GLYCOCONJUGATE PROFILES OF DEVELOPING FIBERS FROM DIFFERENT FRUITING BRANCHES ON THE SAME PLANT

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Abstract

Glycoconjugate analysis is being used to monitor growth and development of cotton fibers. Environmental stress has been detected by glycoconjugate analysis. The present study was undertaken to compare developing bolls at different stages of development under identical environmental conditions. Prior to this study developmental samples have only been obtained from tagged flowers or from bolls at the same stage of development. The differences in glycoconjugate profiles between bolls at the same age could have been due to differences in individual plants due to soil characteristics within the field. In this study glycoconjugate analysis documented the developmental profile of glycoconjugates in bolls of different ages collected from the same plant at the same time to eliminate variability due to individual plants or field soil characteristics.

The results of this study document a drop in sucrose concentration and a concomitant increase in GC-1 glycoconjugates at a time period around 21DPA which corresponds with the onset of secondary cell wall synthesis.

Introduction

Cell Wall

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 of and or thickening of the cell wall. The cotton fiber is an example of a cell which primarily functions to synthesize a massive cell wall. In the cotton fiber the transition from synthesis of primary cell wall to secondary cell wall is a particularly critical time with respect to the mature fiber and the resultant fiber quality. 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.

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. 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.

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. The differences in glycoconjugate profiles of developing cotton fibers have been documented from either tagged flowers or from bolls of various ages. In this present study, bolls from several fruiting branches were collected at the same time to obtain information on the developmental sequence without the possibility of plant to plant variability. The glycoconjugate profiles obtained result in a biochemical plant map for glycoconjugates in the developing fibers. The potential role for glycoconjugate cell wall precursors is shown in Figure 1.

Methods

DP20 was grown at Finch Ranch, Orland, CA. First position bolls from sequential fruiting branches on the same plant were collected on 8/17/96 and immediately placed on "blue ice" and shipped to the laboratory on dry ice at the conclusion of sample collection. 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 30-60 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

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centrifuged in a Z-Spin cartridge, 0.2µm, 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 µCoulombs. Chromatographic analysis was performed using the Dionex Peak Net software. The detector response scales on all chromatograms are normalized for the amount of fiber material extracted.

Results

The chromatograms presented essentially provide a biochemical plant map with respect to glycoconjugates in developing fibers. The plant profiled in Figures 2-4, Plant 2 1RE, was a tall/rank plant which showed a striking decline in sucrose in FB 8 and FB 7, which represent 18 and 21DPA respectively. The sucrose concentration recovered by FB 6, 24DPA, and did not decline until FB 2, 36DPA. Concomitant with the decline in sucrose concentration at FB2 there was a striking increase in the glycoconjugate with a retention time of 14.2 minutes. As a control tissue for the developing fibers, pericarp tissue from the same bolls was also extracted and subjected to glycoconjugate analysis. The results for the pericarp tissue were quite different than for the developing fibers. The sucrose concentration was somewhat lower in FB 8, FB 5 and FB 1 but the differences were not as great as in the fibers. The major glycoconjugate in the pericarp tissue had a retention time of 15 minutes.

The plant profiled in Figures 5 and 6, Plant 3 1RN was a short plant from an area of the field similar to that shown in the photograph. This plant did not demonstrate the decline and then recovery of sucrose in the developing fibers. The sucrose concentration was relatively constant throughout the plant and the differences in glycoconjugates were similarly not as striking as in the tall plants.

Conclusion

The working hypothesis is that these glycoconjugates are cell wall precursors. The decline in sucrose and concomitant increase in specificglycoconjugates coincides with the onset of secondary cell wall synthesis at about 21DPA. The fact that the sucrose concentration in the pericarps does not fluctuate greatly leads to the conclusion that the drop in sucrose concentration in the fibers is due to an overwhelming demand for sucrose that exceeds the supply from translocated photosynthate. The fact that the shorter plant does not demonstrate the fluctuations in sucrose concentration is taken as evidence that its overall slower growth rate results in a rate of fiber development that permits the sucrose demand to remain within the range of the translocated supply. Even though the short stunted plant has a slower growth rate it shows the developmental changes in the GC-1 group of glycoconjugates indicative of the onset of secondary wall synthesis two or three days earlier than in the tall plant. This is also consistent with the observations of other milestones of development.

