Heritability of Fiber Strength in Genetically Engineered Cotton

O.L. May[1], T.J. Wofford[2], and M. E. John[3]
[1]USDA-ARS, , Florence; [2]Monsanto, St. Louis, MO; [3]Monsanto Agracetus Campus, Madison, WI3.

ABSTRACT

Fiber strength must be increased for cotton to remain competitive as a textile fiber. The findings of a 2-yr study of cotton genetically engineered to express increased fiber strength are reported. Particle bombardment transformation of 'Deltapine 50' was accomplished by Monsanto. In field trials, fiber strength was increased by 25-75% over the non-transformed Deltapine 50. However, inconsistent expression of strength may prevent commercialization of cotton germplasm derived from this transformation. These data may have general implications for transgenic breeding in that transformation events imparting inconsistent expression of the value added trait should be discarded early in the breeding process.

Introduction

Global competition in yarn and textile manufacture in the 1990s has engendered the switch from ring spinning to more productive open-end yarn manufacture. Modernization of spinning and textile manufacturing technologies necessitate fiber property improvement, particularly fiber strength, for the new technology to function efficiently. Open-end spun yarns have lower strength than similar size ring spun yarn (Deussen, 1992), but this reduced yarn strength can be ameliorated through better fiber strength.

Improving fiber strength by conventional breeding has been successful (May, 1998), but biotechnology coupled with breeding offers the possibility of more immediate improvement. Monsanto Company is a major player in incorporating value-added traits in cotton cultivars. 'Deltapine 50' (DP 50) plants with increased fiber strength were found after transformation by particle bombardment. Unusual segregation for fiber strength and GUS were noted in some small R_3 and R_4 populations descendent from the original R_0 plant. The objective of this study was to determine the heritability and expression of fiber strength under field conditions by examination of large numbers of parent-offspring relationships.

Materials and Methods

As a result of Monsanto's discovery program, DP 50 plants with increased fiber strength (about 25%) were discovered in the R_2, R_3, and R_4 generations after a single transformation event. Transformation was accomplished by particle bombardment of meristems (McCabe and Martinell, 1993), using the Monsanto proprietary ACCELL technology. The inserted plasmid contained a GUS (B-glucuronidase) marker gene (Jefferson *et al.*, 1987) and a proprietary gene driven by a fiber specific promoter. Unusual segregation for fiber strength was noted in the R_3 and R_4 generations (M.E. John, unpublished data), inconsistent with a single dominant gene genetic model for the GUS and proprietary gene loci. These data prompted further studies with increased population size and numerous parent-offspring relationships to assess heritability of fiber strength.

Populations derived from seed of three R_3 greenhouse plants derived from one transformation event (plants and derived populations designated M_{16}, M_{17}, and M_{18}) that expressed elevated fiber strength were evaluated in 1996, 1997, and 1998 at the Pee Dee Research and Education Centre, Florence, SC with DP 50 as the control. Seeds were planted at 0.3 m spacing into soil prepared for cotton production according to Clemson University Co-operative Extension Service recommendations (Lege *et al.*, 1996). Plants in the M_{16}, M_{17}, and M_{18} populations were assayed for GUS expression prior to anthesis using techniques in which leaf tissue samples from every plant were placed in tissue culture plates and 0.5 ml of GUS-buffered substrate was added. Plates were incubated overnight and tissue samples were rated for appearance of blue colour. Evidence of blue staining was taken to indicate GUS activity (McCabe and Martinell, 1993). Most leaf tissue assays were confirmed through GUS assays of pollen but some plants produced no pollen so there leaf assays could not be confirmed.

Seed from 1996 plants were planted in 1997 as progeny rows. Some plants in 1996 had difficulty setting seed so rows contained as few as two plants to as many as about 700 plants. In total, about 10,000

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plants were evaluated in 1997. GUS assays were conducted as in 1996, except that most assays were in the field, using a simplified technique of McCabe and Martinell (1993). 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 in the laboratory and with microcentrifuge tubes in the field on several progeny rows produced identical results (May, unpublished data); confirming the reliability of the simplified GUS assay.

