2000 Development of trispecies backcross populations using a 2(ADD) hexaploid bridging line to introgress genes from A-genome diploids in upland cotton

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ABSTRACT

Introgression of genes from A-genome diploid *Gossypium* species into tetraploid upland cotton is desirable but post-zygotic breeding barriers, in addition to ploidy differences, make the task difficult. *G. arboreum* L. and *G. herbaceum* L. accessions that had been previously identified as resistant to reniform nematodes were crossed as females to a 2[(AD_1)D_4] hexaploid bridging line used as a male parent. The long-term objectives were to introgress nematode resistance genes and widen the narrow genetic base of upland cotton. Initially, 25 crosses were made with five diploid accessions and the fruit were allowed to develop without embryo rescue, yielding one hybrid with accession A_2-190. In a subsequent ovule culture experiment, 70 crosses were made with six A-genome diploid accessions and the number of seedlings per fruit that germinated in-vitro differed among the accessions, ranging from 0.8 to 5.1. However, seedling growth was weak and only five seedlings from A_2-100 and six from A_2-113 were grown to flowering size in a greenhouse. Like the D_4 parent, the hybrids had purple cup-shaped flowers and were photoperiod sensitive. Pseudobackcross populations were made for the three hybrids using MD51ne and Deltapine 16 upland cultivars. The BC_1 population derived from A_2-190 exhibited diverse and sometimes transgressive segregation for flower and fiber traits and were mostly day neutral. Inbred lines derived from BC_1 or BC_2 generations should be good candidates for replicated field testing to identify novel genes for improved yield, fiber quality, pest resistance and tolerance to abiotic stresses.

Gossypium. hirsutum L. (upland cotton) is one of four cotton species that were independently domesticated, yet it accounts for over 90% of worldwide cotton production. Two of the domesticated species, *G. hirsutum* and *G. barbadense* L., are tetraploids (AADD genomic constitution) and are native to the Americas, whereas the other domesticated species, *G. arboreum* L. and *G. herbaceum* L., are diploids (AA genomic constitution) and are native to Asia and Africa, respectively (Percival et al., 1999). The predominance of upland cotton production was brought about in the early 1900s primarily by the requirements of mechanized spinning for longer fibers, which are less commonly found among A-genome diploid cultivars than among upland cultivars (May and Lege, 1999). In India, where over 2 million ha continues to be planted to the A-genome diploids, primarily in

drought-prone low-input environments, recent efforts to breed these species for improved fiber quality have been reported (Kapoor, 2003; Kulkarni et al., 2003; Patil et al., 2003).

While most modern efforts to improve cotton are focused on upland cotton, the A-genome diploid species could be important sources of genes for increasing resistance to pests and diseases, improving tolerance to abiotic stresses, and increasing yield. More broadly, much of the genetic diversity within the A-genome diploids is likely absent from the A-genome of the tetraploids due to the genetic bottleneck associated with their speciation. Molecular studies have demonstrated that upland cotton has a narrow genetic base (Iqbal et al., 2001; Vafaie-Tabar et al., 2004; Wendel et al., 1992), indicating that crop improvement efforts would benefit from the introgression of new genes. Given the considerable financial and environmental costs of controlling upland cotton pests, introgression of host plant resistance genes would be especially advantageous.

However, breeding barriers hamper the introgression of genes from the A-genome diploids into upland cotton, and thus the use of this germplasm is time-consuming and costly. Post-zygotic breeding barriers typically prevent the production of viable hybrid seed (Beasley, 1940; Pundir, 1972) and sometimes lead to hybrid breakdown of the seedlings that are produced (Avila and Stewart, 2005; Gerstel, 1954). Tissue culture is typically used to rescue hybrid embryos (Gill and Bajaj, 1987; Stewart and Hsu, 1978). The differences in chromosome number and genomic composition between upland cotton and the A-genome diploids are additional barriers to introgression. In spite of these difficulties, hybrids between upland cotton and the A-genome diploids were obtained as early as the 1920s (Zaitzev, 1924). Examples of successful introgression of genes from the A-genome diploids into tetraploid cultivars have included: anthocyanin pigmentation (Harland, 1935), resistance to *Xanthamonas malvacearum* (Knight, 1948), resistance to jassids via increased hairiness (Knight, 1954), resistance to cotton rust (Blank, 1971), and drought resistance and high ginning outturn (Gotmare and Singh, 2004). Perhaps the most notable introgression from diploid to tetraploid cotton has been increased fiber strength from a chromosome-doubled *G. arboreum* × *G. thurberi* progeny crossed with *G. hirsutum*, though the trait is presumed to be derived from *G. thurberi* (Fryxell, 1976). The limited number of documented introgressions is likely a testament to the difficulty and expense of such work.

