Survey of cotton (*Gossypium* sp.) for non-polar, extractable hydrocarbons for use as petrochemicals and liquid fuels

Robert P. Adams
Baylor-Utah Lab, Baylor University, 201 N 5500 W, Hurricane, UT 84737
robert_Adams@baylor.edu

James Frelichowski and Lori L. Hinze
USDA-ARS, PA, SPARC, Crop Germplasm Research, 2881 F&B Road,
College Station, TX 77845

and

Mauricio Ulloa
USDA-ARS, PA, CSRL, Plant Stress and Germplasm Development Research,
3810 4th Street, Lubbock, TX 79415

ABSTRACT

A survey of USDA cotton germplasm accessions, grown with supplemental underground drip irrigation to achieve best yields at College Station, TX, found % HC yields from 7.35 % to 3.14 %. Leaf dry weights (DW) varied about 2-fold from very large leaves: TX-1196 (1.59 g), TX-1757 (1.43), TX-1192 (1.26) to small leaves: SA-1427 (0.59 g), STD-10 (0.64), SA-1232 (0.67). Yields as g HC/ g DW leaf ranged from 0.080 g to 0.028 g. None of the accessions in this survey (2017) were in the 70th percentile of the highest thirty 2016 accessions (7.37 - 13.73 %). It appears that the high HC yielding quantities found in the 2016 survey were atypical and may be due to some unknown factor such as insect and/ or disease damage that caused an induction of defensive chemicals. The 2017 survey seems to be more typical of HC yields in cotton. Additional research is needed to determine the factor(s) that caused the unusually high HC yields in the 2016 test plots. Published on-line www.phytologia.org *Phytologia 100(1): 37-44 (Mar 16, 2018)*. ISSN 030319430.

KEY WORDS: Cotton, *Gossypium* sp., yields of hexane extractable leaf hydrocarbons, petrochemicals, liquid fuels.

The domestication of cotton has a complex history (see Wendel, J. F. and C. E. Grover, 2015). Recently, we (Adams et al. (2017a) reported on hydrocarbon (HC) yields of 30 cotton accessions representing photoperiodic and non-photoperiodic forms of two species grown with supplemental underground drip irrigation to achieve best yields at College Station, TX. They reported very high % HC yields in four accessions yielding 11.34, 12.32, 13.23 and 13.73% (Table 1). Per plant HC yields varied from 0.023 to 0.172 g/ g leaf DW. Hopi had a high % HC yield (10.03%), but it was the lowest per plant yield (0.023 g/ g leaf DW). In contrast, China 86-1 had the second highest % HC yield (13.23%) and also had the highest per plant yield (0.172 g). The correlation between % HC yield and avg. leaf DW was non-significant (0.092). They concluded that it appears that one might breed for both % HC yield and leaf DW in cotton.

Principal Coordinate Analysis (PCoA), utilizing 597 SSR bands, of the 30 accessions revealed the accessions are divided into *G. barbadense* and *G. hirsutum* (Fig. 1, left and right) (see Hinze et al., 2016 for further details on molecular marker analysis). The *G. barbadense* samples (8) are all improved accessions. The samples of *G. hirsutum* contain both wild and improved accessions forming a very loose group, but the wild accessions are mostly found in the upper-right quadrant of the ordination (Fig. 1).

Utilizing the g HC/ g leaf DW data, the high HC yielding accessions are clearly clustered in a tightly grouped set of improved accessions (Fig. 1, dashed oval). Plotting the high and highest yielding samples revealed that all three of the high yielding samples (SA-1181, SA-1403, SA-2269, top 13%) and the highest yielding individual (SA-1419, top 3%) are found in that group (Fig. 1, dashed oval). The discovery of the highest yielding individuals in a group of improved accessions is surprising, in view of the selection for increased cotton seed and fiber yields.

