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The inheritance model for the fiberless trait in upland cotton (*Gossypium hirsutum* L.) line SL1-7-1: variation on a theme

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Abstract Segregating populations were developed to evaluate the inheritance of the fiberless seed phenotype of upland cotton (*Gossypium hirsutum* L.) line SL1-7-1. We report the inheritance of fuzzy, fuzzless and fiberless seed from crosses of SL1-7-1 with wildtype DP5690, Mexican fuzzless seed UA 3-3 (accession 143), Ballard fuzzless seed (accession 243), and MD17. Results from the F_1, F_2 and $F_{2:3}$ progeny derived from the SL1-7-1 X DP5690 indicated that the expression of the fiberless phenotype fit a three loci model with one locus being the dominant fuzzless seed allele N_1. The other two loci were tested to verify whether they were allelic to either recessive fuzzless seed alleles n_2 or n_3. Using the segregation ratios of the F_2 progeny derived from the 143 X SL1-7-1 cross and F_2-derived F_3 families from SL1-7-1 X DP5690 with fuzzy seed (lacked N_1), it is proposed that SL1-7-1 lacks the recessive n_2 allele, but contains the n_3 allele in the genotype of SL1-7-1. The third locus was previously not characterized and has been designated as fl_1 (fiberless), therefore, the genotype for the fiberless phenotype of SL1-7-1 is $N_1N_1fl_1fl_1n_3n_3$. Fiberless lines MD17 X SL1-7-1 were crossed to verify similarities in genotypes between line and the genotype model predictability. Various combinations of the homozygous and heterozygous expression of N_1, n_2, n_3 and fl_1 allele produced plants with lower lint percentages.

Keywords Fiber initiation · Fiberless seed · Fuzzless seed · *Gossypium hirsutum* · Inheritance · Naked seed

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Introduction

Cotton ovular fibers (trichomes) are classified as either lint or fuzz based on the timing of fiber initiation. Initiation of lint fiber begins at anthesis and continues for about two days, whereas, the much shorter fuzz fiber initiates approximately a week later (Stewart 1975). Fuzz and lint likely have identical, or nearly identical, cellular mechanisms involved in fiber initiation but for some unknown reason fuzz fiber development is arrested by the fuzzless seed alleles $N_{1\_}$ and n_2. Both N_1 or n_2n_2 fuzzless seed alleles produce cottonseeds which develop lint, but lack fuzz fibers (Endrizzi et al. 1985; Percy and Kohel 1999). Early work on the fuzzless seed alleles has been reviewed previously by Turley and Kloth (2002). We recently found that a homologous combination of these loci, $N_1N_1n_2n_2$, resulted in the complete elimination of both fuzz and lint fiber (fiberless phenotype; Turley 2002; Turley and Kloth 2002). Presently, the fiberless phenotype of three cotton lines have been associated with the expression

of the fuzzless seed loci, such as MD17 (Turley and Kloth 2002), XZ142w (Du et al. 2001; Zhang and Pan 1991) and L40 (Musaev and Abzalov 1972).

A direct genetic comparison of the inheritance models for the different fiberless lines is needed to establish common patterns between genotypes. Presently, the genotypes of four fiberless lines have been reported in the literature, MD17 fiberless ($N_1N_1n_2n_2n_3n_3$, Turley 2002; Turley and Kloth 2002), MCU5 fiberless (2 to 4 undetermined loci, Nandarajan and Rangasamy 1988; Peter et al. 1984), L40 ($Ft_1Ft_1Ft_2Ft_2Ft_cFt_cN_2N_2$, Musaev and Abzalov 1972) and two different genotypes for XZ142w ($n_1n_1n_2n_2li_3li_3li_4li_4$, Du et al. 2001; $n_2n_2li_3li_3$, Zhang and Pan 1991). With these genotypes, three new loci were proposed which interact with the expression of a fuzzless seed locus n_2 to form either a fuzzless or a fiberless seed phenotype. The n_3 locus interacts with the n_2 locus to produce a fuzzless seed (Turley and Kloth 2002) and the li_3li_3 and possibly the li_4li_4 loci interact with the n_2 locus to form fiberless seeds. No additional phenotypes have been reported for n_3, li_3 and li_4. Because, these loci interact to control fiber initiation, it is imperative to identify and characterize each locus and the type of interaction. It also becomes essential to genetically compare these fiberless lines which were developed in different parts of the world such as MD17 fiberless in the United States, MCU5 fiberless in India, L40 in Uzbekistan, and XZ142w in China, with other uncharacterized fiberless lines.

