STUDYING THE GENES SWITCHED ON DURING FLOODING

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INTRODUCTION

Plants encounter many enviromental stresses such as droughts, flooding, extreme temperatures and high salt concentrations just to name a few. Some plants have been shown to respond to a particular stress by switching on a particular set of genes that then synthesise a set of proteins that assist the plant to survive the stress. Studying these genes and the proteins they encode will give us an understanding of how plants perceive their enviroment and the biochemical action needed for survival in that enviroment. This understanding may then elucidate how genetic manipulation could strengthen a stress response to produce a more stress resistant plant.

When plants encounter flooding or waterlogging, low levels of oxygen are available for respiration, so oxidative carbohydrate metabolism grinds to a halt. When this occurs the plant must now rely on fermentative carbohydrate metabolism for its energy source, so as to stay alive. This switch in carbohydrate metabolism from a oxidative to fermentative pathway is called the anaerobic response.

THE ANAEROBIC RESPONSE IS A SURVIVAL MECHANISM

To date this response has been best characterize in maize where about 10-20 genes are switched on during flooding whilst all other genes are switched off. Several

of these switched on genes have been identified and have found to code for enzymes that are involved in fermentative carbohydrate metabolism. So the response appears to be an adaptation that enhances fermentative metabolism to compensate for the halt of the oxidative pathway, thus enabling the plant to met its energy needs and survive the period of flooding.

AIMS OF THE PROJECT

Work by Arthur Hodgson (Narrabri) has shown that cotton is a flood intolerant plant, being very susceptible to waterlogging. One reason why this is the case may be that cotton has a poor anaerobic response. The aim of my project is to characterize the anaerobic response of cotton i.e. how many genes are switched on, what do these genes code for and how these genes are switched on, and then devise ways in which to improve the response if any weaknesses are found.

COTTON HAS 10-15 GENES SWITCHED ON BY FLOODING

The first aim was to determmine how many genes are switched on during flooding in the cotton seedling. Using molecular biology and protein analysis techniques, it was determined that there are 10-15 genes switched on in cotton seedlings when they are immersed in water. This response is similar in a quantiative sense to the response in the flood tolerant plant maize (10-20 proteins) and greater than the flood intolerant plant, soybean (4-5 proteins), although it is uncertain how the number of genes switched on relates to how flood tolerant the plant is. What this experiment did show was that

cotton does have this gene "switch" when put under flooded conditions. So the next step was to identify some of the proteins that these genes code for and the role they play in the response.

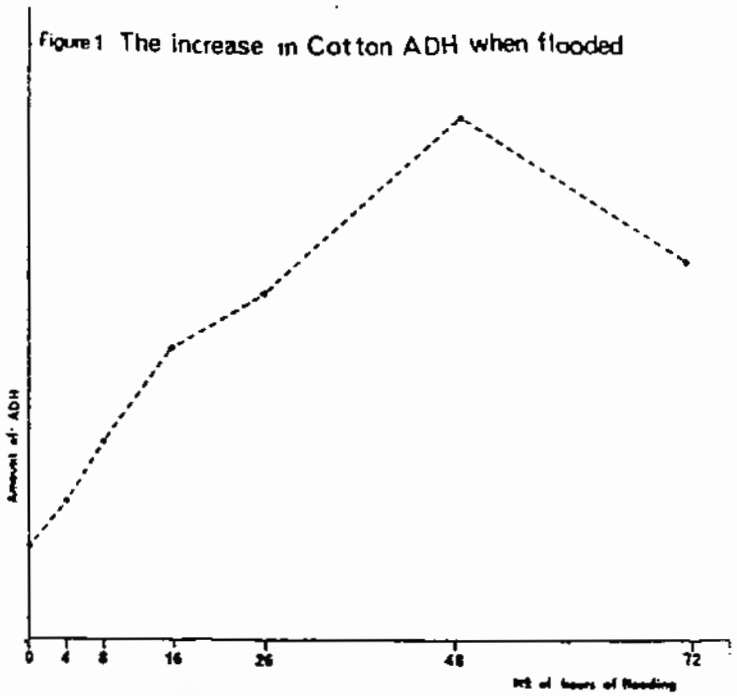
ALCOHOL DEHYDROGENASE IS ONE OF THE FLOOD INDUCIBLE GENES

I have comenced this by examing the protein Alcohol dehydrogenase (ADH) which is the terminal enzyme in the fermentative pathway. In maize this enzyme has shown to be essential in the anaerobic response, without ADH, maize dies immediately upon flooding, whereas maize with ADH can survive 3-5 days of flooding. This fact plus other studies suggests that there might be a correlation between the amount of ADH and the tolerance of the plant to flooding. So the aim was to see whether cotton contains an ADH that is switched on by flooding. Figure 1 displays how the amount of the ADH enzyme increases over time when cotton seedlings are immersed in water. This plus other data I have obtained shows that the level and length of the increase is comparable to ADH's of other plants such as maize and rice that are flood tolerant. This result suggests that cotton sensitivity to flooding isn't due to a lack of ADH inducibility

CLONING OF THE ADH GENE AND ITS CONTROL REGION

Although this result was obtained we are still interested in the flood inducible ADH enzyme because cloning the gene that codes for ADH will enable us to isolate the region of DNA that controls the gene (which will be located directly next to the gene). From studies done on the maize Adh gene, it is known that the control

region is responsible for switching on the gene when the plant is flooded. We suspect that the case will be similar in cotton. Studying this control region should reveal



To clone the gene, a gene libaray must be made. This has been acheived and the libarary has now being screened for the ADH gene and positive clones isolated. I am now characterizing these clones to check whether they do code for ADH.

The gene libarary can also be screened for any other gene of interest. One gene that might be of interest would be the master gene that controls all the genes that are switched on during flooding. We suspect that this gene would encode a protein that binds to the control region of the ADH gene, and this interaction between the protein and the control region permits the Adh gene to produce the ADH enzyme. We suspect that all the genes switched on during flooding in cotton will each have a

similar control region where the master protein can bind, thereby accounting for the response behaving in a co-ordinated manner. Cloning and studying this gene may show us a way to control (and maybe enhance) the whole response.

I would like to thank the CRC for supplying a studentship to allow me to carry out this research.