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CHARACTERIZATION OF COTTON CULTIVARS BY GLYCAN OLIGOMER ANALYSIS

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Abstract

Mature cotton fibers from six cultivars grown in six locations in Brazil have been analyzed by glycan oligomer analysis. The profiles of each of the cultivars are distinct yet contain many similarities. Two of the cultivars which are genetically more distantly related appear significantly different from the other four. These results indicate that such carbohydrate analysis with more refined data analysis, including compounding of different extraction profiles, are promising for development of means for identification of the cultivars of mature cotton fibers or a true variety tracer technology.

Introduction

The potential for utilization of glycan oligomer analysis to distinguish cotton cultivars is based on the observation in this laboratory that glycan oligomer profiles appear to be distinct for plant tissues, species and developmental stages. Six cultivars from six locations in Brazil were analyzed by glycan oligomer analysis utilizing cold water, 0.1N HCl and 80% acetic 1.8N nitric acid extractions. The following are the locations where the cotton was grown: Alto Garças (Mato Grosso); Primavera do Leste (Mato Grosso); Diamantino (Mato Grosso); Uberlandia (Minas Gerais) ; Luis Eduardo Magalhaes (Bahia) ; Chapadao Do Sul (Mato Grosso do Sul).

Materials and Methods

Mature cotton fibers from six locations in Brazil were sampled. Samples from two repetitions were obtained from each location and each sample was then subjected to three analyses. The fibers were cut into 2-3mm segments with a razor blade. A 2.5-6.0-mg sample of fibers was placed in a 2.0 ml screw cap plastic tub and 0.5 ml of water was added. In the refined method, the sample was homogenized with a PRO 200 Homogenizer with a Multi-Gen tip (PRO Scientific, Inc.), 7-mm in diameter, until a uniform homogenate was obtained. Using either sample preparation method, the tube was shaken, then placed in a Branson 85 W sonicator filled with ice water for 15 minutes.. The tubes were centrifuged at 15,000xg for 5 minutes. The supernatant was removed with a Pasteur pipette, 1.0 ml of 0.1 N HCl was added and the tube was mixed on a vortex mixer and placed in a boiling water bath for 30 min to extract the glucose containing oligomers. The supernatant was removed with a Pasteur pipette, 1.0 ml of 80% acetic 1.8N nitric acid (Updegraff, 1969) was added and the tube was mixed on a vortex mixer and placed in a boiling water bath for 30 min to extract the second group of glucose containing oligomers. HPAEC-PAD was performed using a CarboPac PA-1 column. The eluent was 150 mM sodium hydroxide, isocratic from 0 to 5 min then a linear sodium acetate gradient from 5 to 40 min going from 0 to 500 mM in 150 mM NaOH at a flow rate of 1 ml/min. The waveform had a potential of +0.1V from 0 to 0.40 sec, -2.0V from 0.41 to 0.42 sec, +0.6V from 0.43 to 0.44 sec and –0.1V from 0.44 to 0.50 sec with integration from 0.20 to 0.40 sec. Quantification is expressed as µC(microcoulombs)*min/mg fiber. The HCl extract was neutralized with 1.0N NaOH prior to chromatography. The acetic nitric extract was taken to dryness in a Speed Vac to remove the acetic and nitric acids. The samples were then taken up in water prior to chromatography.

Results

The HCl extract glycan oligomer profiles for cultivars A-E are shown in Figure 1. Each figure represents the average content of each of the glycan oligomers for each cultivar from all locations. The acetic nitric extract glycan oligomer profiles for cultivars A-E are shown in Figure 2. Each figure represents the average content of each of the glycan oligomers for each cultivar from all locations. On visual examination similarities and differences unique to each cultivar are apparent. A mathematical model to treat the data

and provide a rapid method of identification of cultivars based on this analysis is the subject of the next phase of this work.