Stewart (1995) described four strategies for introgessing genes from diploid species into upland cotton:

1. Aneuploid Series:

AADD X AA => AAD => chromosome doubling => 2(AAD) X AADD => pentaploid X AADD => aneuploid series

2. Tetraploid X Diploid:

AADD X AA => AAD => chromosome doubling => 2(AAD) X DD => AADD

3. Diploid X Diploid:

AA X DD => AD => chromosome doubling => AADD

4. Diploid X Hexaploid (or reciprocal):

AA X 2(ADD) => AADD

Though the aneuploid series has been a successful approach, it has the disadvantages of reduced fertility in the segregating generations and loss of whole chromosomes. The tetraploid X diploid strategy circumvents the problems of the aneuploid series but is lengthy and has the potential for recombination and loss of desirable genes during meiosis before reaching the tetraploid level. The latter two strategies are more efficient than the former ones. The main advantage of the diploid X diploid strategy is that the initial tetraploid progeny are homozygous, however chromosome doubling is a time-consuming process in which success for a given genotype is not guaranteed (Avila et al., 2004; Avila and Stewart 2005). With the diploid × hexaploid strategy, tetraploid progeny are obtained directly. Only a small number of such 2(ADD) hexaploids are currently maintained in research collections (Maréchal, 1983; Stewart, 1995, Jonathan Wendel, personal communication). In this study, we investigated the utility of the diploid X hexaploid method for introgressing genes from A-genome diploids into upland cotton.

MATERIALS AND METHODS

G. arboreum and *G. herbaceum* accessions that had been previously found to be resistant to reniform nematodes, *Rotylenchulus reniformis* Linford & Oliveira, (Carter, 1981; Stewart and Robbins, 1995; Yik and Birchfield, 1984) were obtained from the USDA National Plant Germplasm System (**http://www.ars-grin.gov/npgs/**) and used as female parents. A 2[(AD_1)D_4] hexaploid, G 371 (*G. hirsutum* cultivar NC8 × *G. aridum* (Rose & Standley) Skovsted accession G 248; Maréchal, 1983), was kindly donated by Jonathan Wendel (Iowa State University, Ames, IA) and used in this study as a male bridging line.

In an initial experiment, 25 crosses were made with five diploid accessions (A_1-024, A_2-019, A_2-087, A_2-113 and A_2-190) as female parents in a greenhouse at Stoneville, MS from 13 February to 12 May 2005. Data on fruit set and seed production were recorded.

In a subsequent experiment, 70 crosses were made with six A-genome diploid accessions (Table 1) in March 2006, and ovules were cultured in-vitro. Four days after pollination fruit were washed in soap and water, then surface sterilized in a laminar flow hood by immersion in an aqueous solution of 2.6% sodium hypochlorite and 0.1% Tween-20 for 10 min with intermittent shaking, followed by immersion in ethanol for 10 min, and then allowed to air-dry. Ovules were placed on a single 100 X 25 mm Petri dish containing 25 ml of modified Murashige and Skoog media with Gambourg's B5 vitamins (M0404, Sigma-Aldrich, St. Louis, MO), plus 1.9 g l^{-1} KNO_3, 0.5 g l^{-1} asparagine, 1.0 g l^{-1} glutamine, 20.0 g l^{-1} glucose, 0.25 g l^{-1} cefotaxime, and 2.2 g l^{-1} gelrite, with a pH of 5.8. Each Petri dish contained ovules from a single fruit. Plated ovules were incubated at 30 °C with 12 h of fluorescent light each day. Data was collected on the number of ovules per fruit, the number of seedlings that germinated per fruit and number of hybrid progenies that were successfully transferred to pots and grown in a greenhouse. For the count data, analyses of variance (ANOVA) and covarviance (ANCOVA) were performed with SAS procedures GLIMMIX using a negative binomial response distribution; means in the original units were obtained with the ILINK option.