A fifth fiberless line, SL1-7-1, was developed in the United States and has been used in numerous physiological experiments which usually utilized the fiberless ovules as a control (no fiber), i.e., in 2D-PAGE comparisons (Turley and Ferguson 1996), in evaluating the role of sucrose synthase in fiber development (Ruan and Choury 1998; Ruan et al. 2000), and in microarray analyses (Wu et al. 2006). However, no information exists in the literature on the genotype of SL1-7-1 or how it was developed. Establishing an inheritance model of SL1-7-1 could help determine linkages between the other fiberless genotypes in cotton and give insights into the process of fiber initiation.

In this study, we determine the inheritance model for fiberless cotton line SL1-7-1. We will describe the inheritance pattern of SL1-7-1 with crosses to wildtype line DP5690, Mexican fuzzless seed UA 3-3 (line 143; Turley and Kloth 2002), Ballard fuzzless seed (line 243; Kearney and Harrison 1927) and MD17 fiberless (Turley 2002; Turley and Kloth 2002). Delta Pine 5690 (DP5690), 143 and 243 were the same test lines used in the inheritance study of MD17 fiberless (Turley and Kloth 2002). We will corroborate that a decrease in lint percent is associated with the accumulation of fuzzless and confederated alleles as was recently suggested by Turley et al. (2007) and propose a modification to the genotype of MD17. We will report a new locus which is designated fl_1 (fiberless 1) which is part of the fiberless genotype of SL1-7-1 along with the first evaluations between two fiberless lines, MD17 X SL1-7-1 and subsequent findings.

Materials and methods

Plant material

Five inbred lines, SL1-7-1 (PI 528807), DP5690 (PVP No. 9100116), 143 (PI 528543), 243 (PI 528610) and MD17 (PI 616493) along with resulting F_1, F_2, F_3 and BC_1 tests crosses were grown at Stoneville, MS. DP5690 was obtained from Delta and Pine Land, Inc. (Scott, MS). SL1-7-1, 143 and 243 were obtained from the National Collection of Cotton Germplasm (Percival 1987), grown in the field, verified for phenotype and the seed increased. MD17 fiberless was developed in Stoneville, MS (Turley 2002). Typically field plots were single row, five meters long and spaced 1.02 m apart. However, some F_3 populations were planted in four consecutive plots (23.4 m in length and spaced 1.02 m apart). Where applicable, plots were over-seeded and after the plants reached the first true leaf stage, seedlings were thinned to 6.5 plants m^{-2}. An exception for thinning occurred in the F_1 population of SL1-7-1 X DP5690 where plants were thinned to 3 plants m^{-2}. Weeds and insects were managed using standard agronomic practices for the Mississippi delta. All self pollination in the field was performed by completely covering the flower bud with muslin bags and closing with a draw string.

Analysis of the crosses, lint percent and outcrosses

The fuzzy, fuzzless and fiberless phenotypes were scored as described by Turley and Kloth (2002).

Plant phenotype was determined by examining seeds from open capsules at the first branch node between main stem nodes 7 through 10. Lint percent and outcrossing were measured by standard means. Lint percents were calculated by dividing the mass of lint ginned by the mass of seed cotton (total weight of lint and seed) and expressed as a percentage of the mass of seed cotton. Lint percentages were calculated for the parents and F_1 plants grown over three separate years with the mean and standard deviation being determined with Stats^{TM} software from Decision Analyst, Inc. Outcrossing of cotton was measured in the field as we previously reported (Turley and Kloth 2002).

Determining the inheritance pattern of SL1-7-1

This genetic study was conducted in both the field and greenhouse. The original cross of SL1-7-1 X DP5690 was produced in the greenhouse the winter of 1992-93. The F_1 generation was grown in the field in 1993 and in 1996, 300 F_2 plants were evaluated in the field. A second evaluation of 278 F_2 plants was performed in the field in 1999. Reciprocal crosses of DP5690 X SL1-7-1 were again made in the summer of 2000, self pollinated in the greenhouse and 352 F_2 plants were examined for fuzzy, fuzzless and fiberless seed. Tests of heterogeneity were performed between these populations. Chi square values were calculated to determine the best fit for all genetic models tested for these and all subsequent experiments. Population sizes were determined by the "Minimum family size required to test a genetic hypothesis for given levels of probability and for varying probabilities of failure (q) of observing a specified result" (Table 1; Hansen 1959). All population sizes in this manuscript fit well within these specified limits and in most cases family sizes exceeded the size suggested for Probability level of 0.05 observations (most met or exceeded Probability levels of 0.001).

