AMINO ACID AND CARBOHYDRATE PROFILES OF COTTON PLANT BIOMASS PRODUCTS Zhongai He **USDA-ARS, Southern Regional Research Center** New Orleans, LA Dan C. Olk USDA-ARS, National Laboratory for Agriculture and the Environment Ames, IA Hailin Zhang Dept. of Plant and Soil Sciences, Oklahoma State University Stillwater, OK Haile Tewolde **USDA-ARS, Crop Science Research Laboratory Mississippi State, MS** Mark Shankle Pontotoc Ridge-Flatwoods Branch Experiment Station, Mississippi State Univ. Pontotoc, MS

<u>Abstract</u>

Nutrition is essential in cotton plant growth and high quality cotton biomass products and byproducts can be used as animal feed and industrial raw materials. To establish the effects of on-farm nutrition on cotton plant biochemistry, we collected field-grown whole cotton plants and separated them into different biomass fractions -- main stems, leaf blades, branches, petioles, roots, and reproductive part (midseason) or bur, peduncles+bracts, and seed cotton (pre-defoliation). The contents of amino acids and selected carbohydrates in these biomass materials were determined. Both essential and nonessential amino acids were enriched in cotton leaf blades and reproductive parts. The distribution pattern of the selected carbohydrates differed from that of amino acids as higher contents of carbohydrate were found in root, main stem and branch parts. Nutritional carbohydrates and amino acids were further cumulated in the reproductive seed part at pre-defoliation. The information reported in this work would be helpful in exploring and optimizing management practices and processing strategies for utilizing these cotton crop biomass materials as valuable and renewable natural resources.

Introduction

Cotton is America's number one value-added crop. Much of the cotton land area in the US is located in the southern and southeastern region (He et al., 2013; Tewolde et al., 2015). The most valuable product of a cotton crop is the lint. Recently, studies have shown that biomass materials from other parts of the cotton plant are also useful as a soil amendment, animal feed, bioenergy sources, and industrial raw materials (He et al., 2016; 2014b; Holt et al., 2014; Ren et al., 2015; Wanjura et al., 2014). In this study, field-grown whole cotton plants collected at mid-season and just before defoliation were analyzed for amino acid and carbohydrate profiles in roots, main stems, branches, petioles, leaf blades, and reproductive parts (burs, peduncles, and seeds). The objectives are (1) to document the compositional characteristics of the individual biomass components and (2) to increase understanding of the accumulation mechanism with plant growth and development. Information on the comparison of the amino acid and carbohydrate profiles of the in-season and pre-harvest samples could be also useful in developing an effective diagnostic tool in monitoring plant biosynthesis for cotton plant physiology and management practices to grow a high-yielding and highquality crop. The relevant data would also be helpful to the cotton industry in making decisions to maximize profitability through better use of cotton biomass resources.

Materials and Methods

Tetraplicate cotton plants were taken from plots of a relatively long-term cropping management trials at the Mississippi Agricultural and Forest Experiment Station near Pontotoc, MS ($34^{\circ}14'$ N, $88^{\circ}99'$ W, 165 m elevation) in an Atwood silt loam soil (fine-silty, mixed, semiactive, thermic Typic Paleudalfs) with $\approx 5\%$ slope (He et al., 2017). Whole cotton plants were fertilized with standard inorganic fertilizers, and collected from border rows at two growth stages: in mid-season (August 2014) and at pre-defoliation (September 2014). The plants were placed in a plastic bag, transported to the lab and stored in a walk-in cooler (~ 42 °F). Next, the root from each plant was cut from the shoot portion at soil level by using hand clippers. The roots were washed in tap water and dried in a forced-air oven at 80

°C to constant weights in paper bags. The remaining shoots were also rinsed with tap water in large plastic tubs, loaded into new plastic bags, and returned to the cooler allowing the water to drain. These shoots were then separated into main stems, leaf blades, branches, petioles, reproductive part (midseason) or bur, peduncles+bracts, and seed cotton (pre-defoliation) (Fig. 1) and dried in the same way as the roots. Following drying, the reproductive parts sampled at near maturity (pre-defoliation) were further separated into (1) cottonseed, (2) burs, and (3) peduncle/bracts. The cottonseed was further separated into seed and lint (fiber) by ginning using a small tabletop gin. The chemical composition of reproductive parts did not include the data of lint. The reproductive parts taken at mid-season were composed of squares (flower buds), flowers, and immature fruits and were not separated into their component parts.

