Registration of RMBUP-C4, a Random-Mated Population with *Gossypium barbadense* L. Alleles Introgressed into Upland Cotton Germplasm

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ABSTRACT

RMBUP-C4 (random-mated barbadense Upland population cycle 4) (Reg. No. GP-961, PI 665950) is a unique random-mated germplasm population of Upland cotton (*Gossypium hirsutum* L.) with introgression of *G. barbadense* L. alleles. This population involved five cycles of random mating beginning with 53 top-crossed F_1 lines. The germplasm was developed through cooperative research by the USDA-ARS, the Mississippi Agricultural and Forestry Experiment Station, and Cotton Incorporated. The cultivars 'Sure-Grow 747' (PVP 9800118), 'PSC 355', and 'FM 966' (PVP 200100209) were each crossed with 18 lines with chromosome substitution from *G. barabadense* (CS-B); however, the seed of PSC 355 × CS-B22sh was lost, resulting in 53 topcross populations. The bulked-pollen method of pollination was used in the development, and there were five cycles of random mating with the intercrossing of F_1 lines considered as cycle zero. After each cycle of random mating, F_1 lines were combined on the basis of the original CS-B parents, thus producing 18 individual populations. These were planted as 18 individual populations and randomly mated among populations each additional cycle. Because the CS-B lines were each euploid chromosome substitution lines with entire chromosomes or arms from *G. barbadense* substituted into Upland, we should have achieved considerable introgression of alleles from *G. barbadense* into this randomly mated population. This population should be of value to cotton breeders and geneticists across the U.S. Cotton Belt as a unique population with *G. barbadense* introgression.

The two cultivated tetraploid species of cotton are *Gossypium hirsutum* L. (Upland cotton) and *G. barbadense* L. Upland cotton comprises the majority of cotton grown worldwide; however, *G. barbadense* has longer, finer, and stronger fibers (Smith et al., 1999). Breeders have had limited success in introgressing *G. barbadense* alleles into Upland, and no Upland cultivar has the fiber quality of modern *G. barbadense* cultivars. Breeders generally have encountered difficulties in introgression due to infertility, cytological abnormalities, and distorted segregation in the F_2 and later generations following interspecific crosses, for example, similar to those reported by Beasley and Brown (1942). From a cross of 'Guazuncho 2' (*G. hirsutum*) and 'VH8-4602' (*G. barbadense*) followed by two backcrosses to Guazuncho 2, researchers reported that fiber length, strength, fineness, and color were influenced by 15, 12, 21, and 16 quantitative trait loci, respectively; that the majority of favorable alleles for fiber length, strength, and fineness came from G. *barbadense*; and that the *G. hirsutum* parent contributed positive alleles for fiber color (Lacape et al., 2005). The *G. barbadense* alleles for these traits mapped to 15 different chromosomes. This illustrates additional complications involved in introgressing alleles for fiber quality from *G. barbadense* into Upland cotton.

An alternative approach to interspecific crossing is the development of chromosome substitution lines with whole chromosomes or arms individually substituted from *G. barbadense* into Upland. Stelly et al. (2005) developed and released 17 lines with chromosome substitutions from *G. barabadense* (CS-B), each having a different chromosome or chromosome arm from *G. barbadense* line 3-79 substituted

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Abbreviations: CS-B, chromosome substitution from *G. barabadense*; HVI, high-volume instrument; UHM, upper-half mean.

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into *G. hirsutum* line TM-1. These CS-B lines are euploid and do not exhibit hybrid sterility or other major detrimental problems in crosses with Upland cultivars. However, parental line CS-B18 exhibits the open-bud duplicate gene phenotype (ob_1 ob_1 ob_2 ob_2). This phenotype was reported by Kohel (1973), and the ob_1 allele was mapped by monosomic analysis to chromosome 18 by Endrezzi (1975). The open-bud phenotype has a flower bud open at the tip, due to shortening of the corolla, with exposure of the stigma and upper anthers. This phenotype was fine mapped to homoelogous chromosomes 13 and 18, with *G. hirsutum* having the genotype (Ob_1 Ob_1 ob_2 ob_2) and *G. barbadense* having the genotype (ob_1 ob_1 Ob_2 Ob_2), with ob_2 and ob_1 being on chromosomes 13 and 18, respectively (Qian et al., 2009).