Acknowledgment

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References

Murray, Allen K., 1996, The use of Glycoconjugate Analysis to monitor Growth and Environmental Stress in Developing Cotton Fibers, 1996 Proceedings Beltwide Cotton Conferences, p. 1255-1259.

Murray, Allen K. and Judy Brown, 1996, Glycoconjugate Analysis of Developing Cotton Fibers From Several Varieties Grown on the Same Site, 1996 Proceedings Beltwide Cotton Conferences, p. 1205-1209.

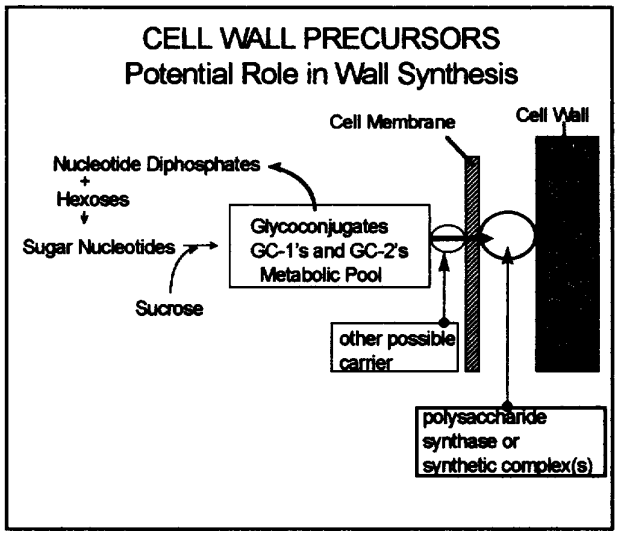

Figure 1. Potential Role of Glycoconjugates in Cell Wall Synthesis. Compounds with retention times of 14-16 min are called GC-1's and compounds with retention times of 18-20 min are called GC-2's.

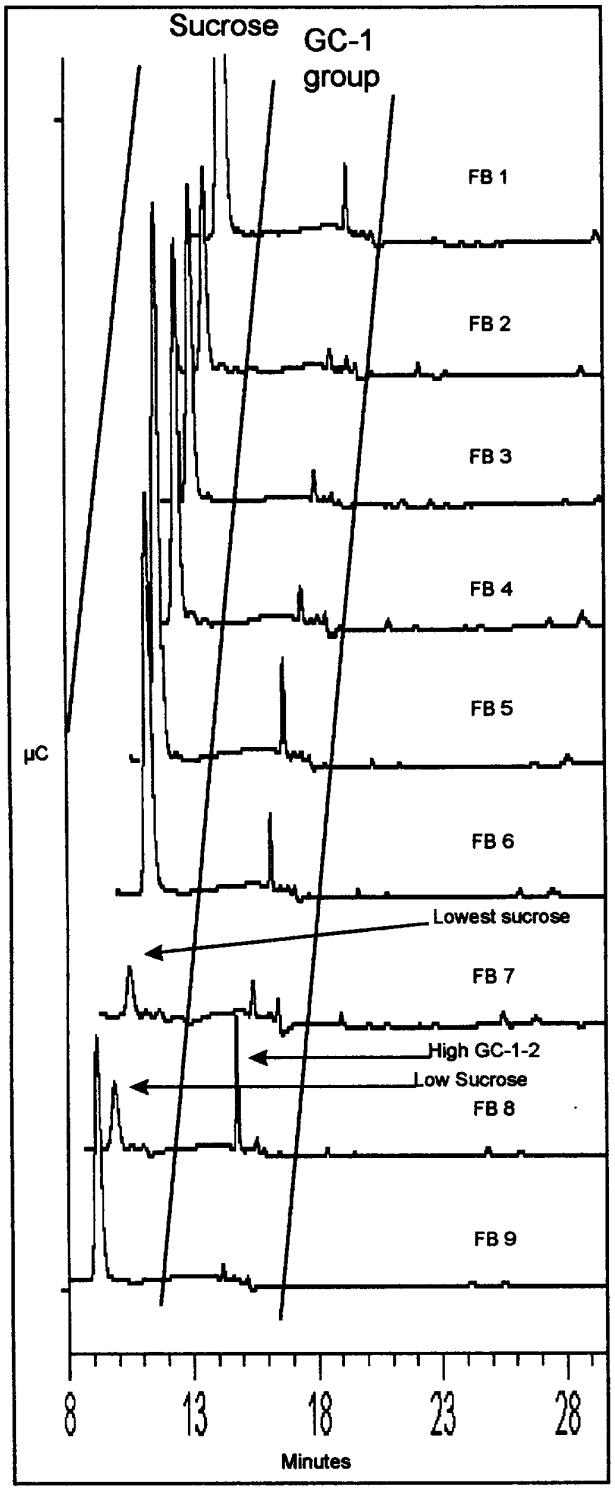

Figure 2. Developing fibers, PLANT 2 1RE
Fruiting branches 9 to 1 representing 15 to 39DPA respectively. Sucrose retention time is 9.3 minutes.

