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Annual Report for Project CSP9C - Tony Millar

The objective of the present research is to see whether altering the level of the enzyme ADH will affect the tolerance of cotton to anaerobic or waterlogged conditions.

An Adh cDNA from cotton was cloned behind the 35S promoter of Cauliflower Mosaic Virus in the sense orientation (to over express) and antisense orientation (to eliminate expression) and transformed separately into cotton. Callus was generated, ADH and anoxia tolerant assays were performed but the callus system was found to be unsuitable to work with. The calli consisted of many different cell types which caused large variations in enzyme measurements from which no meaningful conclusions could be drawn. Also the untransformed control callus was tolerant to anoxia (probably due to the nature of the tissue) thereby making it a useless system to work with. It was then decided that plants would be a better system to analyze.

To date I have almost 40 regenerated plants in soil. These consist of 4 different transformation events for the sense construct and 7 different events for the antisense construct. Although these plants were generated from transformed calli (tested by marker gene assays), this does not guarantee that all plants will be transgenic. Preliminary analysis shows that 5/8 of the sense plants tested express ADH in their leaves, whereas wild-type plants do not express ADH in this tissue. This indicates that I have transgenic plants and that the genetic engineering has in fact worked.

Future experiments will involve further analysing the plants and determining their levels of ADH. Also methods must be developed to test survival of the transgenic plants under anaerobic conditions and therefore see what effects changing the levels of ADH have on the tolerance of cotton to anaerobic conditions.

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