

Genetic Effects of High Fibre Strength Breeding Lines in Crosses with Transgenic Bt Cotton Cultivars (*Gossypium hirsutum* L.)

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Abstract

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Evaluation of genetic effects in cotton (*Gossypium hirsutum* L.) high fibre strength lines is necessary for the genetic improvement of fibre properties. Six breeding lines with high fibre strength and five transgenic Bt cultivars were diallely crossed. Fibre properties and agronomic traits of 11 parents and resultant 30 F₁ hybrids were analyzed by an additive-dominant model with genotype by environment interaction effects. Lint percentage, seed index, fibre length, strength and micronaire were primarily controlled by additive effects. Lint yield was mainly governed by dominance effects. Boll size was equally influenced by additive and dominance effects. A9-1 was a desirable general combiner for lint percentage, micronaire and fibre length. Yumian1 and Jinxing2 were good general combiners for fibre strength. Gk22 was a desirable general combiner for lint yield and boll size but poor for fibre length and strength. Some F₁ hybrids were identified with favourable heterozygous dominant effects for lint yield and various fibre properties. This study revealed that current transgenic Bt cotton cultivars can be improved in fibre quality and lint yield by using some of the lines with high fibre strength in crosses with them.

Keywords: additive dominant model; fibre quality; heterosis; lint yield

Gossypium barbadense L. and *Gossypium hirsutum* L. (Upland) are two planted tetraploid species of cotton with the latter accounting for the majority of world production. Upland cotton has high lint productivity and broad adaptation, while *G. barbadense* exhibits superior fibre properties of length, fineness, and strength. High lint yield is always given a top priority in breeding, to achieve a desirable profitability for cotton growers. Meanwhile, the rapid advances in spinning technology in textile industry require increasingly higher fibre quality for upland cotton. However, no upland cultivar shows the same fibre properties as modern *G. barbadense* cultivars. Developing high yielding upland cotton varieties with enhanced fibre quality is a primary breeding goal for most upland cotton breeding.

Diverse spinning technologies need diverse types of fibre to produce an optimal yarn. High strength

fibres are appropriate for high-speed yarn spinning technology, specifically open-end rotor spinning systems. In comparison with conventional ring spinning, the production capacity of open-end rotor spinning is five times greater and, thus, more economical (BRADOW & DAVIDONIS 2010). Nevertheless, the rotor spinning yarn is 15 to 20% weaker than ring spinning yarn of the same thickness (BRADOW & DAVIDONIS 2010). Thus, open-end rotor spinning requires cotton fibres with greater fibre strength to overcome the intrinsic decrease in yarn strength and to support higher processing speed. On the other hand, fibre length and length uniformity are regarded as the most important fibre quality parameters for ring spinning system (SMITH & ZHU 1999). Most cultivars with high fibre strength have fibre properties desired by the textile industry, but their yield does not satisfy the growers. Currently, transgenic Bt cotton is the

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most widely cultivated cotton in China, accounting for 93% of the national production in 2014 (Science & Technology Daily 2015), but many Bt cotton cultivars have less desirable fibre properties (LIU *et al.* 2008). It is therefore important to explore ways to transfer superior fibre genes from high fibre strength lines to current transgenic Bt cotton cultivars. Our work is aimed at the development of breeding lines with high fibre strength. These lines bred by ourselves have acceptable agronomic performance. Their genetic combining ability with high yielding Bt cotton cultivars has not yet been investigated, but would be helpful for genetic improvement of fibre quality in transgenic Bt cotton.

We crossed four breeding lines and two cultivars with high fibre strength with five transgenic Bt cultivars using line \times tester mating system. The 30 F_1 hybrids and 11 parents were evaluated in successive two years for yield, yield components and fibre traits. The major objectives of this research was to (i) evaluate variance components and genetic effects to identify which parents can be used as good combiners for enhancing cotton fibre quality and yield in breeding projects; (ii) to determine the best-performing crosses of parents with increased fibre quality and lint yield.