Trispecific progenies were crossed to the upland cultivar MD51ne, which was chosen for its good fiber qualities, high lint yield, adaptability to the Mississippi Delta, and its nectariless trait, which confers insect resistance (Meredith, 1993; Schuster et al., 1976). Some trispecific progenies were crossed with Deltapine 16 because this obsolete upland cultivar has been used commonly as a susceptible control in reniform nematode screens and it is in the pedigree of many modern upland cultivars that are adapted to the Mississippi Delta. Observations were made on the phenotypic characteristics of the trispecific progenies, including plant habit, day length sensitivity, flower color, fertility, crossability with upland cultivars and lint production. Over 300 pseudobackcross individuals were observed in a greenhouse for phenotypic variability and potential utility in breeding.

RESULTS AND DISCUSSTION

From the initial crossing experiment, 72% of the pollinations produced fruit that reached maturity but only one normal looking seed was obtained. The normal looking seed, from accession A_2-190, germinated and the plant exhibited characteristics of both parents (Fig. 1). Altman (1988) reported that exogenous application of gibberellic acid alone or in combination with auxin could be used to improve fruit set and obtain hybrids between upland cotton and other *Gossypium* species, though he did not obtain hybrids with *G. arboreum* using this method. Given our initial success with the diploid X hexaploid method, we have initiated studies to improve the efficiency of recovering hybrids based on variations of Altman's method.

In the subsequent ovule culture experiment, significant differences (P = 0.0185) were observed in the average number seedlings per fruit that germinated in-vitro, ranging from 0.8 to 5.1 among the A-genome accessions tested (Table 1). Significant though modest differences (P = 0.0079) among the accessions also were observed for the number of ovules produced per fruit. However, the ANCOVA indicated that the observed differences in initial ovules per fruit did not have a significant affect on the number of seedlings per fruit that were obtained (i.e., the slopes were zero). Thus, the observed differences in crossability were post-zygotic and there was no evidence for pre-zygotic effects.

Though germinating seedlings were obtained for all of the accessions, we observed that subsequent growth, especially root development, was typically weak. Consequently, the number of plants successfully grown in-vitro and acclimated to the greenhouse (five for A_2-100 and six for A_2-113) was considerably lower than the potential based on germination. Thus, further work to identify improvements in tissue culture media and/or gelling agents at the post-germination stage would be valuable.

Like its *G. aridum* parent, G 371 has purple, cup-shaped flowers over a white background with darker purple petal spots, and it sets flower buds only under short days (Fig. 1.). A_2-100 and A_2-190 have open, bright yellow flowers with purple petal spots. A_2-113 is a marker stock that has bright white flowers without petal spots, glabrous stems and leaves and is fiberless when grown in warm environments typical of cotton production. Like most *G. arboreum* accessions, those in this study were day neutral.

The photoperiod sensitivity of G 371 is a disadvantage for a bridging line because it is difficult to use during the summer in northern latitudes such as Stoneville, MS. We have been able to flower G 371 during the summer, only by growing it in a growth chamber with 11 h of light per day. To circumvent the difficulty of working with a short day bridging line,

current efforts are underway to double the chromosome number of $(AD_1)D_{2\text{-}1}$ seedlings derived from a *G. armourianum* Kearney parent that blooms regularly during the summer in a greenhouse at Stoneville.

All of the trispecies hybrids had purple, cup-shaped flowers, either over a yellow or white background depending on the *G. arboreum* parent, and the hybrids were photoperiod sensitive. Evaluation of self progeny from G 371 indicated that genes for purple petals and cup-shaped flowers were fixed in this line. Thus, purple petals and cup-shaped flowers were dominant traits that made excellent markers for confirming that the seedlings were hybrids. The trispecies hybrids shed copious amounts of pollen but few selfed seeds were produced, even when pollinated by hand. However, crossing the trispecies hybrids as females to upland cotton yielded five seeds per fruit on average and the reciprocal crosses also produced seed. Thus, large pseudobackross populations have been readily developed, with our most advanced material from A2-190 being tested for resistance to reniform nematodes.

The long-term goal of the present work is to broaden the genetic base of upland cotton, in addition to selecting for the previously identified resistance to reniform nematodes. Tanksley and Nelson (1996) advocated an inbred backcross method for mining quantitative trait loci from interspecific crosses. The development of interspecific populations for introgression of genes controlling quantitative traits requires a balance between backcrossing enough times to an adapted, relevant and current genetic background to facilitate the selection of superior lines, and leaving enough genetic variability to have a good chance of observing the effects of introduced genes (Dudley, 1982).

