Breeding Transformed Cotton Expressing Enhanced Fiber Strength

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ABSTRACT. New cotton (*Gossypium hirsutum* L.) seeds that can produce fiber with enhanced strength would increase efficiency of the rotor form of open end yarn manufacture. We report results of 3-yr of breeding transgenic 'Deltapine 50' (DP 50) germplasm expressing enhanced fiber strength. The objective was to identify germplasm that could consistently express and transmit enhanced fiber strength to their progeny. Deltapine 50 cotton was transformed via particle bombardment of meristems by Monsanto. The intent of transformation was to insert a β-glucuronidase (GUS) marker gene and a proprietary gene driven by a fiber specific promoter. The R4-R6 generations descendent from three GUS-positive, enhanced strength, R3 greenhouse plants were evaluated in the field from 1996-1998 near Florence, SC. Unusual frequencies of GUS-positive progeny were found upon self-pollination of GUS-positive plants, possibly reflecting altered fitness of transgenic gametes. Fiber strength of most GUS-positive plants was 30-70% greater than the non-transformed DP 50 control plants. After examining numerous parent-offspring relationships, we did not observe any consistent transmission of enhanced fiber strength. Results of this study,

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however, may have application to other transgenic cotton breeding efforts. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: getinfo@haworthpressinc.com <Website: http://www.haworthpressinc.com>]*

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INTRODUCTION

Cotton yarn manufacture is accomplished mainly by two spinning processes, broadly termed open-end and ring. Ring spinning is an established technology capable of manufacturing a range of yarn sizes, certain of which cannot yet be economically produced by open end yarn manufacture. Open end spinning exploits a more productive method of assembling fiber into yarn, hence its economic advantage of producing more yarn per unit of time (Deussen, 1992). The worldwide increase in open-end spinning capacity has been driven by global competition among yarn manufacturers and their need to control production costs (Faerber, 1995). A disadvantage of open-end spun yarn in assembling textile products is they have less fiber-to-fiber contact and thus, lower strength compared with a similar size yarn produced by conventional ring spinning frames (Deussen, 1992); but, this reduction in yarn strength can be ameliorated through enhanced fiber strength. Fiber strength along with measures of length distribution and linear density are fiber characteristics yarn manufacturers consider in assembling cotton bales to produce a certain size yarn and control variation in yarn quality. Fiber strength has been identified as the fiber property with the greatest impact on rotor spun yarn quality; rotor spinning is currently the dominant form of open-end yarn manufacture (Deussen, 1992).

Enhancing fiber strength through conventional breeding has resulted in slow, but steady gains since the early 1900s (May, 1999); but, combining biotechnology and breeding efforts offers the possibility of more immediate gains. Monsanto Company is the leader in the U.S. cottonseed market in the incorporation of input traits into cotton cultivars, as about 52% of the 1999 U.S. cotton hectarage was planted to cultivars with Monsanto developed transgenes. The development of cultivars with output traits, where the value of the harvested crop is enhanced, has lagged efforts to introduce input traits into the cottonseed market. However, output traits such as enhanced fiber strength may ultimately prove more valuable to consumers, producers, and processors of cotton fiber.

The DP 50 plants with increased fiber strength in this study were found

after transformation by particle bombardment. Particle bombardment transformation attempted to insert a common GUS marker gene and a proprietary sequence driven by a fiber specific promoter. Unusual variation for fiber strength and GUS were noted in some small R2 and R3 greenhouse grown populations descendent from the original R0 transformed plant (M.E. John, unpublished data). The objectives of this study were (1) to assess inheritance of fiber strength under field conditions in descendents of the original transformed germplasm; and (2) to determine commercial potential of the germplasm with enhanced fiber strength.

