DETERMINATION OF LIPID CONTENTS IN COTTON CHROMOSOMAL SUBSTITUTION LINES BY FOURIER TRANSFORM INFRARED SPECTROSCOPY

Diwas Kumar Silwal
Nsoki Phambu
Bharat Pokharel
Ahmad N. Aziz
Tennessee State University
Nashville, TN

Abstract

Current studies emphasize the importance of cottonseeds as source of cooking oil for human consumption which had been generally considered only as a by-product. This research analyzed lipid contents in hulls and kernels from seeds of TM-1 (Upland cotton, *Gossypium hirsutum*), 3-79 (Pima cotton, *G. barbadense*) and 17 *G. hirsutum* lines substituted with a whole chromosome or long (lo) / short (sh) chromosomal segments from *G. barbadense* (CS-B): CS-B01, CS-B02, CS-B04, CS-B05sh, CS-B06, CS-B07, CS-B11sh, CS-B12sh, CS-B14sh, CS-B15sh, CS-B16, CS-B17, CS-B18, CS-B22lo, CS-B22sh, CS-B25 and CS-B26lo. Thin pieces of cottonseed samples were placed on a Nicolet^{TM}is^{TM}10 Fourier Transform Infrared Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) to record an average of 16 scans through OMNIC (v8.0) software after correcting against background and smoothened in SPECTRUM (v3.02) for calculating area under curve. Two types of lipids, hydrated and dehydrated were detected in both the hulls and kernels of these cottonseeds under wavenumber range of >1730 to 1750 cm^{-1} and 1700 to 1730 cm^{-1}, respectively. Pima parent (3-79) showed higher lipid content than Upland parent (TM-1) in hull but lower in kernel. CS-B12sh, CS-B17, CS-B22sh and CS-B26lo revealed both the hydrated and dehydrated types of lipid in their hulls while only the CS-B16 kernel had the dehydrated type. All CS-B lines evaluated had potential as source for extracting cooking oil, thus understanding the mechanism of lipid variation will allow us to select suitable candidates in crop improvement programs for improving lipid contents in cotton.

Introduction

As a dual-use crop, cotton (*Gossypium* species) can provide both natural fiber for the textile industries as well as cottonseeds for human food and livestock feed (Wu et al. 2009). Cotton produces 150 kg of seed for every 100 kg of lint fiber, however, the seeds are regarded as by-products (O'Brien et al. 2005). Cottonseeds are directly used as animal feed or processed into oil, meal, hull and linters after ginning and contribute nearly 15% of the net farm value (Cherry and Leffler, 1984; O'Brien et al. 2005). In recent years, the use of cottonseed oil as a biofuel crop has also increased its popularity (Karaosmanoglu et al. 1999; Meneghetti et al. 2007).

European businessmen introduced extraction of edible oil from cottonseeds in America in nineteenth century, which was primarily used as the source of vegetable oil until mid-twentieth century when soybean oil replaced it (O'Brien, 2008). Cottonseed oil is widely used for cooking purposes as well as an ingredient in shortenings, margarines, marinades, pastries and dressings. On dry weight basis cottonseed oil contains twice the energy than starch and protein, so it is the most efficient form of energy storage (Coppock et al. 1987, Cox et al. 1995; O'Brien et al. 2005). This oil is also used in industries for the production of explosives, rubber and Vitamin-E (National Cottonseed Products Association, 2002). Cottonseed oil contains 26% palmitic acid, 15% oleic acid and 58% linoleic acid and its high level of palmitic acid gives it inherent stability for frying applications (Cox et al. 1995; Liu et al. 2002). Hence cottonseed oil does not require hydrogenation before its use in food industry, the process which artificially produces *trans* fatty acids in other oils. However, palmitic acid is associated with increased low-density lipoprotein cholesterol and total plasma cholesterol levels in consumers (Cox et al. 1995; Kris-Etherton et al. 1993).