When these CS-B lines are used as parents with Upland lines, it should be possible to introgress alleles from a particular chromosome or arm into Upland germplasm. General and specific combining ability for agronomic and fiber traits were determined from crosses of 13 of these CS-B lines with five cultivars (Jenkins et al., 2006, 2007) and from nine additional CS-B lines crossed with five cultivars (Jenkins et al., 2012). In these studies, 15 different euploid CS-B lines showed favorable additive effects that were greater than the corresponding chromosome from TM-1 as follows: lint yield (2), boll weight (3), lint percentage (5), fiber strength (7), fiber length (7), micronaire (3), fiber uniformity (3), and fiber elongation (5) (Table 1). These results suggested that developing a random mating population would be a good way to introgress a considerable number of alleles from CS-B lines into an Upland germplasm population. Random mating following crossing of the CS-B lines with cultivars has the advantage of introgressing alleles from many CS-B lines into the same population while avoiding or reducing many of the cytological problems experienced in interspecific crosses of the two species. This manuscript reports the development of a random mated population following the crossing of 18 CS-B lines with three Upland cultivars. The methodology used was bulked pollen after the method of Miravalle (1964) and as shown to be effective for a random-mating procedure (Gutiérrez et al., 2006; Jenkins et al., 2008).

Methods

We topcrossed 18 euploid CS-B lines as males with three cultivars: 'Sure-Grow 747' (PVP 9800118), developed by Sure-Grow Co.; 'PSC 355', developed by the Mississippi Agricultural and Forestry Experiment Station and licensed to Phytogen Seeds; and 'FM 966' (PVP 200100209), developed by Bayer Crop Science. The CS-B lines were CS-B01, CS-B02, CS-B04, CS-B06, CS-B07, CS-B10, CS-B16, CS-B17, CS-B18, CS-B25, CS-B05sh, CS-B11sh, CS-B12sh, CS-B14sh, CS-B15sh, CS-B22sh, CS-B22Lo, and CS-B26Lo. Seed from the cross PSC 355 × CS-B22sh was lost. Lines CS-B06, CS-B07, CS-B05sh, CS-B12sh, and CS-B15sh are known to be CS-B lines, although this has not been confirmed with molecular markers; however, their expected identity is as shown. We have confirmed the chromosome identity of the other 13 CS-B lines using molecular markers.

These 53 F_1 lines were planted in individual 15-hill rows (1590 total plants) in the winter nursery at Tecomán, Mexico, and randomly intercrossed using the bulked-pollen method. Briefly stated, when each line had approximately 15 blooms, 5 unopened blooms were bagged on each row, and the following day pollen from blooms from all 53 rows was bulked and used to pollinate about 10 previously emasculated flowers on each of the 53 rows. This process was continued each day for about 21 d. In this C_0 generation, an average of 171 cross-pollinated bolls per row (total 9089 bolls) were harvested and kept separate by F_1 row × CS-B line, resulting in 18 individual family bulks.

The next four successive cycles of crossing were accomplished by planting a random sample of seed from each of the 18 families from the previous cycle as individual rows and intercrossing among families, using the same bulked-pollen method. We harvested 9089, 1562, 4111, 5103, and 6520 crossed bolls in the C_0, C_1, C_2, C_3, and C_4 generations, respectively. Thirty, 60, and 60 plants were in the rows in the winter nursery at Tecomán in the C_0, C_2, and C_4 cycles, respectively; whereas in the C_1 and C_3 cycles in Mississippi about 240 plants were grown in each row (Table 2). After each cycle of random mating, a random

Table 1. Chromosomes involved in euploid CS-B lines with greater additive effects than corresponding *G. hirsutum* TM-1 chromosome when crossed to five elite cultivars.†

Trait	Chromosomes contributing additive effects
Lint yield	CS-B22sh, CS-B22Lo
Boll weight	CS-B04, CS-B06, CS-B15sh
Lint percentage	CS-B10, CS-B16, CS-B18, CS-B22sh, CS-B 22Lo
Upper-half mean length	CS-B01, CS-B04, CS-B25, CS-B11sh, CS-B14sh, CS-B15sh, CS-B26Lo
Strength	CS-B01, CS-B02, CS-B25, CS-B11sh, CS-B12sh, CS-B15sh, CS-B26Lo
Micronaire	CS-B01, CS-B25, CS-B12sh
Uniformity ratio	CS-B01, CS-B10, CS-B11sh
Elongation	CS-B01, CS-B04, CS-B10, CS-B11sh, CS-B26Lo

†Data from Jenkins et al., 2006, 2007, 2011.