Ion chromatography coupled with amperometric detection was used to measure 17 proteinous amino acids in the cottonseed (He et al., 2014c; Olk et al., 2008). Each sample (20 mg) was mixed with 2 mL of 4 M methanesulfonic acid (MSA) amended with 2 g L⁻¹ tryptamine and autoclaved for 16 h at 136°C (112 kPa). The acid extracts were titrated to pH 4 to 5 with NaOH and centrifuged to remove precipitates. The aliquots were diluted properly with purified water. Concentrations of amino acids in these diluted solutions were analyzed by a Dionex DX-500 (Dionex Corp. Sunnyvale, CA) ion chromatograph equipped with an Amino-Pac PA 10 column (2 mm i.d.). Triple pulsed amperometrric detection was performed using a Dionex ED-40 electrochemical detector. Carbohydrates (fuctose, arabinose, rhamnose, galactose, glucose, xylose, and mannose) were measured in these samples using an improved method with the same instrument for amino acids (He et al., 2015; Olk, 2008).



(A) Plant parts collected mid-season

(B) Plant parts collected pre-defoliation

Fig. 1. Cotton plan biomass parts used in this study

Results and Discussion

Contents of seven carbohydrates are listed in Table 1. The relative abundance of these carbohydrates were similar to those of harvested cottonseed and other agricultural products and byproducts (He et al., 2014a; 2015). Data in this work showed that glucose, xylose were the major carbohydrates with little fucose present in these cotton biomass parts. Content of glucose in the plant biomass decreased with advanced growth stage. Xylose was rich in petioles, branches, main stems, and roots. Contents of xylose in the plant biomass increased with growth stage. Galactose and arabinose were moderate in content, and their contents changed from high in leaf blades to low in roots. Contents of galactose and manniose also increased consistently, but to a lesser extent, in all five parts with growth stage. Thus, we further analyzed the contents of carbohydrates in cotton reproductive parts in mid-season (MS) and pre-defoliation (PD) phases (Fig. 2). In this analysis, the lint (fiber) was separated from the cottonseed at PD but not analyzed because it is mainly cellulose-based (Liu et al., 2016). Data in Fig. 2 showed that content of glucose was higher in reproductive part at mid-season than in boll parts at pre-defoliation.

		Fucose		Rhamnose		Arabinose		Galactose		Glucose		X y l o s e		Mannose		GM/AX	
		MS	ΡD	MS	ΡD	MS	ΡD	MS	ΡD	MS	ΡD	MS	ΡD	MS	ΡD	M S	ΡD
L e a f blades Petioles	A	0.9	1.1	10.9	9.9	17.9	14.0	20.9	23.9	33.8	30.0	12.1	14.3	5.6	8.6	0.89	1.15
	SD A	<u>0.1</u> 1.5	<u>0.1</u> 1.8	<u>2.0</u> 11.8	<u>1.3</u> 13.5	<u>2.2</u> 23.9	<u>1.4</u> 23.7	<u>2.1</u> 17.4	<u>2.0</u> 23.7	<u>3.6</u> 43.0	<u>2.0</u> 22.3	<u>2.4</u> 40.8	<u>1.7</u> 56.5	<u>1.0</u> 7.7	<u>0.2</u> 8.1	0.06 0.40	<u>0.05</u> 0.40
	SD	0.2	<u>0.1</u>	<u>1.2</u>	<u>1.7</u>	<u>4.1</u>	<u>4.9</u>	2.7	<u>3.2</u>	<u>8.0</u>	<u>3.8</u>	<u>10.9</u>	<u>5.8</u>	<u>1.3</u>	<u>0.9</u>	0.05	<u>0.01</u>
Branches	А	0.7	1.0	7.4	8.6	15.5	13.2	12.3	13.2	66.1	23.9	61.9	82.2	5.8	6.1	0.23	0.20
	SD	0.1	0.2	0.6	0.6	1.3	1.8	0.6	<u>1.1</u>	7.3	8.5	3.2	<u>3.4</u>	0.6	0.2	0.00	0.01
Main stems	А	0.3	0.8	4.8	7.1	7.1	9.7	7.0	9.3	56.3	40.4	40.0	71.7	3.2	5.3	0.22	0.19
	SD	<u>0.1</u>	<u>0.2</u>	<u>1.0</u>	<u>1.4</u>	<u>1.0</u>	<u>1.1</u>	<u>0.9</u>	<u>0.9</u>	<u>8.9</u>	14.2	<u>7.6</u>	<u>19.2</u>	<u>1.3</u>	<u>0.5</u>	0.03	0.06
Roots	А	0.2	0.4	5.2	5.0	6.4	7.1	6.1	7.1	65.3	37.9	27.5	65.1	2.8	3.2	0.26	0.14
	SD	0.1	0.1	0.4	0.8	0.7	0.8	0.5	0.5	8.8	9.6	2.3	7.4	0.2	0.3	0.03	0.01