Among the first generation backcross progenies (BC_1) observed in a greenhouse at Stoneville, MS, flower shape and size was highly variable (Fig. 1), indicating that one backcross to upland cotton has likely not greatly diminished genetic variation needed for selection. Indeed, transgressive segregants for flower size were observed, giving reason to look further for novel, agronomically advantageous characteristics in these populations. Transgressive segregation is a common feature of interspecific crosses, especially those involving inbred domesticated plants (Rieseberg et al., 1999).

Initial observations of fiber production indicated that one cross back to upland cotton has lead to considerable gains towards recovering genotypes with good fiber quantity and quality (Fig. 2). Additionally, most of the observed BC_1 progenies set flowers when exposed to 14 h of light per day, indicating that photoperiod sensitivity was eliminated or greatly reduced after only one cross back to an upland genotype. Plant morphology in the BC_1 was typically similar to MD51ne, although some individuals showed greater or lesser vigor. Taken together, these observations suggest that inbred lines derived from BC_1 or BC_2 generations should be good candidates for replicated field testing to identify novel genes for improved yield, fiber quality, pest resistance and tolerance to abiotic stresses.

REFERENCES

Altman, D. 1988. Exogenous hormone applications at pollination for in vitro and in vivo production of cotton interspecific hybrids. Plant Cell Rep. 7:257-261.

Avila, C.A. and J.McD. Stewart. 2005. Germplasm enhancement for cotton improvement. P. 23-27. *In* D.M. Oosterhuis (ed.) Summaries of Arkansas cotton research 2004. University of Arkansas, Agricultural Experiment Station Research Series 533.

Avila, C.A., J.McD. Stewart and R.T. Robbins. 2004. Transfer of reniform nematode resistance from diploid cotton species to tetraploid cultivated cotton. P. 183-186. *In* D.M. Oosterhuis (ed.) Summaries of Arkansas cotton research 2003. University of Arkansas, Agricultural Experiment Station Research Series 521.

Beasley, J.O. 1940. Hybridization of American 26-chromosome and Asiatic 13-chromosome species of Gossypium. J. Agric. Res. 60:175-181.

Blank, L.M. 1971. Southwestern cotton rust. P. 76-77. *In* Beltwide Cotton Production Research Conference Proceedings.

Carter, W. W. 1981. Resistance and resistant reaction of *Gossypium arboreum* to the reniform nematode, *Rotylenchulus reniformis*. J. Nematol. 13:368-374.

Dudley, J.W. 1982. Theory for transfer of alleles. Crop Sci. 22:631-636.

Fryxell, P.A. 1976. Germpool utilization: *Gossypium*, a case history. Agricultural Research Service, U.S. Department of Agriculture Publication ARS-S-137.

Gerstel, D.U. 1954. A new lethal combination in interspecific cotton hybrids. Genetics 39:628-639.

Gotmare, V. and P. Singh. 2004. Use of wild species for cotton improvement in India. ICAC Recorder 12:12-14.

Gill, M.S. and Y.P.S. Bajaj. 1987. Hybridization between diploid (*Gossypium arboreum*) and tetraploid (*Gossypium hirsutum*) cotton through ovule culture. Euphytica 36:625-630.

Harland, S.C. 1935. The genetics of cotton. XII. Homologous genes for anthocyanin pigmentation in new and old world cottons. J. Genet. 30:465-476.

Iqbal, M.J., O.U.K. Reddy, K.M. El-Zik, and A.E. Pepper. 2001. A genetic bottleneck in the 'evolution under domestication' of upland cotton *Gossypium hirsutum* L. examined using DNA fingerprinting. Theor. Appl. Genet. 103:547-554.

Kapoor, C.J. 2003. Genetic improvement of *Gossypium arboreum* for quality cotton. P. 210-212. *In* A. Swanepoel (ed.) Proceedings of the world cotton research conference-3, Cape Town, South Africa. 9-13 March 2003.

Knight, R.L. 1948. The genetics of blackarm resistance VI. Transference of resistances from *Gossypium arboreum* to *G. barbadense*. J. Genet. 48:359-369.

Knight, R.L. 1954. The genetics of jassid resistance in cotton IV. Transference of hairiness from *Gossypium herbaceum* to *G. barbadense*. J. Genet. 52:199-207.