Cottonseed oil production is declining in recent years due to the reduction in cotton acreages, but the flavor and stability still make it a desirable product for human food (O'Brien, 2008). A pound of cottonseed oil was sold at 46 to 50 cents in January of 2014 which was higher than soybean oil's price of 36 to 39 cents per pound (USDA-ERS, 2014). Since cotton varieties are only evaluated on the basis of their fiber yield and this selection process for hundreds of years has narrowed the genetic variation in cotton. Chromosome or chromosome arm substitution (CS) lines were developed in cotton by replacing *G. hirsutum* chromosome/arms from *G. barbadense* (Stelly et al. 2005). This technique was developed to avoid unwanted DNA accumulation that was the major issue in older gene introgression approaches used at whole genome level. Upland parent (TM-1) used in this approach was derived as

an inbred from Deltapine 14 and maintained for 40 generations through self-pollination while the Pima parent (3-79) was a doubled haploid from Pima (*G. barbadense*) germplasm. The 17 progeny lines used in this study are nearly isogenic to TM-1 parent for 25 pairs of chromosomes, except the chromosome/arms substituted from 3-79 parent (Saha et al. 2012; Stelly et al. 2005).

Infrared (IR) spectroscopy is a common and simple technique that can be used under wide variety of environments for analyzing organic compounds with high precision and throughput. It is based on the molecular vibrational stretching and bending of chemical bonds present in the organic compounds where each compound shows a fingerprint alignment and conformation at certain regions. The electromagnetic radiation absorbed by samples being analyzed is directly proportional to the concentration of target molecules and the path length of the measuring cell (Kong and Yu, 2007; Tamm and Tatulian, 1997). Here we present profiling of seed lipids in above mentioned CS-B lines through the use of Fourier Transform Infrared Spectroscopy (FTIR), and comparing these with each other and their two parents. Kohel (1978) reported a wide range of variation in upland cotton around the globe (15-33%) for seed oil content. Varieties with high seed oil content are selected for breeding programs on the basis of phenotypic information (Ash and Dohlman, 2006; Azhar and Ahmad, 2000; Pahlavni et al. 2008). Profiling of lipid distribution in CS-B lines should be useful for improving cottonseed oil during cultivar development. There are only erratic and insignificant efforts in improving cottonseed qualities through breeding and genetics until now (Yu et al. 2012). Understanding the correlation of cottonseed traits with fiber production would help to develop varieties simultaneously with better fiber and higher oil yield (Wu et al. 2009).

Materials and Methods

Mature cottonseed samples, separate for hulls and kernels, were sliced into thin pieces using a razor blade. Selected slices were placed on the sample holder of a Nicolet^{TM} is 10 spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), to be firmly clamped and analyzed through OMNIC (v8.0) software as per the method of Silwal et al (2015). Sixteen scans were collected for each sample, corrected against the background and then smoothened in SPECTRUM (v3.02, Sunnyvale, CA, USA) software three times to improve the quality. Peak resolving was done by Gaussian function after setting full width at half height (FWHH) of 15 and the noise target at 10 to calculate the area under curve in the region of 1700 to 1750 cm^{-1} for each spectra. Data was analyzed in Microsoft Excel and chart diagrams were created for comparing the lipid areas of all CS-B progeny lines among themselves as well as with their two parents.

