GLYCONJUGATE PROFILES OF DEVELOPING FIBERS FROM IRRIGATED AND NON-IRRIGATED PLANTS Allen K. Murray Glycozyme, Inc., Irvine, CA Daniel S. Munk and Jonathan Wroble University of California, Cooperative Extension, Fresno, CA

Abstract

Glycoconjugate analysis is being used to monitor growth and development of cotton fibers. Environmental stress has been detected by glycoconjugate analysis. This study presents glycoconjugate profiles for developing fibers from bolls taken from different fruiting branches on the same day from two fields with identical irrigation schedules up to the end of June 1996. After that date one field was not irrigated. The glycoconjugate profiles from the developing fibers taken from bolls of plants that were not irrigated show a number of peaks that are present after a retention time of 16 minutes which are not predominant in the fibers from the irrigated plants. In addition, there is a difference in the peaks at 14 minutes. The working hypothesis is that these are cell wall precursors which are not utilized in cell wall synthesis or that the inhibition of a rate limiting step in cell wall synthesis results in their accumulation

Introduction

Cotton fiber quality is determined by both genetic and environmental factors. Although different varieties do better in different climates, environmental factors both at a macro and micro level play a large role in determining fiber quality and yield. A great deal is known about fiber development from a biophysical aspect however little is known about the correlation between the biophysical and biochemical characteristics during fiber development. Just as yield affects the producer's income so does the fiber quality as price is based on fiber quality. Since cotton is an indeterminate flowering plant resulting in bolls of various ages on the plant, the timing of irrigation is of great importance since bolls at different stages of development respond differently to environmental stress including drought stress.

Although several methods of determining drought stress are utilized, including water potential as determined using a pressure bomb, none are optimal. The driving force for water transport is the result of a number of membrane potentials and osmotic gradients in addition to transpirational pull. It would seem likely that there may be events that can be measured at the biochemical level that precede the measurable water potentials. In addition to water potentials, other methods of measuring plant stress include soil moisture content, air temperature, leaf temperature, relative humidity. It is interesting to note that presently there is no method in use to evaluate plant stress that utilizes any biochemical analysis of the plant tissue.

Plant cells are surrounded by a rigid cell wall consisting of polysaccharides and a small amount of protein. The primary cell wall is typically synthesized during cell elongation and consists primarily of hemicelluloses and pectic substances. The secondary cell wall is synthesized later, typically during wall thickening, and consists primarily of cellulose. Any growth of a plant cell requires simultaneous expansion and or thickening of the cell wall. Consequently, growth of a plant cell is directly linked to cell wall synthesis. Cell wall synthesis is sensitive to drought stress and other forms of environmental stress which affect cell growth and development. Several glycoconjugates have been identified which appear to function as cell wall precursors and their concentrations can be monitored by glycoconjugate analysis. Aberrations in the normal sequence of appearance and disappearance of these compounds can be taken as indicators of abnormal cell wall synthesis and consequently abnormal growth and development. Such aberrations in the developmental sequence of glycoconjugates have been observed for both low temperature stress and drought stress. Glycoconjugate Analysis (GCA) appears to be a sensitive method by which to monitor cotton fiber development and has the potential to identify abnormal development in cotton fibers. Structural studies currently in progress on the glycoconjugates will greatly facilitate our understanding of the role of these glycoconjugates in cell wall synthesis and significance of abnormal developmental patterns.

Methods

Cotton was grown at the West Side Field Station, Fresno, CA. Both fields received the same irrigation schedule up to June 28, 1996 which was the last irrigation for the nonirrigated samples. The field from which the irrigated samples were obtained received an additional irrigation on July 28, 1996. First position bolls were shipped to the laboratory on dry ice. Frozen bolls were kept in a freezer at -80° until lyophilized. Frozen bolls were cracked open in a vise with a section of angle-iron attached to one jaw by epoxy. Lyophilization was carried out at 200 millitorr at dryness and usually required up to 48 hours to achieve constant weight. In a typical experiment 5mg of cotton fibers were extracted with a volume of 0.5 ml. Following extraction the contents of the vial were centrifuged in a Z-Spin cartridge, $0.2\mu m$, prior to chromatography by HPAEC-PAD. HPAEC-PAD (High pH Anion Exchange Chromatography - Pulsed Amperometric Detection) was performed on a Dionex Bio-LC. Retention times are expressed in minutes and detector response is expressed in μ Coulombs. Chromatographic analysis was performed using the Dionex Peak Net software.

