TRANSGENIC COTTON EXPRESSING A GENE FOR CHITINASE SHOWS IMPROVED TOLERANCE TO VERTICILLIUM WILT IN GLASSHOUSE TRIALS

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Introduction

Conventional plant breeding has done much to improve cotton's tolerance to pests and diseases. However, fungal diseases such as Verticillium and Fusarium wilts remain important factors limiting yield under certain environmental conditions. In particular, the increasing spread of an aggressive strain of Fusarium wilt in cotton-growing areas is causing growers concern. As yet, no genes providing immunity to the vascular wilt diseases have been identified in cotton. Conventional breeding has been used successfully to generate cotton cultivars with good fungal tolerance. However, this tolerance can still be improved and we see the use of transgene-derived antifungal proteins for enhanced host plant tolerance as an important adjunct to the development of cultivars by conventional breeding methods. Our goal is to identify genes that confer improved tolerance to wilt diseases and express these genes in transgenic cotton lines.

When challenged by pathogenic organisms, plants activate a range of inbuilt defence systems. Among these is the increased synthesis of a group of antifungal proteins known as PR (pathogen response) proteins. One group of PR proteins has chitinase activity. Chitinase enzymes may contribute to the destruction of invading fungi by weakening and rupturing their cell walls. In this project, we have used a gene for a basic chitinase from tobacco and generated several transgenic lines of cotton that contain increased amounts of chitinase enzyme. We selected one of these lines for testing and found these plants were less stunted after Verticillium wilt infection than untransformed control cotton plants. Although no reduction in disease incidence was noted, we wish to determine whether reduced severity of wilt symptoms detected under glasshouse conditions is also observed under field conditions. We have therefore submitted an application to GMAC for permission to test this transgenic cotton line in the Verticillium wilt nursery at Narrabri, NSW, and in the Fusarium nursery at Brookstead, Queensland.

Chitinase, like many antifungal proteins, is likely to be active against a broad range of fungi. While this is an advantage, in that transgenic varieties may exhibit improved tolerance to several diseases, it is also possible that chitinase expression may have adverse effects on beneficial mycorrhizal fungi. This field trial will give an opportunity to assess any difference in mycorrhizal associations in the transgenic line.

Methods

Generation of Transgenic Plants

Transgenic chitinase-expressing cotton lines were generated by *Agrobacterium*-mediated transformation of cotton (cv. Coker) using a gene construct developed from a full length tobacco chitinase cDNA gene (Neale et al., 1990). The final construct (Grover et al., 1995) contains the chitinase gene driven by the 35S promoter from the Cauliflower Mosaic virus and a nopaline synthase (*nos*) termination signal. Chitinase gene expression is therefore expected in all tissues of the plant and, on the basis of previous work (Melchers et al., 1993), enzyme accumulation in plant cell vacuoles is anticipated.