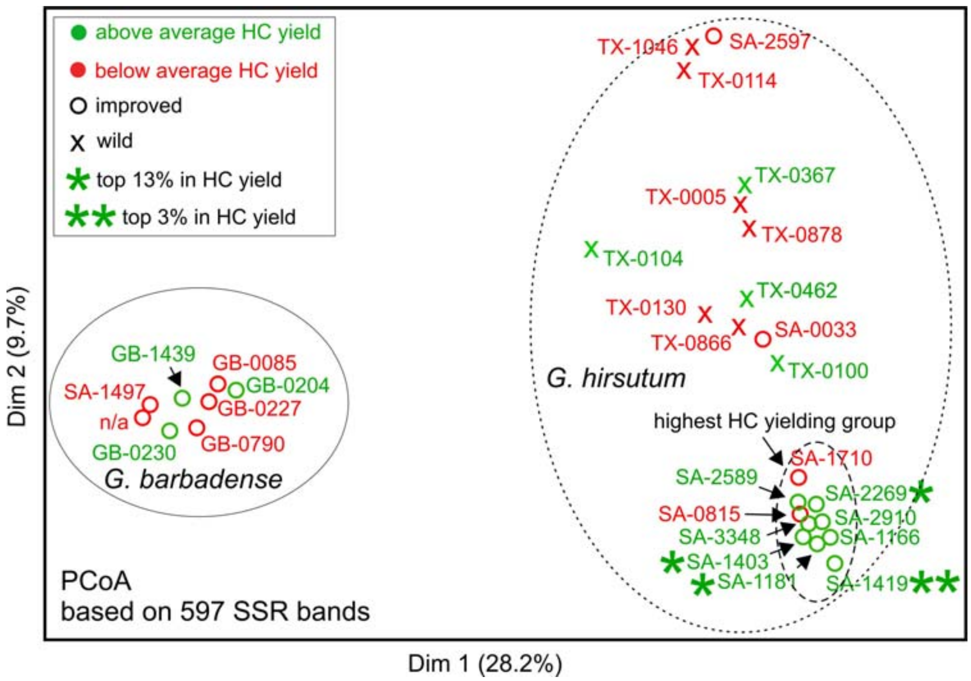

Figure 1. Principal Coordinate Analysis (PCoA) based on 597 SSR bands. The percent of variance accounted for among accessions is given on Dim 1 and Dim 2. See text for discussion.

It is also surprising that none of the wild accessions had high yields, although TX-0100 had a high % yield (10.72%), but having smaller leaves resulted in a moderate total g HC/ g leaf DW yield (Table 1). It is interesting that genetically (by SSR data), TX-0100 is ordinated nearest of any other wild accessions to the high HC yielding group (Fig. 1). It may be that back-crossing TX-0100 with SA-1419 might produce some useful progeny in the future.

Because the high HC yielding accessions were clustered in a small region of *G. hirsutum* (Fig. 1), it seemed promising to grow additional related accessions in the summer of 2017 to determine if other high yielding accessions might be discovered. This paper reports on the HC yields from 26 additional accessions grown in the same plot area as the previous 30 accessions grown in 2016.

MATERIALS AND METHODS

Plant Materials:

Cultivated at the USDA-ARS Southern Plains Agricultural Research Center, College Station, TX, 30 37' 5.00" N, 96 21' 50" W, 354 ft., subsurface drip irrigation, sandy soil, annual rainfall 40". Fifteen total leaves, sampled as 3-4 mature leaves (from the 4^{th} and 7^{th} nodes below the growing point) from each of 4-5 cotton plants, were bulked for an accession sample. The accessions were primarily non-photoperiodic landraces (one landrace was photoperiodic) and obsolete cultivars representing the most commonly grown commercial tetraploid cotton species, *G. hirsutum*. Cotton plants have an indeterminate growth habit and, therefore, vegetative and reproductive development occur at the same time. At the time of sampling, each plant had matured to the formation of green bolls while at the same time the plant had squares and flowers. These accessions were collected worldwide and are maintained by the USDA National Cotton Germplasm Collection in College Station, TX.

Leaves were ground in a coffee mill (1mm). Three grams of air dried material (7% moisture) was placed in a 125 ml, screw cap jar with 20 ml hexane, the jar sealed, then placed on an orbital shaker for 18 hr. The hexane soluble extract was decanted through a Whatman paper filter into a pre-weighed aluminum pan and the hexane evaporated on a hot plate (50°C) in a hood. The pan with hydrocarbon extract was weighed and tared.

