Journal of Genomic Medicine and Pharmacogenomics
JGMP, 3(1): 342-346
www.scitcentral.com

ISSN: 2464-4670

Original Research Article: Open Access

Genetics of Fibre Development in Cotton (*Gossypium hirsutum* L.)

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Received August 17, 2018; Accepted September 30, 2018; Published November 29, 2018

ABSTRACT

A study was undertaken to understand the genetics of cotton fibre development at University of Agricultural Sciences, Dharwad. Segregating F2 populations of the cross between fibred MCU-5 (WT) × fibreless mutant MCU-5 (fl) and its reciprocal cross of upland cotton (*Gossypium hirsutum* L.) were evaluated to study the inheritance of the fibre development. In both, straight and reciprocal crosses the segregation was in the ratio of 15 (fibred): 1(fibreless), indicating duplicate dominant gene interaction signifying that the fibreless trait is controlled by double recessive genes. However, among the fibred plants presence of genetic variability for number of fibres per unit area was observed. It infers that, the presence of individual cell level control over its elongation as a fibre. Scanning electron microscopy (SEM) was performed at fibre initiation stage, 2 DPA, to observe the development of fibre initials on epidermal layer of ovules. As compared to WT, SEM analysis revealed that the presence fibre cell initials in fl mutant found negligible.

Keywords: Fibre initiation, Fibre development, Fibreless mutant

Abbreviations: DPA: Days Post Anthesis; fl: fuzzless-lintless; SEM: Scanning Electron Microscope; WT: Wild Type

INTRODUCTION

Cotton fibres are unicellular trichomes originating from the outer epidermal layer of the seed coat. About 20 to 25% of the seed epidermal cells differentiate into spinnable fibres [1-4]. Fibres are classified into two types, lint and fuzz. Fibre development includes four distinct, but overlapping stages namely initiation, elongation/primary cell wall (PCW) synthesis, secondary cell wall (SCW) synthesis and maturation. The lint fibres initiate growth between anthesis and 2 days postanthesis (DPA) and can elongate 2.5-3.5 cm. Whereas, the fuzz fibres initiate growth between 5 and 10 DPA and are approximately 0.5 cm [4]. However, fast elongation of fibre cell occurs between 5 to 15 DPA. Secondary cell wall synthesis starts at about 20 DPA and continues up to 45 DPA. During this period large amount of cellulose (>90%) deposition takes place and the fibre cell wall becomes thick. In the final maturation stage (45-50 DPA) fibres undergo dehydration and produce mature cotton lint [1,5]. Spontaneous fibreless mutants of cotton have been reported since 1920 [6,7]. They were used to detect and locate QTLs for lint yield [8], fibre quality [9], seed traits [10], pattern of lint initiation [11], to determine differences in gene/protein regulation during development of ovular trichomes, for studying gene functions [12]. Lint yield depends on number of bolls per unit area, number of seeds per boll, number of fibres per seed, number of fibres per unit area and average weight per fibre. Improvements in fibre number per seed and per unit area of seed might results in increased lint yield. Thus, in the present study from the cross WT × fl and fl × WT an attempt was been made to understand the variation for number of fibres per unit area in segregating F2 population besides genetics of fibre development.

MATERIAL AND METHODS

The genetics of fibre development in cotton was studied in F1 and F2 generation of both straight and reciprocal cross

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Citation: Kumar GS, Katageri IS, Kumar NVM & Adiger S. (2018) Genetics of Fibre Development in Cotton (*Gossypium hirsutum* L.). J Genomic Med Pharmacogenomics, 3(1): 342-346.

between fibred MCU5 (WT) and fibreless mutant MCU5 (fl) during kharif 2015-2016 at Main Agriculture Research Station (MARS), University of Agricultural Sciences, Dharwad. MCU5 (WT) is normal linted (fibred) cotton variety with fuzz, whereas fuzzless-lintless (fibre less) MCU5 (fl) (*G. hirsuttum*) is a spontaneous mutant, first identified by Peter et al. [13]. Five bolls per plant were randomly chosen at fully opened boll stage in 800 (straight cross, WT × fl) and 852 (reciprocal cross, fl × WT) F2 plants to take observation for fibred and fibreless trait. Fibre and fuzz development in parents and F1 also recorded. The data was analysed by using chi-square test to understand the genetics of fibre development. Ginning outturn was calculated in all F2 plants. Number of fibres sample, number of fibres per seed and Number of fibres per unit area was calculated as per Bechere et al. [14].

Number of fibers/sample=Lint weight (g) × 1,000,000,000 (correction factor) ÷ Fibre fineness (mT) × Mean length of fibre (mm)

Number of fibers per seed=Number of fibers per sample ÷ Number of seeds per sample

Number of fibres per mm^2=Number of fibres per seed ÷ Surface area of seed (mm^2)

Scanning electron microscopy

At fibre initiation stage (2 DPA) scanning electron microscopy (SEM) was performed to observe the development of fibre initials on epidermal layer of ovules. Ovules excised from two days post anthesis flowers collected in 3% glutaraldehyde and dipped in 1-2% osmium tetroxide for post-fixation. Samples were dehydrated in a graded acetone solution and mounted on stub and allowed for metal coating with Gold using polaron sputter (E5100, Watford England) coater. The SEM images were captured by LEO 435 VP (LEO Electron microscopy Ltd., Cambridge, UK) at 15 kV EHT at Central Food Technological Research Institute (CFTRI), Mysore. The number of fibre initials was quantified in 100 µm2 area using ImageJ software.