Assay for Responses to Verticillium Infection

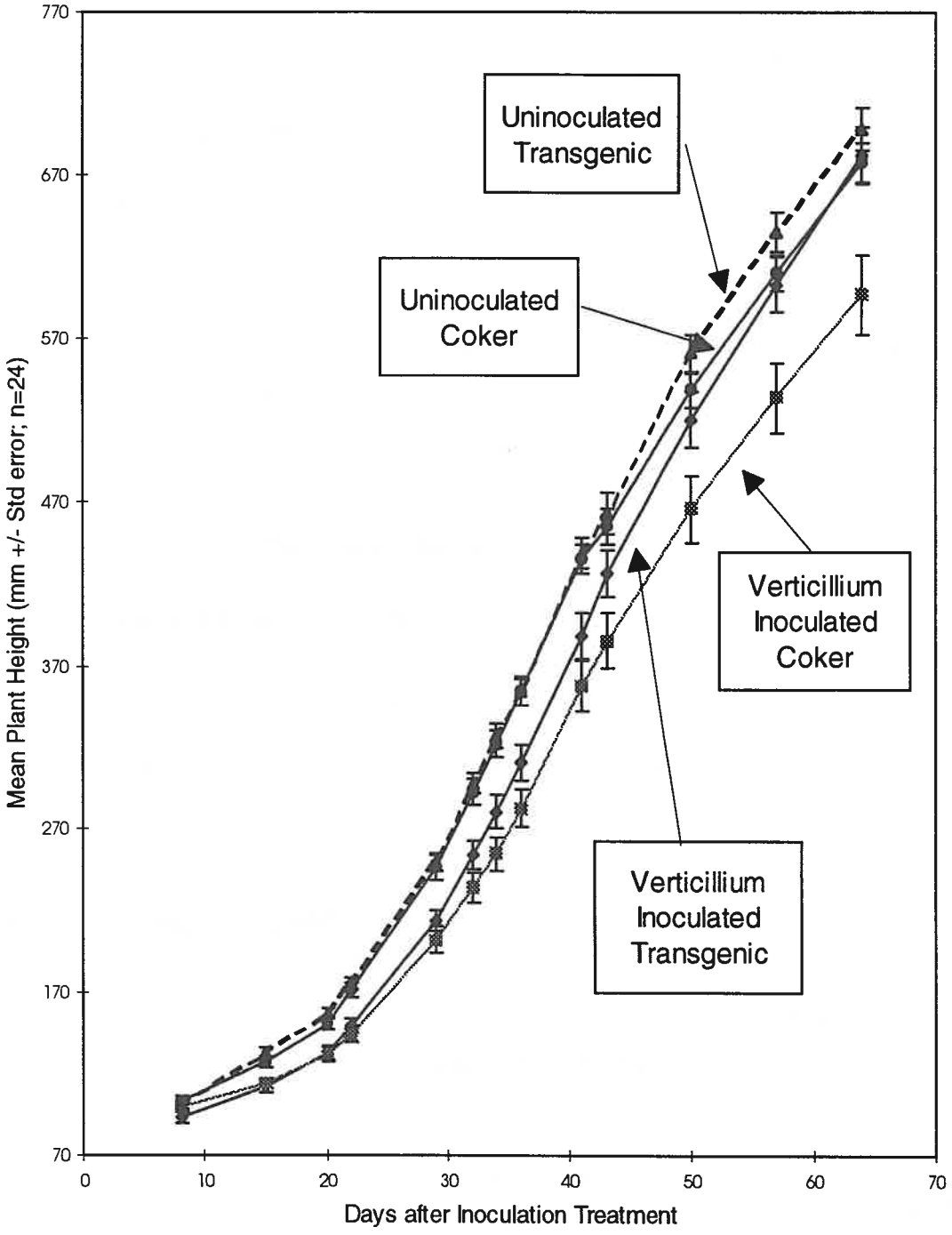
Cotton seeds were germinated in the glasshouse in soil. A field isolate of *Verticillium dahliae* obtained from infected cotton at the Cotton Research Institute, Narrabri, New South Wales (Allen, S., unpublished results), was used for all experiments. A plug of mycelium from a stock plate was transferred to Czapek's agar and incubated for about 2 weeks at 25°C. This plate was flooded with sterile water to obtain a conidial suspension for inoculation of fresh plates, which were incubated for 7 days and conidia harvested, counted and diluted to give 10^6 conidia mL^{-1}. Plants were removed from soil and the roots were washed and then dipped into either the conidial suspension (inoculated) or distilled water (control) for 20 minutes with occasional agitation. The plants were repotted in 15 cm pots in fresh damp soil and assessed regularly.

Results

Chitinase-Expressing Cotton

Increased levels of chitinase enzyme in the transgenic plant were detected using the procedure of Wirth and Wolf, 1990. In leaf extracts from 2-week old plants, a ten-fold increase in chitinase activity was observed compared with that obtained in untransformed plants. In 4-week old transgenic plants the chitinase activity is similar but only negligible chitinase activity can be detected in leaf extracts from control plants. Using a commercial sample of chitinase from *Streptomyces griseus* as a standard, leaf extracts from 4-week old cotton plants were found to contain approximately 50µg chitinase/mg soluble protein or approximately 900 µg chitinase/g fresh cotton leaf. Increased chitinase activity has also been detected in extracts from the transgenic line using electrophoresis on SDS/polyacrylamide gels renatured with Triton X100 (Trudel and Asselin, 1989).

Figure 1. Chitinase-Expressing Transgenic Cotton plants are less stunted by Verticillium wilt than Untransformed Coker Cotton Plants



Response to Verticillium Infection

Plants of the chitinase-expressing line demonstrate less stunting due to Verticillium wilt than untransformed control plants (Figure 1), ie: the Verticillium-inoculated transformed chitinase-expressing line is taller than the Verticillium-inoculated untransformed Coker line. This difference is significant (t-test) from 50 days after inoculation. The reduction in stunting has been observed consistently in three glasshouse trials.

Proposed Field Trials

We wish to test whether this reduction in the severity of the symptoms of wilt disease is also observed under field conditions, and if the level of protection conferred is sufficient to give an improvement in yield when the plants are grown under natural wilt disease pressure from both Verticillium and Fusarium wilt. We have other lines in development that appear to have higher levels of chitinase activity and we anticipate that these will show greater reduction in the severity of *Verticillium* wilt disease symptoms. In addition, we anticipate that other lines containing the chitinase gene together with genes for other putative antifungal proteins will be developed. Prior to any commercial release, these lines would be crossed with elite Australian cultivars.

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References

Grover, A., Llewellyn, D., and Dennis, E.S., 1995, Journal of Plant Biochemistry and Biotechnology, 4:41-42

Melchers, L.S., Sela-Buurlage, M.B., Vloemans, S.A., Woloshuk, C.P., van Roekel, J.S.C., Pen, J., van den Elzen, P.J.M., and Cornelissen, B.J.C., 1993, Plant Molecular Biology 21; 583-593.

Neale, A.D., Wahleithner, J.A., Lund, M., Bonnett, H.T., Kelly, A., Meeks-Wagner, R., Peacock, W.J., and Dennis, E.S., 1990, The Plant Cell, 2:673-684

Trudel, J. and Asselin, A., 1989 Analytical Biochemistry 178: 362-366.

Wirth, S. J., and Wolf, G. A., 1990, Jnl Microbial Methods, 12: 197-205.