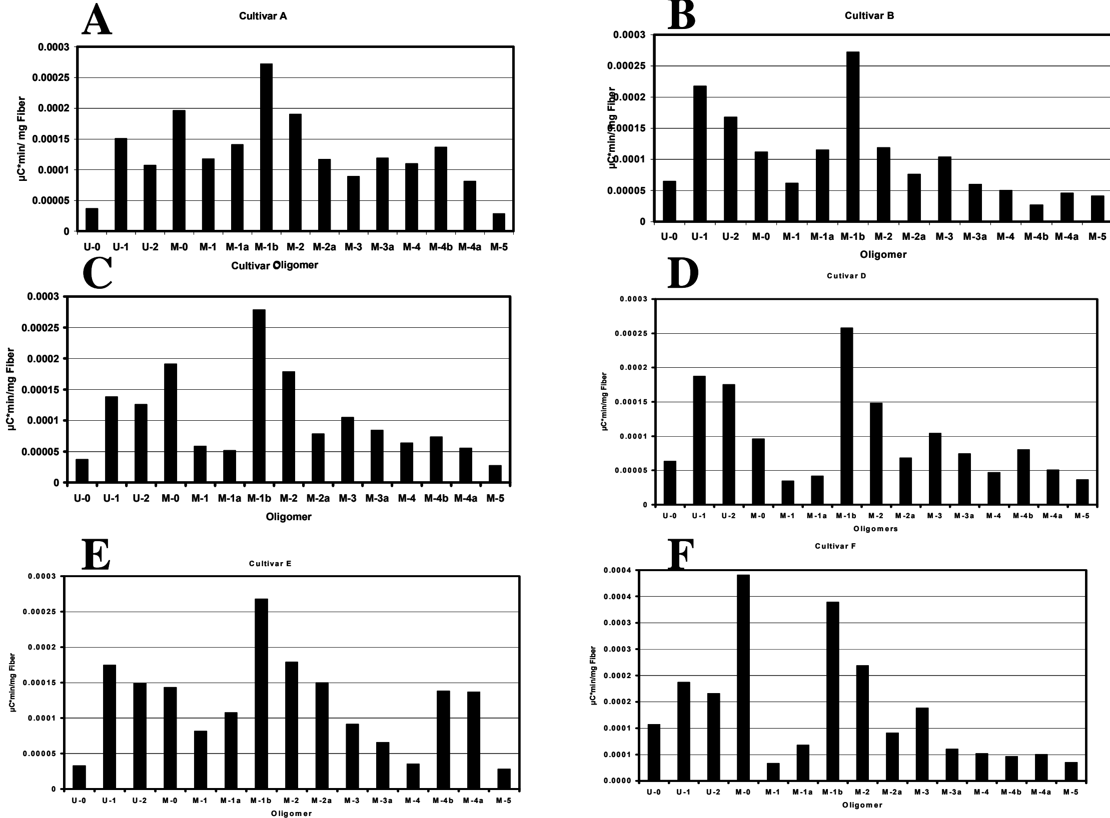

Figure 1. Oligomer Profiles of HCl Extracts of Six Cultivars (A-F). Each figure represents the average for each cultivar , A-F, from all locations. Oligomer contents are expressed on a µC*min/mg fiber.

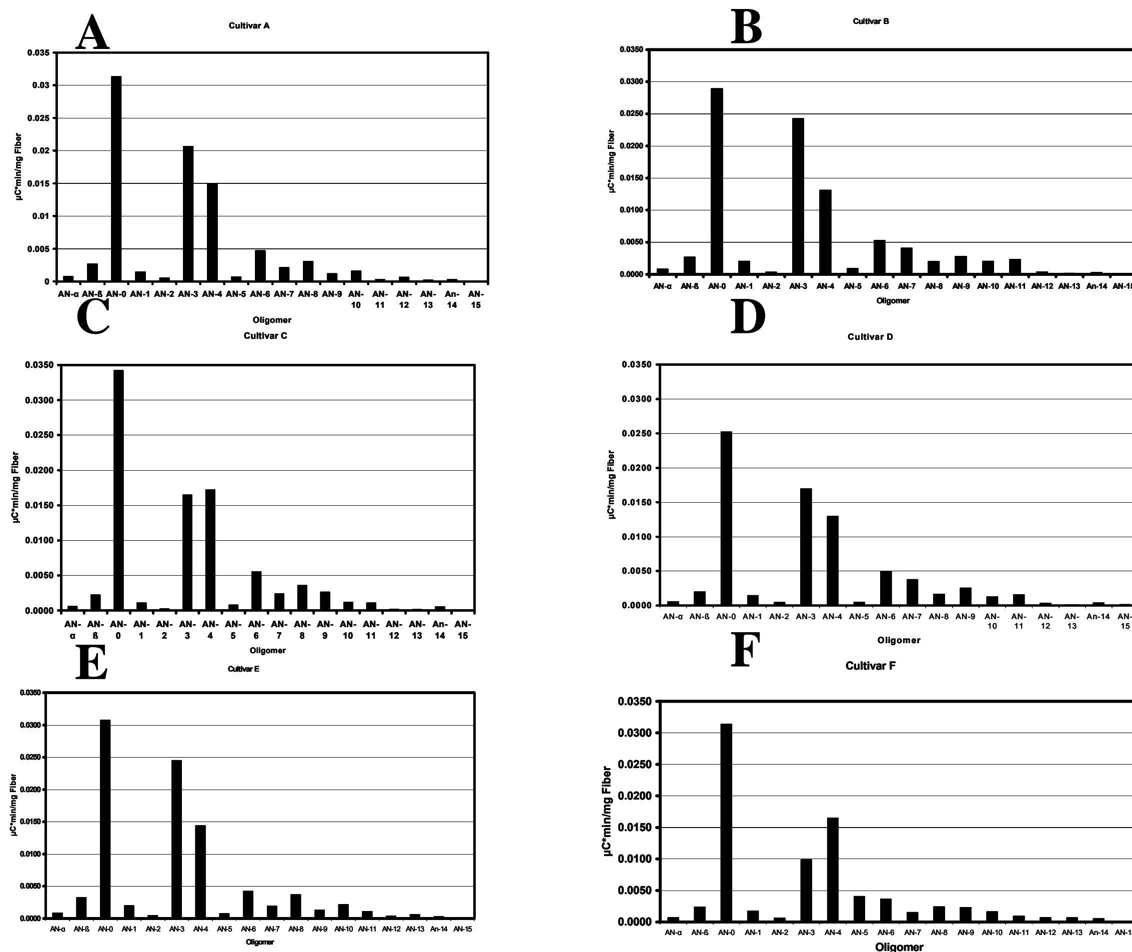

Figure 2. Oligomer Profiles of Acetic Nitric Extracts of Six Cultivars (A-F). Each figure represents the average for each cultivar , A-F, from all locations. Oligomer contents are expressed on a µC*min/mg fiber.

Discussion

Although one can observe differences in the glycan oligomer profiles on visual observation of the profiles for the six cultivars, the next step in the development of this technology involves mathematical models to evaluate the data. This will involve an iterative process assessing the uniqueness of each oligomer in both the HCl extract series and the acetic nitric extract series. It may be possible to make the identification on the basis of a few of the major oligomers rather than the more inclusive array displayed here. Although not shown here, there are some differences between the locations for each of the cultivars. It may be that some of the oligomers are more useful for cultivar identification and others will be more useful for environmental assessment. These are possibilities that will be investigated on further analysis of the data. With development of the model and increased size of the database, the goal is to develop the ability to identify cultivars with a specified degree of certainty. An additional goal would then be to develop the ability to identify the growth environment for a particular sample.

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