Result and Discussion

The FTIR spectrometer used in this study proved to be a reliable, accurate and appropriate technique for analysis of lipid contents in chromosomal substitution lines of cotton. Both the hydrated and dehydrated lipids were detectable in hulls and kernels of these cottonseeds through FTIR. Lipids were detected in all the 19 assayed cottonseed hulls of which seven hull samples showed the evidence for dehydrated type and 16 samples had hydrated type while four hulls (CS-B12sh, CS-B17, CS-B22sh and CS-B26lo) had both the hydrated and dehydrated types of lipid (Table 1 and Figure 1). The Upland parent TM-1 had detectable level of dehydrated lipid while the Pima parent was detected with the hydrated lipid, while the latter having higher lipid area (3.18) than the former (2.73). Seven hulls showed the evidence of dehydrated lipids similar to Upland parent TM-1 (in red, Figure 1), where CS-B05sh had the highest area under curve (4.61) while CS-B22sh had the lowest (1.06). Among these lines, three hulls (CS-B05sh, CS-B07 and CS-B17) had lipid areas higher than TM-1 and three lines (CS-B12sh, CS-B22sh and CS-B26lo) had lower. Sixteen hull samples contained hydrated lipids (in blue, Figure 1) similar to Pima parent 3-79, of which CS-B12sh had the highest area (7.34) while CS-B04 had the lowest (1.20). Among these 16 lines, seven progenies (CS-B01, CS-B06, CS-B12sh, CS-B14sh, CS-B15sh, CS-B22lo and CS-B26lo) had higher lipid area than 3-79 while seven lines (CS-B02, CS-B04, CS-B11sh, CS-B16, CS-B17, CS-B18) had lower, and the last remaining CS-B22sh had the area under curve equal to that of 3-79.

Table 1. Lipid profiles in cottonseed hulls

Hydrated Lipid			Dehydrated Lipid		
Sample	**Wavenumber (cm^{-1})**	**Area under curve**	**Sample**	**Wavenumber (cm^{-1})**	**Area under curve**
3-79	1746.88	3.18	TM-1	1720.65	2.73
CS-B01	1738.01	5.45	CS-B05sh	1721.45	4.61
CS-B02	1739.32	2.10	CS-B07	1716.79	4.27
CS-B04	1744.22	1.20	CS-B12sh	1719.13	2.51
CS-B06	1742.84	5.46	CS-B17	1706.18	3.18
CS-B11sh	1740.93	2.22	CS-B22sh	1725.86	1.06
CS-B12sh	1744.32	7.34	CS-B26lo	1718.99	1.07
CS-B14sh	1742.89	5.44			
CS-B15sh	1742.43	5.78			
CS-B16	1744.92	2.65			
CS-B17	1742.23	2.99			
CS-B18	1746.44	1.35			
CS-B22lo	1739.41	4.06			
CS-B22sh	1746.02	3.18			
CS-B25	1741.58	3.14			
CS-B26lo	1744.45	4.28			

Hydrated lipids (1750 to >1730 cm-1) and Dehydrated lipids (1700 to 1730 cm^{-1}) as in cotton lines detected by FTIR

