Development of mapping population segregating for sucking pest resistance in cotton

Abstract

The current study was conducted to identify the best parents with good agronomical features and contrasting characters for sucking pest resistance to develop a population that can be used for QTL mapping of resistance component traits. Initial attempts at this university have enabled to identify five potential parents (*viz*., KC3, NDLH 1938, CO18, K12 and RG8) with improved sucking pest resistance and yield characters and they were crossed with the recently released high-yielding variety CO-17, which is also suitable for high-density planting. All these six parents were evaluated for fiber yield and quality traits besides anatomical characters such as trichomes density, length, width and sharpness that confers sucking pest resistance and genotyped with 200 SSR markers that span the entire genome. Efforts have been dedicated to the careful selection of optimal parents, namely CO17 and KC3, each possessing distinct traits related to resistance against sucking pests and also fiber quality characters in addition. This strategic approach aims to combine their contrasting genetic components for sucking pest resistance, with the intention of developing a new generation of plants that exhibit heightened resilience to these types of pests. CIR139, a polymorphic SSR marker of these two parents was used to fix the true-hybrids which were advanced to generate F_2 mapping population. This population is believed to be useful for QTL mapping of anatomical features that confers sucking pest resistance such as trichome length, density, breadth, and sharpness besides fiber yield and quality traits.

Comment [A1]: Quantitative trait loci

Comment [A2]: Write completely Simple Sequence Repeats

Keywords: Cotton, Sucking pest, Trichomes, SEM, SSR, hybrid validation

Introduction

Cotton (*Gossypium* spp) is one of the most important commercial crops grown in India. According to the data published by the Ministry of Textiles, Government of India. In 2022, India accounted for approximately a quarter of the global cotton production, ranking first in cotton acreage globally with 120.69 lakh hectares, but it ranked 38th in terms of cotton productivity. The average productivity of cotton is markedly low at about 510 kg/ha as compared to world average of 808 kg/ha (https://texmin.nic.in/sites/default/files/Cotton%20Sector.pdf).

This yield loss in cotton is mainly attributed (up to 30%) to biotic and abiotic stresses (Kamburova *et al.*, 2022); among them insect pest infestations pose the biggest threat to cotton production as there are around 162 insect pest species that attack cotton at various stages of development (Kannan *et al*., 2004). Especially, after the introduction and adoption of Bt hybrid cotton, the yield losses caused by bollworms / defoliators (major pest) become minimized but damage due to sap suckers / sucking pest complex (which were considered as minor pests) has increased (Men *et al*., 2005). Many pests once considered minor previously have attained major pest status. For example, whitefly (*Bemisia tabaci*), a notorious pest drains the cell sap

continually and resulting in to 50% boll drop (Ahmad *et al.,* 2002). Another sucking pest cotton jassid (*Amrasca biguttula),* is a polyphagous pest that causes significant crop loss not only by sucking the cell sap but also by introducing poisonous chemicals into the leaves (Bhat *et al.,* 1986) and it caused reduction in seed cotton yield to a tune of 114 kg /ha (Sidhu and Dhawan, 1986). Attique and Ahmad (1990) have indicated that the combined assault of thrips (14.6 individuals/leaf) and jassid (4.6 individuals/leaf) caused a 37.6% decrease in seed cotton production. To tackle the yield loss due to these sucking pests, cotton is extensively treated with pesticides. Although spraying pesticides efficiently suppresses insect pests, it is neither economically profitable nor safe for human health (Unsar Naeem-Ullah *et al.,* 2020). Consequently, aside from these temporary solutions, this problem requires an eco-friendly and cost-effective solution which can be sustainably accomplished by evolving -resistant cultivars.

In this sense, it is vital to breed resistance types by discovering resistant genes and/or quantitative trait loci (QTL), introducing them into popular susceptible cultivars, and making them resistant to sucking pest complex. The key step in QTL mapping of pest resistance genes is to construct a mapping population that is derived from contrasting parents for the investigated traits. Such mapping population provide immense benefit to genetically dissect the sucking pest tolerance traits which would help to fast-track the future molecular breeding program. Hence, this study was conducted with an objective to find notable and resistant genotype(s) against sucking pests and creating a mapping population for sucking pest resistance. To this end, three species of cotton *Gossypium hirsutum*, *G. barbadense*, *G. arboreum* were studied for their anatomical and agronomical traits that confer resistance to the sucking pests.