MATERIALS AND METHODS

Specific details about the fiber strength gene inserted into DP 50 cannot be divulged in order to maintain confidentiality of Monsanto business information. We can state that as a result of Monsanto's molecular discovery program, DP 50 plants with increased fiber strength (about 25%) were recovered after one transformation event (M.E. John, unpublished data). Transformation was accomplished by particle bombardment of meristems (McCabe and Martinell, 1993), using the Monsanto ACCELL™ technology. Transformation attempted to insert a plasmid contained a GUS marker gene driven by the 35s CaMV promoter (Jefferson et al., 1987) and a proprietary gene driven by the E6 fiber specific promoter (John and Keller, 1996). A single GUS-positive R0 plant derived from one round of particle bombardment transformation set seed in the greenhouse to yield 23 GUS-positive R1 plants (M.E. John, unpublished data). No molecular data was collected from this R0 or subsequent R1 and derived generations as to the number of GUS or fiber strength gene loci introduced through transformation. One GUS-positive R1 plant with elevated fiber strength (compared with non-transformed DP 50) was advanced for R2 greenhouse testing. Similarly, a single GUS-positive R2 plant with elevated fiber strength was advanced for an R3 greenhouse trial. Unusual variation for fiber strength among the GUS-positive R2 and R3 progeny was noted, inconsistent with a single dominant gene genetic model for the fiber strength gene (M.E. John, unpublished data). These data prompted further studies of the inheritance of GUS and fiber strength under field conditions with more numerous parent-offspring relationships.

Under terms of a Cooperative Research and Development Agreement (No. 58-3K95-6-465), Monsanto supplied ARS with R4 seed derived from three GUS-positive R3 plants (designated M16, M17, and M18 along with populations derived thereof). The three GUS-positive R3 plants with elevated fiber strength that set the R4 seed for our trials were selected from the R3 greenhouse trials conducted by M.E. John and described above. The R4 and R5-through R6-derived M16, M17, and M18 populations were evaluated in

the field at the Pee Dee Research and Education Center, Florence, SC in 1996, 1997, and 1998, respectively. Non-transformed DP 50 served as the control in all experiments. Each year, seeds were hand planted on 0.3 m spacing within rows (rows spaced about 1 m apart) into soil prepared for cotton production according to Clemson University Cooperative Extension Service recommendations (Lege, 1995). The uniform 0.3 m spacing within rows facilitated GUS phenotypic analysis and manual self-pollination. In 1996, R4 plants within the M16, M17, and M18 populations were assayed for GUS expression prior to anthesis using a histochemical technique outlined by Jefferson et al. (1987). Briefly, leaf tissue samples from every plant were brought from the field and placed into tissue culture plates with 0.5 ml of GUS-buffered substrate. Plates were incubated at the prescribed 37°C overnight and blue colored tissue samples were scored as GUS-positive, while absence of blue staining was scored as GUS-negative. Most leaf tissue assays were further confirmed through GUS assays of pollen (some plants were infertile, producing no pollen which precluded reconfirmation of those leaf assay results). The other objective of GUS analyses of pollen was to assess putative GUS genotype based on visual assay of the amount of blue-stained pollen. Pollen assays were conducted on flowers that had been manually closed by paper clips to prevent introduction of foreign pollen by pollinating insects the day preceding anthesis until transported to the lab. Pollen from a flower on each plant was assayed for GUS activity using techniques described by McCabe and Martinell (1993).

In 1996 during the boll set period, every effort was made to enforce self-pollination of each R4 plant. The density of local populations of bees (*Bombus* spp.) is sufficiently high that they can effect cross-pollination of cotton, a confounding factor when examining parent-offspring relationships. Self-pollination entailed paper clip closure of flowers the day before anthesis followed by tagging of bolls after pollination. Prevention of cross-pollination of cotton by wire or paper clip closure of flowers the day before anthesis has been a common breeding procedure since the early 1900s (Brown, 1938). At maturity, bolls were harvested from R4 plants that produced fiber. Bolls from 25 DP 50 plants were harvested for the purpose of providing experimental control. Bolls were preferentially harvested from the middle to the upper portion of the plant fruiting zone, to minimize fruiting site as a factor in plant-to-plant variation in fiber strength (Lewis, 1996). Seedcotton was ginned by plant on a 10-saw laboratory model gin. Fiber samples were submitted to Starlab, Knoxville, TN for fiber strength analysis. Starlab measured fiber strength twice on each sample with the stelometer, using International Calibration Standards to calibrate the instrument. The two strength measurements for each plant were then averaged together to provide a data

point. Standard errors of M16, M17, and M18 population means were calculated.