Crosses to test for potential recessive fuzzless seed alleles in SL1-7-1

To test for recessive fuzzless seed alleles in SL1-7-1, seeds from 57 of the 70 fuzzy seed plants harvested from the F_2 progeny of the SL1-7-1 X DP5690 were planted in 23.4 m plots in 2000. The other 13 lines were not evaluated because they did not produce sufficient seed to plant the 23.4 m plots. Seed was collected from all fuzzless seed plants in the $F_{2:3}$ populations and the $F_{3:4}$ families were evaluated for segregation of fuzzy, fuzzless and fiberless seed. Additionally, 20 fuzzless F_3 seed plants from the $F_{2:3}$ populations were transplanted in the greenhouse (only five plants survived transplanting) and were crossed with DP5690 (pollen parent). The F_1 progeny from the crosses were evaluated. Crosses of 143 X SL1-7-1 and 243 X SL1-7-1 were made in the field and all plants in all crossing plots were sequentially numbered and individual crosses recorded on the tags attached to each cross for later verification of the phenotype of the parent plant at harvest. All F_1 plants from these crosses were self pollinated in the greenhouse and the F_2 progeny were grown in the field in the summer of 1997. Two hundred individual F_2 plants from the 143 X SL1-7-1 and 243 X SL1-7-1 F_1 seed were sequentially numbered, harvested and analyzed for seed fuzz. Seed from individually harvested F_2 plants were planted plant-to-row in 30 single row plots in 1997 and 50 single row plots in 1999.

Crossing fiberless lines MD17 X SL1-7-1

The MD17 X SL1-7-1 cross was performed in the greenhouse. The F_1 population was self pollinated in the greenhouse and the F_2 plants were consecutively numbered and harvested every two days in the fall. Seed were collected when the capsules were mature, preferably before completely opened (fiberless seed does not remain in the capsule after it opens). After all the samples were collected and combined, the phenotype was assessed for each plant.

Results

SL1-7-1 fits a three allele inheritance model

A summary of phenotypes and the expected and proposed genotypes of SL1-7-1, DP5690, 143, 243, MD17 and XZ142w are listed in Table 1. One hundred or more plants of each parental line (SL1-7-1, DP5690, 143, 243, MD17 and XZ142w) were scored at boll opening to insure uniformity of

Table 1 A summary of reported and proposed genotypes of DP5690, 143, 243 and MD17, XZ142w, and SL1-7-1

Variety	Accession no.	Genotype		Phenotype
		Reported[a]	Proposed	
DP5690	PVP9100116	$n_1n_1N_2N_2N_3N_3$	$n_1n_1N_2N_2Fl_1Fl_1N_3N_3$	Normal
143	PI528543	$n_1n_1n_2n_2n_3n_3$	$n_1n_1n_2n_2Fl_1Fl_1n_3n_3$	Fuzzless
243	PI528610	$N_1N_1N_2N_2n_3n_3$	$N_1N_1N_2N_2Fl_1Fl_1N_3N_3$	Fuzzless
MD17	PI616493	$N_1N_1n_2n_2n_3n_3$	$N_1N_1n_2n_2Fl_1Fl_1N_3N_3^{b}$	Fiberless
XZ142w	–	$n_1n_1n_2n_2li_3li_3$	$n_1n_1n_2n_2li_3li_3n_3n_3^{bc}$	Fiberless
SL1-7-1	PI528807	Unknown	$N_1N_1N_2N_2fl_1fl_1n_3n_3^{b}$	Fiberless

[a] Reported in Turley and Kloth (2002)

[b] The fiberless genotypes are $N_1N_1n_2n_2$ for MD17, $n_2n_2li_3li_3$ for XZ142w and $N_1N_1fl_1fl_1n_3n_3$ for SL1-7-1

[c] Line XZ142w is proposed to have n_3n_3 because it has fuzzless seed plants in the F2 progeny (Zhang and Pan 1991)

phenotype and correspondence between phenotypes and genotypes. Abbreviations for fuzzy seed coat (F), fuzzless seed coat (N) and fiberless seed coat (fls) are used in the text.

The F_2 progeny from the cross SL1-7-1 X DP5690 were used to determine the inheritance model for SL1-7-1. The second generation filial plants were grown and scored in three different planting seasons (1996, 1999 and 2002). Data was combined across years after a test of heterogeneity between years demonstrated the results were homogeneous. The observed phenotypic segregation ratios and inheritance models for the F_2 population data are listed in Table 2. We used previously known loci N_1, n_2 and n_3 to design and test various inheritance models for the SL1-7-1 genotype. We also preferred the designation of fl_1 and fl_2 (fiberless 1 and 2) for any new locus contributing to the expression of the fiberless phenotype, instead of using the *li* (lintless) designation. The *li* designation is problematic in that it indicates the lack of lint but is non-descriptive with regard to the presence or absence of fuzz. In Table 2, two identical trihybrid segregation ratios of 16(F):47(N):1(fls) fit the data. These two models are very similar, however, with the recessive locus n_3n_3 being replaced with fl_2fl_2. Neither of these models can be rejected at this point with the observed segregation ratio from the SL1-7-1 X DP5690 F_2 population.