Materials and Methods:

Plant material

The study was carried out at the Department of Cotton, CPBG, Tamil Nadu Agricultural University, Coimbatore. Six cotton genotypes *viz*., CO17*(G. hirsutum)*, KC3*(G. hirsutum)*, NDLH1938*(G. hirsutum)*, CO18(*G. barbadense)*, RG-8*(G. arboreum)*, K-12*(G. arboreum)* that were shown to possess field tolerance to sucking pests during previous field experiments at this center (Table 1) were sown during winter 2022 (September to February) in Randomized Block Design in such a way that each genotype was sown in six rows each (which has a length of 4.5 m) with a spacing of 60 cm (row to row) and 45 cm (plant to plant) and replicated thrice. Recommended package of practices of were followed for better crop growth and stand. Besides collecting data on anatomical traits, these genotypes were also used for developing mapping populations. CO17, a high yielding popular mono-sympodial variety was chosen as the female parent and it was crossed individually with five male parents *viz.*, KC3, NDLH1938, CO18, RG-8 and K-12 by employing manual emasculation and pollination following Doak's method (Doak, 1934). The crossed bolls were harvested during February, 2023 and F_1 seeds from each cross combination were sown in (Feb) 2023 by following above agronomic practices.

Table 1: Varietal information of the parents investigated in this study

Varieties	Parentage	Year of release	Name of Institute	Salient Features
CO17 *(G. hirsutum)*	Khandwa 2 x LH 2220	2020	Tamil Nadu Agricultural University Coimbatore.	High yielder, Compact plant type, suitable for mechanized harvest and high-density planting system., Possesses zero monopodia with short sympodial length,
KC3 *(G. hirsutum)*	TKH 497 x KC 1	2006	Tamil Nadu Agricultural University, Kovilpatti.	Moderately resistant to leaf hopper, Resistant to Grey mildew and Alternaria blight and tolerant to drought Suitable for rainfed black soil tracts
NDLH 1938 *(G. hirsutum)*	-	-	Regional Agricultural Research Station, Nandyal.	High yielder, Resistant to Leafhopper and other sucking pests. Suitable for high density planting system.
CO18 (*G. barbadense)*	TCB selection from EC 101786	2020	Tamil Nadu Agricultural University Coimbatore.	high yielding with extra-long staple cotton, moderately resistant to leaf hoppers and Alternaria leaf blight and root

				rot.
RG8 *(G. arboreum)*	-	1986	Rajasthan Agricultural University, Sriganganagar.	Moderately resistant to bollworm, aphids, Resistant to root rot and tolerant to drought. Suitable for low input cultivation practices.
K12 *(G. arboreum)*	K 11 x K 9	2017	Tamil Nadu Agricultural University, Kovilpatti.	Resistant to Leafhopper and other sucking pests Suitable for Winter rainfed under black soil tracts of southern districts of Tamil Nadu

Field and Anatomical observations

The average yield of the parents investigated in this study was calculated from the single plant and a minimum of 10 grams of lint sample collected from each parent was analyzed using the high-Volume Instrument (HVI) (User model: HVC classic 900) for fiber quality parameters such as fiber strength, fiber length, ginning out turn and spinnability was measured by Quickspin® machine.

Statistical analysis

All the trichome characters data were statistically scrutinized in GRAPES (version 1.0.0) software. The analysis of variance was performed for assessed traits, and LSD test was performed to reveal the statistical differences among the treatments.

Comment [A3]: Latin Square Design

Grading: each character was grouped into a,b,c,d,e and f based on their mean values. the superiority of grouping order is given as

Comment [A4]: T -Captial

$$a > b > c > d > e > f$$

(Treatments with same letters are not significantly different).

Trichome analysis

Data on leaf trichome density were recorded in parental lines and individual plants of the F_1 population by following the procedure described by Wright *et al.*, (1999). For measuring plant anatomical characters such as the number of gossypol glands/glandular trichome, trichome density, sharpness and length of trichome on leaf lamina, midrib and veins, three plants were randomly selected from each row, and one leaf from each plat's top portions (preferably 3rd or 4th leaf from the top) was used for analysis with Scanning Electron Microscope (Sohail *et al*., 2003; Amjad and Aheer, 2007). The upper side of the leaf was transferred to specimen stubs and taped with carbon and lyophilized and palladium-sputter coated before examining under a Field Emission Scanning Electron Microscope (CARL ZEISS FESEM, Germany) model Gemini 300. Trichome characteristics were measured using scaling software that was already installed in the above FESEM.