MATERIAL AND METHODS

Plant material. Male parents were six high fibre strength breeding lines and cultivars including 2870, Yumian1, Jinxing2, A801-5, AX and A9-1. Yumian1 and Jinxing2 are two commercial cultivars and the other four breeding lines were bred by Jiangxi Agriculture University (JXAU), Nanchang, China. Female parents were five transgenic Bt cotton cultivars. 33B and 99B are two alien Bt cotton cultivars. Y22, 9007 and GK22 are three domestic Bt cotton cultivars. These genotypes represent a range of fibre properties and yield components.

Experimental design. Six females were crossed with five males to form 30 crosses in the summer of 2006 and 2007 following line \times tester mating system. The 30 F_1 and 11 parents were planted at the experimental station of JXAU, Nanchang during the growing seasons (April–November) of 2007 and 2008. Seeds were sown in fertile-soil cubes on 28 March in 2007 and 18 April in 2008, and raised in a warm room covered by a plastic film. Seedlings were transplanted to the field on 7 May in two years. The experimental design was a randomized complete

block with three replications. Plots consisted of single 9.3 m rows spaced 1.05 m. Plants within the row were spaced 0.45 m. Field management followed the local conventional techniques.

Trait measurements. A 50-boll sample was harvested by hand from each plot, which was composed of first or second position bolls from approximately the middle five fruiting branches. These samples were ginned using a laboratory roller gin and used to determine lint percentage (LP), boll size (BS) and seed index (SI). Cotton in each plot was hand-harvested four times at 3-weeks intervals. Lint yield (LY) was determined after ginning and expressed as kilogram per hectare (kg/ha). Twenty grams of lint from each boll sample was submitted to the Supervision Inspection and Testing Center of Cotton Quality, Ministry of Agriculture, Anyang, Henan, for high volume instrument (HVI) fibre analyses. Fibre quality data determined by HVI testing included upper half mean (UHM) fibre length, fibre bundle strength (Str), micronaire (Mic), length uniformity index (UI) and elongation at break (El).

Genetic model and statistical methods. Analysis of variances (ANOVA) was performed first on the two years data set for four agronomic traits and five fibre properties using the general linear model (GLM) procedure of SPSS 10.0 software. Mean separation among the 11 parents was conducted using Fisher's LSD test at a probability level of 0.05. Means of the 11 parents were compared to those of F_1 using *t*-tests. Mean squares for genotype effects were greater than mean squares for genotype \times environment interactions for all traits except El (data not shown), thus means for parents and hybrids are reported across 2-year.

Data analysis was conducted by an additive-dominance (AD) genetic model with genotype by environment interaction effects (ZHU 1998). Variance components were estimated using a mixed model, minimum norm quadratic unbiased estimation (MINQUE) approach (RAO 1971; WU *et al.* 2010a). Genetic effects were estimated by an adjusted unbiased prediction (AUP) (ZHU 1993). Jackknifing over blocks within environments was employed for calculating standard errors of variance components and genetic effects (MILLER 1974; WU *et al.* 2008). The significance of each parameter was evaluated using an approximate *t*-test.

RESULTS

Phenotypic means for parents. Females generally had higher fibre quality and lower lint yield than males (Table 1). A9-1 performed best in fibre quality

Table 1. Means of yield and fibre traits in the parents in 2007 and 2008

Genotypes	BS (g)	LP (%)	SI (g)	LY (kg/ha)	UHM (mm)	UI (%)	Mic	El (%)	Str (cN/tex)
2870	4.5	39.9	11.0	1 030.8	30.0	85.3	4.7	6.5	33.0
Yumian1	3.5	38.0	11.7	1 114.9	30.8	85.2	4.1	6.5	38.6
Jinxiang2	4.2	40.9	10.7	639.4	30.5	85.9	4.5	6.6	37.9
A801-5	4.2	37.1	13.3	1 283.9	30.6	86.0	5.6	6.5	33.9
AX	3.4	39.4	11.7	1 135.7	31.1	86.2	5.0	6.5	35.4
A9-1	3.9	43.4	9.8	921.4	32.3	85.2	3.6	6.4	33.3
Y22	4.2	41.0	10.2	1 128.1	29.4	85.1	5.3	6.5	29.8
9007	5.6	38.8	10.2	1 212.4	29.8	85.2	5.1	6.4	30.5
33B	4.2	40.7	10.2	1 138.7	30.2	84.6	4.5	6.6	31.6
99B	4.6	36.2	13.6	626.4	31.5	85.9	3.9	6.6	35.5
GK22	5.2	38.9	10.4	1 357.7	29.1	84.9	4.8	6.5	29.7
LSD _{0.05}	0.7	2.4	0.9	362.6	1.1	1.0	0.3	0.1	2.0
Average									
F ₁ ^a	4.6*	39.9	11.0	1 380.4*	30.5	85.6	4.7	6.5	33.2
Parents	4.3	39.5	11.2	1 053.6	30.5	85.4	4.6	6.5	33.5