The 1996 R4 plants were advanced to 1997 R5 progeny rows in a typical pedigree breeding system. Within M16, M17, and M18 germplasm, R4 plants with highest fiber strength were planted in 1997 as R5 progeny rows for the purpose of observing transmission of strength from parent to offspring. Several R4 plants appeared to produce little pollen, therefore affecting seed production. Thus, R5 progeny rows contained as few as two plants to over 600 plants. Seed was hand sown as in the 1996 R4 experiment. GUS assays were conducted as in 1996, except that many assays were conducted in the field using a simplified technique due to the large number of plants to be tested (ca. 5500). Microcentrifuge tubes were filled with 0.5 ml of GUS-buffered substrate and clipped to the plant, in the process immersing a piece of leaf tissue in the buffer. Tubes were allowed to incubate overnight at ambient temperatures and then read for presence of blue staining. Duplicate runs in tissue culture plates incubated at 37°C compared with GUS data from microcentrifuge tubes on several progeny rows produced identical results (May, unpublished data) and thus, we have confidence in the simplified GUS assay. GUS-positive R5 plants were self-pollinated in the same manner as that of 1996 R4 plants. At maturity, fiber strength was measured on GUS-positive plants and 10 DP 50 control plants in the same manner described for the 1996 R4 experiment. Some GUS-positive plants failed to produce sufficient fiber for strength analysis, therefore in Tables 1-3 we show the number of plants tested for fiber strength in addition to the number assayed for GUS expression. Unusual GUS segregation ratios in the 1997 R5 experiment (Tables 1-3), combined with the cost of fiber strength analysis ($4U.S. per sample), caused us to decide not to obtain fiber strength analysis on all R5 progeny rows. One M16, one M17, and four M18 R5 progeny rows were not assayed for fiber strength. We feel that this was a reasonable cost/benefit compromise in examining parent-offspring relationships, given the similarity in GUS expression among the M16, M17, and M18 R5 progeny rows. Lastly, GUS-positive and GUS-negative plants were harvested from one M16 and one M18 1997 R5 progeny rows for the purpose of observing co-segregation of GUS and fiber strength.

The 1998 experiment included only those R6 progeny derived from GUS-positive plants within M16 R5 progeny row #34, M17 R5 progeny row #30, and M18 progeny row #19. These R5 progeny rows were chosen for advance to the R6 experiment based on their high mean fiber strength and comparatively low plant-to-plant standard deviation for fiber strength. Despite the R4 and R5 experiments that showed inconsistent expression of fiber strength between parents and progeny, we conducted the R6 experiment on the basis of a hypothesis that stable introgression of an exxon introduced by such a

violent approach as particle bombardment might not occur for several generations. As in the R4 and R5 experiments, R6 seed was hand sown in the field. GUS assays were run in the field with microcentrifuge tubes as in the R5 experiment. Bolls were harvested only from GUS-positive plants within the two M16 #34 R6 progeny rows that produced more GUS-positive than GUS-negative plants (ca. 3:1 ratio of GUS-positive to GUS-negative plants). We felt that only these progeny rows had any promise as to consistent expression of enhanced fiber strength, because all other progeny rows produced more GUS-negative than GUS-positive plants. Fiber strength analysis was conducted as in the R4 and R5 experiments.