Table 1. Contents of seven carbohydrates in cotton plant parts collected at mid-season (MS) and pre-defoliation (PD) phases. GM/AX: (Galactose+Mannose)/(Arabinose+Xylose). Data are shown in g kg⁻¹ of dry matter with average (A) and standard deviation (SD, n=4).

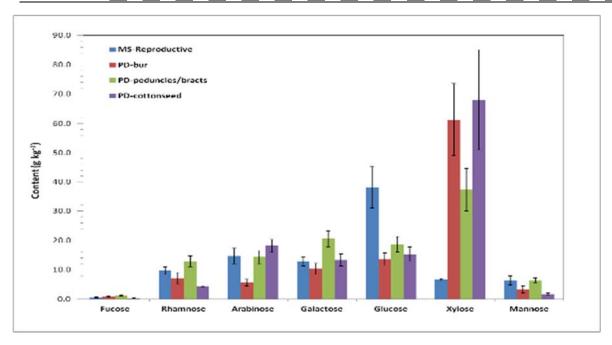


Fig. 2. Contents of carbohydrates in cotton reproductive parts at mid-season (MS) and pre-defoliation (PD) growth phases.

Contents of essential amino acids and non-essential amino acids are shown in Fig. 3 and 4, respectively. Those data showed that Leu, Lys, Phe, and Arg were the major essential amino acids, of which the highest content was >10 g kg⁻¹ each. Glu, Ala, and Asp were the major non-essential amino acids, of which the highest content was around 10 g kg⁻¹ or higher each. All biomass samples have shown similar orders in relative abundances of the 10 essential amino acids and of the 7 non-essential amino acids. The distribution patterns of amino acids among the different biomass samples were different from those of carbohydrates. Highest contents of both essential and non-essential amino acids were found in leaf blade and reproductive biomass samples. At mid-season, contents of amino acids in leaf blades were greater than those in reproductive part. All amino acids increased in the reproductive parts with advanced growth, leading to higher contents of amino acids in seed cotton than in leaf blade at the pre-defoliation stage.

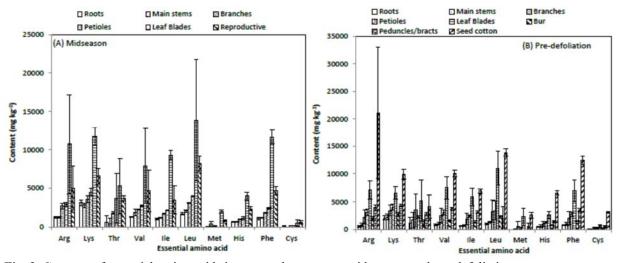


Fig. 3. Contents of essential amino acids in cotton plant parts at mid-season and pre-defoliation stages.