BS – boll size; LP – lint percentage; SI – seed index; LY – lint yield; UHM – upper half mean fibre length; UI – uniformity index; Mic – fibre micronaire; El – fibre elongation; Str – fibre strength; *significant at ≤ 0.05 ; ^a*t*-tests were made between the mean values of the parents and the mean values of the F₁ hybrids

averaging 32.3 mm for UHM, 33.3 cN/tex for Str and 3.6 for Mic across both years. Moreover, the highest LP was recorded in A9-1. Yumian1 and Jinxiang2 had the strongest fibres among 11 parents (38.6 and 37.9 cN/tex, respectively), and finer fibres within a premium range of 4.1 to 4.5 for Mic. AX had desirable fibre length and strength at 31.1 mm and 35.4 cN/tex, respectively, but poor fibre fineness with Mic at 5.0. GK22 ranked first in lint yield at 1357.7 kg/ha, and produced heavier bolls than all parents except 9007. The means of F₁ hybrids were greater than those of parents for BS and LY, indicating positive mid-parent heterosis for these traits in some F₁ hybrids (Table 1).

Variance component analysis. Variance components for agronomic and fibre traits were expressed as proportions to phenotypic variance and are listed in Table 2. Additive effects were significant for all traits except UI and El. Significant dominance effects were detected for BS, SI, LY, UHM and UI. Additive effects were greater than dominance effects for LP, SI, UHM, Mic and Str, indicating these traits could be effectively selected in early generations. Dominance effects were greater than additive effects for LY and UI, indicating that the two traits can be improved by heterosis breeding. The two were equally important for BS. Additive by environment variances

Table 2. Variance components as proportions to phenotypic variance for yield and fibre traits

	BS	LP	SI	LY	UHM	UI	Mic	El	Str
V _A /V _P	0.19**	0.23**	0.32**	0.04**	0.28**	0.00	0.34**	0.00	0.46**
V _D /V _P	0.19**	0.00	0.10**	0.26**	0.03*	0.19**	0.01	0.00	0.00
V _{AE} /V _P	0.05**	0.17**	0.21**	0.09**	0.01	0.03*	0.16**	0.45**	0.00
V _{DE} /V _P	0.00	0.14**	0.00	0.00	0.03	0.05*	0.17**	0.15**	0.20**
V _e /V _P	0.57	0.45	0.37	0.60	0.65	0.72	0.32	0.40	0.34

BS – boll size; LP – lint percentage; SI – seed index; LY – lint yield; UHM – upper half mean fibre length; UI – uniformity index; Mic – fibre micronaire; El – fibre elongation; Str – fibre strength; V_A – additive variance; V_D – dominance variance; V_{AE} – additive by environment variance; V_{DE} – dominance by environment variance; V_e – error variance; V_P – phenotypic variance; *significant at ≤ 0.05 ; **significant at ≤ 0.01

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Table 3. Predicted additive effects for yield and fibre traits of 11 parents

Parents	BS (g)	LP (%)	SI (g)	LY (kg/ha)	UHM (mm)	Mic	Str (cN/tex)
2870	–0.03	0.30	–0.03	–15.9	–0.11	0.07	0.09
Yumian1	–0.31**	–0.87**	0.12	–27.4	–0.15	–0.20**	1.88**
Jinxing2	–0.05	0.18	0.05	–50.8	0.14	–0.06	1.91**
A801-5	0.01	–0.86**	0.64**	19.5	0.18	0.29**	0.21
AX	–0.30**	0.10	0.13	–7.9	0.04	0.09	0.72**
A9-1	–0.01	1.60**	–0.41**	4.2	0.91**	–0.35**	–0.01
Y22	–0.01	0.61*	–0.28*	24.3	–0.06	0.19**	–0.90**
9007	0.46**	–0.32	–0.30**	6.5	–0.37**	0.14**	–1.37**
33B	–0.09	0.31	–0.36*	29.6	–0.14	0.02	–1.09**
99B	0.07	–1.33**	0.89**	–94.9*	0.22	–0.23**	0.30
GK22	0.26**	0.28	–0.45**	112.7*	–0.66**	0.03	–1.73**

