Development of interspecific hybrids between upland cotton and D-genome diploid species of *Gossypium*

Erik J. Sacks[1], Mauricio Ulloa[1], James McD. Stewart[2], Pablo Murillo Navarrete[3] & Sebastian Acosta Nunez[3]
erik_sacks@lycos.com
[1]USDA-ARS, [2]University of Arkansas, [3]INIFAP

Introduction:

Upland cotton, *G. hirsutum*, is a tetraploid species (AADD genomic constitution) that has become the predominant cotton of commerce worldwide. Upland cotton is one of four domesticated cotton species, which also include the new world tetraploid species (AADD) *G. barbadense* and two old world A genome diploid species, *G. arboreum* and *G. herbaceaum*. Bottlenecks during speciation and domestication have resulted in limited genetic diversity among upland cotton cultivars. The undomesticated D genome diploid species are native to the Americas and represent a vast germplasm resource that has the potential to be used for improving upland cotton. Though the D genome cotton species produce little or no fiber, prior introgression efforts have already resulted in upland cotton cultivars with improved fiber strength. In spite of this important success, few efforts have been made to introgress genes from the D genome species because the work is difficult and time-consuming.

One approach to introgressing genes from the D genome species into upland cotton is to start by crossing upland cotton with a D genome species to obtain an ADD triploid. To restore fertility, the triploid is treated with colchicine to obtain a 2(ADD) hexaploid. The hexaploid is subsequently crossed with an A genome diploid to obtain a tetraploid AADD trispecies hybrid that is used in further crosses with upland cotton. Thus, the AADD/DD crosses are an initial step towards introgression of novel D-genome genes into upland cotton. Additionally, the 2(ADD) hexaploids are also useful as bridging lines for introgressing genes from the A-genome diploid species.

The D genome species are mostly short-day plants and many are also large shrubs or small tress. In addition some D genome species have a long juvenile period, and some require a strong dry season in which to induce dormancy. Thus, it is challenging to grow and flower many of the D genome species in the United States, especially during the summer growing season. However, some success can be had by using growth chambers to manipulate day length and by confining the plants to pots to control their size.

The purpose of this study was to obtain hybrids between upland cotton and D genome species, and to assess differences among the parents in the relative difficulty of obtaining hybrids.

Materials & Methods:

Two field crossing experiments were conducted; one in Stoneville, MS and the other in Iguala, Mexico. Both experiments were factorial designs with upland cotton genotypes as female parents and accessions of the D genome species as male parents. To promote fruit set, 250 ppm gibberellic acid was applied to the floral bracts on the day a given cross was made.

In the summer of 2006 at Stoneville, three upland cotton cultivars were crossed with one accession of *G. armourianum* and three accessions of *G. thurberi*. The *G. armourianum* genotype was relatively day-neutral and flowered in a greenhouse during the summer. To obtain flowers from three accessions of *G. thurberi*, they were grown in a growth chamber with 11 h of light per day. The upland cultivars were grown in a field and flowers were emasculated in the morning prior to pollen dehiscence (dawn until about 9:30 a.m.). Crosses were made before noon. In addition to the field crosses during the summer, we also crossed upland cotton grown in a greenhouse with *G. raimondii* grown in the growth chamber during the autumn of 2006. For the field crosses, data was taken on the number of normal-appearing seed obtained from each fruit. All of the seed was subsequently germinated in a growth chamber at 28 C and then planted in a greenhouse for evaluation. The number of triploid hybrid progeny per fruit, as determined by infertility and phenotypic characteristics (e.g. petal spot, pubescence and leaf shape), was recorded.

In December of 2006 at Iguala, six perennial field-grown upland cotton lines that were previously collected in Mexico were crossed with ten field-grown accessions of the D species. Data was taken on fruit set and number of fruit containing at least one normal-appearing seed. As of this time, the Iguala seeds have not yet been germinated to confirm if they are hybrid.

Results & Discussion:

Stoneville:

From 31 *G. hirsutum*/*G. armourianum* crosses, 226 seedlings were obtained, of which 90% were hybrid (Table1). In contrast, 105 *G. hirsutum*/*G. thurberi* crosses produced only 143 seedlings, and only two were hybrid. The hybrid seedlings were intermediate in phenotype to the parents and were sterile, as expected for triploids. The non-hybrid seedlings were identical to the upland cotton parents and were fertile. Given that one worker emasculated all of the flowers without prior knowledge of pollen source, chance accidental self-pollinations alone would not likely account for the difference between the *G. armourianum* and *G. thurberi* crosses.

The different outcomes of the *G. hirsutum*/*G. armourianum* crosses and *G. hirsutum*/*G. thurberi* crosses might be explained by differences in the pollen competitiveness for the D genomes species with any upland cotton pollen that may have been present after emasculation, induced apomixes or the development of seed from unreduced gametes. Because the upland cotton parents were highly inbred, the above hypotheses cannot be tested with the seedlings already obtained. However, if new crosses are made with an F_1 upland cotton that is heterozygous for a trait in which the three phenotypic classes are readily distinguishable (e.g. purple leaf or okra leaf), the above hypotheses can be simply tested because selfing will produce progeny that segregate 1:2:1, apomixes will result in all heterozygotes , unreduced gametes will result in only homozygotes, and a combination of explanations will result in greater than expected numbers for some of the phenotypic classes.

Table 1. Cross compatibility for upland cotton cultivars crossed as female parents in a factorial design with accessions of *G. thurberi* and *G. armourianum* as male parents during the 2006 field season in Stoneville, MS.