Simple Sequence Repeat (SSR) assay

Total genomic DNA was isolated from the tender leaves of the six parents by Cetyl Trimethyl Ammonium Bromide (CTAB) method (Saghai-Maroof *et al*., 1984). The quantity and quality of DNA were assessed in a nano-drop spectrophotometer and 0.8% agarose gel electrophoresis, respectively. Cotton-specific simple-sequence repeat (SSR) / microsatellite markers that span the entire cotton genome were obtained from CottonGen (https://www.cottongen.org/) (Table 2). Amplification of SSR marker was performed *in vitro* by Polymerase Chain Reaction (PCR) in an Applied Biosystems thermocycler (proflex PCR system). The PCR reaction mixture (11 µL) consisted of 2.5 µL template DNA (50 ng/ µL), 1µL Taq buffer 10 X, 1 µl dNPTs, 0.5µl Taq DNA polymerase, 5 µL autoclaved water and 1 µL each of forward (0.5 µM) and reverse primer (0.5 µM). Thermal profile used in this study for PCR amplification was as follows: initial denaturation at 94°C for 4 min followed by 35 cycles of denaturation at 94°C for 30 sec; annealing at 51–55°C (depending on the primer pair) for 45 sec and extension at 72°C for 1 min; final extension at 72°C for 10 min. After PCR, 2.5% agarose gel was prepared using 0.5 × TBE buffer and those amplified PCR products were resolved in a gel electrophoresis unit. The gels were examined under UV illumination using a UVITEC Essential V6 gel documentation system. Polymorphic SSRs that differentiate all the investigated parental genotypes were identified manually with the help of differential movement of the amplified PCR products from each parent.

Results and Discussion

Agronomical and fiber quality characteristics of the investigated parents

The study was initiated by identifying the contrasting parents with various agronomical, quality and anatomical parameters. Agronomical parameters such as average seed cotton yield and quality parameters such as fiber length, fiber strength, ginning outturn and spinnability among the parents employed in this investigation were observed and studied (Table 2). Among

the six investigated cultivars, CO17 stands out with the highest seed cotton yield of 2361 kg/ha, while KC3 has the lowest yield at 1081 kg/ha. With respect to fiber strength and fiber length, CO18 had the maximum fiber strength (36.2 g/tex) and fiber length (31.02 mm), whereas RG-8 and KC3 had the lowest fiber length and strength. Ginning Out Turn is a critical factor for cotton processing, which indicates the percentage of lint obtained after ginning. KC3 has the highest ginning out turn (37.0%), while RG8 has the lowest (22.3%). In case of spinnability to a higher count (which suggests finer and more desirable cotton for spinning) CO17, KC3, and NDLH 1938 were rated with 40s' count which highlighted that these lines have excellent spinnability. On the other hand, CO18 had a slightly lower count of 35', and RG8 and K12 have coarser fibers with only 30s' counts.

Table 2: Agronomical parameters of parents investigated in this study

Parents	**Average seed cotton yield**	**Fiber length**	**Fiber strength**	**Ginning out turn**	**Spinnability**
CO17	2361 kg/ha	27.0 mm	26.8 g/tex	35.0%	40s' count
KC3	1081 kg/ha	26.4 mm	21.5 g/tex	37.0 %	40s' count
NDLH 1938	1886 kg/ha	25.8 mm	24.6 g/tex	32.0%	40s' count
CO18	1720 kg/ha	31.02 mm	36.2 g/tex	35.2 %.	35' count
RG8	1142kg/ha	16.4 mm	19.2 g/tex	22.3%	30's count
K12	1193 kg/ha	27.7 mm	22.1 g/tex	37.0%	30s' count

Trichome analysis

Trichome characters of the investigated parents were measured, compared and presented in Table 4. Analysis of varience table (table 3) suggests that the treatments were highly significant for all the traits such as trichome density, length, width, sharpness and number of glandular trichomes. Among the studied varieties, K12 exhibits the highest trichome density with 6 trichome per milli meter square respectively. It is believed that trichomes provide a robust physical barrier against herbivores and potentially making it more resistant to herbivore damage.

