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BREEDING AND GENETICS

A New Ligon-Lintless Mutant (li_y) in Upland Cotton

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ABSTRACT

Cotton fiber mutants are useful tools for understanding the genetics and physiology of cotton fiber development. Currently, there are two naturally occurring, dominant lintless mutant lines, Ligon-lintless-1 (Li_1) and Ligon-lintless-2 (Li_2), and one man-made mutant line, Ligon-lintless-x (Li_x), that exhibit extremely short lint fibers. Here we report a new lintless mutant that is the result of artificial chemical mutagenesis. In 2008, the cotton line MD 15 (PI 642769) was mutagenized with 3.2% v/v ethyl methane sulfonate (EMS). In 2010, a single Ligon-lintless-type plant was identified among the 2,000 M_2 mutant progeny plants and was designated li_y. This plant was crossed with the wild-type MD 15 with the objective of determining the genetic control of the lintless trait. Unlike Li_1, Li_2, and Li_x, which are controlled by a single dominant gene, this new lintless trait is controlled by a monogenic recessive gene designated as li_y. The li_y plant is short and stunted and has an okra-leaf phenotype. The li_y gene is not allelic to either Li_1 or Li_2. The genetic loci controlling these four Ligon-lintless mutations are located on four different chromosomes. This new lintless mutant will be useful in further investigating fiber elongation in cotton.

Cotton fibers are highly elongated single-celled seed trichomes that emerge from the outer epidermal cells of ovules on or around the day of anthesis. Cotton fiber length is one of the key determinants of fiber quality in the textile industry. Cotton fiber mutants are a powerful resource for the elucidation of fiber development mechanisms owing to the morphological and biochemical variances in their fiber cells (Liang et al., 2015). Currently, there are two naturally occurring fiber mutant lines, Ligon-lintless-1 (Li_1) and Ligon-lintless-2 (Li_2), and one man-made mutant line, Ligon-lintless-x (Li_x), that exhibit extremely short lint fibers of approximately 6 mm on mature seeds (Cai et al, 2013; Kohel, 1972; Narbuth and Kohel, 1990). The Li_1 and Li_2 mutants have been studied extensively as a model to understand the molecular and cellular basis of fiber elongation, and consequently to devise a strategy for improvement of fiber length for cotton fiber development (Gilbert et al., 2013; Hinchliffe et al., 2011; Naoumkina et al., 2015; Thyssen et al., 2017). Cotton fiber mutants also are useful tools for understanding the genetics and physiology of cotton fiber development (Bolton et al., 2009; Ding et al., 2014; Kwak et al., 2009; Thyssen et al., 2014). Both the Li_1 and Li_2 mutations are located in the D_T sub-genome of *G. hirsutum* L. (Naoumkina et al., 2015). The Li_1 gene is on chromosome 22 (Gilbert et al., 2013; Karaca et al., 2002; Rong et al., 2005) and the Li_2 gene is on chromosome 18 (Hinchliffe et al., 2011; Kohel et al., 2002; Rong et al., 2015; Thyssen et al., 2014). The Li_x gene is located on chromosome 4 according to Cai et al. (2013). Our most recent sequence data indicate that li_y is on chromosome 12 (Fang et al., 2020). Recently, Thyssen et al. (2017) identified the causative gene of Li_1 mutation as a single Gly65Val amino acid substitution in a polymerization domain of an actin gene, GhACT_LI1 (Gh-D04G0865). Fang et al. (2020) reported that an EMS-induced mutation in a tetratricopeptide repeat-like superfamily protein gene (Ghir_A12G008870) on chromosome A12 is responsible for the li_y short fiber phenotype in cotton. Unlike Li_1, Li_2, and Li_x that are monogenic, dominant short fiber mutations (Cai et al., 2013; Kohel et al., 1992; Narbuth and Kohel, 1990), the new man-made Ligon-lintless mutation is controlled by a monogenic recessive gene designated li_y. The mechanism of li_y to cause short fiber remains to be investigated.