BS – boll size; LP – lint percentage; SI – seed index; LY – lint yield; UHM – upper half mean fibre length; UI – uniformity index; Mic – fibre micronaire; El – fibre elongation; Str – fibre strength; *significant at ≤ 0.05 ; **significant at ≤ 0.01

were significant for all traits except UHM and Str and accounted for 9% or more of the phenotypic variances for LP, SI, LY, Mic and El, indicating a considerable effect of environment upon these traits, especially El and SI. Similarly, dominance effects were also environmentally dependent for LP, Mic, El and Str with dominance by environment interaction variance at 14%, 17%, 15% and 20%, respectively. This indicated that the potential of heterosis in these traits depended on environment. Error variances were high for all agronomic and fibre traits, with 32 to 72% of the total variance being due to residual (Table 2).

Additive genetic effects. Additive effects under an AD model are equivalent to general combining ability effects (GCA), by which desirable general combiners could be indentified for improving traits of interest (McCARTY *et al.* 2007; WU *et al.* 2010b). The predicted additive effects for all agronomic and fibre traits are presented in Table 3. GK22 exhibited positive additive effects for LY, suggesting that it can be used as a general combiner for improving LY, but large negative additive effects for UHM and Str. A9-1 contributed the greatest positive additive effects to LP and UHM and the negative(favourable) additive

Table 4. Predicted homozygous dominance genetic effects for yield and fibre traits of 11 parents

Parents	BS (g)	SI (g)	LY (kg/ha)	UHM (mm)	UI (%)	Mic
2870	0.05	0.01	–185.4**	–0.15	–0.29*	–0.04
Yumian1	–0.15	0.33	–164.4*	0.22	0.01	–0.04
Jinxing2	–0.16	–0.44*	–385.2**	–0.17	0.11	–0.01
A801-5	–0.27	0.68**	–69.1	–0.09	–0.07	0.09
AX	–0.42*	0.24	–100.8*	0.19	0.07	0.03
A9-1	–0.60**	–0.31*	–355.5**	0.01	–0.42*	–0.11
Y22	–0.47	–0.07	–176.9**	–0.36	–0.45	0.06
9007	0.15	–0.23	–92.8	0.01	–0.02	0.02
33B	–0.05	–0.08	–240.1*	–0.02	–0.60**	–0.06
99B	–0.51**	0.17	–275.4**	–0.07	0.23	–0.08
GK22	0.19	0.20	–319.8**	–0.05	–0.22	0.02

BS – boll size; SI – seed index; LY – lint yield; UHM – upper half mean fibre length; UI – uniformity index; Mic – fibre micronaire; *significant at ≤ 0.05 ; **significant at ≤ 0.01

effects to Mic. Positive additive effects for Str were detected for Yumian1, Jinxing2 and AX. Negative (favourable) additive effects for Mic were detected for Yumian1, A9-1 and 99B. All males except 99B exhibited negative additive effects for Str and UHM and positive (unfavourable) additive effects for Mic.

Dominance genetic effects. Homozygous (Table 4) and heterozygous dominance genetic effects (Table 5) were estimated. The former is pertinent to inbreeding depression for hybrids when selfed. Generally, parents with high negative dominance effects are

more likely to produce great heterosis (Wu *et al.* 2010b). Nine of the eleven parents showed negative homozygous dominance effects for LY with Jinxing2 and A9-1 exhibiting the largest negative dominance effects at –385.2 and –355.5 kg/ha, respectively. Negative homozygous dominance effects for BS were predicted for three of the eleven parents with the largest negative effect for A9-1. Jinxing2 and A9-1 showed negative homozygous dominance effects for SI, while A801-5 showed positive homozygous dominance effect. Negative homozygous dominance

Table 5. Predicted heterozygous dominance genetic effects for yield and fibre traits of 30 F1 hybrids

