Cotton defences against Fusarium wilt disease

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Introduction

Fusarium wilt is a disease of cotton (*Gossypium hirsutum*) caused by the soil-borne fungal pathogen, *Fusarium oxysporum* sp. *vasinfectum* (Fov). This disease was first reported in Australia in 1993 in the Darling Downs, and since then it has been steadily spreading to other cotton growing areas. There is currently no effective control for Fov in infected soils; spores can survive in soil for up to 10 years and can be spread over long distances in infected soil. Fusarium wilt is of serious concern to the cotton industry. At present, commercial cotton cultivars range from susceptible to moderately resistant and no completely resistant cultivars are available. Little is known about the interactions between cotton and Fov and it is not clear what factors are involved in the expression of resistance or susceptibility to this disease. New sources of resistance or increased tolerance to this disease are needed urgently by cotton growers.

The aim of this study is to investigate cotton's defence responses to Fov infection, and to identify genes whose expression is associated with improved resistance. Plant pathogen interactions are complex and plants will respond to infection with an arsenal of different defences. Plant responses will be reflected in the expression changes of certain plant genes. Gene expression changes due to pathogen attack are thought to be involved in defence against that pathogen. By examining the gene expression changes in cotton plants infected with Fov we hope to discover some of the defence response strategies used by cotton. We have used microarray technology to examine the gene expression changes occurring in infected cotton. The power of this tool is the ability to examine expression changes of thousands of genes simultaneously.

Methods

Microarrays are made by spotting several thousand cDNA clones onto glass slides. The gene expression differences of two samples are then compared by labelling the cDNA samples with different fluorescent dyes, combining the samples and hybridising them to the microarray slides. By analysing the fluorescence of the hybridised spots we can assess the gene expression changes between samples.

We produced microarrays using two cotton gene libraries. The cotton libraries were made from infected and uninfected cotton tissues, both root and hypocotyl. 4000 clones from the infected library, 2000 clones from the uninfected library and various control clones were included on the microarray slides. Infected and uninfected cotton samples were labelled and hybridised to the arrays. We therefore determined what plant gene expression levels changed in response to the infection. We used an *in vitro* infection system with cotton seedlings infected with Fov using root dip inoculation and then grown in agar. We examined the gene expression changes in cotton root and hypocotyl tissues at different time

points during infection and 3-4 biological replicates were examined for each timepoint. Reisolation of Fov from infected plants at different stages of infection was also performed in order to track the movement of the pathogen into the plant.

Results

Gene expression changes in Fov infected cotton roots.

In our system, Fov forms a mycelial mat on the root surface during the first 24 hours after inoculation (hpi). Fungal penetration occurs around 24 hpi. Changes in gene expression patterns are first observed at about the time of fungal penetration. Gene expression changes due to infection steadily increase over time with over 350 clones affected in the later stages of infection (Fig.1a). Many of these genes have been sequenced and analysed.

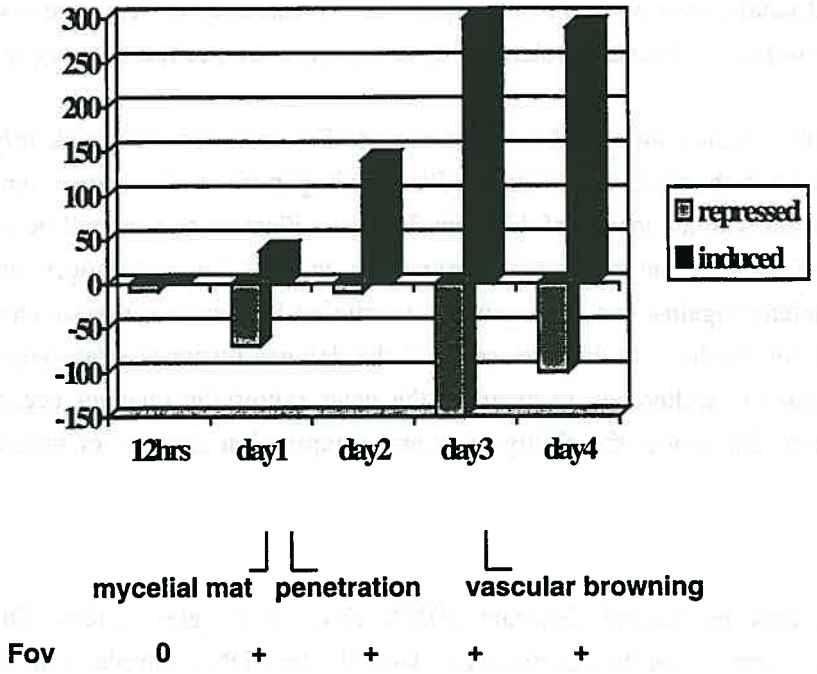

Fig 1. Number of clones with differential expression over a time course of Fov infection in roots. The presence (+) or absence (0) of Fov, determined by reisolation, is also shown.