RESULTS AND DISCUSSION

The cultivar DP 50 transformed to express enhanced fiber strength in this study was once the most widely planted picker-type upland cotton in the USA (USDA-AMS, 1995). Despite the popularity of DP 50 with growers, it has low inherent fiber strength that limits its use to production of low strength cotton yarns, and textile products manufactured with such yarns. Indeed, the enhancement of its fiber strength would greatly increase the utility of DP 50 fiber for yarn and textile manufacturers and potential return to growers. Fiber strength of M16, M17, and M18 GUS-positive R4 plants was increased from about 30-75% over non-transformed DP 50 (Tables 1-3), confirming under field conditions that transformation or an associated effect on native genes has increased strength. Because we had no molecular evidence that the fiber strength gene was extant or functioning in the GUS-positive DP 50 germplasm, the enhancement of fiber strength could reflect some sense and/or antisense effect of the original transformation on native DP 50 genes. Based on GUS pollen expression, the M16, M17, and M18 R3 greenhouse plants that produced the R4 seed for the 1996 field experiment were putatively GUS homozygotes (M.E. John, unpublished data). However, variation for fiber strength was noted among their R4 GUS-positive progeny (Tables 1-3). For example, GUS-positive M16 R4 plants ranged in fiber strength from 232 kN m kg^{-1} to 314 kN m kg^{-1} (DP 50 average T1 = 171 $\pm$ 11 kN m kg^{-1}). Reasons for the apparent segregation in GUS-positive R4 plants derived from the three high strength R3 greenhouse parents are not known. Southern and Northern blot analyses of developing fiber might have indicated if the fiber strength gene was present and functioning, respectively, in the variable fiber strength GUS-positive plants, but, such analyses were not feasible given the number of plants in the field trial. We found three GUS-negative plants in the M16 population expressing higher fiber strength (ca. 25-35%) than the DP 50 controls (Table 1). The lack of GUS expression in these plants was confirmed with pollen assays (data not shown), so we feel that they were not mis-scored

TABLE 1. R4 and R5 segregation for fiber strength (T_1) under field conditions in M16 progeny derived from transformed 'Deltapine 50' evaluated in 1996 and 1997 field trials near Florence, SC.

		1996 R4 Generation		1997 R5 Generation				
Population	Plant #[a]	GUS	T_1	GUS		T_1 Segregation		
		Phenotype	kN m kg^{-1}	Segregation		GUS-POSITIVE Mean Range		Standard[b]
				+	−	---- kN m kg^{-1} -----		Deviation
M16	1	+	292	15	0	225 (12)	179-271	33
	5	+	243	----------		---	--------	--
	6	+	314	2	0	235 (2)	209-261	--
	7	+	260	205	120	211 (184)	152-282	16
	8	+	299	304	326	---	--------	--
	11	+	314	2	0	188 (2)	175-201	--
	12	+	298	11	0	206 (9)	162-269	35
	13	+	266	----------		---	--------	--
	21	+	317	21	1	236 (20)	180-304	31
	23	+	257	----------		---	--------	--
	34	+	301	276	302	253 (259)	181-300	16
	41	+	310	7	0	250 (7)	194-289	34
	44	+	298	2	0	---	--------	--
	47	−	232	----------		---	--------	--
	48	+	298	131	126	---	--------	--
	49	−	216	----------		---	--------	--
	52	+	232	----------		---	--------	--
	53	+	247	----------		---	--------	--
	54	+	238	----------		---	--------	--
	56	+	238	----------		---	--------	--
	63	+	244	----------		---	--------	--
	64	−	234	----------		---	--------	--
	67	+	240	----------		---	--------	--
	72	+	272	165	213	---	--------	--
	73	+	314	207	269	255 (202)	186-294	15
		SD	3.3					

1996 Deltapine 50 control T_1 = 171 ± 11 kN m kg^{-1}; 1997 average 192 ± 11 kN m kg^{-1}.
[a] Some R4 plants in 1996 trial failed to produce fiber, explaining gaps in sequence of plant numbers. Missing 1997 data (denoted by ----) reflects plant not advanced to a R5 progeny row.
[b] Standard deviation in 1997 fiber strength is indicative of amount of plant-to-plant variation in strength within a progeny row.
( ) indicates number of GUS-positive plants tested for fiber strength in 1997.

for GUS phenotype. It is possible that recombination rendered these plants transgenic at the fiber strength locus, but not the GUS locus, albeit with altered expression of the gene imparting enhanced fiber strength.