SL 1-7-1 has the dominant N1 seed locus

The crosses SL1-7-1 X DP5690 and 243 X SL1-7-1 were used to demonstrated that SL1-7-1 was homozygous for the dominant fuzzless seed locus N_1. Ninety eight F_1 plants of SL1-7-1 X DP5690 were scored for fuzz and fiber phenotypes. The first proof for inheritance of the N_1 locus is that all F_1 plants produced fuzzless seed (Table 2). The F_2 progeny would be expected to show a phenotypic segregation of three fuzzless-seeded plants (fiberless seeded plants are pooled into this class) to one plant producing fuzzy seed. The F_2 plants from the cross SL1-7-1 X DP5690 showed a phenotypic segregation of 3(N + fls):1F (699N + fls:231F; Table 2). The second proof is demonstrated in the 243 X SL1-7-1 cross. As expected, seed from the entire F_1 (52 plants), F_2 (195 plants) and F_3 (1,842 plants) of the 243 X SL1-7-1 progeny were fuzzless. The seed of the F_2 and F_3 progeny were all fuzzless indicating that the dominant N_1 locus from SL1-7-1 and 243 were allelic.

Genetic tests for n_2 and n_3 in SL1-7-1

To test whether the SL1-7-1 line was homozygous for the n_2 and the n_3 loci, two hypotheses were tested. The first test is, if 143 and SL1-7-1 shared n_2 and n_3 loci, all F_2 progeny of the 143 X SL1-7-1 cross would have fuzzless seed (3N:1fls). Some 35 of the 195 plants in the F_2 population had fuzzy seed. The second test that the SL1-7-1 line was homozygous for the n_2 and the n_3 loci was to evaluate the 57 fuzzy-seeded plants from the F_2 progeny of SL1-7-1 X DP5690. Selecting the fuzzy seed progeny eliminated complications of expression of fuzzless seed due to the N_1 locus. If both n_2 and n_3 loci are present in SL1-

Table 2 Observe and expected segregation ratios and inheritance models for F_2 progeny[a] of the cross SL1-7-1 X DP5690

Cross, generation and inheritance models	F_2 segregation ratio[b]	X^2	P	Conditions
SL1-7-1 X DP5690[c]				
F_1 observed	0F:98N:0fls			
F_2 observed[d]	231F:680N:19fls	1.3968	0.49737	
Inheritance models of SL1-7-1				
$N_1N_1n_2n_2$	4F:11N:1fls[b]	28.9267	0.00000	No n_3 allele in either DP5690 or SL1-7-1. Genotype $N_1N_1n_2n_2$ is fiberless
$N_1N_1n_2n_2n_3n_3$	15F:45N:4fls	28.1561	0.00000	The n_3 allele only in SL1-7-1. Genotype $N_1N_1n_2n_2$ is fiberless
$N_1N_1fl_1fl_1n_3n_3$	16F:47N:1fls	1.3968	0.49737	The n_2 allele in SL1-7-1. Genotype $N_1N_1fl_1fl_1n_3n_3$ is fiberless
$N_1N_1fl_1fl_1fl_2fl_2$	16F:47N:1fls	1.3968	0.49737	No n_2 or n_3 alleles in SL1-7-1. Genotype $N_1N_1fl_1fl_1fl_2fl_2$ is fiberless
$N_1N_1fl_1fl_1fl_2fl_2n_3n_3$	64F:191N:1fls	65.2917	0.00000	Genotype $N_1N_1fl_1fl_1fl_2fl_2n_3n_3$ is fiberless

[a] Genotype $N_1\_$ and $n_2n_2n_3n_3$ produce fuzzless seed

[b] Abbreviations include: F for fuzz covered seed; N for fuzzless seed; and fls for fiberless

[c] The genotype of DP5690 is assumed to be homozygous for n_1, N_2, N_3, FL_1

[d] Populations totals are the sums of plants from multiple crossings and years

7-1, the F_2 fuzzy seed families should segregate into 7 families with progeny showing phenotypic ratios of 1(F):0(N), 4 families with phenotypic ratios of 15(F):1(N) and 4 families with phenotypic ratios of 3(F):1(N). Of these 57 families (5,624 total plants), 32 families segregated in a 1(F):0(N) and 19 segregated in a 15(F):1(N) ratio. No families fit the expected 3(F):1(N) segregation ratio. Six families (total of 58 plants) had fuzzless seed types, but did not fit the 15(F):1(N) ratio as determined by chi square. After extensive testing (including test crosses and scoring of $F_{3:4}$ families) we concluded that the fuzzless-seeded plants were the result of the introduction of the N_1 allele via out crossing at a rate of 1.03% and not a unique interaction of fuzzless and fiberless determining loci.