Kulkarni, V.N., B.M. Khadi, L.A. Deshpande, S. Sreenivasan and M.S. Bisankopp. 2003. Hirsutisation of diploid cultivated cotton. P. 86-94. *In* A. Swanepoel (ed.) Proceedings of the world cotton research conference-3, Cape Town, South Africa. 9-13 March 2003.

Maréchal, R. 1983. Une collection d'hybrides interspécifiques du genre *Gossypium*. Cot. Fib. Trop. 38(2):240-246.

May, O.L. and K.E. Lege. 1999. Development of the world cotton industry. p. 65-97. *In* C.W. Smith and J.T. Cothren (eds.) Cotton: Origin, history, technology, and production. New York, John Wiley & Sons, Inc.

Meredith, W.R., Jr. 1993. Registration of 'MD51ne' cotton. Crop Sci. 33:1415.

Patil, S.S, R.S. Patil, C.A. Deepak, D. Somashekhar and B.C Kolhar .2003. Genetic evaluation and comparison of *G. herbaceum* and *G. hirsutum* varieties in different levels of rainfed and intensive management situations. P. 152-161. *In* A. Swanepoel (ed.) Proceedings of the world cotton research conference-3, Cape Town, South Africa. 9-13 March 2003.

Percival, A.E., J.F. Wendel and J.M. Stewart. 1999. Taxonomy and germplasm resources. p. 33-63. *In* W. C. Smith (ed.) Cotton: Origin, history, technology, and production. New York, John Wiley & Sons, Inc.

Pundir, N.S. 1972. Experimental embryology of *Gossypium arboreum* L. and *G. hirsutum* L. and their reciprocal crosses. Bot. Gaz. 133:7-26.

Rieseberg, L.H., M.A. Archer and R.K. Wayne. 1999. Transgressive segregation, adaptation and speciation. Heredity 83:363-372.

Schuster, M.R., M.J. Lukefahr and F.G. Maxwell. 1976. Impact of nectariless cotton on plant bugs and natural enemies. J. Econ. Entom. 69:400-402.

Stewart, J.M. 1995. Potential for crop improvement with exotic germplasm and genetic engineering. P. 313-327. *In* G.A. Constable and N.W. Forrester (eds.) Challenging the future: Proceedings of the world cotton research conference-1, CSIRO, Melbourne, Australia.

Stewart, J.McD. and C.L. Hsu. 1978. Hybridization of diploid and tetraploid cottons through in-ovulo embroyo culture. J. Hered. 69:404-408.

Stewart, J.M. and R.T. Robbins. 1995. Evaluation of Asiatic cottons for resistance to reniform nematode. P. 165-168. *In* D.M. Oosterhuis (ed.) Proceedings of the 1994 cotton research meeting and 1994 summaries of cotton research in progress. Arkansas Agricultural Experiment Station Special Report 166.

Tanksley, S.D. and J.C. Nelson. 1996. Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor. Appl. Genet. 92:191-203.

Vafaie-Tabar, M., S. Chandrashekaran, M.K. Rana, and K.V. Bhat. 2004. RAPD analysis of genetic diversity in Indian tetraploid and diploid cotton (*Gossypium* spp). J. Plant Biochem. Biotech. 13:81-84.

Wendel, J.F., C.L. Brubaker and A.E. Percival. 1992. Genetic diversity in *Gossypium hirsutum* and the origin of upland cotton. Am. J. Bo. 79:1291-1310.

Yik, C-P. and W. Birchfield. 1984. Resistant germplasm in *Gossypium* species and related plants to *Rotylenchulus reniformis*. J. Nematol. 16:146-153.