The shaker-hexane extracted HC yields are not as efficient as soxhlet extraction, but much faster to accomplish. To correct the hexane yields to soxhlet yields, one sample was extracted in triplicate by soxhlet with hexane for 8 hrs. The soxhlet correction factor (sCF) was determined to be 1.14. All shaker extraction yields were corrected to oven dry weight (ODW) by multiplication of 1.085. Thus, the total CF was 1.24 (1.14 x 1.08).

RESULTS

The survey of USDA germplasm cotton accessions grown with supplemental irrigation at College Station, TX, found (Table 2) that % HC yields ranged from 7.35 % (STD-08) to 3.14 % (SA-2356).

Leaf dry weights (DW) varied about 2-fold (Table 2) from very large leaves: TX-1196 (1.59 g), TX-1757 (1.43), TX-1192 (1.26) to small leaves: SA-1427 (0.59 g), STD-10 (0.64), SA-1232 (0.67).

Yields as g HC/ g DW leaf ranged from 0.080 g (TX-1192), 0.079 (STD-08), 0.076 (TX-1196) to about 1/3 as much: SA-1427 (0.029 g) and SA-2356 (0.028 g).

None of the accessions in this survey (2017) were in the 70th percentile of the thirty 2016 accessions (7.37 - 13.73 % HC, Table 1). However, 2016 appears to be an unusual year for HC production at College Station. Four accessions were grown at College Station, TX in both 2016 and 2017 (Table 3). Notice (Table 3) that in 2016, leaf DW was larger for SA-1403 and SA-1419, but smaller for SA-1181 and SA-2269. The % HC yields were all higher in 2016 and often, much higher, cf. S-1181, 12.31 % (2016) vs. 6.41 % (2017). Due to the much higher % HC yields and small differences in leaf weights, the mg HC/ leaf wt was also much higher in 2016. We have yet to find a factor to explain these differences between the years. It seems likely that 2016 was a time of stress induction of HC by damage from insects, disease or some other vector more recently, greater focus has been on inducible plant defenses (Chen 2008; Opitz, Kunert and Gershenzon, 2008; Pare and Tumlinson, 1997, 1998; Turlings, et al. 1995).

It seems relevant to examine the work of Stipanovic, Bell and Benedict (1999) who reviewed the defensive role of pigment gland constituents in cotton. They found that cotton gland constituents (sesquiterpenoids, gossypol, and gossypol derivates, etc.) are a constitutive defense resource for cotton resistance to insects and diseases. Stipanovic, Bell and Benedict (1999) also discussed that these gland constituents can be rapidly synthesized in response to pathogens.

Chen (2008) also found that some constitutive chemicals may be increased to even higher levels after insect attack. Opitz, Kunert and Gershenzon (2008) analyzed the response of stored (constitutive) terpenoids in cotton subjected to mechanical damage, herbivory and jasmonic acid treatments. They showed that terpenoid levels increased successively from control to mechanical damage, herbivory, and jasmonic acid treatments. In addition, they reported that herbivory or mechanical damage in older leaves led to terpenoid increases in younger leaves. It might be noted that normally plants lose their lower (older) leaves and these leaves are usually damaged by insects and diseases. Opitz et al. (2008) found the composition of the terpenoid mixture did not significantly differ in response to herbivore, mechanical damage or jasmonic acid treatments.

Table 3. Comparison g DW/ leaf, % HC yield, and mg HC/ g DW leaf for SA-1181, SA-1403, SA-1419 and SA-2269 grown in a common test plot at USDA, College Station in successive years (2016, 2017).

	g DW/ leaf		% HC yield		g HC/ g DW leaf	
accession	2016	2017	2016	2017	2016	2017
SA-1181	0.96	1.21	12.32	6.41	0.119	0.078
SA-1403	1.46	1.11	9.08	7.45	0.133	0.080
SA-1419	1.30	0.99	13.23	6.41	0.172	0.063
SA-2269	1.24	1.36	11.09	5.94	0.138	0.082

Turlings et al. (1995) showed that plants injured by herbivores emit chemical signals that attract and guide the herbivores' natural enemies to the damaged plants. Thus, indirectly, injured plants send out a "SOS" signal for help against herbivores. Pare and Tumlinson (1997) also confirmed this phenomenon in a series of experiments on cotton using beet army worms and mechanical damage to leaves.