Examination of 17 R4-R5 parent-offspring relationships in 1997 similarly found inconsistent expression of enhanced fiber strength between parents and progeny. The M16 R4 plants 1, 6, 11, 12, 21, 41, 44, M17 R4 plant 1, and M18 plant 58 produced high strength and were apparent GUS homozygotes, but they produced R5 GUS-positive progeny with wide ranges in fiber strength (Tables 1-3). Typical of the variation in fiber strength observed, M16 plant 1 produced 15 R5 GUS-positive progeny that varied in strength from 179 to 271 kN m kg^{-1}. The remaining high strength M16, M17, and M18 R4

TABLE 2. R4 and R5 segregation for fiber strength (T_1) under field conditions in M17 progeny derived from transformed 'Deltapine 50' evaluated in 1996 and 1997 field trials near Florence, SC.

		1996 R4 Generation		1997 R5 Generation				
Population	Plant #[a]	GUS	T_1	GUS		T_1 Segregation		
		Phenotype	kN m kg^{-1}	Segregation		GUS-POSITIVE Mean Range		Standard[b]
				+	−	---- kN m kg^{-1} ----		Deviation
M17	1	+	306	25	1	230 (21)	174-286	34
	7	+	317	----------		---	-------	--
	15	+	265	130	104	213 (120)	163-251	13
	18	+	244	----------		---	-------	--
	25	+	314	100	133	256 (99)	221-302	15
	27	+	247	----------		---	-------	--
	29	+	240	----------		---	-------	--
	30	+	287	121	152	257 (118)	180-304	18
	32	+	246	----------		---	-------	--
	41	+	305	93	141	---	-------	--
		SD	30					

1996 Deltapine 50 control T_1 = 171 ± 11 kN m kg^{-1}; 1997 average 192 ± 11 kN m kg^{-1}.
[a] Some R4 plants in 1996 trial failed to produce fiber, explaining gaps in sequence of plant numbers. Missing 1997 data (denoted by ----) reflects plant not advanced to a R5 progeny row.
[b] Standard deviation in 1997 fiber strength is indicative of amount of plant-to-plant variation in strength within a progeny row.
( ) indicates number of GUS-positive plants tested for fiber strength in 1997.

plants were apparent GUS heterozygotes, but they also produced GUS-positive progeny with variable fiber strengths (Tables 1-3).

Some GUS-negative plants within R5 M16 progeny row 73 (n = 262, mean = 200 kN m kg^{-1} ± 11 kN m kg^{-1}, range 172-233 kN m kg^{-1}) and R5 M18 progeny row 55 (n = 153, mean = 206 kN m kg^{-1} ± 11 kN m kg^{-1}, range 178-237 kN m kg^{-1}) produced fiber strengths (about 230 kN m kg^{-1}) similar to that of some R5 GUS-positive plants within the same progeny rows. Without molecular evidence of genotype at the fiber strength and GUS loci, we do not know if the similarity in fiber strength of the GUS-positive and GUS-negative plants reflects recombination between the GUS and strength gene loci, or perhaps silencing of the GUS gene. Additional evidence that the elevated strength GUS-negative plants might have been transgenic at the strength locus was that none of the 59 DP 50 control plants evaluated from 1996-1998 produced a fiber strength about 230 kN m kg^{-1} (3-yr control DP 50 mean = 187 kN m kg^{-1} ± 10 kN m kg^{-1}). Therefore, a reasonable conclusion is that the GUS-negative plants expressing elevated fiber strength were transgenic at the strength locus, but that recombination has disassociated the GUS and strength gene loci. Alternatively, it is possible that the GUS gene has been silenced in these plants.

We considered that some portion of the variation in fiber strength observed among GUS-positive progeny of plants with elevated fiber strength might not

TABLE 3. R4 and R5 segregation for fiber strength (T_1) under field conditions in M18 progeny derived from transformed 'Deltapine 50' evaluated in 1996 and 1997 field trials near Florence, SC.