Hybrids	BS	SI	LY (kg/ha)	UHM (mm)	UI (%)	Mic
	(g)					
2870 × Y22	−0.24	0.03	−68.3	0.37	0.71*	0.04
2870 × 9007	0.38*	−0.18	50.2	−0.17	−0.26	−0.04
2870 × 33B	−0.36**	0.13	−51.1	0.27	0.57*	0.05
2870 × 99B	0.22	0.32*	127.2*	0.05	−0.08	0.05
2870 × GK22	−0.14	−0.35	281.2	−0.28	−0.32	0.01
Jinxing2 × Y22	0.17	0.08	173.9*	0.18	0.34	0.01
Jinxing2 × 9007	−0.79*	0.49	−25.2	−0.15	−0.61**	0.00
Jinxing2 × 33B	0.09	−0.13	34.6	−0.26	0.67*	0.05
Jinxing2 × 99B	0.29*	−0.57*	12.2	0.03	−0.87**	−0.06
Jinxing2 × GK22	0.16	−0.39*	78.9	−0.33	0.25	0.01
Yumian1 × Y22	0.26*	−0.64**	153.6	−0.28	−0.22	−0.05
Yumian1 × 9007	0.14	0.42**	169.7	0.17	0.25	0.07
Yumian1 × 33B	−0.19	0.10	−4.8	0.11	0.24	−0.02
Yumian1 × 99B	−0.11	0.48	13.5	0.17	−0.10	0.01
Yumian1 × GK22	0.16	0.57**	337.5*	0.23	−0.28	−0.01
A801-5 × Y22	0.21	−0.02	−230.8	0.24	−0.38	−0.10
A801-5 × 9007	−0.18	−0.34**	−90.8	−0.10	−0.02	−0.04
A801-5 × 33B	0.43*	−0.28	184.1*	0.07	−0.25	0.02
A801-5 × 99B	0.07	0.02	160.1	−0.12	0.31	0.10
A801-5 × GK22	0.01	−0.08	154.4	0.18	0.72	−0.05
AX × Y22	0.39	0.14	60.7	0.17	0.18	−0.03
AX-5 × 9007	0.32	0.00	67.0	−0.30	0.26	0.08
AX-5 × 33B	−0.35	0.29	102.9	−0.03	−0.10	−0.01
AX × 99B	0.36*	0.02	−52.4	0.07	0.30	−0.03
AX × GK22	−0.24	−0.80**	7.8	−0.26	−0.56	−0.03
A9-1 × Y22	0.13	0.25	313.0	0.01	0.31	0.06
A9-1 × 9007	0.39*	−0.23	27.6	0.35	0.26	−0.06
A9-1 × 33B	0.36	−0.33	273.5	−0.18	−0.03	0.04
A9-1 × 99B	0.27*	0.31	101.4	0.03	−0.03	0.01
A9-1 × GK22	−0.01	0.19**	3.9	0.23	0.42*	0.04*

BS – boll size; SI – seed index; LY – lint yield; UHM – upper half mean fibre length; UI – uniformity index; Mic – fibre micro-
naire; *significant at ≤ 0.05 ; **significant at ≤ 0.01

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effects were predicted for parents, 2870, A9-1, and 33B for UI.

Heterozygous dominance effects in an AD model measure the specific combining ability (SCA) of parents in specific crosses (McCARTY *et al.* 2007). High heterosis in the F_1 or F_2 hybrids are associated with high heterozygous dominance effects between two parents (JENKINS *et al.* 2006). Significant positive dominance effects for LY were detected in four of the 30 F_1 hybrids with the highest dominance effect in Yumian1 \times GK22. There were seven and four F_1 hybrids detected with significant positive heterozygous dominance effects for BS and SI, respectively. Only one hybrid (A9-1 \times GK22) showed significant positive dominance effect for Mic. There were four and two hybrids showing significant positive and negative dominance effects, respectively, for UI. Four hybrids with significant positive dominance effects for LY, such as Yumian1 \times GK22, Jinxing2 \times Y22, A801-5 \times 33B, and 2870 \times 99B had no unfavourable heterozygous dominance effects for UHM, UI and Mic. These four crosses can be used in hybrid production to promote heterosis for lint yield while maintaining higher fibre quality.