To examine the environmental component of % HC yields, Adams, et al. (2017b) compared % HC yields of SA-1181, SA-1403, SA-1419 and SA-2269 grown in a greenhouse to the yields from 2016 samples from College Station, TX. Adams et al. (2017b) concluded that the lower % yields (Fig. 2) in protected conditions (i.e., greenhouse) seems to imply the genotype is particularly affected by insects, diseases, water stress, etc. that apparently induced increased HC production in the field at College Station in 2016. We have added the % HC yields from the 2017 field plot samples grown at College Station (Fig. 2, crosshatched bars). For SA-1181, SA-1419, and SA-2269, the 2017 field yields are more like the greenhouse yields than the 2016 field yields (Fig. 2). However, SA-1403 has the field yields more similar (9.08, 7.45) than the greenhouse yield (Fig. 2). These data suggest that 2016 was an atypical year for the production of HC in the College Station plot.

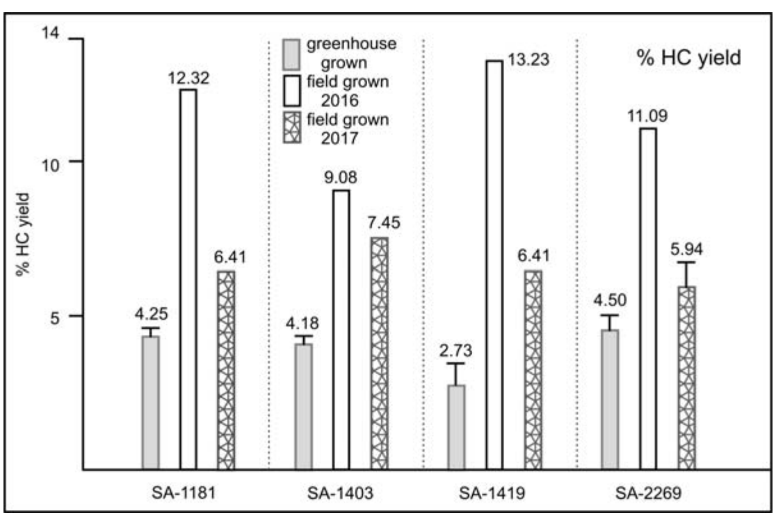

Fig. 2. Comparison of % yield of HC from greenhouse vs. field grown plants (2016 and 2017, College Station, TX). Modified from Adams et al. (2017b).

We considered that plant maturity might have been different between the samplings in 2016 and 2017, so a second harvest was conducted in 2017 for accession SA-2269. There was a very highly significant lowering of g DW leaf weight in the second sampling (10/2/2017, Table 4). The % HC yields were non-significant between sampling dates (Table 4). The mg HC/ g DW leaf was very highly significantly lower in the later (10/2/2017) harvest (Table 4, 0.082 mg, 0.047 mg) and this is near the magnitude seen in the 2016-2017 data. However, neither of these sampling dates (Table 4) explains the very large HC yields in the 2016 samples.

Table 4. Effects of plant maturity on the biomass (g DW/ leaf), % HC yield, and mg HC/ g DW leaf for SA-2269 harvested on 6/28/2107 (no bolls opened) vs. 10/2/2017 (most bolls opened). *** = very highly significant, ns = non significant.

accession date harvested	maturity stage	g DW/ leaf	% HC yield	g HC/ g DW leaf
SA-2269 6/28/2017	no bolls opened	1.36	5.97	0.082
SA-2269 10/02/2017	most bolls opened	0.89	5.39	0.047
	t-value	6.45	0.90	3.89
	probability	0.0001	0.387	0.0025
	significance	***	ns	***

It is instructive to graph leaf biomass, % HC yields, and g HC/ g leaf DW for SA-2269 onto a graph (Fig. 3) of these variables for commercially grown Fibermax 1320 (Adams et al. 2017b). Leaf biomass and g HC/ g leaf DW both decline from bolls maturing to bolls opened stages (Fig. 3) as also found in Fibermax 1320. However, the % HC yield for SA-2269 was not significantly different during that period, whereas, Fibermax 1320 had a barely significant increase (Fig. 3).