Table 2. Number of plants and cross pollinated bolls harvested by cycle.

Cycle	Growing period (mo/yr)	Number of plants	Location of plants	Number of open bolls harvested
Cycle 0	10/08–4/09	1590	Mexico	9089
Cycle 1	5/09–9/09	4320	Mississippi	1562†
Cycle 2	10/09–4/10	1080	Mexico	4111
Cycle 3	5/10–9/10	4320	Mississippi	5103
Cycle 4	10/10–4/11	1080	Mexico	6520

†An extremely wet harvest season with excessive boll rot resulted in fewer harvested bolls than desired in the cycle-1 generation in 2009.

sample of crossed seed from each of the 18 families were planted as individual plots and selfed to produce C_xS_1 seed. An equal number of C_4S_1 seeds from each of the 18 families were bulked to compose the germplasm for release and registration.

An equal number of these self-pollinated seed from each of the 18 families after each cycle of random mating was bulked and called C_xS_1. These four C_0S_1, C_1S_1, C_2S_1, and C_3S_1 bulks plus the 21 parents were planted in 2011 in replicated plots for the determination of agronomic and fiber properties. The entries were planted in a randomized complete block design with four replications. Each plot was a single row 12 m long with a row spacing of 96.5 cm and within-row plant spacing of 10 cm. Plots were planted at two locations on the Plant Science Research Farm at Mississippi State (33.4° N 88.8° W) in 2011, and yield and fiber data were obtained. Soil type at location 1 was a Leeper silty clay loam (fine smectitic, non-acid thermic Vertic Epiaquept). Location 2 was a Marietta loam (fine-loamy, siliceous, active Fluvaquentic Eutrudepts) soil type. Yield was determined by harvesting with a two-row mechanical cotton picker equipped with load cells for individual rows. Before machine harvest, a 25-boll sample was hand harvested from the center of the plant for determining data on fiber traits at Cotton Incorporated. Fiber traits were measured with high-volume instruments (HVI), and included the upper-half mean length of fiber (UHM; the average length of the longer one-half of fibers sampled), uniformity of fiber lengths (ratio between the mean length and UHM length of fibers), fiber bundle strength (force required to break a bundle of fibers one tex unit in size), elongation of fibers before breakage (percentage elongation of a bundle of fibers before breaking), and micronaire (measure of fiber fineness or maturity by an instrument that measures the air permeability of a constant mass of cotton fibers compressed to a fixed volume).

Characteristics

In the ANOVA (Table 3), mean squares were significant for genotypes for all traits, and environments were significant for lint yield, fiber micronaire, and fiber elongation. Mean agronomic and fiber properties of the parental CS-B lines and three Upland cultivars, as well as C_0S_1, C_1S_1, C_2S_1 and C_3S_1 bulks are shown in Table 4. The yield of the cycle C_0S_1 bulk (4 cycles of random mating) was greater ($P < 0.05$) than all the parents except for CS-B10 and the three cultivars. None of the S_1 bulks were different from any of the cultivar parents in UHM fiber length, although two of the CS-B substitution lines, CS-B25 and CS-B14sh, exhibited longer fibers than the best cultivar, FM 966. None of the S_1 populations or any of the CS-B lines exceeded FM 966 in fiber bundle strength and all other HVI properties were similar to the commercial parents. Correlations among traits have also been changed by the random mating and many correlations in C_3S_1 are not significantly different than zero, Table 5.