Another proof for the absence of the n_2 locus in SL1-7-1 results from the re-evaluation of a large F_2 population from MD17 X DP5690 which segregated 178F:522N:58fls. This ratio fit a 2 loci model with a ratio of 15F:45N:4fls with a $X^2_{15:45:4,\ 2df} = 2.6093$, $P = 0.27126$ (Table 2). This modifies an earlier model of the genotype by Turley and Kloth (2002). The new model for the MD17 genotype is $N_1N_1n_2n_2$ and indicates that if SL1-7-1 had both the N_1 and n_2 loci it should also segregate in a 15F:45N:4fls ratio. An evaluation of the F_2 population of the SL1-7-1 X DP5690 cross of 231F:680N:19fls would have a $X^2_{15:45:4,\ 2df} = 28.1561$, $P = 0.00000$. With the 35 fuzzy seeded plants listed above for the F_2 segregation of the 143 X SL1-7-1 cross and all fuzzless seeded plants in the $F_{2:3}$ populations from the SL1-7-1 X DP5690 cross shown to be the result from outcrossing, SL1-7-1 lacks the n_2 locus. See Table 2 for other genotypes tested for SL1-7-1.

With the absence of the n_2 allele verified our next objective was to test for the presence of n_3 in the F_2 progeny of the 143 X SL1-7-1 cross (Table 3). Consequently, models which included the n_3 locus in the expression of the fiberless phenotype fit the observed ratios of 12F:45N:7fls and 12F:42N:10fls (Table 3). Backcross populations were used to confirm that n_3 is a shared locus between line 143 and SL1-7-1. The F_1 X SL1-7-1 resulted in a 98(N):41(fls) plants with a $X^2_{6:2,\ 1df} = 1.4988$, $P = 0.22086$ for the segregation of three loci (N_1, n_2 and fl_1) with n_3 as a shared locus, and $X^2_{28:4,\ 1df} = 36.7122$, $P = 0.00000$ for five loci (N_1, n_2, fl_1, n_3 and fl_2) with fl_2 being the third allele responsible for the fiberless phenotype in SL1-7-1. The F_1 X 143 resulted in 43(F):126(N) plants which would segregate 3N:1F with a $X^2_{6:2,\ 1df} = 0.0178$, $P = 0.89401$ for the segregation of three loci (N_1, n_2 and fl_1) and a $X^2_{20:12,\ 1df} = 10.4809$, $P = 0.00121$ for five loci (N_1, n_2, fl_1, n_3 and fl_2). These data indicate that n_3 is a shared locus between line 143 and SL1-7-1 and is involved in both the development of the fuzzless phenotype in line 143 (Turley and Kloth 2002) and in

Table 3 Observed and expected segregation ratios and inheritance models for F_2 progeny[a] of the cross 143 X SL1-7-1

Cross, generation and inheritance models	F_2 segregation ratio[b]	X^2	P	Conditions
143 X SL1-7-1				
F_1 observed	0F:48N:0fls			
F_2 observed	35F:134N:26fls			
Inheritance models of SL1-7-1				
$N_1N_1fl_1fl_1$ n_2n_2	12F:36N:16fls	16.0724	0.00032	143 and SL1-7-1 share n_2, but not n_3. Genotypes $N_1N_1fl_1fl_1n_3n_3$ and $N_1N_1n_2n_2$ are fiberless
$N_1N_1n_2n_2$	9F:27N:28fls	75.8647	0.00000	143 and SL1-7-1 share n_2, but not n_3 and the fl_1 is the same allele as li_3li_3 allele reported by Zhang and Pan (1991). Genotypes $N_1N_1fl_1fl_1n_3n_3$, $N_1N_1n_2n_2$ and $n_2n_2li_3li_3$ are fiberless
$N_1N_1fl_1fl_1n_3n_3$	12F:45N:7fls	1.1607	0.55972	If n_3 is a shared fiberless locus and fl_1 and li_3 are not the same locus in SL1-7-1. Genotypes $N_1N_1n_2n_2$ and $N_1N_1fl_1fl_1n_3n_3$ are fiberless
$N_1N_1fl_1fl_1n_3n_3$	12F:42N:10fls	1.0064	0.60458	If n_3 is a shared fiberless locus in SL1-7-1 and fl_1fl_1 is identical to the li_3li_3 allele. The fiberless genotypes are $N_1N_1n_2n_2$, $N_1N_1li_3li_3n_3n_3$ and $n_2n_2li_3li_3$
$N_1N_1fl_1fl_1fl_2fl_2$	240F:708N:76fls	11.6930	0.00289	If n_3 is unique to 143 and $N_1N_1n_2n_2$, $N_1N_1fl_1fl_1n_3n_3$ and n_2n_2 $fl_1fl_1n_3n_3$ are fiberless
$N_1N_1fl_1fl_1fl_2fl_2n_3n_3$	238F:698N:88fls	7.4569	0.02403	If n_3 is unique to 143 and $N_1N_1n_2n_2$, $N_1N_1fl_1fl_1n_3n_3$ and n_2n_2 $fl_1fl_1n_3n_3$ are fiberless and n_2n_2 $fl_1fl_1n_3n_3$ are fiberless

[a] Genotype $N_1\_$ and $n_2n_2n_3n_3$ produce fuzzless seed

[b] Abbreviations include: F for fuzz covered seed; N for fuzzless seed; and fls for fiberless

[c] The genotype of 143 has been reported to be $n_2n_2n_3n_3$ by Turley and Kloth (2002)

the development of the fiberless phenotype in line SL1-7-1.

