myo-INOSITOL, SUCROSYL OLIGOSACCHARIDE METABOLISM AND DROUGHT STRESS IN DEVELOPING COTTON FIBERS, *IN VIVO*, *IN VITRO* AND *IN PLANTA*

A. K. Murray
Glycozyme, Inc.
Irvine, CA
Daniel S. Munk and Jonathan Wroble
University of California, Cooperative Extension
Fresno, CA
Gretchen F. Sassenrath-Cole
USDA, ARS, APTRU
Stoneville MS

Abstract

Sucrosyl oligosaccharides appear to function as cell wall precursors. A decrease in sucrose concentration and a concomitant increase in raffinose concentration characterize maximal secondary wall synthesis in cotton fibers. The relative concentrations of sucrosyl oligosaccharides were found to vary fibers from plants subjected to high stress in irrigation experiments. In this work, various substrates were added to developing fibers and found to be consumed over a 72 hour period. The ratios of substrates altered the oligomers (~mers) which were extracted by dilute acid. The *in planta* administration of *myo*-inositol gave similar results to the *in vitro* incubations.

Introduction

The differences in glycoconjugate profiles of developing cotton fibers have been documented from either tagged flowers or from bolls of various ages (Murray, 1996, Murray & Brown, 1996, 1997, Murray *et. al*. 1997). The experiments presented here lead to a conclusion that the sucrosyl oligosaccharides could serve as precursors to cell wall polysaccharides, in vitro. Sucrose, cellobiose, raffinose, glycerol and *myo*-inositiol are consumed on prolonged incubation with developing fibers. Differences in the concentration of these substrates can alter the relative distribution of cell wall polymers extracted from the fibers following incubation. Since the sucrosyl oligosaccharides have been observed to undergo Interconversion during the synthesis of wall polymers, *in vitro*, one goal of this investigation was to determine if administration of *myo*-inositol *in planta* has the same effect as *in vitro*. Raffinose is formed from sucrose by the addition of an α-1,6 linked galactose and subsequent α-1,6-galactose residues are added to form the other oligosaccharides in the series (Figure 1). The galactosyl donor for the synthesis of these oligosaccharides is galactinol, which is formed from UDP-galactose and *myo*-inositol (Kandler and Hopf, 1982, Lehle and Tanner, 1973, Tanner and Kandler, 1968).

Methods

Cotton plants were DP-50 grown in the Mississippi Delta region for the time of day samples which were collected at 7am, noon and 7pm at 25 DPA. Plants for the *myo*-inositol, *in planta*, experiments were grown at the West Side Research and Extension Center in the San Joaquin Valley. Each locule was injected with 200μl of 0.5M *myo*-inositol at 6pm. The bolls were harvested and placed on dry ice at 9am the following morning. The night was unusually cool with a minimal temperature about 14°C.

Cotton fibers were subjected to aqueous extraction and analysis of the soluble carbohydrates by high pH anion chromatography with pulsed amperometric detection (HPAEC-PAD) (Murray, 1998). Additional extraction of the ~mers was achieved under conditions of dilute acid and elevated temperature prior to HPAEC-PAD. In vitro, incubations were carried out with rehydrated fibers from freeze-dried bolls. The incubations were done at 37°C under toluene to prevent microbial growth. All substrate concentrations were 25mM unless otherwise indicated.

Results

The presence of the sucrosyl oligosaccharides in developing cotton fibers has been observed under varying conditions of environmental stress. Since differences in the relative concentrations of raffinose, stachyose and verbascose have been observed between fibers from low stress (irrigated) and high stress (non-irrigated) plants, the ability to metabolize these oligosaccharides, in vitro was investigated. Essentially no difference was found between fibers from low and high stress plants indicative of a simple substrate effect causing the observed differences in the field.

Fibers were incubated with glycerol, *myo*-inositiol, sucrose, cellobiose and raffinose for up to 72hours. The relative concentrations of these substrates at zero time, one hour, two hour and 72 hour time points is shown in Figure 2. The substrates are removed from the medium and are not hydrolyzed to monosaccharides, which are shown with the shorter retention times up to about 6 minutes. The higher order oligosaccharides which are formed and then removed from the medium are shown in Figure 3, which is the same chromatogram as Figure 2 shown at an expanded scale.

Oligomers (~mers) extracted from fibers following a 72 hour incubation with varying concentrations of substrates are shown in Figure 4. The control has no substrates added other substrates are as indicated in Methods with the 2 designation indicating 50mM. The ratios of substrates can alter the profile of ~mers extracted from the fibers following incubation. The reduction in ~mers which can be extracted may either be due to a lack of formation or the

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:518-520 (1999)
National Cotton Council, Memphis TN

stimulation of subsequent metabolism which converts them to a form which can not be extracted by the dilute acid. Since more ~mers are extracted from the control, the latter explanation is attractive but further work must be done to determine if this is, *in fact*, the case.