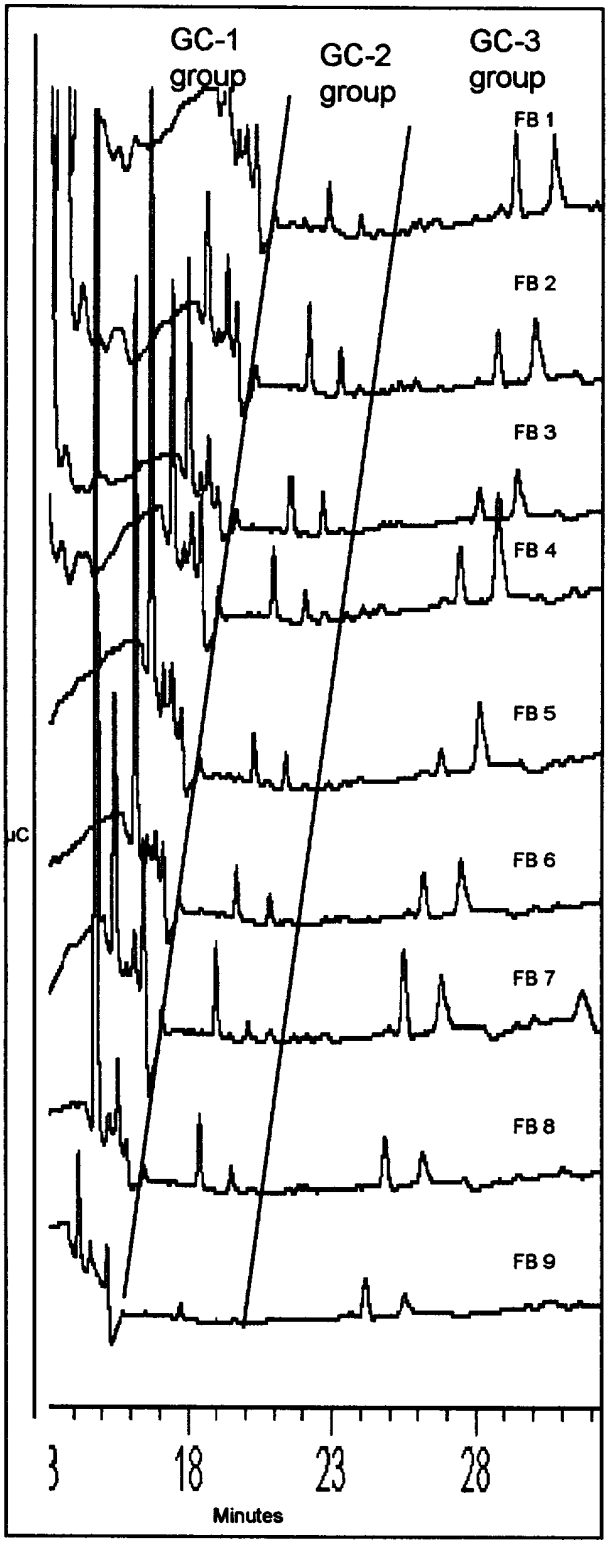

Figure 3. Developing fibers, PLANT 2 1RE
Expanded scale. Fruiting branches 9 to 1 representing 15 to 39 DPA respectively.