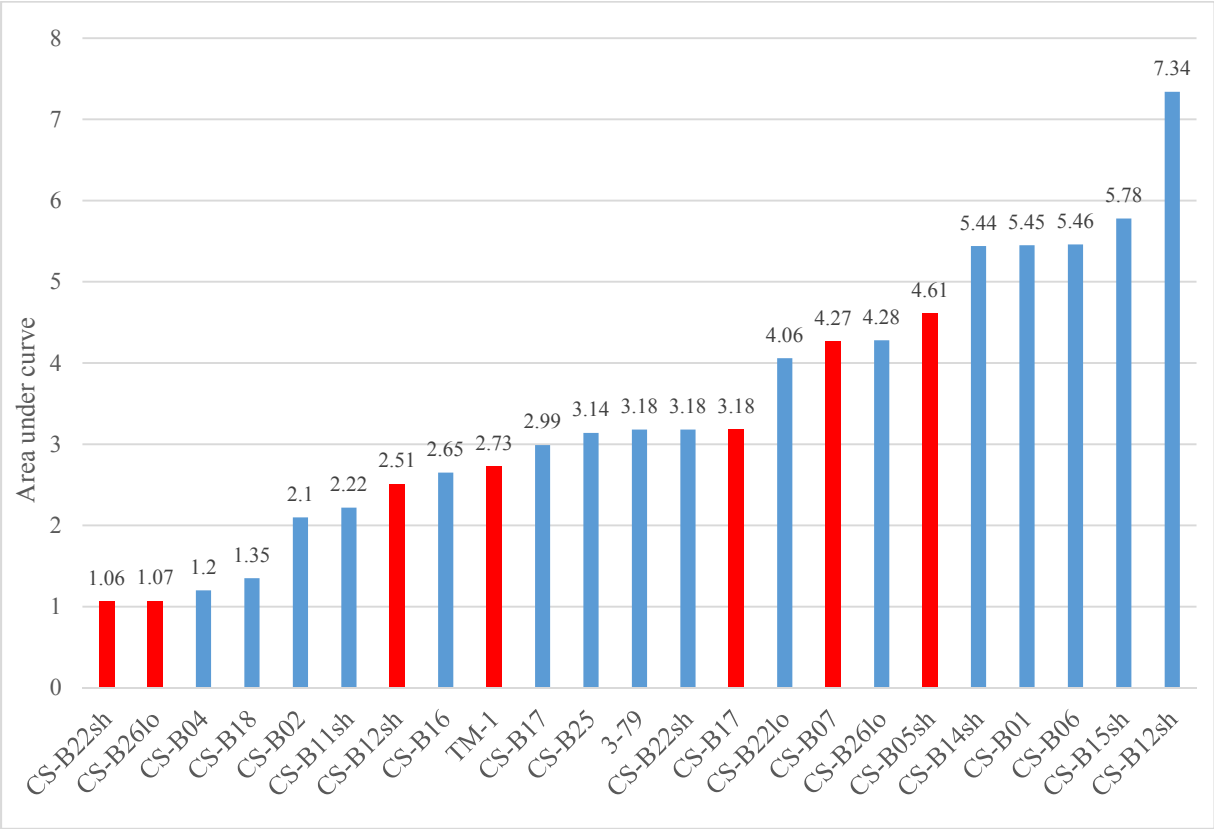

Figure 1. Hydrated lipids (blue) and Dehydrated lipids (red) in cotton lines as detected by FTIR

All 19 kernel samples assayed showed the presence of lipids, of which 18 lines showed the evidence of hydrated lipids while only CS-B16 had the evidence for dehydrated lipid with an area of 5.69 (Table 2 and Figure 2). Both the parent TM-1 and 3-79 contained hydrated lipids, while the former having higher lipid area (10.28) than the latter (8.07). Among the 18 lines with detectable levels of hydrated lipids, CS-B01 had the highest area of 12.52 and CS-

B02 had the lowest (6.89). Eight lines (CS-B01, CS-B05sh, CS-B11sh, CS-B12sh, CS-B14sh, CS-B22sh, CS-B25 and CS-B26 lo) had higher lipid areas than both their parents while three lines (CS-B02, CS-B15sh and CS-B16) had lower.

Table 2. Lipid profiles in cottonseed kernels

Sample	Wavenumber (cm^{-1})	Type of lipid	Area under curve
TM-1	1743.13	Hydrated	10.28
3-79	1744.16	Hydrated	8.07
CS-B01	1743.06	Hydrated	12.52
CS-B02	1743.92	Hydrated	6.89
CS-B04	1744.28	Hydrated	8.60
CS-B05sh	1742.45	Hydrated	10.41
CS-B06	1742.62	Hydrated	10.10
CS-B07	1743.27	Hydrated	9.86
CS-B11sh	1742.78	Hydrated	11.89
CS-B12sh	1742.89	Hydrated	10.87
CS-B14sh	1742.83	Hydrated	10.51
CS-B15sh	1739.34	Hydrated	7.82
CS-B16	1717.83	Dehydrated	5.69
CS-B17	1741.94	Hydrated	9.12
CS-B18	1742.18	Hydrated	9.61
CS-B22lo	1740.72	Hydrated	8.86
CS-B22sh	1742.71	Hydrated	10.45
CS-B25	1742.78	Hydrated	11.51
CS-B26lo	1743.23	Hydrated	10.31

