GENETIC ENGINEERING FOR WATERLOGGING TOLERANCE IN TRANSGENIC COTTON

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Background and Aim of Project

Waterlogging is a persistent problem associated with the cultivation of cotton in heavy soils such as the cracking grey clays found in the cotton growing regions of north-western New South Wales. In these regions waterlogging leads to reduced yields even in the best of years and can occasionally cause yield losses up to 40%.

Waterlogged cotton plants are faced with a variety of diverse factors in the root environment the most important of which is limiting oxygen concentrations. Under such conditions energy production generated through aerobic respiration cannot proceed and the plant instead must rely on the anaerobic fermentation of sugars with the production of ethanol.

Alcohol fermentation is a simple biochemical pathway, consisting of a two-step conversion of pyruvate catalysed by the enzymes Pyruvate decarboxylase (*Pdc*) and Alcohol dehydrogenase (*Adh*). The ability to carry out ethanol fermentation has been demonstrated to be a key adaptation to waterlogging and anaerobic conditions in a number of species (notably wheat, rice and barley). Cotton stands out as having extremely low levels of ethanol fermentation and this may significantly contribute to this plants poor tolerance of waterlogged soils. The

aim of this project is to produce cotton with increased levels of the two enzymes in the alcohol fermentation pathway through the insertion of extra copies of the genes using *Agrobacterium* transformation. In addition, existing ethanol fermentation rates will be experimentally further reduced by targeting the ADH enzyme with antisense constructs. The resulting transgenic plants can then be tested in controlled conditions for altered anaerobic tolerance.

Achievements and Status of the Project

Constructs have been engineered for the overproduction and antisense-inhibition of the cotton *Adh* gene by Tony Millar during the course of his PhD in our laboratory. One of these constructs was shown to lead to an increased ADH enzyme activity when inserted into transgenic cotton. The number of individual transgenic lines obtained from this work, however, was not sufficient for a statistically valid evaluation of the physiological responses of the transgenic plants to waterlogging. In addition, no transgenic plants with increased *Pdc* expression were available at the start of the current project.

A plasmid aimed at increasing PDC enzyme levels was constructed using a gene available from rice. The rice *Pdc* gene, was fused to the CaMV 35S promoter and other sequences that will allow its insertion and expression in the cotton genome at all times and in all tissues.

In the past year the *Adh* gene constructs (sense and antisense) and the *Pdc* gene constructs have been inserted into cotton using *Agrobacterium tumefasciens* as a vector. Lines of transformed tissue were selected on the basis of kanamycin resistance also conferred by the transforming plasmids. Plants were regenerated

from these calli and grown to flowering in a containment glasshouse. The number of transgenic plants obtained for each construct so far is tabulated below.

Construct	No. of transgenic lines				
	Total	Fertile	Sterile	Not yet known	T1 Seeds
35S-Cotton Adh	17	10	3	4	8
35S-Antisense Adh	14	5	1	8	2
35S-Rice Pdc	20	13	3	4	11

The number of independently transformed, fertile plants obtained is therefore well within the objectives set at the beginning of the project (10 lines per construct) and should provide sufficient material to evaluate the effect of increased expression of the two genes on the tolerance of cotton to waterlogged conditions.

The transgenic lines are currently being characterised by Southern blotting to confirm the presence of the inserted DNA and to monitor the copy number of gene insertions. Seeds from some the earliest transgenic lines have been harvested (T1 generation seeds) and are being grown for the next stage in the analysis. The segregation of the inserted sequences is easily monitored by qualitatively assaying for the NPTII marker that confers kanamycin resistance to the plants. In the case of *Adh* transformants, the enzyme produced by the transgene can easily be detected on native gels stained for ADH activity. Gel staining for PDC activity is

unfortunately not possible. To circumvent this problem, the rice PDC protein is being purified following expression in bacterial cells. This protein will be used to raise antibodies against PDC for the detection of the protein on Western blots of protein extracts from the transgenic plants.

In parallel to the work on cotton, studies have been undertaken aimed at testing the effect of genetically altered ADH and PDC levels in the model plant *Arabidopsis thaliana*. For this purpose, an assay for anaerobic tolerance in *Arabidopsis* has been optimised. This approach is yielding interesting results concerning anaerobic tolerance in plants. Although it is clear that comparisons between one species to the other are limited, the biochemical and physiological tools developed in the course of the work on *Arabidopsis* will be invaluable in the next stages of the analysis of transgenic cotton.

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