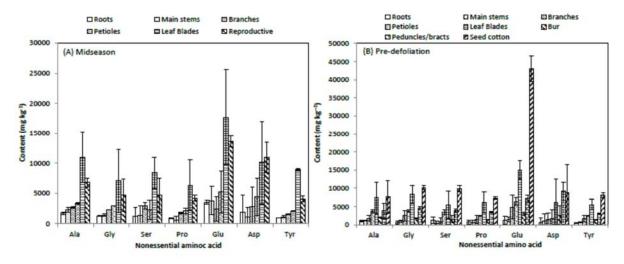


Fig. 4. Contents of non-essential amino acids in cotton plant parts at mid-season and pre-defoliation stages

Summary

The distribution pattern of the selected carbohydrates differed from that of amino acids, as higher contents of carbohydrate, especially the polymeric cellulose, hemicelluloses, and lignin (data not shown), were found in root, main stem and branch parts. Growth stage affected the relative contents of some, but not all, measured parameters. The general trend was that nutritional carbohydrates and amino acids had further accumulated in the reproductive seed part with advanced growth. The information reported in this work would be helpful in exploring and optimizing management practices and processing strategies in utilizing these cotton crop biomass materials as valuable and renewable natural resources.

Acknowledgements

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

References

He, Z., D.C. Olk, and H.M. Waldrip. 2014a. Soil amino compound and carbohydrate contents influenced by organic amendments, p. 69-82, *In Z.* He and H. Zhang, eds. Applied Manure and Nutrient Chemistry for Sustainable Agriculture and Environment. Springer, Amsterdam, the Netherland.

He, Z., H. Zhang, and D.C. Olk. 2015. Chemical composition of defatted cottonseed and soy meal products. PLoS One 10(6):e0129933. DOI:10.1371/journal.pone.0129933.

He, Z., H. Zhang, H. Tewolde, and M. Shankle. 2017. Chemical characterization of cotton plant parts for multiple uses. Agric. Environ. Lett. 2:110044. doi:10.2134/ael2016.11.0044.

He, Z., S.M. Uchimiya, and M. Guo. 2016. Production and characterization of biochar from agricultural by-products: Overview and use of cotton biomass residues, p. 63-86, *In* M. Guo, et al., eds. Agricultural and Environmental Applications of Biochar: Advances and Barriers. Soil Science Society of America, Inc., Madison, WI.

He, Z., H.N. Cheng, D.C. Chapital, and M.K. Dowd. 2014b. Sequential fractionation of cottonseed meal to improve its wood adhesive properties. J. Am. Oil Chem. Soc. 91:151-158.

He, Z., Z.N. Senwo, H. Zou, I.A. Tazisong, and D.A. Martens. 2014c. Amino compounds in poultry litter, litteramended pasture soils and grass shoots. Pedosphere 24:178-185.

He, Z., M. Shankle, H. Zhang, T.R. Way, H. Tewolde, and M. Uchimiya. 2013. Mineral composition of cottonseed is affected by fertilization management practices. Agron. J. 105:341-350.

Holt, G.A., P. Chow, J.D. Wanjura, M.G. Pelletier, and T.C. Wedegaertner. 2014. Evaluation of thermal treatments to improve physical and mechanical properties of bio-composites made from cotton byproducts and other agricultural fibers. Ind. Crop. Prod. 52:627-632.

Liu, Y., Z. He, M. Shankle, and H. Tewolde. 2016. Compositional features of cotton plant biomass fractions characterized by attenuated total reflection Fourier transform infrared spectroscopy. Ind. Crop. Prod. 79:283-286.

Olk, D.C. 2008. Improved analytical techniques for carbohydrates, amino compounds, and phenols: tools for understanding soil processes. Soil Sci. Soc. Am. J. 72:1672-1682.

Olk, D.C., A. Fortuna, and C.W. Honeycutt. 2008. Using anion chromatography-pulsed amperometry to measure amino compounds in dairy manure-amended soils. Soil Sci. Soc. Am. J. 72:1711-1720.

Ren, J., N. Li, L. Li, J.-K. An, L. Zhao, and N.-Q. Ren. 2015. Granulation and ferric oxides loading enable biochar derived from cotton stalk to remove phosphate from water. Bioresour. Technol. 178:119-125.

Tewolde, H., M.W. Shankle, T.R. Way, A. Adeli, J.P. Brooks, and Z. He. 2015. Enhancing management of fall-applied poultry litter with cover crop and subsurface band placement in no-till cotton. Agron. J. 107:449-458.

Wanjura, J.D., E.M. Barnes, M.S. Kelley, G.A. Holt, and M.G. Pelletier. 2014. Quantification and characterization of cotton crop biomass residue. Ind. Crop. Prod. 56:94-104.