However,KC3, RG-8 and NDLH 1938 were found to possess moderate trichome densities of 5,4 and 4 per milli meter square and. CO17 had the least trichome density of 2 per milli meter square. Notably, CO18

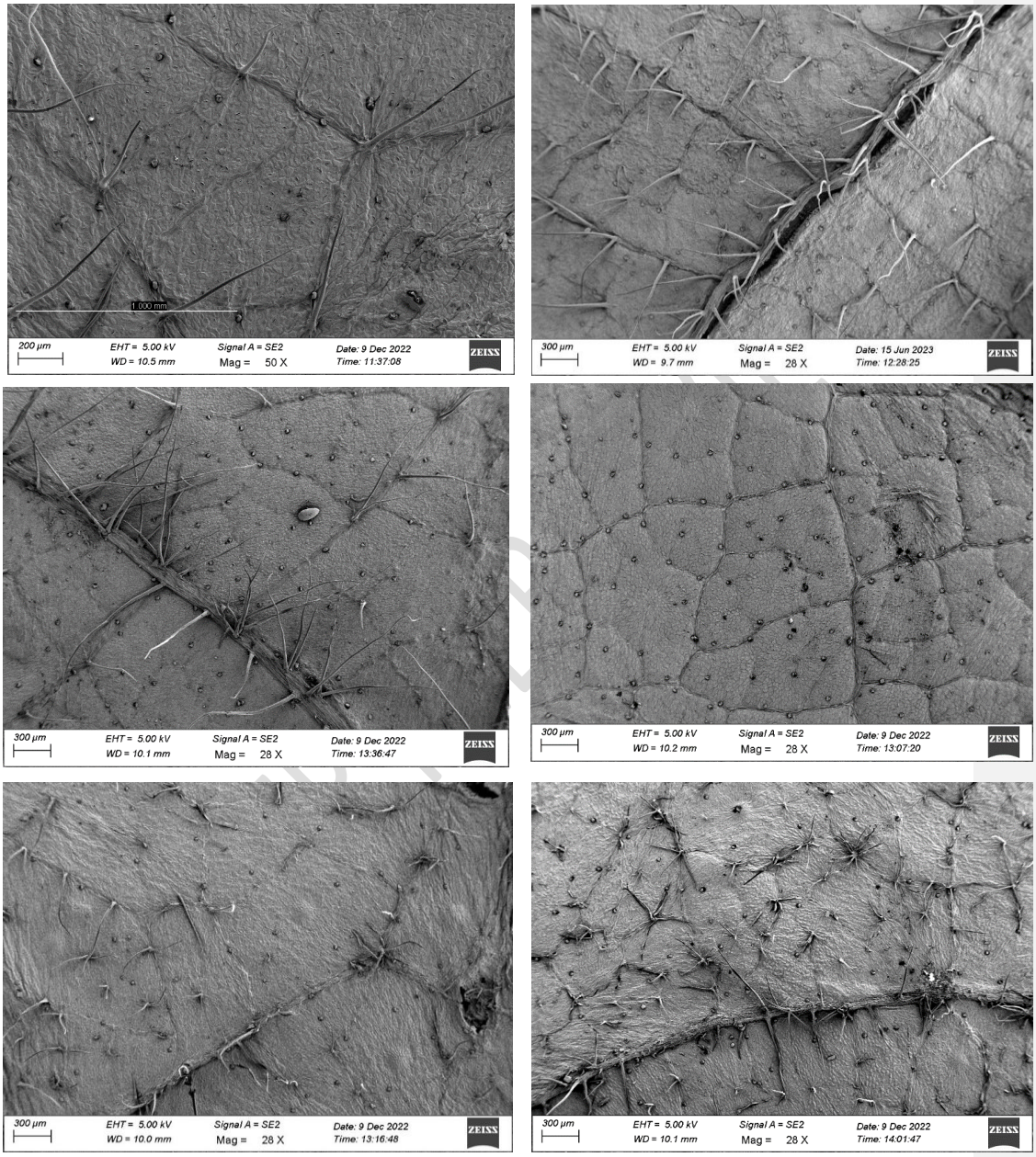

Fig. 1 - Scanning electron microscope pictures of abaxial leaf surface showing trichomes density of six parents A) CO17, B) KC3, C) NDLH1938, D) CO18, E) RG-8, F) K-12