The fl_1 locus is in SL1-7-1

The MD 17 line was derived from a 243 X 143 cross and determined to have the genotype $N_1N_1n_2n_2N_3N_3$ (Table 1). A cross between the fiberless lines MD17 X SL1-7-1 resulted in an F_2 population consisting of 136(N):49(fls) plants. Using the genotypes of $N_1N_1n_2n_2N_3N_3$ and $N_1N_1fl_1fl_1n_3n_3$ for the expression fiberless seeds, the F_2 progeny would segregate in a phenotypic ratio of 45N:19fls (Table 4). The deviation from this model was not significant (X^2, 1df = 0.981) and, therefore, the results are consistent with the segregation of three loci (n_2, fl_1 and n_3) between the MD 17 and SL 1-7-1 lines. Other genotypic conditions which result in differing segregation ratios are evaluated in Table 4.

It is possible that the fl_1 could be equivalent to the li_3 locus. The only genetic test performed in this manuscript is the cross between 143 X SL1-7-1 (Table 3). If fl_1 and li_3 were the same locus, the segregation of the F_2 progeny would have a $X^2_{12:42:10,\ 2df} = 1.0064$, P = 0.60458, and a $X^2_{12:45:7,\ 2df} = 1.1607$, P = 0.55972 if they were not the same locus. The population is not large enough to use as a proof one way or another. Future crosses of line 143 with the fuzzy F_2 progeny of SL1-7-1 X DP5690 would allow a preliminary proof whether fl_1 and li_3 are the same locus or different. Any fiberless plants produced in the F_2 progeny of the 143 cross would then be backcrossed onto SL1-7-1 and XZ142w. Table 5 is a comparison of the deduced genotype of each parental line and the resultant F1 progeny from parental crosses with their measured lint percentages. Table 5 illustrates that the increase in fiberless and fuzzless seed alleles fl_1, n_2, N_1 and n_3 in the respective genotypes produced plants with much lower lint percentages. When one fuzzless seed locus was homozygous and the other heterozygous, lint percentages dropped dramatically below 10% as in the F_1 population of 143 X MD17, 243 X MD17 and MD17 X SL1-7-1 (Table 5). This should come as no surprise, as the homozygous combinations of N_1 or n_2 resulted in

Table 4 Observe and expected segregation ratios and inheritance models for F_2 progeny[a] of the cross MD17 X SL1-7-1

Cross, generation and inheritance models	F_2 segregation ratio[b]	X^2	P	Conditions
MD17 X SL1-7-1[c]				
F_1 observed	58N:0fls	1N:0fls		
F_2 observed	136N:49fls			
Inheritance model for MD17				
$N_1N_1n_2n_2n_3n_3$	9N:7fls	22.4042	0.00000	Shared N_1 and n_3 with segregating n_2 and fl_1 loci. Genotype $N_1N_1n_2n_2$ and $N_1N_1fl_1fl_1n_3n_3$ are fiberless
$N_1N_1n_2n_2N_3N_3$	48N:16fls	0.2180	0.64055	Shared N_1 with segregating n_3, n_2, and fl_1 loci. Genotype $N_1N_1n_2n_2$ is fiberless
$N_1N_1n_2n_2N_3N_3$	45N:19fls	0.9081	0.34062	Shared N_1 with segregating n_3, n_2, and fl_1 loci. Genotype $N_1N_1n_2n_2$ and $N_1N_1fl_1fl_1n_3n_3$ are fiberless

[a] Genotype $N_1\_$ and $n_2n_2n_3n_3$ produce fuzzless seed

[b] Abbreviations include: F for fuzz covered seed; N for fuzzless seed; and fls for fiberless

[c] The genotype of SL1-7-1 is proposed to be assumed $N_1N_1fl_1fl_1n_3n_3$

Table 5 A list of varieties or crosses, expression of alleles N_1, n_2, fl_1 or n_3, and lint percentages

