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Studies on Time-Course Expression of Defence Genes in Banana against *Pratylenchus coffeae* for the Creation of a Subtractive cDNA Library

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Abstract

Identification of genes that confer nematode resistance is important for banana improvement. These can either be used as molecular markers for marker-assisted selection or directly by guiding the design of transgenic plants with high resistance. Cultivars with differential reaction to nematode infection and knowledge of time-course expression of defence genes after nematode infection are a prerequisite for the isolation of resistance genes in banana through functional genomics. Based on pot culture screening and biochemical studies, cultivars 'Karthobiumtham' (ABB) and 'Nendran' (AAB) were identified as resistant and susceptible, respectively, to the root-lesion nematode *Pratylenchus coffeae*. The semi-quantitative RT-PCR analysis revealed that mRNA levels of the chalcone synthase gene, the first enzyme in the pathway for flavonoid biosynthesis, was constitutively higher in roots of resistant 'Karthobiumtham' than in the susceptible 'Nendran'. The transcript level of this defence gene was found to increase up to 6 days after nematode inoculation (DAI) and start declining from 7 DAI onwards in both resistant as well as susceptible root samples. From these preliminary studies, it is inferred that for identifying and isolating differentially expressed genes due to nematode infestation, a subtractive library of the interaction of *Musa* with *P. coffeae* should be created by subtracting the cDNA of uninoculated root samples from cDNA of inoculated root samples within 6 DAI. A cDNA library is being created through Suppression Subtractive Hybridisation (SSH) for isolating the genes that are differentially activated during the nematode-host interactions.

INTRODUCTION

Plant-parasitic nematodes are one of the major constraints to sustainable banana production. In India, crop losses in banana due to the root-lesion nematode were reported to be 44.4% (Sundararaju and Cannayane, 2003). Cultivation of nematode-resistant cultivars is a promising strategy for low-cost, environmentally friendly control of nematodes (Speijer and De Waele, 1997). A lack of knowledge of resistance mechanisms in banana complicates transformation of susceptible bananas with resistance genes (Van den Berg et al., 2007). Cultivars with differential reaction to nematode infection and knowledge on time-course expression of defence genes after nematode infection are a prerequisite for studying the tolerant/resistant host - nematode interaction at molecular level. The present study was undertaken with the objective of identifying suitable nematode-resistant banana cultivars and the optimum time at which root samples should be collected for isolation of root RNA from nematode-infected plants.

MATERIALS AND METHODS

Pot Culture Screening

Suckers of nine banana cultivars, namely 'FHIA-01' (AAAB), 'Karthombiumtham' (ABB), 'Nendran' (AAB), 'Anaikomban' (AA), 'Kunnan' (AB), 'Pisang Jari Buaya' (AA), 'Pisang Lilin' (AA), 'Yangambi Km 5' (AAA), 'Rasthali'(AAB), and the wild *Musa acuminata* ssp. *burmannicoides* 'Calcutta-4'(AA) were planted in pots containing sterilised potting mixture. Plants were inoculated with active *Pratylenchus coffeae* at 3000 nematodes per pot at 30 days after planting. Root mass, root vigour, number of healthy and infested roots, root length and visual observations on root-lesion indices were recorded as described by Pinochet (1988).

Enzymes Activity Assay

Activity of peroxidase, polyphenol oxidase, β-1,3-glucanase and total phenol content were assessed in 'Karthombiumtham' and 'Nendran' according to Dalisay and Kuc (1995), Mozzetti et al. (1995), Pan et al. (1991) and Zieslin and Ben-Zaken (1993) respectively.

Semi-Quantitative Reverse Transcript-PCR

First strand cDNA was synthesised from 'Karthombiumtham' and 'Nendran' root samples of 0, 2, 4, 6 and 7 days after inoculation (DAI) of *P. coffeae* for amplifying the chalcone synthase and 28S rRNA genes through PCR. The intensity of bands was determined by densitometric analysis of gels using AlphaEase FC software.

RESULTS AND DISCUSSION

Pot Culture Screening

In the pot culture trials, maximum root length and weight was recorded in nematode-inoculated plants of 'Pisang Jari Buaya', followed by 'Karthombiumtham' whereas minimum root length and weight was recorded in 'Nendran'. The maximum number of healthy roots was recorded in 'Calcutta 4' (85%), followed by 'Karthombiumtham' (74%), whereas less than 10% of healthy roots were recorded in 'Nendran'. The same trend was observed for the root-lesion index.

Biochemical Analysis

In uninoculated plants, higher quantities of phenol were observed in the resistant 'Karthombiumtham' than in the susceptible 'Nendran'. Gowen (1995) also reported that cell phenol contents are related to decreased nematode susceptibility. A similar trend was observed for the other enzyme activities, namely peroxidase, polyphenol oxidase and β-1,3-glucanase. The peroxidase activity of the resistant cultivar was more than twice that of the susceptible cultivar.

Reverse Transcript-PCR analysis of Chalcone Synthase

The transcript of the chalcone synthase gene (CHS) was found in both resistant and susceptible cultivars of uninoculated root samples, indicating that the CHS gene expresses constitutively. This proved that the defence gene is expressed in planta, in both compatible and incompatible interactions. The steady increase of chalcone synthase transcript levels up to 6 DAI was noticed in nematode-infected resistant and susceptible

roots. A similar result was also found in alfalfa (*Medicago sativa*) to the root-lesion nematode *Pratylenchus penetrans* by Baldridge et al. (1998). The semi-quantitative RT–PCR analysis of the CHS gene revealed that for construction of cDNA-Subtractive Hybridisation libraries from the inoculated nematode-resistant banana cultivar, mRNA should be isolated from the root samples before 6 DAI.

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Figures

Fig. 1. Expression profiles of *Musa* chalcone synthase in root samples of 'Nendran' (left) and 'Karthombiumtham' (right). Equal concentration of first strand cDNA synthesised from the total RNA isolated at 0, 2, 4, 6, 7 DAI of *Pratylenchus coffeae* were used as templates. 28S rRNA was used to normalise the amount of templates added in PCR reactions.