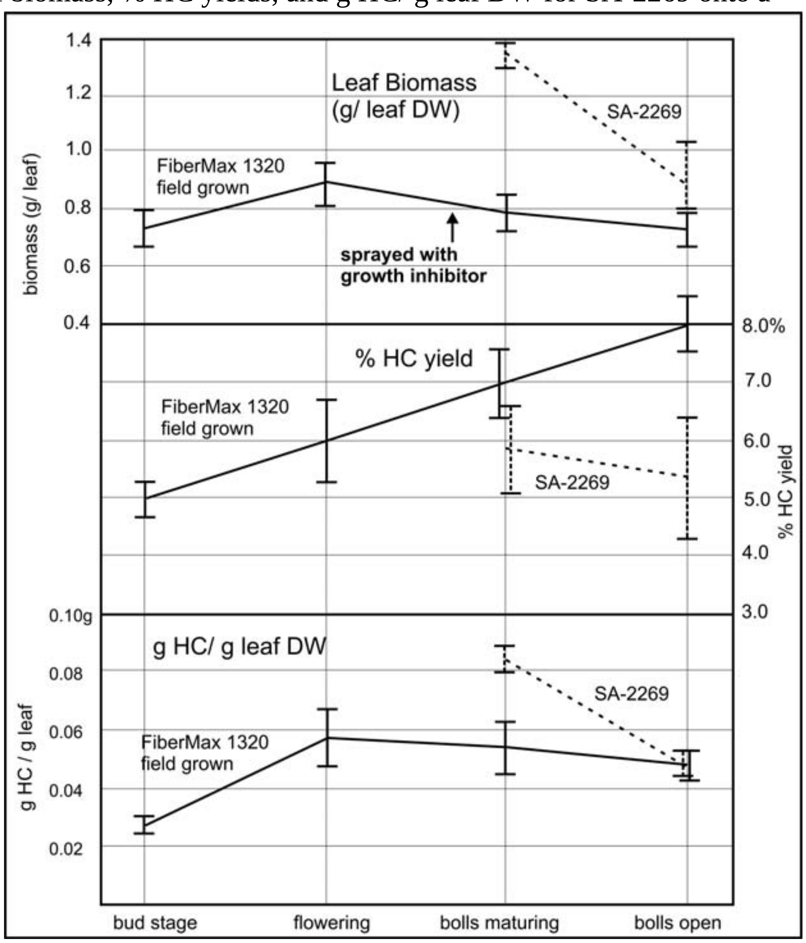

Figure 3. Ontogenetic variation in leaf biomass, % HC yields, and g HC/ g leaf DW in commercially grown Fibermax 1320 (from Adams et al. (2017b). These variables are also graphed for SA-2269 grown at USDA, College Station, TX (dashed lines). The bar lines are 2 standard errors of the mean.

There was considerable variation among the SA-2269 plants (Table 5) . Among the plants sampled with no bolls opened (the normal time to sample) the % HC yields ranged from 4.840 % to 7.44 %. This is similar to range of variation found in samples with most bolls opened (3.72 % to 7.65%, Table 5).

Table 5. Variation among plants for leaf weight, % yield HC, and g HC/ g leaf weight.

accession	source name	g DW 1 leaf	% yld	g/ g 1 leaf	# plants	coll date
SA-2269	SA-2269/TM1 (1-1)	1.44	6.53	0.094	5	06/28/2017
SA-2269	SA-2269/TM1 (1-2)	1.47	6.32	0.093	5	06/28/2017
SA-2269	SA-2269/TM1 (1-3)	1.47	7.44	0.109	5	06/28/2017
SA-2269	SA-2269/TM1 (1-4)	1.21	4.62	0.056	5	06/28/2017
SA-2269	SA-2269/TM1 (2-1)	1.23	4.84	0.060	5	06/28/2017
SA-2269	SA-2269/TM1 (2-2)	1.26	5.62	0.071	5	06/28/2017
SA-2269	SA-2269/TM1 (3-1)	1.45	6.41	0.093	5	07/06/2017
SA-2269	no bolls opened, Avgs =	1.36	5.97	0.082		
SA-2269	SA-2269/TM1 (1-1)	0.69	5.99	0.041	5	10/2/2017
SA-2269	SA-2269/TM1 (1-2)	0.80	4.71	0.038	5	10/2/2017
SA-2269	SA-2269/TM1 (1-3)	1.08	3.72	0.040	5	10/2/2017
SA-2269	SA-2269/TM1 (1-4)	0.94	5.24	0.049	5	10/2/2017
SA-2269	SA-2269/TM1 (2-1)	0.85	7.65	0.065	5	10/2/2017
SA-2269	SA-2269/TM1 (2-2)	1.00	5.00	0.050	5	10/2/2017
SA-2269	most bolls opened, Avgs =	0.89	5.39	0.047		

CONCLUSION

This survey found a few moderately high HC yielding cotton accessions, but not as promising as found in the 2016 survey. However, it should be noted that growth of four of our best accessions in successive years (2016, 2017) failed to generate consistent yields of HC. The atypically high HC yields in the College Station plot of 2016 are under further investigation.

