IDENTIFICATION OF MARKERS OF THE DEFENCE RESPONSE IN SUGARCANE

By

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Abstract

In sugarcane, markers for the defence response are yet to be identified. In order to identify markers for the defence response in sugarcane, sugarcane roots were treated with the plant defence regulator methyl jasmonate and the roots, stem and leaf tissue harvested. A cDNA library was constructed from RNA isolated from the roots and 821 clones were sequenced. Northern hybridisations revealed that the PR-10 gene is induced in roots within 8 hours after application of methyl jasmonate to the roots and in the stem and leaf 120 hours after application of salicylic acid to the roots. These findings indicate that both the salicylate and jasmonate signalling pathways are active within sugarcane and that it may be possible to induce resistance to pests and pathogens using defence-inducing compounds.

Introduction

Many economically important pathogens attack sugarcane root systems, yet little is known about defence responses within the roots. A greater understanding of defence compounds in sugarcane roots will be useful in developing strategies to control root pathogens. In Arabidopsis, defence responses have been analysed by examining the expression patterns of genes known to be involved in different defence response pathways. Markers for the defence response are yet to be identified in sugarcane.

Two known defence pathways in plants involve the signal molecules, salicylic acid and jasmonic acid. Jasmonic acid and its methyl ester appear to be involved in the signalling responses of most plant species to pathogen attack and wounding (Meyer et al., 1984; Creelman et al., 1992; Staswick, 1992). After wounding or treatment with an elicitor, jasmonates have been found to rapidly accumulate in the surrounding tissue (Creelman et al., 1992). Exogenous application of jasmonates to plant tissue has been shown to induce the expression of genes that encode for proteins involved in the response to pathogen attack and wounding such as the PDF1.2 gene from Arabidopsis (Penninckx et al., 1996). Salicylic acid (SA) has been shown to play a role in signal transduction leading to defence gene activation after pathogen attack. Endogenous SA levels increase in response to pathogen attack, and exogenous application of SA causes activation of plant defence genes (Ward et al., 1991; Yalpani et al., 1991).

We have commenced to identify genes that are expressed in the roots of sugarcane in response to treatment by plant defence regulators. Analysis of the genes involved in defence response may lead to the identification of molecular markers for use in breeding programs, identify promoters that are pathogen induced, wound induced, or root specific that could be used in genetic engineering approaches to enhance disease resistance. Markers for the defence response will be useful in identifying compounds that are capable of inducing systemic acquired resistance. This may allow identification of compounds that when applied to the foliage, induce defence genes within the root system. Enhanced resistance against pathogens may reduce the need for pesticide application, which is becoming less accepted by society as their negative impact on the environment is realised.

Materials and methods

cDNA library construction

Roots of 3 month old sugarcane plants, cultivar Q117, were treated by pouring 100 mL of 200 μM methyl jasmonate into the pot. The treatment was applied each day for 4 days and roots were harvested each day, 3 hours after the treatment. RNA from the treated roots was extracted, pooled and reverse transcribed into cDNA. A directional cDNA library was constructed using the Lambda ZipLox system (Life Technologies).

Sequencing and processing of clones

Plaques from the primary library were randomly picked and the inserts amplified by PCR. Inserts greater than 500 base pairs in size were sequenced using the ABI PRISM® BigDye™ sequencing kit with the T7 sequencing primer, and sent to the Australian National Genome Information Service (ANGIS) for processing. Processing at ANGIS involved vector clipping, blastn (EST) blastn (DNA) and blastx searches. The resulting files were annotated for putative function within the sugarcane root database residing at CSIRO.

Additional plant treatment

100 mL of 5 mM salicylic acid was poured into pots of 3 month old sugarcane plants grown in glasshouses. Root, stem and leaf tissues were harvested 8, 24, 72 and 120 hours post treatment.

KEYWORDS: Functional Genomics, EST.
Northern analysis

15 μg of total RNA from each time point, from leaf, stem and root was run on a denaturing gel and transferred to nylon membrane. Probes were radioactively labelled using the Strip EZ DNA labelling kit (Ambion) and hybridised overnight in UltraHyb solution (Ambion) to manufacturers’ specifications.

Results and discussion

Classification of clones

821 sequences passed the processing and were entered into the sugarcane root database. Of these 334 have no significant homology to other sequences in public databases, and 124 sequences have significant homology to other sequences but have no known function.

Northern analysis

In order to determine if the methyl jasmonate and salicylic acid treatment had an effect on gene expression, northern analysis was performed. Clones homologous to genes induced by the defence regulators in other plant species were used as probes.

A PR-10 gene was found to be induced in roots within 8 hours by methyl jasmonate application (Figure 1), and was found to be induced in both leaf and stem tissue 120 hours after application of salicylic acid to the roots (Figure 2). Induction of the PR10 gene by both methyl jasmonate and salicylic acid indicates that these two signalling pathways are active within sugarcane and that application of salicylic acid to the roots can induce gene expression in aerial parts of the plant.

Future work

The 821 clones that are present in the sugarcane root database will be screened by reverse northern experiments using RNA samples from the two treatments as probes. This may identify other clones whose expression is either induced or repressed by the application of the defence regulators and may therefore play a role in the defence response. This information may be useful in determining putative functions for those clones that have no homology to clones within the public databases.

REFERENCES