Gene expression changes in Fov infected hypocotyls.

Fov was first reisolated from hypocotyl tissue 3 days after inoculation. Vascular browning was also seen at this time. In the early stages of infection, 2 days after inoculation, gene expression changes largely involved repression (reduced expression) of certain genes. Once Fov was present in the tissue, at 3 and 4 days after inoculation, larger numbers of genes were shown to have increased expression levels in infected tissue.

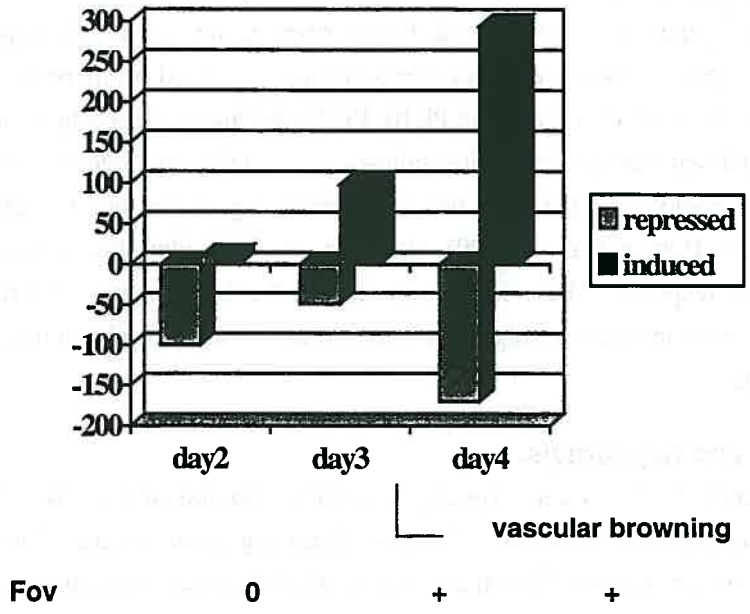

Fig 2. Number of clones with differential expression over a time course of Fov infection in hypocotyls. The presence (+) or absence (0) of Fov, determined by reisolation, is also shown.

Discussion

Gene responses to Fov infection in roots.

Many of the genes induced by Fov have previously been found to be induced by other stresses such as cold, drought, low oxygen or wounding. Therefore, these are not specific to pathogen attack. Several plant genes were repressed during fungal invasion, such as catalase and chitinase. Chitinase is known to

be induced in response to Verticillium wilt (Hill *et al*, 1999) and its repression in roots after Fov inoculation may represent suppression of plant defence responses by the pathogen. Other processes whose genes are down regulated include the structural genes of the cytoskeleton and metabolic genes involved in protein synthesis and breakdown and sugar metabolism.

Gene responses to Fov infection in hypocotyls.

In the hypocotyls, genes involved in metabolism were found to be repressed. The genes repressed were from sugar metabolic pathways, photosynthesis and pyruvate synthesis. Many of the general stress response genes observed to be induced in root tissue were induced in the hypocotyl tissues. However, an additional set of defence-related genes was also induced. In this respect, the hypocotyls responded quite differently to infection and appear to mount a defence response not observed in the roots. Many PR (pathogenesis-related) proteins were induced including PR10, PR2 (glucanase), PR3 (chitinase) and PR5 (thaumatin). Genes associated with phytoalexin biosynthesis (gossypols) and lignin deposition were also induced. Some of these responses have been observed previously as defence responses in cotton to vascular wilts (Bell, 1994, Bianchini *et al* 1999). However, we have identified several new genes associated with the defence response. These include genes for the biosynthesis of terpenoid alkaloids and lignans. Both of these compounds are highly anti-fungal and thought to play a role in the defence of plants against pathogens.

Common responses in roots and hypocotyls.

A marked response observed in both the roots and hypocotyls is the repression of a many different genes for membrane water channel proteins. This observation is interesting as switching off of these genes in other plants results in immediate wilting. Thus there may be a link between repression of water channel proteins and the development of wilt disease symptoms.

Regulatory proteins, such as transcription factors and ethylene responsive genes were induced, indicating that ethylene may be involved in signalling between the plant and pathogen in both root and hypocotyl tissues.

Conclusions

Our results have led us to a better understanding of the complex interactions occurring between cotton and Fov. We found that hypocotyls and roots respond quite differently to infection. Plant defence responses to the pathogen occur mainly in the hypocotyl. We have discovered many plant genes involved in cotton/Fov interactions. Some of these genes have been previously associated with vascular wilt disease, such as genes involved in gossypol synthesis or pathogenesis related proteins (Bell, 1994). However, we have discovered many new genes related to cotton's defence responses to Fov, which have not previously been identified as involved in this type of disease. Future work will include comparing the gene expression changes in a susceptible and a moderately resistant cultivar to determine

whether there are any gene expression differences that correlate with differences in resistance to Fov. New defence related genes discovered represent possible avenues for engineering resistant cotton by improving cotton's defence responses to Fov.

Acknowledgements

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