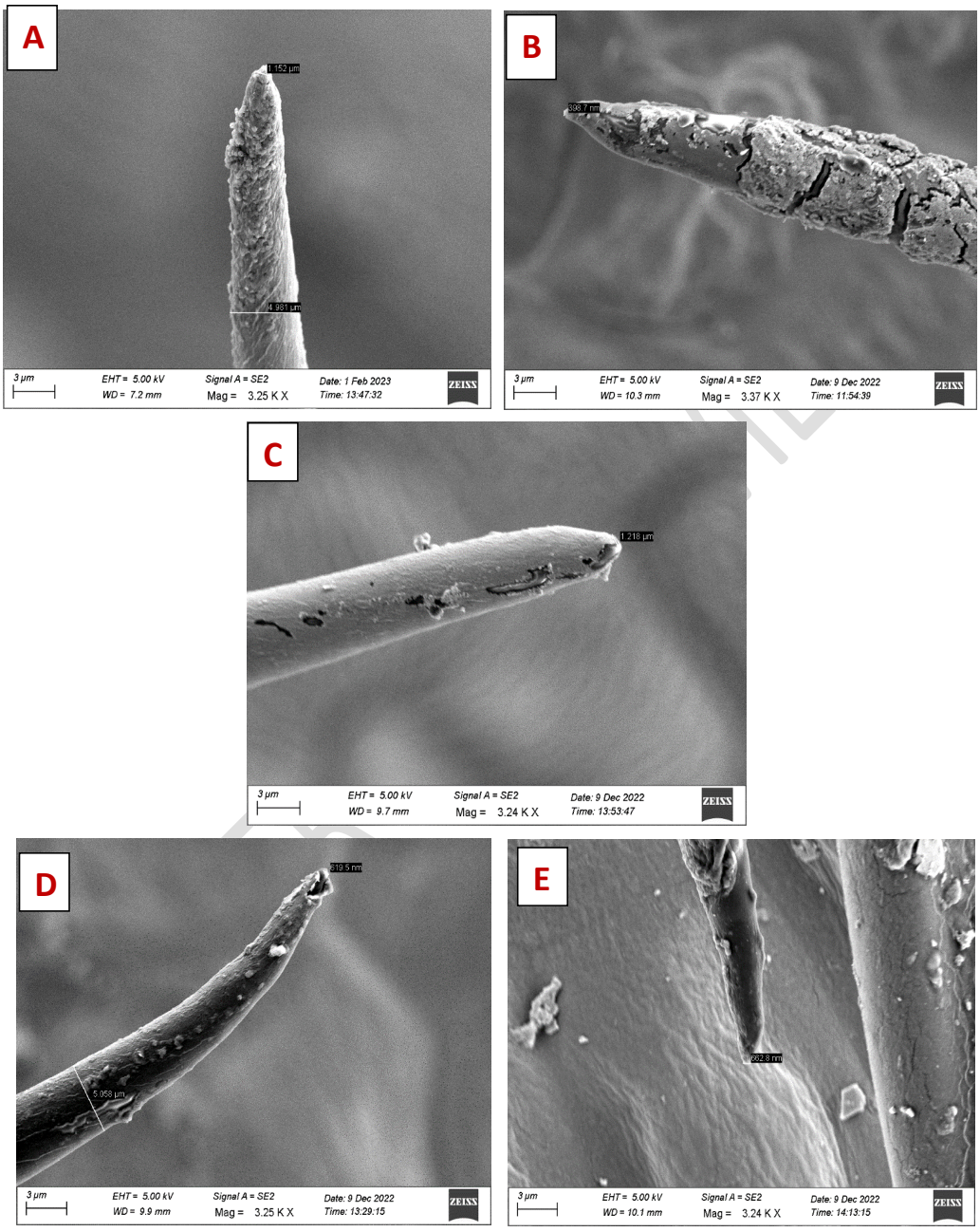

Fig 2. - Scanning electron microsope pictures of abaxial leaf surface showing trichomes sharpness of 6 parents A) CO17, B) KC3, C) NDLH1938, D) RG-8, E) K-12

is a glabrous type and it completely lacks trichomes; this showed that CO18 might be vulnerable to herbivore attacks. Various studies have suggested that increased trichome density improves resistance to jassids (Tidke and Sane, 1962; Yadav et al., 1967), and *Aphis grandis* by preventing easy movement (Cook, 1906); nevertheless, increases the susceptibility of the plants to bollworms since it attracts female moths for ovipositioning (Kadapa et al., 1983 and 1987).

