

Transgenic cotton for the control of fusarium wilt

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Introduction

Fusarium wilt continues to be an increasing problem in almost all major cotton growing regions of Australia. The severe impact of the disease is a major concern due to the absence or low resistance to this disease in most cotton cultivars. Control of *Fusarium oxysporum* f.sp. *vasinfectum* (Fov), presents a key challenge to researchers and breeders, particularly with the difficulty involved in the reduction of disease loads in infected soils.

Since the appearance of Fov in 1992 there is a pressing need to identify resistance/antifungal genes in either *Gossypium* or other plant species, that can be transferred to elite cultivars. We identified a protein called NaD1 which protects the flowers of the ornamental tobacco, *Nicotiana alata* from damage by fungal pathogens (Lay et al. 2002). The protein is a member of the plant defensin family, a group of small, cysteine-rich proteins with anti-microbial activity. NaD1 inhibits the spore germination and hyphal growth of Fov in *in-vitro* assays (fig.1). The gene coding for NaD1 has been cloned (Lay et al. 2002) and transferred into cotton.

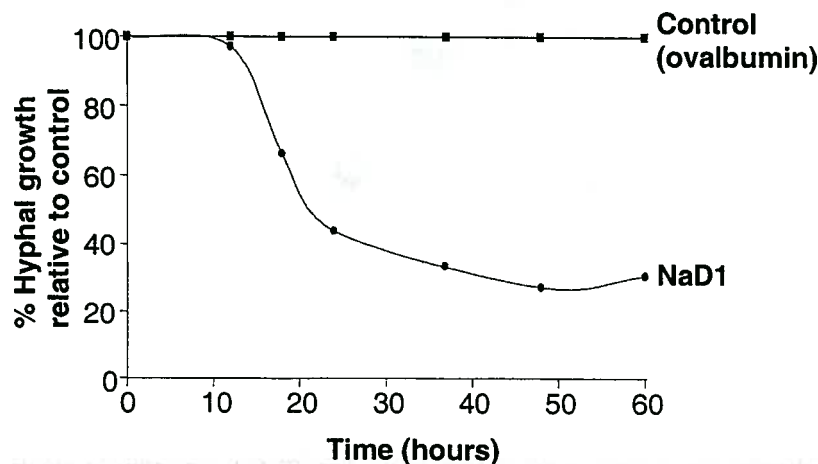


Figure 1. *In vitro* inhibition of Fov VCG01111 hyphal growth by NaD1 (20ug/ml)

Transgenic cotton

The initial transgenic cotton lines to be assessed for Fov resistance were produced by Hexima Ltd. Cv Coker 315 was transformed with the *NaD1* gene under the control of CaMV 35S promoter and 35S terminator (fig.2). Three transgenic lines with high to medium expression of the NaD1 protein were selected and homozygous seed produced.

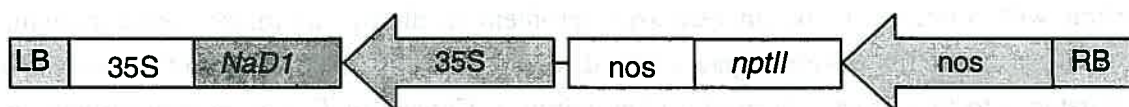


Figure 2. The pHEX3 construct used for Agrobacterium-mediated transformation of cotton. Arrows denote promoters, *nptII* is the antibiotic selectable marker gene.

Gene stability

The three transgenic lines are being analyzed to determine whether a single copy of the gene has been inserted and whether the insertion is stable from one generation to the next (Fig.3).

1 2 3 4 5 6 7



Figure 3. Southern hybridization of homozygous transgenic line 35.9.1 indicating a single insertion. Lanes 1-3 untransformed Coker 315 digested with *Nhe* I, *Bcl* I and *Bgl* II. Lanes 4-6, line 35.9.1 digested with *Nhe* I, *Bcl* I and *Bgl* II. Lane 7 pHEX 3 plasmid digested with *Bgl* II.

Protein expression

NaD1 is expressed in all tissues of 14 day old transgenic cotton plants. Expression is highest in the hypocotyl (approximately 0.3% of total soluble protein) and lower in roots and cotyledons (approximately 0.1% of total soluble protein).

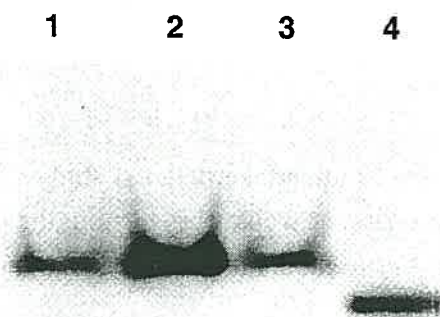


Figure 4. Protein blot of NaD1 expression in 14 day old homozygous transgenic cotton (line 35.9.1) Lane 1: roots, lane 2: hypocotyl, lane 3: cotyledons, lane 4: 25 ng purified NaD1.

New promoters

As Fov infects the plant through the roots, we are also investigating the use of another promoter (pXero2) which may give higher expression of NaD1 in the root cells invaded by the hyphae (figure 5). Transformation of Coker 315 with this construct is in progress.

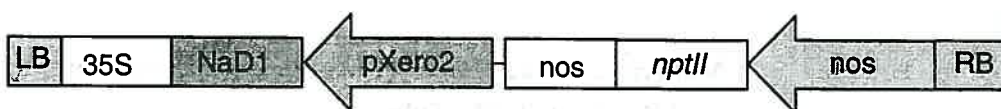


Figure 5. The pHEX14 construct containing the pXero2 promoter. Arrows denote promoters, *nptII* is the antibiotic selectable marker gene.

Fov assessment

The transgenic cotton lines are being assessed for resistance to Fov in glasshouse trials at the University of Melbourne and at CSIRO Plant Industry, Canberra in collaboration with Dr Helen McFadden.

Two week old cotton plants are removed from soil and root dipped for five minutes in a spore suspension (1×10^7 spores/ml) prepared from Fov grown in potato dextrose broth and filtered to remove fungal hyphae. The cotton plants are then grown in soil for a further six weeks. We have been using VCG01111 isolate 24500 as this has been found to be the more vigorous and pathogenic in our hands. The height and condition of the plant is recorded weekly and vascular browning is measured at week 6.

Conclusions

The future aims of this project are to undertake detailed studies of the transgenic plant-Fov interaction during the infection process, as well as broadening the project scope to include two other fungal pathogens of cotton: *Verticillium dahliae* and *Thielaviopsis basicola*.

While this project is still in its infancy it is hoped that in the long term the *NaDl* gene will be incorporated into elite cotton cultivars to enhance fungal resistance. Recently, a field trial of transgenic potato expressing a related defensin gene from alfalfa (owned by Monsanto), showed enhanced resistance to the fungal pathogen *Verticillium dahliae* (Gao et al. 2000, Gianessi et al. 2002). Similarly, if resistance to Fov is demonstrated in the glasshouse trials with the transgenic cotton then the next step would be to test the plants in the field.

Acknowledgements

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References

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