		Male parent				
		G. thurberi			*G. armourianum*	
Female parent (*G. hirsutum*)		D1-10	D1-17	D1-36	D2-1-6	Totals
Coker 100	No.of hybrid seedlings	0	0	0	66	66
	Total No. of seedlings	21	11	32	81	145
	No. of fruit	12	4	17	11	44
DP90	No.of hybrid seedlings	0	0	0	51	51
	Total No. of seedlings	25	2	29	58	114
	No. of fruit	12	4	20	10	46
Sealand1	No.of hybrid seedlings	1	1	0	87	89
	Total No. of seedlings	10	6	7	87	110
	No. of fruit	13	5	18	10	46
Totals	No.of hybrid seedlings	1	1	0	204	206
	Total No. of seedlings	56	19	68	226	369
	No. of fruit	37	13	55	31	136

From six subsequent *G. hirsutum*/*G. raimondii* crosses in the greenhouse, 20 seedlings were produced, of which 19 were hybrid. Triploid hybrids from the *G. hirsutum*/*G. armourianum* crosses were treated with chromosome doubling compounds and fertile plants have been recovered. Thus, we can conclude that obtaining hybrids between upland cotton and *G. thurberi* is more difficult than with *G. armourianum* and *G. raimondii*. Of the upland cotton cultivars, Sealand1 appeared to be more cross compatible than Coker 100 and DeltaPine90, confirming an earlier observation that Sealand1 is a relatively good parent for interspecific cotton crosses.

Iguala:

Differences in percent fruit set and number of set fruit that produced at least one normal appearing seed differed among the female and male parents (Table 2). Fruit set was typically lower in the Iguala trial than in the Stoneville trial, but this may have been due to dry season stress at the former location, though the plants were irrigated. The most cross-compatible upland cotton parents were US6 and US131. The most cross-compatible D species appeared to be *G. raimondii*, *G. aridum* and *G. gossypiodes*.

The germplasm collection in Iguala is invaluable. There is a need to secure stable long-term funding for outstanding resource.

Conclusion:

This study demonstrated that it is feasible, with some effort, to initiate an introgression pipeline to move genes from the D genome cotton species into upland cotton. The hybrids produced in this study will be a source of new genes for upland cotton improvement. The 2(ADD) hexaploids produced from these hybrids will also facilitate introgression of genes from the domesticated diploid A genome species. Given the narrow genetic base of upland cotton, introgression efforts should be increased.


G. hirsutum US6 in Iguala, Mexico


G. lobatum


G. gossypiodes

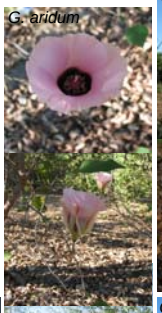
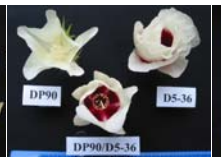

G. aridum

Table 2. Cross compatibility for 6 upland cotton lines from Mexico crossed as female parents in a factorial design with 10 accessions of D genome species as male parents during December 2006 in the field at Iguala, Mexico.

			Male parent										
			Percent of set fruit with 1 or more seed										
	Female parent		*G. trilobum*/*G. thurberi*	*G. laxum*	*G. lobatum*	*G. raimondii*	*G. armourianum*	*G. davidsonii*	*G. aridum puebla*	*G. aridum*	*G. aridum*	*G. gossypioides*	
Species	Collection location	Id	D8-6/D1-35	US70	US104	D5-4	D2-19	D3d-26	US5	US72	US122	M#43	Average
G. hirsutum palmeri	Puebla	US6	50	81	90	80	71	70	82	91	70	85	77
G. hirsutum palmeri	Puebla	US89	38	71	100	57	25	25	29	29	0	77	45
G. hirsutum	Nayarit	US131	60	76	100	50	100	100	92	100	100	67	84
G. hirsutum	Jalisco	US134	0	0	0	88	67	50	60	100	86	33	48
G. hirsutum	Coahuilla		67	50	—	83	50	75	67	56	67	79	66
G. hirsutum	Colima	US127	25	40	0	89	50	20	67	100	89	90	57
		Average	40	53	58	74	61	57	66	79	69	72	63
			Percent fruit set										
G. hirsutum palmeri	Puebla	US6	40	76	67	67	47	67	73	73	67	76	65
G. hirsutum palmeri	Puebla	US89	53	47	47	47	27	27	47	47	27	81	45
G. hirsutum	Nayarit	US131	33	77	7	67	53	53	80	33	60	40	50
G. hirsutum	Jalisco	US134	7	13	0	53	20	13	33	7	47	40	23
G. hirsutum	Coahuilla		20	67	—	40	40	27	60	60	40	56	45
G. hirsutum	Colima	US127	80	67	0	60	80	33	20	67	60	67	53
		Average	39	58	24	56	44	37	52	48	50	60	47
			# of pollinations										
G. hirsutum palmeri	Puebla	US6	15	21	15	15	15	15	15	15	15	17	
G. hirsutum palmeri	Puebla	US89	15	15	15	15	15	15	15	15	15	16	
G. hirsutum	Nayarit	US131	15	22	15	15	15	15	15	15	15	15	
G. hirsutum	Jalisco	US134	15	15	15	15	15	15	15	15	15	15	
G. hirsutum	Coahuilla		15	21	—	15	15	15	15	15	15	25	
G. hirsutum	Colima	US127	15	15	15	15	15	15	15	15	15	15	




G. raimondii