DISCUSSION

Currently, high yield Bt cotton cultivars are desired by commercial cotton producers, but are characterized by either medium or low fibre quality. However, simultaneous genetic improvement of fibre yield and quality traits has been very difficult due to negative correlations. An alternative approach to break negative linkages is to introduce new genes from exotic germplasm and/or establish epistatic interactions by multiple generations of intermating or multiple backcrosses (HINZE *et al.* 2011). For this goal, selection of appropriate parents and evaluation of their hybrids are important for achieving progress. We have developed some breeding lines with high fibre strength and acceptable agronomic performance. However, very little is known about the genetic combining ability of those breeding lines with high fibre strength and high yielding Bt cotton cultivars. Such knowledge would help to use these lines for further improvement of fibre quality in transgenic Bt upland cotton cultivars.

Additive effects contributed the most to UHM, Mic and Str, indicating main fibre properties were controlled primarily by additive genetic effects. Similar results were also reported by JENKINS *et*

al. (2007), ZENG *et al.* (2011), and TANG and XIAO (2014). Additive and additive by environment interaction effects were more important than other effects for LP and SI, suggesting a possible improvement by multiyear screening. This is well in accordance with our previous findings (TANG & XIAO 2013). Dominance effects were greater than additive effects for LY, as shown in previous studies by McCARTY *et al.* (2004), WU *et al.* (2010a, b), and SAHA *et al.* (2013). Nevertheless, contradictory results were presented by JENKINS *et al.* (2006, 2012) who stated that additive genetic effects played a major role in lint yield. The differences in results from these studies may be due to different genetic backgrounds of parents used in combinations.

Several parents were identified to have positive additive effects for LY and various fibre properties. GK22 was a desirable general combiner for LY and BS but undesirable for improving fibre properties. Although A9-1 had the second lowest lint yield among six female parents, it contributed positive but non-significant additive effects to LY while other females except A801-5 exhibited negative but non-significant additive effects to LY. This result indicated that parental values did not generally translate to additive effects rankings. In a previous study of 11 diverse cotton lines evaluated for combining ability by CHEATHAM *et al.* (2003), two lines both had high fibre strength, but one exhibited a much greater GCA effects for strength than did the other one. Yumian1 and Jinxing2 were two good general combiners for increased Str and decreased Mic. A9-1 is the favored genotype for increasing UHM (0.91 mm) and LP (1.60%) and decreasing Mic (−0.35) when it is used as a parent for crossing. It is noteworthy that better-parent heterosis for LY was detected for all hybrids except A801-5 \times Y22, A801-5 \times 9007 and AX \times 99B (data not shown). Those combinations with the three above-mentioned genotypes as parents did not result in any reduced lint yield value. For example, Jinxing2 \times Y22 had 92.9% mid-parent heterosis and 51.1% better-parent heterosis for LY, respectively. Furthermore, the hybrid had a combination of favorable mid-parent heterosis for UHM, Mic and Str at 2.5%, −6.1% and 3.4%, respectively. Yumian1 \times GK22 had negative mid-parent heterosis, with fibres 4.2% weaker than the parental average, but remained the desirable Str (32.7 cN/tex). In addition, the hybrid performed well for improving LY (1856.6 kg/ha). Five hybrids with A9-1 as a parent exhibited significant lint yield improvement from their parental lines and maintained

good fibre quality with UHM ranging from 30.8 to 31.8 mm, Str readings of 31.1 to 33.7 cN/tex, and Mic readings of 4.1 to 4.8. Consequently, the fibre quality and LY could be simultaneously improved for transgenic Bt cultivars in this research by combining better-parent heterosis in LY and middle-parent heterosis in fibre properties.

CONCLUSION

In summary, fibre properties are primarily controlled by additive genetic effects, while lint yield is mainly governed by dominance effects. A9-1 was a desirable general combiner for UHM, Mic and LP. Yumian1 and Jinxing2 were good general combiners for Str. Gk22 was a desirable general combiner for LY and BS but poor for UHM and Str. Some F_1 hybrids were identified with favourable heterozygous dominant effects for lint yield and various fibre properties. Current transgenic Bt cotton cultivars can possibly be improved in fibre quality and lint yield by crossing with some of the tested lines with high fibre strength.

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