Variety or cross	Generation	Fiberless seed genotypes		Lint percent
		Homozygous	Heterozygous	
DP5690	Parent	–	–	38.7 ± 0.7
143	Parent	n_2n_3	–	23.3 ± 0.5
243	Parent	N_1	–	12.3 ± 1.2
MD17	Parent	N_1n_2	–	0.00 ± 0.0
SL1-7-1	Parent	$N_1fl_1n_3$	–	0.00 ± 0.0
DP5690 X 143	F_1	–	n_2n_3	33.2 ± 3.1
DP5690 X 243	F_1	–	N_1n_3	32.4 ± 1.2
DP5690 X MD17	F_1	–	N_1n_2	27.4 ± 1.3
DP5690 X SL1-7-1	F_1	–	$N_1fl_1n_3$	29.0 ± 1.4
143 X 243	F_1	–	$N_1n_2n_3$	20.5 ± 0.7
143 X SL1-7-1	F_1	n_3	$N_1n_2fl_1$	23.6 ± 3.4
243 X SL1-7-1	F_1	N_1	fl_1n_3	12.9 ± 2.4
143 X MD17	F_1	n_2	N_1n_3	7.1 ± 2.9
243 X MD17	F_1	N_1	n_2	4.7 ± 2.4
MD17 X SL1-7-1	F_1	N_1	$n_2fl_1n_3$	2.7 ± 2.0

Lint percentages were calculated with 3 or more reps over multiple years. Lint percent is expressed as the percent of fiber mass of total seed cotton mass (fiber and seed mass)

fiberless phenotype of MD17 and N_1, fl_1 and n_3 resulted in fiberless phenotype of SL1-7-1. Variation in the lint percentages was also observed in 14 N_1 lines recently evaluated by Turley et al. (2007). The lint percents of these 14 lines ranged from 0.7 to 23.6% and were likely the result of additional deleterious or enhancing loci which interact with the N_1.

Discussion

The inheritance model of SL1-7-1 ($N_1N_1fl_1fl_1n_3n_3$) is the sixth model presented in the literature for a fiberless cottonseed (Du et al. 2001; Musaev and Abzalov 1972; Nadarajan and Rangasamy 1988; Turley and Kloth 2002; Zhang and Pan 1991). The

genetic analyses reported herein indicate that three loci were involved in the expression of the fiberless phenotype in SL1-7-1 (Table 2). The F_1 and F_2 progenies of SL1-7-1 X DP5690 and 243 X DP5690 indicated that the dominant fuzzless seed mutant N_1 was experimentally determined to be one of the three loci responsible for the fiberless phenotype of SL1-7-1 (Table 2). The second locus was found to be shared between lines 143 and SL1-7-1 and eventually deduced to be n_3 (Table 3). After reviewing all the test crosses reported in Tables 2 through 4, the third locus appears to be a unique locus which has not been reported in the literature. We have designated this new locus as fl_1 (fiberless 1). Consequently, we propose the genotype of SL1-7-1 to be $N_1N_1fl_1fl_1n_3n_3$ which results in the complete inhibition of fiber initiation and development on cotton ovules. At this point, no known phenotypes exists for the solitary, homozygous expression of li_3li_3, fl_1fl_1 and n_3n_3. However, when these loci are combined with either N_1N_1 or n_2n_2 they remove all fuzz or fiber on the cotton ovule.

With the reported genotype of SL1-7-1, we have demonstrated common connections which link the genotypes of four of the five fiberless lines (Du et al. 2001; Musaev and Abzalov 1972; Nadarajan and Rangasamy 1988; Turley and Kloth 2002; Zhang and Pan 1991). Figure 1 was designed to show a genotypic theme which is emerging from a global effort to understand the genetics of fiber initiation. The fiberless lines SL1-7-1, MD17 and XZ142w share at least one of the fuzzless seed alleles N_1 or n_2 (Fig. 1). These relationships becomes more intriguing when considering that N_1 and n_2 may be homoeologous loci on the homeologous chromosome 12 and 26 (Endrizzi and Ramsay 1980; Samora et al. 1994). A recent genetic map, however, reports that the n_2 locus was not located on chromosome 26, but on chromosome 12 (Rong et al. 2005). However, Rong et al. (2005) report that the use of Pima S7 may nullify this finding if the Pima S7 shared the n_2 locus with their n_2 test line. They postulated, if both lines shared the n_2 locus, that they have likely identified the location and effects of a new mutant in fiber analysis (Rong et al. 2005). Turley and Kloth (2002) reported that the expression of the recessive fuzzless seed trait was dependent on two loci, n_2 and n_3. It is possible that they mapped the n_3 locus, however, further work is needed for verification.