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MATERIALS AND METHODS

In 2008, the cotton line MD 15 (PI 642769) was mutagenized with 3.2% v/v ethyl methane sulfonate (EMS). MD 15 was developed by USDA-ARS, Stoneville, MS and released for its enhanced fiber strength (Meredith, 2006). It is an okra-leaf germplasm line, and the presence of outcross show as heterozygous sub-okra leaves. In each generation, plants resulting from outcrosses were removed from the mutant population by selecting for the okra-leaf trait. Approximately 5,000 MD 15 seeds were imbibed for 16 to 20 h in aerated distilled water. The seeds were then rinsed and placed in aerated water, and EMS was pipetted into the water to achieve a final concentration of 3.2% v/v and mixed with the seed for 2 h to produce the M_0 generation. The seeds were then removed and rinsed several times to remove any residual EMS (Auld et al., 1998; Bechere et al., 2009a, b).The treated seeds were hand planted immediately in the field at Lubbock, TX in 2008 to produce the M_0 plants. In December 2008, M_1 seeds were harvested by collecting one boll per plant from the surviving 2,000 M_0 mutant plants (40% survival). The bolls were ginned, the seeds were mixed, and a sample of seeds from this bulk (approximately 2,000 seeds) was planted at Stoneville in 2009 to produce the M_1 plants. One boll per plant was harvested. In 2010, the M_2 seeds were planted to produce the M_2 plants from which a single Ligon-lintless-type plant was identified and named Ligon-lintless-y and seed were kept separately. This plant was grown at Stoneville during 2011 to 2014 to stabilize this trait. In each generation, the Ligon-lintless-type mutant with okra leaf was selected and maintained. In 2015, crosses were made between the Ligon-lintless mutant and the original wild-type parent MD 15 with the objective of determining the genetic control of this mutation. The F_1 and F_2 of these crosses were grown at Stoneville, MS and New Orleans, LA in 2016. Results from 15 separate F_1s (one from New Orleans and 14 from Stoneville) were grown at these locations to produce 172 F_2s and 192 to 253 F_2 plants, respectively. These F_2 plants were used to study the inheritance of the Ligon-lintless mutation. At maturity, the li_y phenotype was short and stunted with short fiber (Ligon-lintless), whereas MD 15 had normal growth and the bolls were fully and normally developed. The F_2 plants segregated into one of these classes.

Data Analysis. Chi-squares were calculated to determine the best fit for all genetic models tested. Tests of homogeneity were conducted between the values of the 15 F_1s before the data were combined (χ^2 at $p = 0.05$).

Allelism Test. Allelism test was conducted to test if the li_y gene is allelic to the Li_1 or Li_2 genes. Two crosses, namely Li_1 x li_y and Li_2 x li_y, were made in the field at Stoneville, MS in 2018 and the F_1 grown in the greenhouse during 2018. The F_2 was grown in the field in 2019 and the segregants were scored for the fiber lintless trait. A total of 377 and 381 F_2 plants were scored for Li_1 x li_y and Li_2 x li_y crosses, respectively.

Fiber Quality Measurement. Wild-type MD15 and homozygous li_y plants (approximately 50 plants each) were grown in the field during summer 2015 in New Orleans, LA. Naturally opened bolls were manually harvested from the central part of the plant (approximately 25-30 bolls for each line). The cotton fibers were ginned in a laboratory gin at the Cotton Fiber Testing Lab, USDA-ARS-SRRC, New Orleans, LA. Fiber mean length, short fiber content (SFC), fineness, and maturity ratio were measured by the Advanced Information System (AFIS) (USTER Technologies Inc.). AFIS requires a smaller sample size (~500 mg) to obtain fiber quality measurements of length and fineness. Fiber mean length is the average length of a fixed weight (w) or number (n) of fibers expressed in mm. SFC is the percentage of fibers less than 12.7 mm in a fixed weight (w) or number (n) of fibers. Fiber fineness is given as millitex, which is a measure of linear density derived by the weight of fibers in a micrograms per length of fibers in meters. Maturity ratio indicates fiber maturity in terms of the degree of thickening of the secondary cell wall relative to the diameter or fineness of the fiber. A higher maturity ratio indicates a more mature fiber. Micronaire values were measured by the Fibronaire instrument, which requires 3.24 g of fibers to obtain micronaire values. A higher micronaire value indicates a more mature, coarser fiber.

RESULTS AND DISCUSSION

Phenotypic Descriptions. Li_1 and Li_2 are naturally occurring mutants, whereas Ligon-lintless-y (li_y) is induced by EMS chemical mutagenesis. The fiber phenotypes of the seed cotton are similar in Li_1 and Li_2 (fibers shorter than 6 mm, Fig. 1B and C), whereas Ligon-lintless-y plants showed noticeable variations in fiber length on mature seeds within the

boll and between bolls on the same branch (Fig. 1D and E). Figure 1F represents examples of minimal and maximum (up to 12 mm) length of lint fibers on mature Ligon-lintless-y seeds. Endrizzi et al. (1984) reported relatively thick fiber and stunted and deformed vegetative morphology for Li_1 (Fig. 1B and G). Li_2 develops into a normal plant with normal leaves. Homozygous Ligon-lintless-y plants exhibited stunted vegetative growth, reaching approximately half the height of the wild-type plants (Fig. 1G.). Seedlings of Li_2 had normal survival rate but seedlings of Li_1 had lower survival rate when compared to seedlings of Li_2 (Kohel et al. 1992). Both Li_1 and Li_2 had better survival rates than li_y. Fiber elongation between Li_1 and Li_2 were similar and followed similar patterns (Kohel et al. 1992). An overview of phenotypic feature comparisons is presented in Table 1, Fig. 1, and Fig. 2.