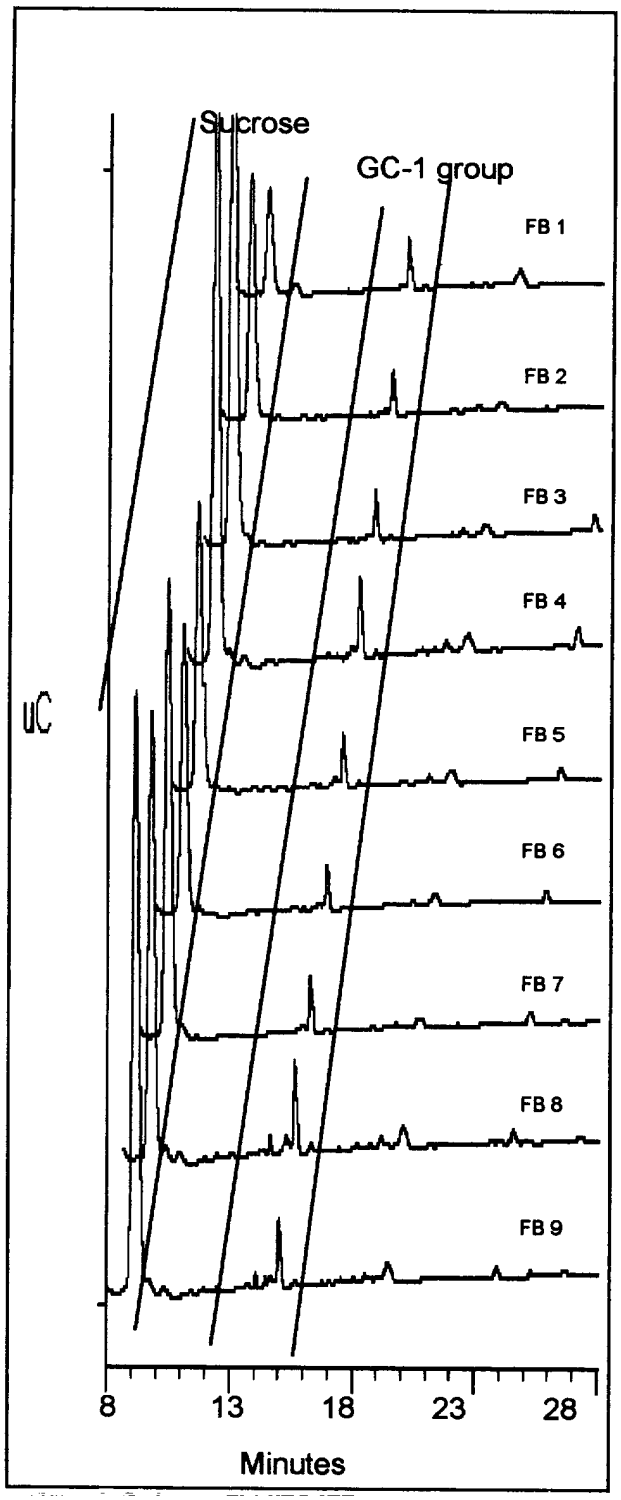

Figure 4. Pericarps, PLANT 2 1RE
Fruiting branches 9 to 1 representing 15 to 39 DPA respectively. Sucrose retention time is 9.3 minutes.