The results of the *myo*-inositol injection, *in planta*, are shown in Figure 5. The upper two chromatogram plots are for the cold water extracts and the lower two plots show the ~mers extracted with dilute acid. It is interesting to note that the fibers from *myo*-inositol injected bolls released a lower concentration of ~mers the same as was observed, in vitro, with the higher *myo*-inositol concentration. Obviously, one can not tell just what the ratios of substrates were *in planta*. Although, the figure shown is for Pima S-7, similar results were obtained with Maxxa.

Summary

Several of the sucrosyl oligosaccharides have been reported in cotton fibers previously (Jaquet, *et. al*. 1982). Glycerol, *myo*-inositol, sucrose, cellobiose and raffinose are consumed by rehydrated cotton fibers *in vitro*. Their disappearance from the incubation medium is not accompanied by an increase in monosaccharides indicative of their incorporation into the fibers. The relative concentrations of these substrates does influence the ~mers which can be extracted from the fibers with dilute acid. The *in planta*, administration of *myo*-inositol results in a similar alteration in the ~mers which can be extracted from the fibers.

Discussion

The consumption of the substrates glycerol, *myo*-inositol, sucrose, cellobiose and raffinose by fibers incubated for 72 hours indicates that they must be consumed in some manner and not hydrolyzed to monosaccharides. This is suggestive that these substrates are incorporated into the fibers. The fact that the ratios of substrates alters the ~mers which are extracted from the fibers with dilute acid is also suggestive of a role in fiber development. Since *myo*-inositol plays an ephemeral role in cell wall biosynthesis the administration *myo*-inositol, *in planta*, was attempted to determine if the effects were the same as *in vitro*. The fact that similar results were obtained indicates that further experiments will be conducted to elucidate its role in fiber development.

References

Jaquet, Jean Pierre; Buchala Anthony J; Meier Hans, . 1982, Changes In The Nonstructural Carbohydrate Content Of Cotton (Gossypium Spp.) Fibers At Different Stages Of Development.
PLANTA. 156. p481-6.

Kandler, O. and H. Hopf, 1982. Oligosaccharides Based on Sucrose (Sucrosyl Oligosaccharides), Plant Carbohydrates I, Intracellular Carbohydrates, Encyclopedia of Plant Physiology, New Series, Vol 13A, p 348-383.

Lehle, Ludwig and Widmar Tanner, 1973, The Function of *myo*-Inositol in the Biosynthesis of Raffinose [Purification and Characterization of Galactinol:Sucrose 6-Galactosyltransferase from *Vicia faba* Seeds, Eur. J. Biochem. 38, 103-110.

Murray, A. K., 1998, Method For Monitoring Growth And Detection Of Environmental Stress In Plants , U.S. Patent No. 5,710,047

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.

Murray, Allen K., Daniel S. Munk and Jonathan Wroble, 1997, Glycoconjugate Profiles of Developing Fibers from Irrigated and Non-Irrigated Plants, 1997 Proceedings Beltwide Cotton Conferences, p. 1439-1441.

Murray, Allen K. and Judy Brown, 1997, Glycoconjugate Profiles of Developing fibers from Different Fruiting Branches on the Same Plant, 1997 Proceedings Beltwide Cotton Conferences, p. 1496-1499.

Tanner, W. and O. Kandler, 1968, Myo-Inositol, a Cofactor in the Biosynthesis of Stachyose, Eur. J. Biochem. 4, 233-239.

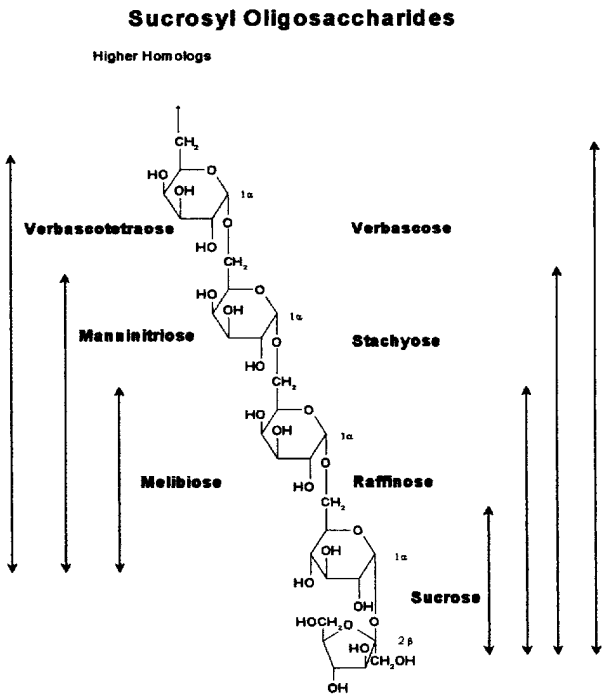

Figure 1. Sucrosyl oligosaccharides.