Apart from the number of trichomes, the length and width of trichomes was found to vary among the six cotton varieties: NDLH 1938 displayed the longest and widest trichomes, suggesting strong potential for physical defense (Figure 1). CO17 also had relatively long trichomes, followed by KC3, K12 and RG8 were found to have moderately long trichomes but with slightly less width. Hair length, density and position are three important qualities to be considered for sucking pest resistance in cotton (Wannamaker, 1957). KC3 was observed to have the sharpest trichomes (0.398 µm) among the six investigated parents. K-12 and RG-8 recorded s relatively sharp trichomes while CO17 followed by NDLH1938 recorded trichomes with lower sharpness values (Figure 2). CO18 had the highest number (35 nos) of glandular trichomes, followed by NDLH 1938 (25 nos) and KC3 to the least of 12 numbers per 1mm. Glandular trichomes can contribute to a plant's defense mechanisms by producing chemical compounds that deter herbivores or serve other functions related to stress responses (Dan Ma et al.,2016).

Overall, KC3 appears to have relatively lengthier, broader, denser and sharper trichomes besides possessing significant numbers of glandular trichomes. These characteristics may collectively contribute for its pest resistance behavior and making it a promising choice for use as a donor parent. On the other hand, though CO17 has shown higher yield and fiber quality parameters, it showed poor trichome characteristics (Table 2). Hence, it has been decided to start the mapping population development program with these two contrasting parents (CO17 and KC3). It is anticipated that mapping population derived from these two parents would segregate for sucking pest resistance. The F_1 plants evolved from crossing these two parents were fixed for their truthfulness using polymorphic SSR markers identified between CO17 and KC3.

Table 3: Analysis of Variance for trichome characteristics

*- significance at 5% level, **-significance at 1% level

Characters	**Mean sum of squares**		
	Treatment (df = 5)	**Replication (df = 3)**	**Error (df =15)**
Trichomes Density (1mm)	22.242**	0.042	0.975
No. of Glandular Trichomes (1mm)	299.300**	0.444	1.611
Trichomes Length (µm)	329065.535**	236.289	231.597
Trichomes Width (µm)	446.907**	0.065	0.342
Trichomes Sharpness (µm)	0.848**	0.000	0.000

Table 4: Variation in trichome characteristics of the investigated parents along with grouping

SSR Assay to Fix True Hybrids

Parents	Trichomes Density (1mm)	No. of Glandular Trichomes (1mm)	Trichomes Length (µm)	Trichomes Width (µm)	Trichomes Sharpness (µm)
CO17	2(±0.65) [c]	15d	746.3a	24.11b	1.152b
KC3	5b	12e	419b	24.13b	0.398e
NDLH 1938	4b	25b	763.35a	30.01a	1.218a
K12	6a	17c	361c	15.69c	0.662c
RG8	4b	14d	326.7d	14.99c	0.619d
CO18	0d	35a	0e	0d	0f

The F_1 plants derived from CO17 and KC3 were found to be anatomically (trichomes characteristics) better performing and they were analyzed for the hybridity using polymorphic SSR markers.

Confirmation of F_1 hybrids was done with the help of molecular markers. A total of 200 cotton specific SSR primers, as shown in (Table 4), were utilized to investigate the polymorphism between CO17 and KC3. Only the polymorphic SSRs, which amplified bands specific to the male or female parent, were considered for analyzing the F_1 hybrids. Simple sequence repeats (SSRs) or microsatellites are widely accepted and reliable molecular marker systems because they are abundant, co-dominant, robust, detect high levels of allelic diversity, easy scoring of alleles, reproducibility, and transferability to laboratories, and can be analyzed by a convenient PCR-based method, making it easy to screen a large number of individuals (Paniego et al., 2002).

Among the 200 SSR markers employed in this study, CIR 139 alone was discovered as polymorphic marker as it permitted to differentiate and identify hybrids from their parental lines. A similar kind of SSR marker to confirm F_1 hybridity in cotton has been shown earlier (Muthuraj and Mahalingam *et al*., 2019). A low rate of SSR polymorphism is common in cotton and such poor polymorphism has also been shown previously (Dongre et al., 2010).