Thus, it appears that we have improved agronomic and fiber properties and changed linkage relationships among alleles by random mating for four cycles. Data for the fifth cycle of random mating was not available because the S_1 of this cycle was only produced in 2012; however, it is not expected that these mean values will vary much from the fourth cycle of random mating. Since the S_1 of a random-mating population is a segregating generation, breeders should be able to extract individual lines that have agronomic and fiber properties superior to the mean of the segregating generation. Random mating should have introgressed properties from the CS-B lines into the random-mating population. Theoretically many alleles from *G. barbadense* should be introgressed into this random-mated population. No reports were found in the literature of these CS-B lines being used in crosses with cultivars followed by the development of a random-mated population. Thus, the random-mated population, RMBUP-C_4 (Reg. No. GP-961, PI 665950), is unique and should have great genetic and breeding value to the cotton industry. This introgressed population can be used for direct plant-to-row selections, or one boll or lock could be bulk harvested from each plant and planted and individual plant selections made in the S_2 or later generations. Breeders who plant this population should harvest a large random sample of bolls and maintain this bulk for further research and cultivar development.

Availability

Until seed supply is exhausted, seeds (1000) from RMBUP-C_4 are available to cotton breeders, geneticists, and other research personnel on written request to johnie.jenkins@ars.usda.gov. It is requested that appropriate recognition of the source be given when this germplasm contributes to the development of a new breeding line, hybrid, or cultivar. Genetic material of this released population has been deposited in the National Plant Germplasm System, where

Table 3. ANOVA for agronomic and fiber properties in RMBUP-C3S1.

Source	df	Boll weight	Lint percentage	Lint yield	Seed index	UHM† length	Strength	Micronaire	Uniformity ratio	Elongation
Environments	1	0.159	1.804	3682512**	0.056	0.0001	5.825	0.855**	0.066	6.480**
Replications (environments)	6	0.173	1.324	158161**	3.807**	0.0010	3.045	0.291**	0.997	1.826**
Genotype	24	1.115**	84.172**	667928**	6.250**	0.0081	19.388**	0.688**	4.292**	5.141**
Entry × environment	24	0.559**	0.973	76398*	0.612	0.0008	2.490	0.135**	0.733	0.226
Error	144	0.094	0.799	44591	0.558	0.0007	1.656	0.068	0.613	0.145

*significant at the 0.05 probability level.

**significant at the 0.01 probability level.

†UHM, upper-half mean.

Table 4. Agronomic and fiber properties of four cycles of random mating, chromosomal substitution lines, and cultivar parental lines.

Entry	Boll weight	Lint	Lint yield	Seed index	UHM† length	Strength	Micronaire	Uniformity ratio	Elongation
	g	%	kg ha^{-1}	g	mm	kN m kg^{-1}		%	%
Cycle 0 S1	5.02	36.03	1242	10.95	29.53	314	4.59	83.94	6.79
Cycle 1 S1	5.15	36.36	1089	11.03	28.77	296	4.80	83.70	6.51
Cycle 2 S1	4.98	36.59	1074	10.62	29.56	311	4.53	83.63	6.66
Cycle 3 S1	4.68	36.78	1277	11.31	29.69	310	4.62	84.25	6.64
CS-B01	4.81	29.81	731	11.56	29.37	285	4.03	84.03	7.98
CS-B02	4.61	33.14	854	10.79	28.10	287	4.52	82.45	5.54
CS-B04	5.45	32.20	788	10.97	26.69	282	4.47	83.09	7.34
CS-B06	5.12	33.06	900	11.09	28.92	289	4.45	83.81	6.60
CS-B07	4.82	30.34	1032	9.88	29.43	295	4.43	84.09	7.44
CS-B10	4.92	35.46	1124	11.68	28.99	292	4.85	83.70	7.54
CS-B16	5.41	35.23	555	10.52	28.39	289	4.72	82.71	7.03
CS-B17	5.11	29.43	746	12.55	28.19	283	4.29	83.08	7.73
CS-B18	4.30	31.90	618	10.96	29.18	309	5.03	83.18	6.85
CS-B25	4.65	29.55	743	12.62	30.51	323	3.89	84.94	5.76
CS-B05sh	4.56	32.88	883	10.30	28.61	279	4.31	82.78	6.41
CS-B11sh	5.01	29.36	662	12.26	29.34	306	4.53	84.51	8.08
CS-B12sh	4.89	31.94	913	11.14	28.77	289	4.27	83.61	6.38
CS-B14sh	4.30	31.19	879	10.93	30.35	304	4.39	84.20	6.36
CS-B15sh	4.92	32.07	888	11.07	29.78	312	4.29	84.66	7.21
CS-B22Lo	3.89	36.27	1043	9.39	27.88	294	4.80	83.40	6.73
CS-B22sh	4.59	35.75	843	10.17	26.80	279	4.69	82.13	6.15
CS-B26Lo	4.48	32.98	808	10.18	29.05	290	5.08	84.09	6.93
SG 747	4.46	40.68	1478	9.23	28.42	278	4.31	83.04	7.43
PSC 355	4.58	38.45	1750	9.87	28.35	325	4.98	84.69	8.14
FM 966	5.34	39.63	1462	11.11	29.18	331	4.59	84.25	4.76
Entry F sig	**	**	**	**	**	**	**	**	**
Entry × Env F sig	ns‡	ns	*	ns	ns	ns	**	ns	ns
Entry LSD 0.05	0.30	0.86	208	1.10	0.69	13	0.26	0.69	0.38
Mean CS-B	4.77	32.36	834	11.00	28.96	294	4.50	83.58	6.89
Mean cultivars	4.79	39.59	1563	10.07	28.65	312	4.63	83.99	6.78