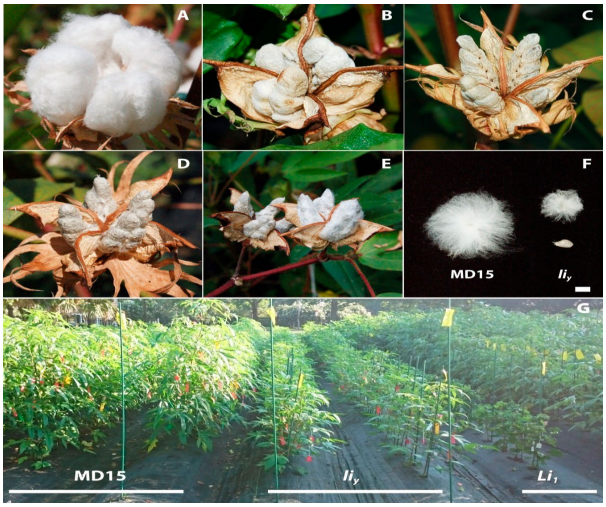

Figure 1. Comparison of Ligon-lintless mutant phenotypes of *Gossypium hirsutum*. Open bolls of: A. MD 15 (WT); B. Li_1; C. Li_2; D. and E. li_y; F. mature seeds of MD 15 and li_y; G. MD 15, li_y, and Li_1 plants in the field.

Table 1. Comparison of Ligon-lintless-1 (Li_1), Ligon-lintless-2 (Li_2), Ligon-lintless-li_y, and Ligon-lintless-Li_x

Li_1	Li_2	li_y	Li_x
Monogenic & dominant gene (Cai et al., 2013; Kohel et al., 1992;Narbuth and Kohel, 1990)	Monogenic & dominant gene (Cai et al., 2013; Kohel et al., 1992;Narbuth and Kohel, 1990)	Monogenic & recessive gene	Monogenic & dominant gene (Cai et al., 2013; Kohel et al., 1992;Narbuth and Kohel, 1990)
Naturally occurring mutant	Naturally occurring mutant	Induced by chemical mutagenesis	Somatic mutation occurring during the tissue culture process
Fuzzy seed and short fiber (< 6 mm)	Fuzzy seed and short fiber (< 6 mm)	Fuzzy seed and short fiber (< 6 mm)	Fuzzy seed and extremely short fiber
Pleiotropy in the vegetative phase	Normal vegetative growth	Pleiotropy in the vegetative phase	---
Relatively thick fiber & stunted & deformed vegetative morphology	Normal leaf and normal plant	Okra leaf and normal plant	Normal leaf and normal plant
Mapped on Chromosome D04 (Griffee and Ligon, 1929)	Mapped on Chromosome D13 (Hinchliffe et al., 2011; Kohel et al., 2002); Nabruth and Kohel, 1990; Thyssen et al., 2014)	Mapped on Chromosome A07 (Naoumkina et al., 2017)	Mapped on Chromosome A04 (Cai et al., 2013)
Seedlings have lower survival rate when compared to Li_2	Seedling has normal survival rate	Seedlings have low survival rate as compared to its wild type. Seedlings are stunted and short	Seedling has normal survival rate
Weight of fiber significantly greater than Li_2	Ligon-lintless-2 had the least fiber weight	---	---
Phenotype of seed cotton similar to Li_2	Phenotype of seed cotton similar to Li_1	Phenotype of seed cotton similar to Li_1 and Li_2	---
Fiber elongation similar to Li_2	Fiber elongation similar to Li_1	---	---

Li$_1$ *Li*$_2$ *Li*$_y$ *Wild type*

Figure 2. Phenotypes of *Li*$_1$, *Li*$_2$, and *Li*$_y$ in the greenhouse at Stoneville, MS.