CIR 139 has amplified an allele of 180 bp in the female parent (CO17) and 190 bp in male parent (KC3). As expected, in hybrid plants, the SSR marker CIR139 has amplified two alleles of size 180 and 190 bp. (Figure 3). The expression of both parental alleles in 40 hybrid plants (Figure 3) provided evidence of the hybrid plants authenticity as well as their ancestry

from the two parents employed in the study; those F_1s which were not shown to have the two alleles of these two parents were discarded (F_1 lines *viz*., 1, 11, 19 and 29) and not used further in generation advancement. As a result, 38 of the 40 F_1 plants were tagged as true hybrids and they were advanced to generate F_2 mapping population. This population is believed to be useful for QTL mapping of anatomical features that confers sucking pest resistance.

Table 5: List of SSR markers used for the polymorphic survey on CO17 and KC3

S.No	SSR primers name	Primer sequence (5'- 3')	Linkage group	Annealing temperature	Monomorphic (M)/Polymorphic(P) B/W CO17 X KC3
1	CIR246	F: TTAGGGTTTAGTTGAATGG R: ATGAACACACGCACG	AD_ch14_Dt.02	51°	M
2	BNL2884	F: TCAACTCATACCAAATCAATTCC R: CCCTGTTTTGTTCAATGGGT	AD_ch06_At.06	51°	M
3	BNL1693	F: CCCTTGGGAATAGCAGGTG R: CATGTGTCTCCGTGTGTGTGTG	AD_ch15_Dt.01	51°	M
4	CIR014	F: AGCTTGCCTCTTTCTG R: ACATTAGAACTCCCTGCT	NA	51°	M
5	BNL1153	F: CTTTATCCGGAGACGGAACA R: CTAACTTTTGCTCACCCCCA	Chr25	51°	M
6	CIR316	F: TTACAGGCACTACCACC R: CCTTTCTGGCGACTT	AD_ch11_At.11	51°	M
7	BNL1059	F: CCTTCTCTGACACTCTGCCC R: TGTATTCTCTTCTTTTCCTTATACTTTT	AD_ch14_Dt.02 AD_ch03_At.03	51°	M
8	BNL2568	F: GGGAAGAGAGGGAGACTAACG R: ATTTTGATAGGTTGGTTTTGTCC	AD_ch24_Dt.08	51°	M
9	BNL3147	F: ATGGCTCTCTCTGAGCGTGT R: CGGTTCAGAGGCTTTGTTGT	AD_ch24_Dt.08	51°	M
10	BNL1964	F: AGGGAGGGGGAGGTTCTC R: CGGTAGTCCTCCACCATGTT	AD_ch16_Dt.07	51°	M
11	BNL1122	F: TCGATAACGGCTATAGTAATCTCTC	AD_ch16_Dt.07	51°	M

		R: CAACAAATAAGCAGCCAAGAAA			
12	BNL1440	F: CCGAAATATACTTGTCATCTAAACG R: CCCCCGGACTAATTTTTCAA	AD_ch25_Dt.06	51°	M
13	BNL1417	F: TTATTCTAACCACCGCCTCC R: TGAGTGGATATGCTTGGCCT	AD_ch25_Dt.06	51°	M
14	BNL1878	F: TGCTTCAACTGCTCTTGCAT R: TCGATATCTGGAACACCCAC	AD_ch19_Dt.05	51°	M
15	BNL1513	F: TTTCACAAGCACAACCATAGG R: AATACAGGTTCAAAGTTGATAGGG	AD_ch18_Dt.13	51°	M
16	BNL3599	F: TTTAGCCCCAGTAACATGCC R: ACTGCAAGCTCTGCCCTAAA	NA	51°	M
17	JESPER 153	F: GATTACCTTCATAGGCCACTG R: GAAAACATGAGCATCCTGTG	AD_ch18_Dt.13	51°	M
18	JESPER 127	F: GATTTGGGTAACATTGGCTC R: CTGCAGTGTTGTGTTGGGTAGA	AD_ch24_Dt.08	51°	M
19	CIR307	F: GACTTGAAAAGATTACACAC R: GAATTTGCTGGCTCT	AD_ch15_Dt.01	51°	M
20	BNL3255	F: GACAGTCAAACAGAACAGATATGC R: TTACACGACTTGTTCCCACG	AD_ch04_At.04	51°	M
21	CIR316	F: TTACAGGCACTACCACC R: CCTTTCTGGCGACTT	AD_ch11_At.11	51°	M
22	MUCS277	F: TATACCTCCACTCCCTCCCC R: CCAATACCCTCTTCTCACCG	AD_ch21_Dt.11	55°	M
23	NAU3485	F: GTTCAAAGTCGGGTTATTGG R: AGTGCAACGGCTTAGGATAC	NA	55°	M
24	MUCS459	F: TAAGACACGCAAGCCATTTC R: CGCAAGCCACTCCCTTTAC	AD_ch02_At.02	55°	M
25	MUCS359	F: GAGCTTGGTTGGTTTCAAGC R: CCCCCTGTGATCTTTTTACG	AD_ch14_Dt.02	55°	M
26	BNL3971	F: CACATATTTTTGCCTCACGC R: TGTGGACCCAAAAAGGAAGA	AD_ch14_Dt.02	55°	M
27	NAU29	F: CGATGCATCCATATCCATTA	A02	55°	M