*significant at the 0.05 probability level.

**significant at the 0.01 probability level.

†UHM, upper-half mean.

‡ns, not significant at the 0.05 probability level.

it will become available for distribution 5 yr after the date of publication. Seed of this population is not warranted by USDA-ARS to be free of transgenes although no transgenic lines were used in their development.

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Table 5. Correlation coefficients among traits in parents, C0S1, and C3S1 cycles of random mating.

	Lint percentage	Lint yield	Seed index	UHM† length	Uniformity ratio	Strength	Elongation	Micronaire
Boll weight								
Cultivars	0.04	0.19	0.61**	0.24	0.23	0.35	−0.66**	0.28
CS-B	−0.04	0.09	0.53**	0.15*	0.08	0.07	0.29**	0.13
C0S1	−0.42	−0.75*	0.28	−0.11	0.45	−0.29	0.20	0.62
C3S1	−0.86**	−0.61	0.61	0.06	−0.23	−0.08	0.12	0.65
Lint percentage								
Cultivars		−0.18	−0.33	−0.40*	−0.59**	−0.55**	−0.17	−0.44*
CS-B		0.29**	−0.41**	−0.41**	−0.34**	−0.21**	−0.23**	0.40**
C0S1		0.20	−0.40	−0.18	−0.34	0.33	0.47	−0.12
C3S1		0.41	−0.47	−0.23	0.23	0.33	0.29	−0.41
Lint yield								
Cultivars			0.00	−0.19	0.25	0.18	0.27	0.56**
CS-B			−0.11	0.06	0.08	0.10	−0.05	0.22**
C0S1			0.03	0.18	−0.48	0.13	−0.48	−0.25
C3S1			0.01	0.10	−0.25	−0.08	−0.07	−0.10
Seed index								
Cultivars				0.73**	0.49**	0.68**	−0.51**	0.50**
CS-B				0.37**	0.32**	0.35**	0.25**	0.12
C0S1				0.73*	0.54	0.32	0.27	0.20
C3S1				0.38	−0.41	0.30	0.08	0.68
UHM								
Cultivars					0.40*	0.58**	−0.42*	0.07
CS-B					0.71**	0.65**	0.14	−0.14
C0S1					0.42	0.71*	0.33	−0.04
C3S1					−0.24	0.53	−0.21	−0.10
UI								
Cultivars						0.76**	0.06	0.61**
CS-B						0.59**	0.21**	−0.08
C0S1						0.42	0.49	0.14
C3S1						−0.34	−0.44	−0.58
Strength								
Cultivars							−0.26	0.52**
CS-B							0.01	0.04
C0S1							0.52	0.11
C3S1							0.46	0.05
E1								
Cultivars								0.30
CS-B								0.09
C0S1								0.06
C3S1								0.54

* significant at the 0.05 probability level.

**significant at the 0.01 probability level.

†UHM, upper-half mean.

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