RESULTS AND DISCUSSION

Number of fibre initials in parents

The literature says that fibre initiation starts from epidermal layer between -2 DPA to +2 DPA [5,15,16]. The cells which show elongation of initials by 2 DPA were further elongate into either fuzz (0.5 cm) or lint (2.5 to 3.5 cm). Only 20% of such initials going to elongate into fibres [1-4]. In the present study fibre initials were counted at 2 DPA stage. MCU5 (WT) normal fibred parent recorded 118 fibre initials per 100 μm^2 whereas, fibre less mutant MCU (fl) found negligible fibre initials that is only two fibre initials per 100 μm^2, indicating fibre less trait in mutant and scanning electron micrograph of 2 DPA ovules of both genotypes are depicted in **Figure 1**.

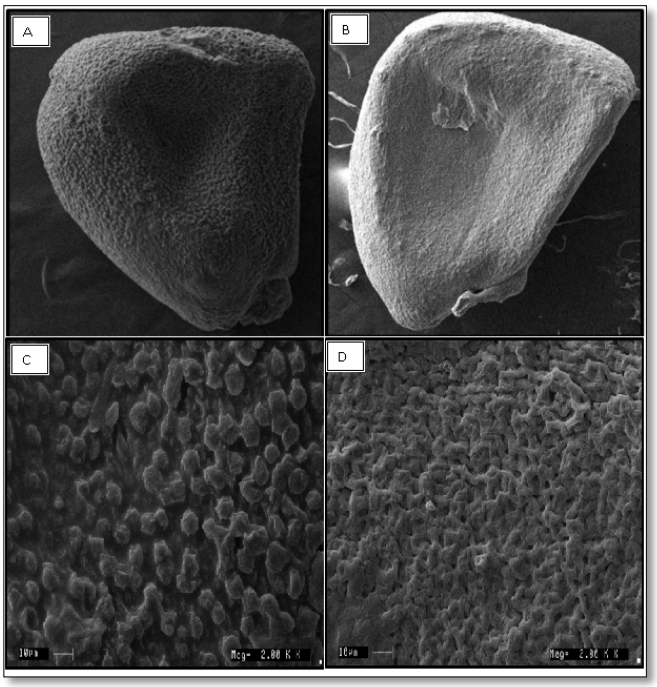

Figure 1. A and **B** are 2 DPA ovule of MCU5 (WT) and MCU5 (fl) mutant respectively. **C** and **D** are magnified SEM micrograph ((bar is 10 µm) of ovule surface at chalazal end of MCU5 (WT) and MCU5 (fl) mutant, respectively.

Genetics of fibre development in tetraploid cotton

The F1's obtained from both straight (WT × fl) and reciprocal (fl × WT) cross showing only fibred, indicates that fibred trait is governed by dominant genes. In the direct cross 749 and 51 F2 plants, and in reciprocal cross 797 and 55 F2 plants were recorded fibred and fibre less traits, respectively. This observed results from both cross F2 populations followed 15 (fibred):1(fibreless) segregation ratio and it was confirmed by chi-square test **(Table 1)**. This indicates that the presence of duplicate dominant gene interaction and fibreless trait governed by two recessive genes. Similarly, Kumar et al. [17] concluded double recessive genes controls the lintless trait and they also reported presence of duplicate dominant gene action, from the study of inheritance pattern for fibreless trait in the diploid F2 populations of cross between Fuzz linted (FL) × Fuzz-lintless (fl) mutant. Nadarajan and Rangaswamy [18] studied the inheritance pattern for fibreless trait in F2 populations by crossing fibreless line with five different G. hirsutum lines. They observed 15(F):1(fls), 63(F):1(fls) and 255(F):1(fls) segregation ratios and concluded fibred trait controlled by two to four genes.

Table 1. Genetics of fibre development, chi-square test for duplicate gene action (15:1).

SL. No.	Cross	Fibred		Fibreless		Total Number of plants	χ^2 Value	Table χ^2 Value
		Observed	Expected	Observed	Expected			
1	MCU5(WT)× MCU5(fl) mutant	749	750	51	50	800	0.8838	3.84
2	MCU5(fl) mutant× MCU5 (WT)	797	796	55	56	852	0.9717	