Results and Discussion

Fiber strength of GUS + R_4 plants was increased from 25% to nearly 75% over the DP 50 controls (Table 1), further illustrating the more immediate crop improvement possible with biotechnology. Unfortunately, wide segregation for fiber strength was noted among GUS+ plants within the M_{16} population (due to space limitations, only findings from the M16 cotton population are discussed), indicating lack of fit of fiber strength segregation to a simple genetic model. GUS+ plants ranged in fiber strength from about 15 g/tex to as much as 30 g/tex (DP 50 average T_1=17.1 ±1.1 g/tex; Table 1). We have no explanation for the segregation in fiber strength among the GUS+ plants. Southern and Northern blot analysis might have indicated if the proprietary gene was present and functioning in all GUS+ plants, but such analyses were not available. We found three GUS- plants in the M16 population. The origin of the GUS- plants is unclear, as the R3 plant with elevated fiber strength from which M16 was derived was putatively a GUS homozygote (based on GUS expression in pollen; M.E. John, unpub. data). The three GUS- plants could reflect an inadvertent seed mixture, gene elimination, silencing, or some other cryptic molecular event.

Based on these unusual segregation data, our goal was then to further observe segregation for GUS and fiber strength among progeny (particularly GUS+ homozygotes) derived from selfing M_{16} plants, and those we derived from outcrossing GUS+ plants to various cultivars. The R_5 progeny of 1996 M_{16} plants exhibited wide segregation for fiber strength (Table 1), similar to what we observed in the R_4 generation. For example, M16 plant #1 was a high strength R_4 plant, and an apparent GUS+ homozygote, that produced 15 GUS+ progeny that segregated into a wide range (17.9-27.1 g/tex) of fiber strength. Other GUS+ apparent homozygotes such as plants 6, 11, 12, 21, 41, and 44 similarly segregated into a wide range of fiber strengths. Plants that were apparently GUS heterozygotes 7, 8, 34, and 73, also produced a wide range in fiber strength (strength data in Table 1 is only for the GUS+ plants). The data from M_{16} plant #73 GUS- plants (n=262, mean=20.0 +1.1 g/tex, range 17.2-23.3 g/tex) indicates that none produced a fiber strength near that of the high strength (>25 g/tex) GUS+ plants (suggesting that the GUS gene and strength gene have not been disassociated through recombination), and that the plant-to-plant standard deviation was of a similar small magnitude as that observed in DP 50. The R_4 and R_5 data from M_{16} populations suggest that it may not be possible to extract a true breeding genotype that consistently expresses increased strength.

Instrument measurement variation may be insufficiently large to explain strength segregation among GUS+ plants. Some of the fiber strength variation among these plants could reflect where the boll samples were taken from the plants (Lewis, 1996). In a detailed study of fiber strength variation as affected by fruiting zone within DP 50, Lewis (1996) found that strength as measured by HVI varied by up to 1.6 g/tex but that in a higher strength cultivar, strength varied as much as six g/tex. Experiments have not been conducted yet with a high strength transformed plant similar to Lewis (1996), thus we are not aware of how much variation exists within a transformed plant for fiber strength.

If a true breeding line cannot be isolated from the descendants of the original DP 50 transformant, the goal may be reached by observing transgene behaviour in different genetic backgrounds. The impact of genetic background on the efficacy of genetic engineering has been demonstrated (Jenkins *et al.*, 1997; Sachs *et al.*, 1998), rendering the colculsio based on these M16 data only preliminary. Sufficient numbers of populations containing GUS and the proprietary gene homozygotes have to be sampled to determine segregation for strength. Two F_1 populations of crosses with plant #34 and one F_1 population involving plant #48 gave data (high mean strength and low plant-to-plant standard deviation; data not shown) suggesting that fiber strength expression might be more stable in a different genetic background than the original transformant. F_2 progeny rows from these F_1 plants are being evaluated in the field.

Although no commercial product is imminent from this research, we are continuing breeding efforts to isolate a germplasm with stable expression of increased strength.

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