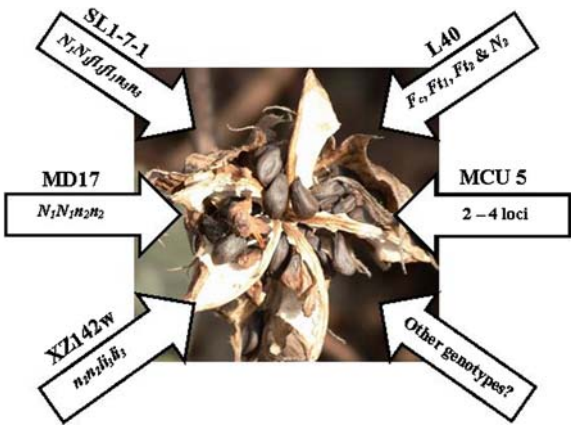

Fig. 1 Diagram of the genotypes of different fiberless lines of cotton. The expression of the fiberless phenotype of SL1-7-1, MD17 fiberless, XZ142w and likely L40 involves at least one of the fuzzless seed alleles, N_1 or n_2. Any connections which may exist between MCU 5 fiberless and the other three fiberless lines has not been identified

Conflicting results have been reported for the genotype of XZ142w with Zhang and Pan (1991) proposing the $n_2n_2li_3li_3$ model and Du et al. (2001) proposing the $n_1n_1n_2n_2li_3li_3li_4li_4$ model. Du et al. (2001) also proposed a change in the nomenclature and functions of fuzzless seed alleles, N_1 and n_2, as originally described by Kohel (1973). This change included the complete elimination of the recessive allele n_2. This modification is proposed despite the fact that both N_1 and n_2 loci have been well characterized genetically and mapped to chromosomes (Endrizzi et al. 1985; Samora et al. 1994; Rong et al. 2005). After preliminary studies with F_2 populations of XZ142w X DP5690, we accept the genotype model reported by Zhang and Pan (1991) and is therefore used in the Table 1 and Fig. 1.

Other genotypes may also program a fiberless phenotype. One of the five reported fiberless lines MCU 5 has a complete lack of the fuzzless seed in either the F_1 or F_2 progeny (Fig. 1, Nadarajan and Rangasamy 1988). The F_2 population from a cross of MCU 5 fiberless with a normal fiber producing line resulted in a F_2 population consisting entirely of either fuzzy-linted or fiberless plants. No plants with lint and fuzzless seed were reported (Nandarajan and Rangasamy 1988). The absence of the fuzzless seed phenotype in the data from Nandarajan and Rangasamy (1988) was a major deviation from our results and other reports on the genetics of the fuzzless seed

alleles. One possible explanation could be that the MCU 5 line has either the n_2 or n_3 locus along with other loci reported and therefore do not have the fuzzless phenotype. The crossing MCU 5 with common fuzzless seed and fiberless lines and evaluation of the F_1 and F_2 progeny would allow this to be determined. Further research is in progress to identify new genotypes that express the fiberless phenotype.

The biological role for the fuzzless seed alleles in fiber production cannot be described at this time. However, it is documented that N_1 eliminates all fuzz fiber and has a greatly reduced percentage of lint (Kearney and Harrison 1927; Ware 1940). Similarly, the recessive n_2 allele eliminates fuzz fiber formation and has lower lint percentages (Turley and Kloth 2002). The lower lint percent of the recessive fuzzless seed line has not been shown to be the result of the n_2 allele. However, the heterozygous and homozygous expression of N_1, n_2 and fl_1 alleles, as shown in Table 5, likely reduce lint percentage. In homozygous combination they completely eliminate all fiber implicating their important role in fiber initiation (Turley and Kloth 2002).

An effort is now being made to identify all the loci responsible for the fiberless seed phenotype and to develop near iso-genic lines possessing these alleles. These lines will be extremely advantageous in identifying and verifying specific genes associated with the different loci. The fuzzless seed phenotype allows us to visually follow these alleles through different crosses, facilitating a correlation with gene/protein expression. Further work is in progress to elucidate the genotypic similarities and differences between the fiberless lines XZ142w, MD17 and SL1-7-1. Included in this study will be the evaluation of various new possibilities for the fiberless genotypes, $N_1N_1li_3li_3$, $n_2n_2fl_1fl_1n_3n_3$ and others. Identifying these genotypes is essential to propose correct models which explain segregation patterns for all fiberless X fiberless crosses. With the identification of new loci with apparently no atypical phenotype, the genetics become more complex to decipher.

An emphasis in cotton research has been placed on identifying genes during fiber development in fuzzless seed and fiberless lines. Various groups have included both fuzzless seed and fiberless lines in microarray studies (Lee et al. 2002, 2006; Shi et al. 2006; Taliercio and Boykin 2007; Wu et al. 2006). Identifying the genes associated with each locus is a prerequisite to molecularly improve fiber quality. By identifying these loci and associated genes, we will have insights into the biology of fiber initiation, better fiber quality and increasing lint percentage. Lint percentage is a major component of fiber yield. In recent years cotton yields have reached a plateau, stagnating both the growth and development of the cotton industry in the United States. Any improvements in lint percentage could, theoretically, increase yields of cotton fiber production.

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