Genetic Segregation of the Ligon-lintless-y Mutation. A total of 15 F_2 populations derived from 15 individual F_1 plants from the cross of Ligon-lintless-y mutant x MD 15 (WT) were grown at Stoneville (14 populations) and New Orleans (1 population) during 2016. The number of F_2 plants in each population ranged from 172 to 253. (Table 2). Five of the populations had *p* values ranging from 0.50 to 0.90, nine populations had *p* values ranging from 0.10 to 0.50 and one population had *p* value < 0.99. Degrees of freedom used was 1 (2 classes minus 1). The probability of obtaining a value as large as or larger than the *p* values at one degree of freedom was not significant for all 15 F_2 populations. Hence the hypothesis for a good fit of three (wild type):one lintless type was confirmed and a one monogenic recessive gene controlling the lintless trait was accepted. This gene is named *li*$_y$ (Ligon-lintless-y).

A test of heterogeneity was also carried out to determine whether the samples have been drawn from the same population. Heterogeneity Chi-square was calculated as total Chi-square minus pooled Chi-square. Because total Chi-square is 16.36 and pooled Chi-square is 8.7, heterogeneity Chi-square is 6.5 with 13 degrees of freedom (Table 3). Reference to the Chi-square table shows *p* value of 0.50 to 0.90. The probability is approximately 90 to 95 % that a Chi-square of this size or larger could come from a homogeneous sample set just by chance. We have no evidence of heterogeneity and we conclude that we are dealing with a homogeneous set of progenies and that our best estimate of the true ratio is 2508:734 (Table 3).

Table 2. Segregation of 15 F_2 populations of *li*$_y$ x MD 15 grown at Stoneville, MS and New Orleans, LA

Location	F_1 Progenies	F_1	No. of F_2 plants	No. of wild types		No. of *li*$_y$ types		Chi-square *P* Values	df	Value	Difference	Hypothesis
				Obs	Exp (3:1)	obs	Exp (3:1)					
STVz	1	WT[z]	210	162	157.5	48	52.5	0.3857	1	0.50-0.90	NS[z]	accept[y]
STV	2	WT	209	163	156.8	46	52.3	0.7476	1	0.50-0.90	NS	accept
STV	3	WT	228	177	171.0	51	57.0	0.6316	1	0.50-0.90	NS	accept
STV	4	WT	226	180	169.5	46	56.5	1.9513	1	0.10-0.50	NS	accept
STV	5	WT	215	165	161.3	50	53.8	0.2616	1	0.50-0.90	NS	accept
STV	6	WT	243	193	182.3	50	60.8	1.9023	1	0.10-0.50	NS	accept
STV	7	WT	227	167	170.3	60	56.8	0.1861	1	0.10-0.50	NS	accept
STV	8	WT	213	160	159.8	53	53.3	0.0012	1	<0.99	NS	accept
STV	9	WT	220	157	165.0	63	55.0	1.1636	1	0.10-0.50	NS	accept
STV	10	WT	214	169	160.5	45	53.5	1.3505	1	0.10-0.50	NS	accept
STV	11	WT	226	180	169.5	46	56.5	1.9513	1	0.10-0.50	NS	accept
STV	12	WT	194	156	145.5	38	48.5	2.2732	1	0.10-0.50	NS	accept
STV	13	WT	253	202	189.8	51	63.3	2.3725	1	0.10-0.50	NS	accept
STV	14	WT	192	142	144.0	50	48.0	0.0833	1	0.50-0.90	NS	accept
NO[z]	15	WT	172	135	129.0	37	43	1.1163	1	0.10-0.50	NS	accept

[z] WT = Wild Type, NS=not significant, STV=Stoneville, NO=New Orleans

[y] Accept Hypothesis: Ratio is a good fit to 3:1

Table 3. Summary of data from fifteen F_1 progenies based on 3:1 ratio

Source	No. of wild type observed	No. of *li*$_y$ types observed	Degrees of Freedom	Chi-square
Total			14	16.36^{***z}
Pooled	2508	734	1	8.7***
Heterogeneity			13	6.5 NS^{y}

z*** Significant at 0.001 probability level

y NS = not significant

Allelism Test. In both crosses the allelism tests (Tables 4 and 5) indicated approximately one-third of the segregants in each cross were wild types (fuzzy types). The *Li*$_1$ and *Li*$_2$ types were the dominant segregants in both crosses when compared to the *li*$_y$ types, thus indicating that the *Li*$_1$ and *Li*$_2$ are dominant to the *li*$_y$ type. A total of 377 and 382 plants were scored for *Li*$_1$ x *li*$_y$ and *Li*$_2$ x *li*$_y$, respectively. Out of these F_2 plants, 106 were fuzzy (wild type) in the first cross and 96 were fuzzy (wild type) in the second cross (Tables 4 and 5). The occurrence of the fuzzy types, which were not part of the parental phenotypes, indicates that these three mutant loci are different and not allelic.