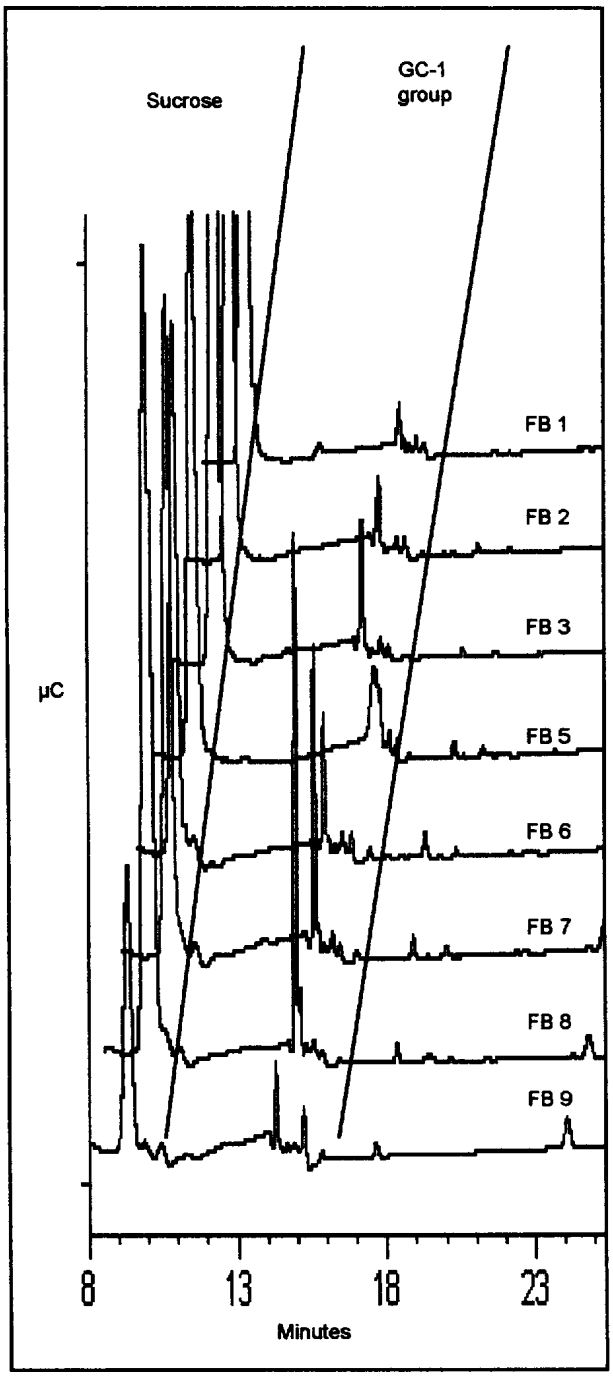

Figure 5. Developing fibers, PLANT 3 1RN SHORT
Fruiting branches 9 to 1 representing 15 to 39DPA respectively. Fruiting Branch 4 is missing. Sucrose retention time is 9.3 minutes.

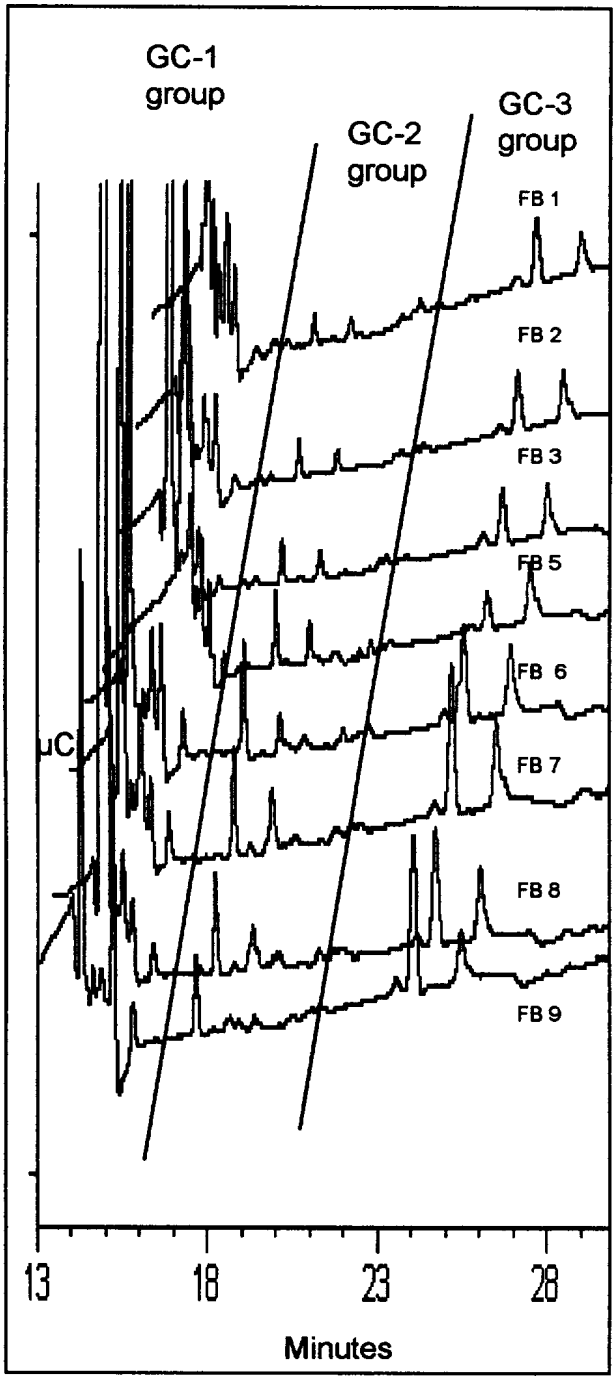

Figure 6. Developing fibers, PLANT 3 1RN SHORT
Expanded scale, Fruiting branches 9 to 1 representing 15 to 39 DPA respectively. Fruiting Branch 4 is missing.