		1996 R4 Generation		1997 R5 Generation				
Population	Plant #[a]	GUS Phenotype	T_1 kN m kg^{-1}	GUS Segregation +	GUS Segregation −	T_1 Segregation GUS-POSITIVE Mean Range ---- kN m kg^{-1} ----		Standard[b] Deviation
M18	1	+	295	99	131	----------		--
	8	+	256	----------		----------		--
	9	+	296	114	128	----------		--
	12	+	299	106	139	----------		--
	14	+	260	----------		----------		--
	19	+	284	115	145	262 (110)	214-294	16
	23	+	245	----------		----------		--
	28	+	254	----------		----------		--
	30	+	314	105	158	----------		--
	34	+	228	----------		----------		--
	36	+	226	----------		----------		--
	39	+	256	----------		----------		--
	51	+	300	123	156	250 (121)	210-289	15
	55	+	306	132	152	256 (129)	217-295	16
	58	+	346	3	0	234 (3)	213-272	33
	59	+	277	----------		----------		--
	60	+	244	----------		----------		--
	71	+	242	----------		----------		--
		SD	3.3					

1996 Deltapine 50 control T_1 = 171 ± 11 kN m kg^{-1}; 1997 average 192 ± 11 kN m kg^{-1}.
[a] Some R4 plants in 1996 trial failed to produce fiber, explaining gaps in sequence of plant numbers. Missing 1997 data (denoted by ----) reflects plant not advanced to a R5 progeny row.
[b] Standard deviation in 1997 fiber strength is indicative of amount of plant-to-plant variation in strength within a progeny row.
( ) indicates number of GUS-positive plants tested for fiber strength in 1997.

be segregation. The variation could reflect instrument measurement error and/or where the fiber was borne on the plants. Considering the small plant-to-plant standard deviation (10 kN m kg^{-1} average over 3-yr) for fiber strength among control DP 50 plants, instrument measurement variation would seem too small to account for apparent fiber strength segregation among transgenic plants. Alternatively, it has been demonstrated that fiber properties vary by fruiting position within the cotton plant (Lewis, 1996); and as the inherent level of fiber strength increases, variability among fruiting zones within the plant increases proportionately. In a detailed study of fiber strength variation as affected by fruiting zone within DP 50, Lewis (1996) found that strength varied by up to 16 kN m kg^{-1} (fiber strength by high volume instrument measurement), but that in a higher strength cultivar strength varied as much as 60 kN m kg^{-1}. However, in that study about half of the range in fiber strength (ca. 30 kN m kg^{-1}) variation in the high strength cultivar was due to the low fiber strength of bolls harvested at fruiting sites borne low on the cotton plant. Our protocol for harvesting bolls

is to preferentially choose those borne in the middle to upper portion of the plants fruiting zone. According to Lewis (1996), this procedure should help minimize within plant variation for fiber strength and therefore, make it unlikely that apparent segregation is an artefact of harvesting bolls at different fruiting zones among plants within a progeny row.

After finding the segregation for fiber strength in the R4 and R5 experiments, we concentrated efforts in the R6 experiment on descendents of R5 plants in three progeny rows that expressed high mean strength and had comparatively low plant-to-plant standard deviations. This strategy is consistent with the finding by Sachs et al. (1998) that expression of a single-gene trait such as transgenic protein production to confer insect tolerance in cotton can exhibit variation similar to that of a quantitatively inherited trait. Consequently, expression and breeding value for the value-added trait might vary by the individual plant even in self-progeny of parents with desirable levels of expression. The R5:6 progeny rows derived from M16 R4 plant 34, M17 R4 plant 30, and M18 plant 19 produced about 40% GUS-positive progeny, compared with an expected 75% or more depending on the number of GUS loci extant in the R5 parents (Table 4). Only seven of the 81 R5:6 progeny rows we examined in 1998 produced more GUS-positive than GUS-negative plants (data not shown). Two M16 R5:6 progeny rows that produced about a 3:1 ratio of GUS-positive to GUS-negative plants segregated for fiber strength in a manner similar to that found in the R5 and R6 experiments (Table 5). Overall, selection within the transformed DP 50 germplasm was ineffective at identifying germplasm with the desired breeding value for enhanced fiber strength.