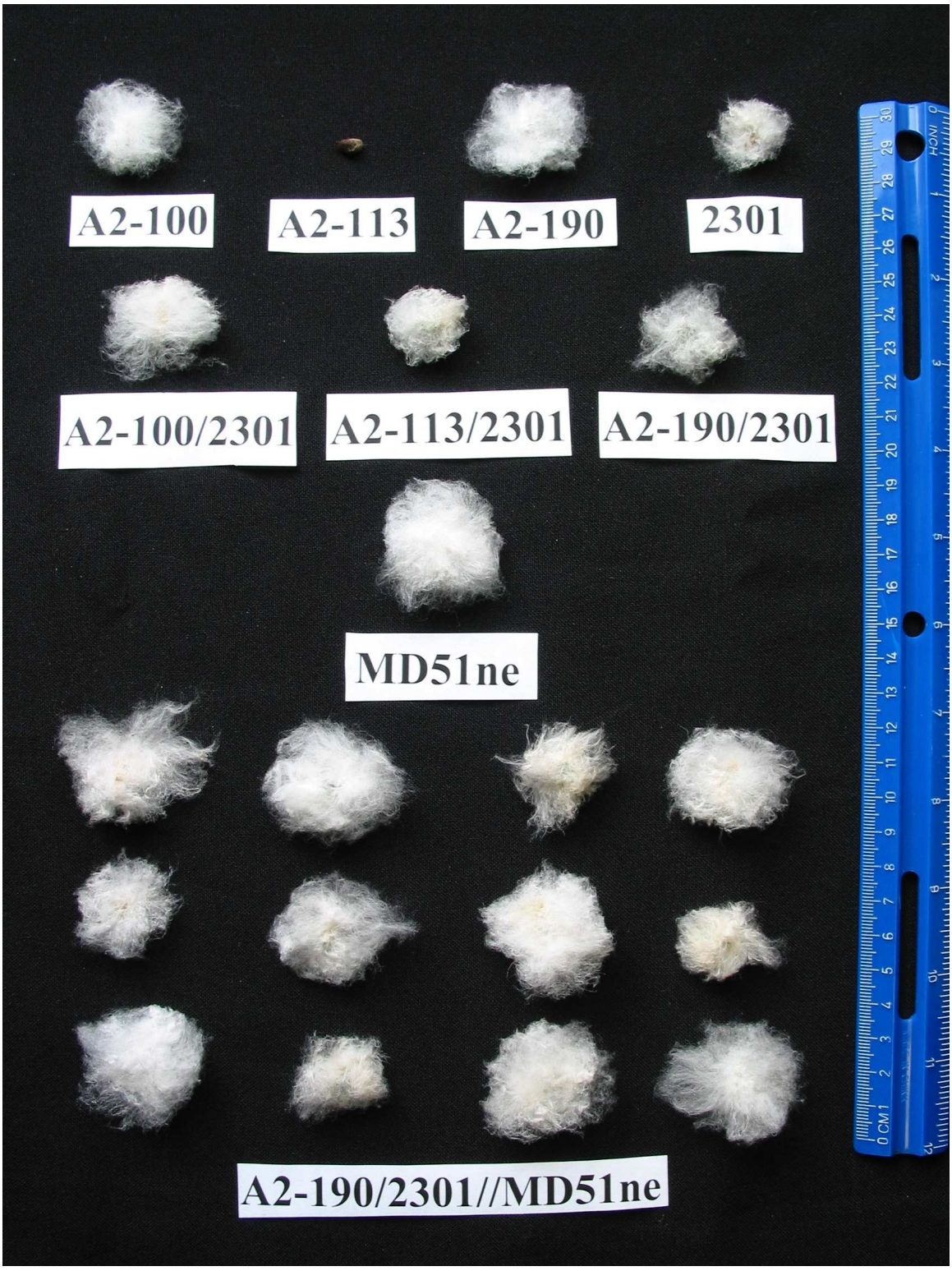
Zaitzev, G.S. 1924. A hybrid between the Asiatic and American cotton plants *Gossypium herbaceum* L. and *Gossypium hirsutum* L. Bulletin of Applied Botany and Plant Breeding 13:132-134.

Table 1. Ovule culture production of hybrid seedlings from crosses between accessions of *G. arboreum* or *G. herbaceum* and G 371, a 2[$(AD_1)D_4$] hexaploid.

		No. ovules per fruit			No. seedlings per fruit	
Entry	N	Mean	SE		Mean	SE
A1-024	8	22.9	1.7		0.8	0.4
A2-019	15	27.5	1.4		5.1	1.4
A2-087	8	20.0	1.6		2.1	0.9
A2-100	9	25.2	1.7		2.6	1.0
A2-113	17	27.4	1.3		4.9	1.3
A2-194	13	23.9	1.4		2.3	0.8
Over all entries	70	24.5	1.5		3.0	1.0


A
A2-100
B
A2-113
C
A2-190
D
2301
E
A2-100/2301
A2-113/2301
A2-190/2301
F
G
H
I
A2-190/2301//MD51ne
A2-190/2301
MD51ne
A2-190/2301//DP16

1


A2-100
A2-113
A2-190
2301
A2-100/2301
A2-113/2301
A2-190/2301
MD51ne
A2-190/2301//MD51ne

1