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Results

The chromatograms for fruiting branches 3 & 4(29DPA) and 5 & 6 (23DPA)show a series of peaks between retention times of 16 minutes and 26 minutes which are not as predominant in the irrigated samples (Figure 1) as in the non-irrigated samples(Figure 2.) In the region of retention times past 16 minutes, the fibers from the irrigated plants have a relatively predominant peak at 17.5 minutes. Although the same peak is present in the non-irrigated sample in Figure 2, it is not a predominant peak. The expanded scale chromatograms, Figures 3 and 4, demonstrate similar results however there are many more peaks present in the non-irrigated samples. The predominant peaks are at 17.5, 18.7, 24.1, and 25.5 minutes. The expanded scale chromatograms of the 14 min region in Figures 5 and 6 show a pair of peaks, one just preceding and one just after a retention time of 14 minutes in the nonirrigated samples. The double peak is only present in the irrigated samples from fruiting branch 5 & 6 (23DPA).

Conclusion

Drought stress appears to result in a group of glycoconjugates that are present in greater concentrations than in the irrigated plants. The working hypothesis is that these are cell wall precursors which are not utilized in cell wall synthesis or that the inhibition of a rate limiting step in cell wall synthesis results in their accumulation. The difference between irrigated and non-irrigated plants is greater at younger stages of boll development.

Acknowledgment

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References

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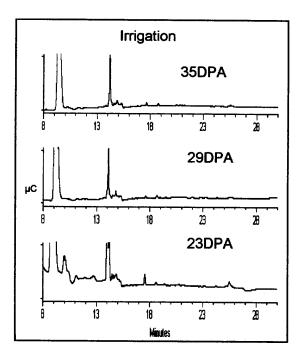


Figure 1. Glycoconjugate Profiles with Irrigation. The pattern of peaks appearing after 16 min has major peaks at 17.8 and 18.7 min.

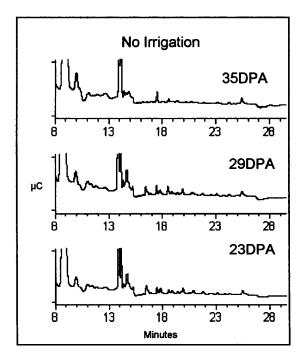


Figure 2. Glycoconjugate Profiles with No-Irrigation. A number of peaks past 16 min in the 23 and 29DPA samples are present which were not present in the irrigated samples in Figure. 1.

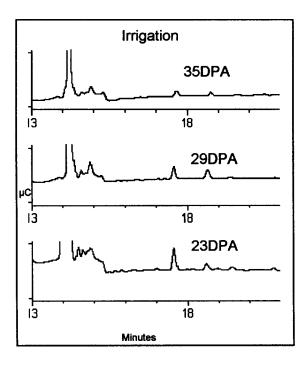


Figure 3. Glycoconjugate Profiles with Irrigation. Expanded scale. Past 16 min only peaks at 17.6 and 18.8 min are present in fibers from irrigated plants.

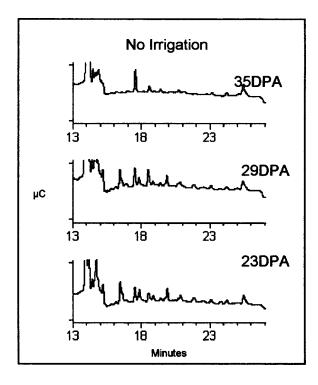


Figure 4. Glycoconjugate Profiles with No-Irrigation. A number of peaks past 16 min are present at 29DPA and 23DPA which are not present at 35DPA.

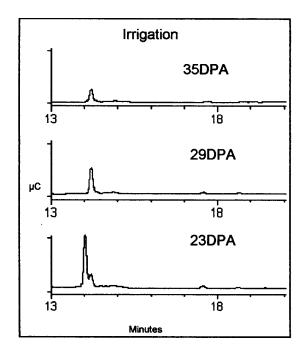


Figure 5. Glycoconjugate Profiles with Irrigation. Expanded scale to show peaks around 14 min. Single peak is observed with only a small second peak at 23DPA.

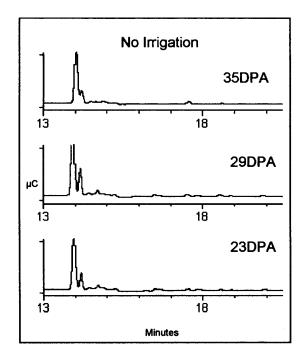


Figure 6. Glycoconjugate Profiles with No-Irrigation. Expanded scale to show peaks around 14 min. Second peak is observed in all chromatograms with only a small one at 35DPA.