Comparison of Gene Expression in *Solanum bulbocastanum* Infected with Virulent and Avirulent Isolates of *Meloidogyne chitwoodi*

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Abstract

Resistance to root knot nematode *M. chitwoodi* has been identified in the wild tuber-bearing *Solanum* species, *S. bulbocastanum*. Three pathotypes were identified suggesting at least two different genetic factors for virulence and resistance in the pathogen and the host species, respectively. Roots of *S. bulbocastanum* were infested with two isolates of *M. chitwoodi* differing in virulence. The infection process was monitored by histological examination of roots allowing time points to be identified. cDNA libraries were constructed from infected root tissue using Suppression Subtractive Hybridisation (SSH) to enrich for transcripts from either compatible or incompatible interactions, at three days and seven days post infection. Both plant and nematode genes, which may be important during the host/parasite interaction, were identified.

Keywords: *Meloidogyne chitwoodi*; *Solanum bulbocastanum*; suppression subtractive hybridisation; virulence genes

INTRODUCTION

Suppression subtractive hybridisation (DIATCHENKO *et al.* 1996) is a powerful technique that enables researchers to compare two populations of mRNA and obtain clones of genes that are expressed in one population but not in the other. It has been used to isolate genes expressed in the gland cells of *Heterodera glycine* (GAO *et al.* 2001) and for the identification of defence related host genes in fungal infections (BIRCH *et al.* 1999). In this project the technique is being used to isolate nematode genes and identify those associated with virulence and avirulence.

MATERIALS AND METHODS

The first subtractive hybridisation was carried out by subtracting the cDNA of uninfected *Lycopersicon esculentum* Miller (cv. Moneymaker) roots (Driver) from the cDNA of roots infected with *M. chitwoodi* Golden, O'Bannon, Santo & Finley (Tester). 400 clones were sequenced from this library.

In the second experiment, two sets of 75 *Solanum bulbocastanum* Dun plants were inoculated with 800J2s of either the avirulent *M. chitwoodi* population Cbd-V5 or 800J2s of the virulent *M. chitwoodi* population Cbd-V6. After harvesting at 3 days, 7 days, 10 days, 17 days and 21 days, roots of 3 plants were stained with acid fuchsin (DAYKIN & HUSSEY 1985) and 7 roots were stored at –80°C. After 8 weeks the remaining plants were harvested and egg masses counted.

cDNA was synthesised (Clontech PCR-Select cDNA Subtraction Kit) size fractionated and cloned. In this way libraries from 3 days and 7 days post infection, enriched for either the virulent and avirulent interaction were made. The resulting colonies were blotted and probed with unsubtracted driver, unsubtracted tester, subtracted tester and subtracted driver. Only clones that hybridised with the subtracted tester were sequenced. Blast searches were then carried out identify the possible designation of the differentially expressed genes.

RESULTS

Histological observations. Observations of the infected root tissue from different time points were successful in identifying differences in the infection process between the two isolates. After 8 weeks

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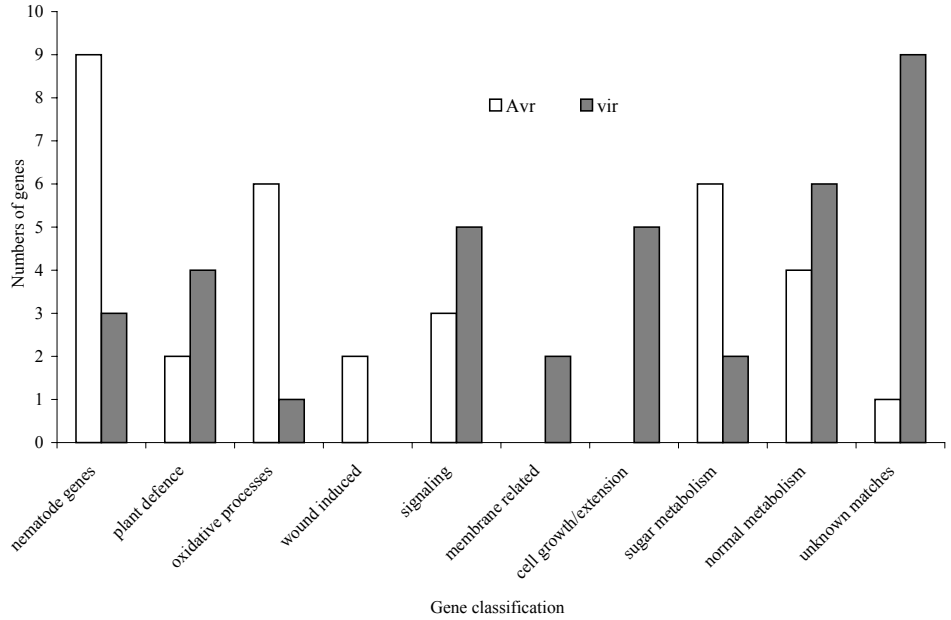

Figure 1. Comparison between the sequences obtained from the 3 day SSH enriched libraries when the tester was the avirulent Cbd-V5 (Avr) or virulent Cbd-V6 (vir)

there were on average 13 egg masses/plant from the incompatible interaction whereas from the compatible interaction there were 165 egg masses/plant.

SSH enriched cDNA libraries. The results of searches for homology to database revealed that approximately 67% of sequences were homologous to known plant ESTs, 13% to nematode sequences and 20% of indeterminate origin. Of those that could be classified, they represented a wide range of genes from both plants and nematodes and included a range of functional groups that differed in proportion between the compatible and incompatible interactions (Figure 1). Of particular interest are all the nematode sequences and those related to plant defence.

CONCLUSIONS

The selected lines of *M. chitwoodi* Cbd-V5 and Cbd-V6 differ in their ability to reproduce on *Solanum bulbocastanum* 93-60-2 with resistance upheld in Cbd-V5 infections but broken in Cbd-V6 infections.

Genes associated with oxidative processes commonly found during a hypersensitive response were identified from the avirulent/incompatible libraries while genes associated with cell growth and signalling were identified only from the virulent/compatible libraries. Genes associated with sugar metabolism were identified with a higher incidence of such genes being seen in the avirulent interaction. Previously unknown nematode genes were identified. These genes will now be characterised to determine their role in the plant pathogen interaction and to determine whether they are differentially expressed in each isolate.

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