LITERATURE CITED

Adams, R. P., A. K. TeBeest, J. Frelichowski, L. L. Hinze, R. G. Percy, M. Ulloa and J. Burke. 2017a. Survey of Cotton (*Gossypium* sp.) for non-polar, extractable hydrocarbons for use as petrochemical feedstocks. Phytologia 99: 54-61.

Adams, R. P., A. K. TeBeest, M. Ulloa, T., J. Burke and J. Frelichowski and L. L. Hinze. 2017b. Comparison of hydrocarbon yields in cotton from field grown vs. greenhouse grown plants. Phytologia 99: 200-207.

Hinze, L.L., E. Gazave, M.A. Gore, D.D. Fang, B.E. Scheffler, J.Z. Yu, D.C. Jones, J. Frelichowski and R.G. Percy. 2016. Genetic diversity of the two commercial tetraploid cotton species in the *Gossypium* Diversity Reference Set. Journal of Heredity 107: 274-286.

Opitz, S., G. Kunert and J. Gershenzon. 2008. Increased terpenoid accumulation in cotton (Gossypium hirsutum) foliage is a general wound response. J. Chem. Ecol. 34: 508-522.

Pare, P. W. and J. H. Tumlinson. 1997. *De novo* biosynthesis of volatiles induced by insect herbivory in cotton plants. Plant Physiol. 114: 1161-1167.

Pare, P. W. and J. H. Tumlinson. 1998. Cotton volatiles synthesized and released distal to the site of insect damage. Phytochemistry 47: 521-526.

Stipanovic, R. D., A. A. Bell and C. R. Benedict. 1999. Cotton pest resistance: The role of pigment gland constituents. pp. 211-220. in: Biologically active natural products: Agrochemicals. H. G. Cutler and S. J. Cutler, eds., CRC press, Boca Raton, FL.

Turlings, T. C. J., J. H. Loughrin, P. J. McCall, U. S. R. Rose, W. J. Lewis and J. H. Tumulinson. 1995. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. Proc. Natl. Acad. Sci. 92: 4169-4174.

Wendel, J. F. and C. E. Grover. 2015. Taxonomy and evolution of the cotton genus, *Gossypium*. In: Cotton, 2nd ed., D. D. Fang and R. G. Percy, eds., Agronomy Monograph 57.

Table 1. Screening results of 30 cotton accessions, grown in 2016 in a test plot at USDA, Crop Germplasm Research, College Station, TX. From Adams et al. (2017).

