38]. Physical barriers are formed by the plant to prevent further ingress of the invading organisms [39]. Major biochemical changes following resistance induction include accumulation of secondary metabolites such as phytoalexins [40,41], and production of PR proteins such as β -1,3-glucanases and chitinases [20,21,23]. β -1,3-Glucanase is reported to release elicitors for phytoalexin synthesis [42]. Chitinase and peroxidase enzymes are known to be induced during endophyte colonization [43].

There is evidence that non-pathogenic *F. oxysporum* endophytes can act against *R. similis* through induced resistance. In plant colonization studies, Paparu et al. [44]

reported limited *F. oxysporum* endophyte colonization in the root epidermis, speculating that physical barriers prevented further ingress into the root cortex. In screen-house split-root experiments where the pest and endophyte were inoculated separately onto spatially separated roots, reduced penetration and multiplication of nematodes was observed, demonstrating induced resistance [45]. Also, an increase in production of phenolic compounds was observed in roots inoculated with endophytes and later challenged with *R. similis* [45].

The current study was designed to compare expression of defense-related genes in roots of a susceptible East African Highland banana (EAHB) cultivar (cv. Nabusa, genomic group AAA-EA) and a tolerant banana cultivar (cv. Kayinja, genomic group ABB), following inoculation with endophytic *F. oxysporum* and challenge with *R. similis*. While the biochemical and structural responses of endophyte colonization of plant roots have previously been investigated [45,46], little is known regarding the endophyte-induced resistance pathway and the molecules involved.

2. Materials and methods

2.1. Fungal inoculum and nematode preparation

A non-pathogenic endophytic *F. oxysporum* strain V5w2 was isolated from EAHB plants by Griesbach [3] and is currently stored at the facilities of the International Institute of Tropical Agriculture (IITA) in Kampala, Uganda in soil, on filter paper and in 15% glycerol [47]. Strain V5w2 was chosen because of its demonstrated ability to suppress *R. similis* in screenhouse experiments [45]. From filter paper, the fungus was sub-cultured on half strength potato dextrose agar (PDA) (19 g PDA and 19 g agar l⁻¹ distilled water) and a fungal spore suspension prepared 7 days after growth at $\pm 25^\circ\text{C}$. Spore densities were subsequently determined under a light microscope (100 \times magnification) using a haemocytometer. Spore concentrations were adjusted to 1.5×10^6 spores ml⁻¹ with sterile distilled water.

Radopholus similis nematodes were cultured on carrot discs according to Speijer and De Waele [48]. The nematodes were originally isolated from banana roots and maintained at 27 $^\circ\text{C}$ on sterile carrot discs at IITA, Kampala, Uganda. A nematode suspension was prepared by rinsing nematodes from the carrot discs and from the edge of the Petri dishes using sterile distilled water. A final volume of 110 ml sterile distilled water containing 178 female and juvenile *R. similis* ml⁻¹ was obtained.

2.2. Plant material

EAHB cv. Nabusa (highly susceptible to *R. similis*) and cv. Kayinja (tolerant to *R. similis*) were inoculated with the non-pathogenic endophytic *F. oxysporum* strain V5w2. Tissue culture banana plants were propagated using

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