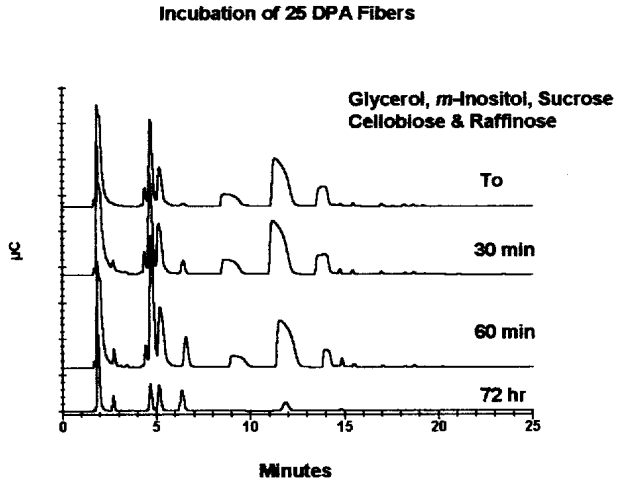

Figure 2. Substrates consumed during incubation of fibers. (Approximate retention times: glycerol: 1.7 min, myo-inositol: 1.8 min, sucrose: 9 min, cellobiose: 11.5 min, raffinose: 13.8 min.)

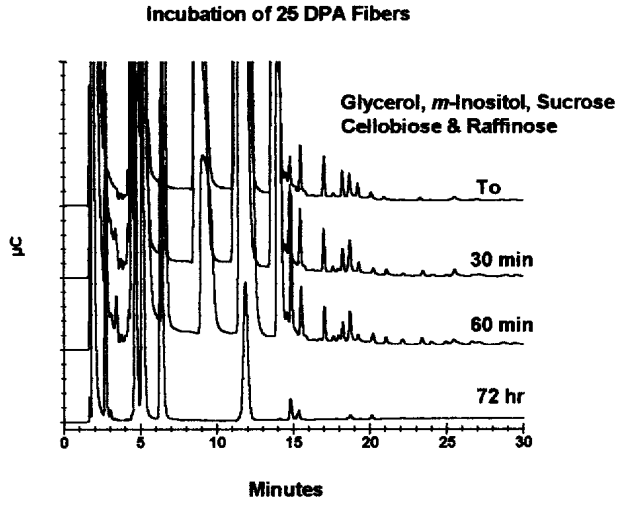

Figure 3. Substrates consumed and oligosaccharides metabolized during incubation of fibers.

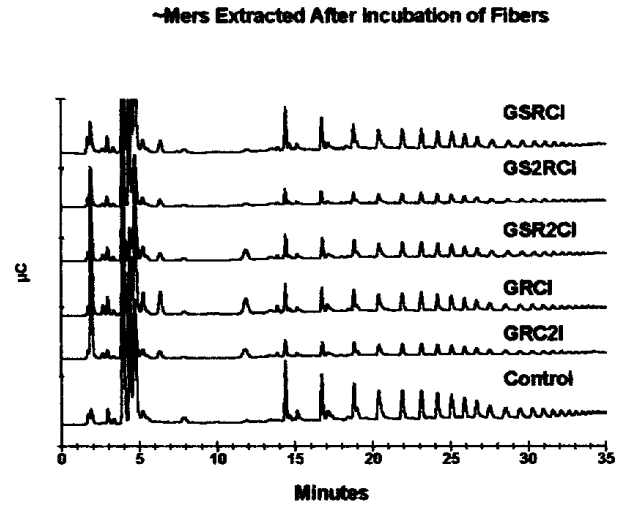

Figure 4. Oligomers (~mers) extracted from fibers following a 72 hour incubation.

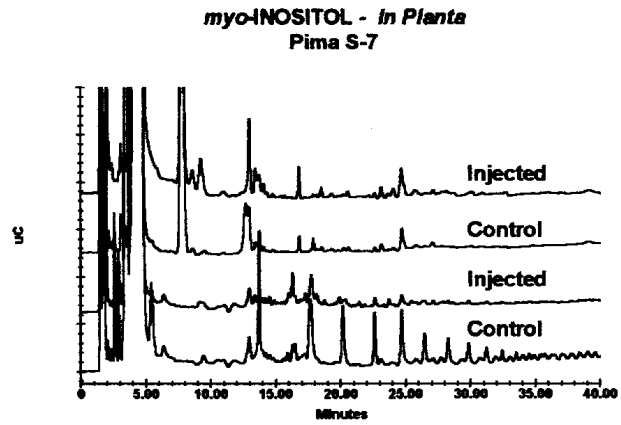

Figure 5. The results of *myo*-inositol injection, *in planta*. The upper two chromatogram plots are for the cold water extracts and the lower two plots show the ~mers extracted with dilute acid.