Lab acc Source	USDA identifier	g avg leaf DW (# plants)	% yield HC	g HC yield/ g leaf DW
14994, U12, Christidis 53D7	SA-1166	0.706 (4)	13.73 ++Hi	0.097
14997, U15, China 86-1	SA-1419	1.300 (4)	13.23 ++	0.172 ++Hi
14995, U13, Acala SJ-1	SA-1181	0.962 (4)	12.32 ++	0.119 +
15002,U20,Vir-7080Col.Macias17	SA-3348	0.896 (4)	11.34 ++	0.102
14998, U16, TM 1	SA-2269	1.244 (4)	11.09 ++	0.138 +
15001, U19,TAM 91C-34	SA-2910	1.006 (4)	10.85 +	0.109
15004, U22,Latifolium, wild	TX-0100	0.894 (5)	10.72 +	0.096
14985, U3, Nevis 81	GB-0227	0.728 (4)	10.36 +	0.041
14999, U17, KL 85/335	SA-2589	0.812 (4)	10.25 +	0.083
14992, U10, Hopi	SA-0033	0.266 (4)	10.03 +	0.023 Low
15009, U27,Richmondi, wild	TX-0462	0.973 (5)	9.93	0.097
14988, U6, Tadla 2	GB-1439	1.106 (4)	9.70	0.107
15005, U23,Latifolium, wild	TX-0104	0.967 (5)	9.25	0.089
14996, U14, 3010	SA-1403	1.463 (4)	9.08	0.133 +
15000, U18, KLM-2026	SA-2597	0.802 (4)	9.02	0.072
15007, U25,Morrili, wild	TX-0130	0.830 (5)	8.67	0.072
15010, U28,Marie-galante, wild	TX-0866	0.511 (5)	8.05	0.041
14990, U8, Pima S-5	SA-1497	0.995 (4)	7.92	0.079
14993, U11, Mexican #68	SA-0815	0.994 (4)	7.92	0.079
15003, U21,Palmeri, wild	TX-0005	0.398 (5)	7.92	0.032
14984, U2, Mono 57	GB-0204	1.360 (4)	7.37	0.100
14986, U4, Ashmouni Giza 32	GB-0230	1.128 (4)	7.37	0.083
15008, U26,Marie-galante, wild	TX-0367	1.289 (5)	7.37	0.095
14989, U7, 3-79	na	0.720 (4)	7.06	0.051
14987, U5, Ashabad 1615	GB-0790	0.866 (4)	7.01	0.061
14991, U9, TAM 87N-5	SA-1710	0.764 (4)	6.64	0.051
15006, U24,Punctatum, wild	TX-0114	0.815 (5)	6.33	0.052
14983, U1, Tanguisw LMW 12-40	GB-0085	1.335 (4)	5.97	0.080
15011, U29,Marie-galante, wild	TX-0878	0.692 (5)	4.50	0.031
15012, U30,Yucantanense, wild	TX-1046	0.728 (5)	3.29 Low	0.024 Low

Table 2. Screening results of 26 cotton accessions, grown in 2017 in a test plot at USDA, Crop Germplasm Research, College Station, TX

accession	source name	g DW 1 leaf	% HC yield	g/ g 1 leaf	# plants	collection date
STD-08	AllTexAtlas	1.07	7.35 HI	0.079 Hi	4	06/28/2017
SA-2260	PD 93007	.94	7.28 HI	0.068	5	07/06/2017
TX-1205		.75	6.66	0.050	5	06/28/2017
SA-0965	PLAINS	1.10	6.66	0.074	5	07/06/2017
STD-10	CAMDE	.64 Low	6.66	0.042	5	06/28/2017
SA-1212	MEXICO 910	1.06	6.49	0.069	5	07/06/2017
SA-0369	D AND PL 10-1	1.07	6.41	0.069	5	07/06/2017
TX-1192		1.26 Hi	6.32	0.080 Hi	5	06/28/2017
SA-0300	ROWDEN #2	.93	6.29	0.059	5	07/06/2017
STD-06	Acala1517-99	.83	5.87	0.049	5	06/28/2017
SA-2330	MARICO (SMOOTH)	1.15	5.83	0.067	5	07/06/2017
SA-3777	PAYMASTER 892	1.04	5.74	0.060	5	07/06/2017
SA-1349	IAC 18	1.11	5.74	0.063	5	07/06/2017
SA-1232	AC 134 CB 4029	.67 Low	5.36	0.036	4	07/06/2017
STD-02	FM832	.88	5.33	0.047	5	06/28/2017
SA-0825	MEXICAN #102	.82	5.24	0.043	5	07/06/2017
STD-01	AcalaMaxxa	1.08	5.24	0.057	5	06/28/2017
SA-1427	LONG FIBER #2	.59 Low	4.90	0.029	5	07/06/2017
TX-1196		1.59 Hi	4.84	0.076	5	06/28/2017
SA-1184	COKER 310	1.20	4.84	0.058	5	07/06/2017
SA-2910	TAM 91C-34	.80	4.34	0.035	5	07/06/2017
SA-1230	73 CB 3992	.76	4.25	0.033	5	07/12/2017
SA-1465	DES 422	1.00	4.13	0.041	5	07/06/2017
STD-07	SG747	.92	3.59	0.033	5	06/28/2017
TX-1757		1.43 Hi	3.50	0.050	4	07/12/2017
SA-2356	FUNTUA FT-5	.90	3.14 Low	0.028 Low	5	07/06/2017