Variability for ginning outturn in tetraploid cotton

Presence variation for ginning outturn was recorded in F2 of crosses, MCU5 (WT) × MCU5 (fl) mutant and its reciprocal cross (fl × WT). The ginning outturn was in the range of 0 to 43.75% in WT × fl population and 0.77 to 43.90% in fl × WT F2 population **(Figure 2)**. It was also evident from the visual observation of seeds being covered by number of fibres. As the number of fibres per seed increased, the increase in ginning outturn was noticed. For example, seeds of plant with ginning outturn 0% were not covered by any fibres, whereas the seeds of plant with ginning outturn of 9% were partially covered by fibres with 1525.90 fibres per seed and plant with ginning outturn 36.66% was fully covered by fibres with 20631.46 fibres per seed **(Table 2)**. The ginning outturn recorded higher positive correlation with number of elongated fibres per seed (0.949), fibres per unit area (0.952) compared to surface area of seed (0.213) **(Table 3 and 4)**. These results indicated that, as the increase in number of fibres per unit area there is increase in GOT percent. Previously, Li et al. [19] suggested that the number of fibre initials can be used as predictor of lint percentage in cotton breeding. Similarly Gabriela et al. [20] reported that fibre initial density alone can be used as easy and early predictor of lint percent or potential yield. In normal fibred cotton genotype the potential cells are elongating into fibre is said to be about 20 to 25% [1-4]. Bechere et al. [14] revealed that the number of fibres per seed and fibre density were lower in $9023n_4$ mutant than in the wild type, which explains low lint yield in mutant line. Thus, it is evident that the number of elongated fibres determines the ginning outturn and in turn it is possible to increase the ginning outturn percent by increasing number of elongating fibres per unit area.

Table 2. Fibres per unit area (per mm^2) and number of fibres per seed.

Sl. No.	Genotype	GOT (%)	Number of fibres/sample	Number of fibres per seed	Surface area of seed (mm^2)	Fibres/mm^2 of seed surface
1	MCU-5(WT)	36.66	42,08,817.01	20631.46	143.50	143.77
2	MCU-5 (fl)mutant	0.00	0.00	0.00	140.60	0.00
F_2 Plants (below)						
3	G-482	0.00	0.00	0.00	134.50	0.00
4	G-473	1.56	1,38,854.72	373.27	140.60	2.65
5	k-374	2.80	2,32,421.89	606.85	133.70	4.54
6	K-675	3.76	2,16,653.65	606.87	140.70	4.31
7	K-707	4.73	3,28,120.98	861.21	139.90	6.16
8	K-361	5.46	3,67,062.88	1122.52	139.70	8.04
9	K-272	6.79	5,18,763.08	1313.32	140.50	9.35
10	K-36	8.51	6,97,627.39	1459.47	139.80	10.44
11	G-278	9.00	5,72,211.40	1525.90	132.00	11.56
12	K-629	11.20	7,19,703.72	2110.57	138.70	15.22
13	G-292	13.78	8,51,554.50	2333.03	130.30	17.91

Table 3. Number of fibre initials per 100 µm^2 area of 2 DPA ovules.

Sl. No.	Genotype	Fibre initials per 100 µm^2
1	MCU-5(WT)	118
2	MCU-5(mutant)	2

Table 4. Correlation coefficients among GOT related traits.

	GOT	No. of fibres per seed	Surface area of seed (mm^2)	Fibres/mm^2 of seed surface
GOT	1.000			
No. of fibres per seed	0.949**	1.000		
Surface area of seed (mm^2)	0.213	0.363*	1.000	
Fibres/mm^2 of seed surface	0.952**	1.000**	0.354*	1.000

** significant at 5%LOS. ** significant at both 5% and 1% LOS*

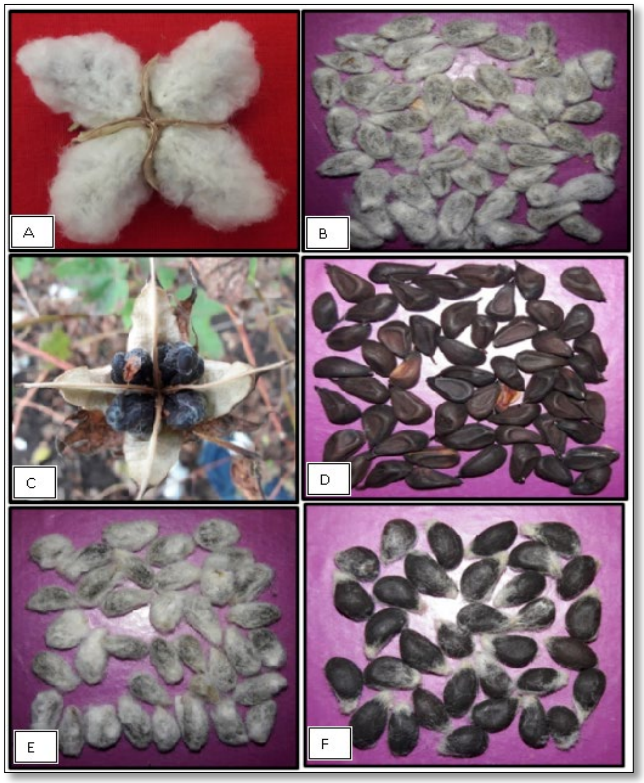

Figure 2. A-B and **C-D** represents opened boll and seeds of MCU5 (WT) and MCU5 (fl) mutant respectively **E** and **F** represent difference for fuzz in F1 of cross (WT × fl) and (fl × WT), respectively.

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