We found that GUS-positive plants produced either few progeny or an unusual ratio of GUS-positive to GUS-negative progeny upon self-pollination (Tables 1-3). Apparent R4 GUS-homozygotes produced fewer R5 progeny compared with putative R4 GUS-heterozygotes, and putative R4 GUS-

TABLE 4. Segregation for GUS marker gene summed over R5:6 progeny rows within M16, M17, M18 cotton populations evaluated in 1998 field experiment at Florence, SC.

	N	GUS Segregation	
		+	–
M16 34	50	149	251
M17 30	17	69	90
M18 19	14	69	96

N = number of R5:6 progeny rows examined for GUS expression in 1998. The R6 progeny rows in this table were derived by self-pollination of R5 plants within the indicated 1997 progeny row.

Gus segregation is GUS-positive and GUS-negative plants summed over all R6 progeny rows within each of the M16, M17, and M18.

TABLE 5. Fiber strength (T_1) segregation among GUS-positive R6 M16 progeny exhibiting mendelian segregation for GUS reporter gene when evaluated in the field in 1998 near Florence, SC.

Population	GUS Segregation	T_1		SD
	+/−	Mean	Range	
		---------- kN m kg^{-1} ----------		
M16 34-9	6:2	276	219-299	30
M16 34-144	7:2	265	227-297	29

34-9 and 34-144 refer to 1996 M16 R4 plant #34 grown as a R5 progeny row in the 1997 R5 experiment from which plants 9 and 144 produced mendelian segregation for GUS in 1998 R6 experiment.

Mean and range to fiber strength data from only GUS-positive plants. Deltapine 50 control average T_1 = 197 ± 8 kN m kg^{-1} (N = 24).

heterozygotes produced more R5 GUS-negative than GUS-positive progeny. Because we do not know how many GUS loci existed in these plants, we did not attempt to fit the data to a mendelian model. Additionally, we do not know whether these phenomenon reflect fitness of transgenic gametes or some post-fertilization event that affects germination or survival. No discernable pattern of reduced germination or altered survival was observed in the 1996 through 1998 experiments (data not shown), therefore we speculate that the reduced recovery of transgenic plants reflects fitness of transgenic gametes, in particular pollen because of its recalcitrance at transmitting chromosomal abnormalities (Endrizzi et al., 1984). The GUS-positive pollen may not germinate and/or grow through the style at the same rate as GUS-negative pollen, which could result in preferential fertilization of ovules by GUS-negative pollen. Additionally, we did observe in the R4 through R6 experiments plants with abnormal andreocium development that appeared to result in poor pollen production. These data combined with the reported difficulty in obtaining consistent expression of transgenic traits such as insect tolerance (Jenkins et al., 1997; Sachs et al., 1998), indicate that transgenic cotton breeding may require alternate strategies. In particular, increased population sizes may be necessary to recover sufficient frequency of transgenic types with the desired level of expression of the value-added trait that simultaneously allows selection for agronomic characteristics. Additionally, purity of seed at transgenic loci from cultivar increase generations produced under open pollination could be affected by pollen competition from non-transgenic sources. Finally, in a separate paper we describe the behavior of the GUS gene and enhanced fiber strength phenotype in our study as influenced by various genetic backgrounds derived from a forward crossing program.

CONCLUSIONS

Unstable expression of fiber strength in the transformed DP 50 germplasm precludes commercialization. However, the degree of fiber strength enhancement of a cultivar such as DP 50 with low inherent fiber strength argues for further efforts at engineering enhanced fiber properties. Results of our study suggest that transformation events imparting unfavorable inheritance of a marker and/or a value-added trait should be discarded early in the breeding process rather than expending resources to try to extract a true-breeding type. These resources could more effectively be directed at deriving additional germline transformants that generally occur at low frequency from particle bombardment. Finally, the reduced recovery of GUS-positive progeny in our study has implications for transgenic cotton breeding in general. Larger populations may need to be evaluated to select for the desired level of expression of the value-added trait plus agronomic characteristics.

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