Fiber Quality. Fiber quality measurements obtained from mature fibers of WT and *li*$_y$ revealed significant differences in several fiber traits tested by AFIS (Table 6). All evaluated parameters, including fiber length, short fiber content, fineness, maturity ratio, and micronaire were significantly inferior in *li*$_y$ fibers compared to that of the wild-type fibers. It appears that the *li*$_y$ mutation affected multiple traits, including the height of the plant and length and maturity of fiber.

Table 4. Marker results for the F_2 population of the cross *Li*$_1$ x *li*$_y$

li$_y$ marker genotype^{z}	*Li1* marker genotype^{y}	Fiber phenotype prediction	Phenotype score	Total F_2 plants
B	+	Short due to both *li*$_y$ and *Li*$_1$	Short	49
H	+	Short due to *Li*$_1$	Short	112
A	+	Short due to *Li*$_1$	Short	72
B	-	Short due to *li*$_y$	Short	38
H	-	Wild type (Fuzzy)	Wild type (Fuzzy)	68
A	-	Wild type (Fuzzy)	Wild type (Fuzzy)	38
				377

z Marker genotype for *li*$_y$ locus. A = homozygous dominant, H = heterozygous, B = homozygous recessive.

y Marker genotype for *Li*$_1$ locus. +indicates the presence of *Li*$_1$ gene, -indicates absence of *Li*$_1$ gene.

Table 5. Marker results for the F_2 population of the cross *Li*$_2$ x *li*$_y$

li$_y$ marker genotype^{z}	*Li*$_2$ marker genotype^{y}	Fiber phenotype prediction	Phenotype score	Total F_2 plants
B	+	Short due to both *li*$_y$ and *Li*$_2$	Short	45
H	+	Short due to *Li*$_2$	Short	139
A	+	Short due to *Li*$_2$	Short	80
B	-	Short due to *li*$_y$	Short	21
H	-	Wild type (Fuzzy)	Wild type (Fuzzy)	62
A	-	Wild type (Fuzzy)	Wild type (Fuzzy)	34
				381

z Marker genotype for *li*$_y$ locus. A = homozygous dominant, H = heterozygous, B = homozygous recessive.

y Marker genotype for *Li*$_2$ locus. + indicates the presence of *Li*$_2$ gene, - indicates absence of *Li*$_2$ gene.

Table 6. Average fiber quality values of mature fibers measured by the Advanced Fiber Information System (AFIS)

Line	Fiber mean length L(w) (mm)	Fiber mean length L(n) (mm)	SFC (w)[z] (%)	SFC (n) (%)	Nep[y] size (µm)	Neps per gm	UQL[x] (mm)	Fineness[w] (millitex)	IFC[v] (%)	Maturity[u] ratio (%)	Micronaire
li$_y$ (Mutant)	20.0	16.5	14.6	29.7	696	823	23.7	119.1	7.5	0.9	< 2.4
MD 15 (WT)	27.8	24.9	2.4	8.8	674	38	32.0	192.0	1.5	1.1	4.6
t-test *p*-value	1.6e-0.8*[t]	9.0e-09*	3.7e-0.5*	2.6e-05*	--	--	--	1.7e-15*	--	5.6e-12*	--

[z] SFC = Short fiber content. Percent of fibers shorter than ½ inch.

[y] Nep = A small knot of entangled fibers that will not straighten to a parallel position during processing. Nep-um = Nep size.

[x] UQL = Upper quartile length

[w]Fine = fineness, a relative measure of size, diameter, linear density or weight per unit length.

[v] IFC = Immature fiber content

[u] MR= maturity ratio, the degree of cotton fiber wall development relative to the diameter of the fiber. Higher value indicates advanced maturation level.

[t] * Significant at the 0.05 probability level

CONCLUSIONS

Unlike the *Li*$_1$, *Li*$_2$, and *Li*$_x$ fiber mutants that are controlled by monogenic dominant genes, the new Ligon-lintless fiber mutant is controlled by a monogenic recessive gene *li*$_y$. The *li*$_y$ phenotype is short and stunted, whereas fiber length on mature seeds varied from a few mm to 12 mm. Further research is currently underway to elucidate the genetic mechanisms controlling the *li*$_y$ mutation. In the allelism test, the occurrence of wild types in the crosses of *li*$_y$ with both *Li*$_1$ and *Li*$_2$, their separate chromosomal locations, their different phenotypes (Fig. 2), and that *li*$_y$ is recessive, whereas *Li*$_1$, *Li*$_2$, and *Li*$_x$ are dominant, indicates that *li*$_y$ is unlikely to be allelic to the dominant *Li*$_1$, *Li*$_2$, and *Li*$_x$ genes.

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DISCLAIMER

Mention of trade names or commercial products in the publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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