Hydrated lipids (1750 to >1730 cm-1) and Dehydrated lipids (1700 to 1730 cm^{-1}) as in cotton lines detected by FTIR

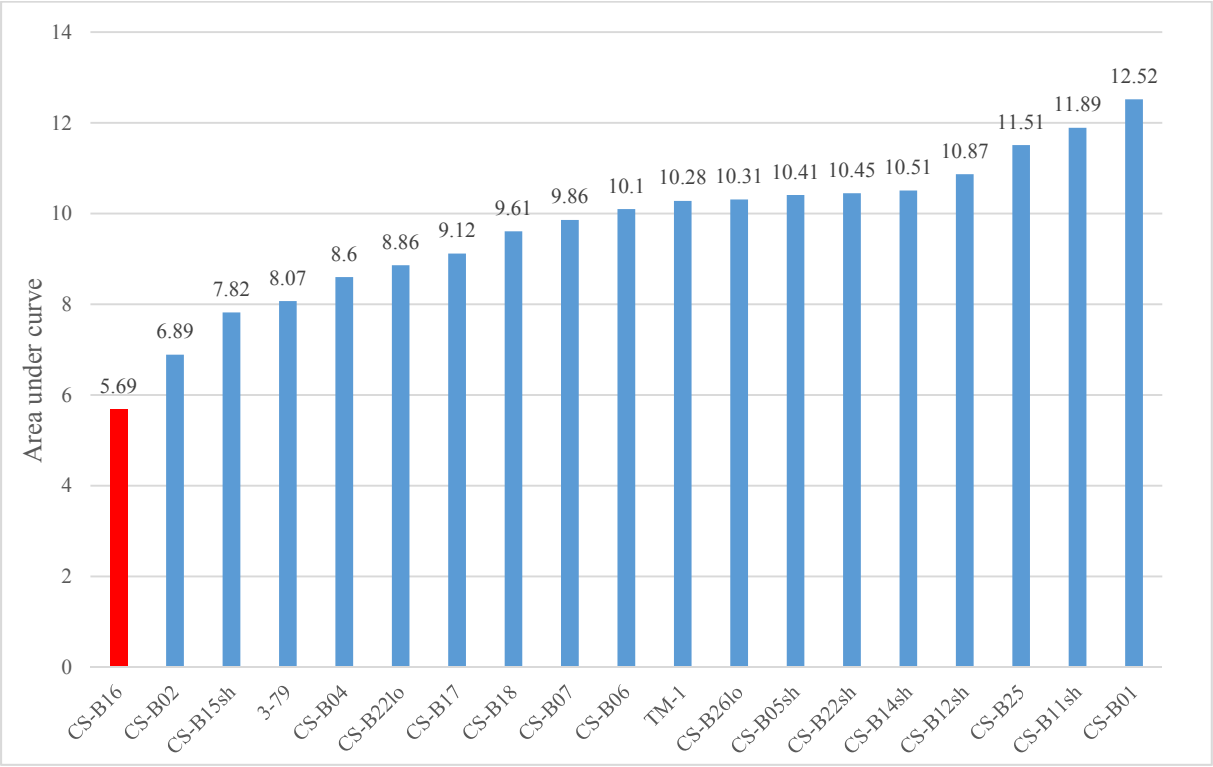

Figure 2. Hydrated lipids (blue) and dehydrated lipid (red) in cottonseed kernels

Conclusion

Findings from this study warrant future research works about hydrated and dehydrated lipids to establish their roles and importance in the aspects of seed physiology. Usually the kernels were found with higher lipid areas than their respective hulls, and kernels are primarily utilized to extract cooking oil for human consumption. Understanding the mechanism of lipid variation between these CS-B lines and the deviations from their two parents would help us to utilize suitable candidates in plant breeding research programs in improving the oil contents. Two lines CS-B01 and CS-B12sh were promising with higher lipid areas than both their parents in hulls as well as kernels, suggesting that these lines could be used for breeding trails for seed oil quality.

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