	51	R: ATTGGCTTCAATTTGTGATG			
28	TMF17	F: TTTGCGCCCTTTACTCAATC R: CAATGTATGAATCTTTTCCACAA	AD_ch02_At.02	55°	M
29	NAU1300	F: CAGGAGAATTAAAGGCAAGG R: TTACGGTGCAAATCATCAAC	A.09	55°	M
30	NAU3284	F: CGAACACTATGATTGCTGGA R: CCCACCCCTACCACATTT	AD_ch09_At.09	55°	M
31	NAU3478	F: ATGGCACTTCCTCATCATCT R: TGTTGGTTGACTGGAATGAC	A.10	55°	M
32	STV138	F: AACTCACCAACCGTTATCATGTCA R: TTGGGGTGATGAAGAGAAAGGTAA	AD_ch11_At.11	55°	M
33	BNL1607	F: TCCAAAAAGGAAGGGTTGTG R: ATGTGGCACCTCGAGATCAC	AD_ch11_At.11	55°	M
34	TME20	F: CGCAAACGAACCAGTACAGA R: GCGTCTACATTAGCGCCATA	A.12	55°	M
35	MUCS329	F: CTGATTTTCTTTGCGTGTGG R: TGACAATCAAAGCCAAAACC	AD_ch14_Dt.02	55°	M
36	JESPR58	NA	AD_ch19_Dt.05	55°	M
37	NAU3482	F: GTGGGTGCAACAAAAATGTA R: TTCTTCAAGCTCCCATGAAT	NA	55°	M
38	MUCS379	F: CAGGACTAGCCGATTGAACC R: AACAGACACCATTGTGATTCG	NA	55°	M
39	NAU453	F: CCACCACCACTCTCCTTCTC R: TCTTCTGCTGGTGCAATGAC	AD_ch08_At.08	55°	M
40	BNL218	F: AGGAAGGCATGTCATGGGTA R: TTCGCTTTGCAGGAAGACT	AD_ch05_At.05	55°	M
41	NAU2730	F: TATAAGCAAAGGGGTCAAGG R: GTACCATGGAACAGGGAAAG	AD_ch13_At.13	55°	M
42	NAU3475	F: CCTTTGACTTCGAGAAGAGG R: CCTTCTTTTCCCCCTTTTAC	A.05	55°	M
43	NAU3166	F: AACTGGTTTTTCTGGCTTTG R: GTATTGCAGGCCTCATTTTG	AD_ch25_Dt.06	55°	M
44	NAU4943	F: TCCTCTAAGAACCCAACTCG R: TTTCTGTGCCTTCTTCATCA	AD_ch16_Dt.07	55°	M

45	NAU5068	F: CCAGATTTTGAAGTCCAACC R: ATAGCAGCTAACGACGCTCT	A.07	55°	M
46	BNL3147	F: ATGGCTCTCTCTGAGCGTGT R: CGGTTCAGAGGCTTTGTTGT	AD_ch11_At.11	55°	M
47	MUCS452	F: CCAACAGGAGGAGCATTTG R: CAATCACGGCTTTCACGAG	A.11	55°	M
48	BNL4053	F: TGAAGGCTTTGAAGCAAACA R: AAGCAAGCACCAAGTTAGCC	AD_ch09_At.09	55°	M
49	NAU3275	F: CTCGTGATCCGTTCTCTTCT R: TTTTGTTTCCCCAAAAGGTA	A.04	55°	M
50	TMC05	F: ATCAGCCAATCACCGAGAAC R: CTCAATGGCTGCATGAACAG	AD_ch19_Dt.05 AD_ch10_Dt.10	55°	M
51	MUCS240	F: TCCCTAGGATAAGCACTGCC R: TTACACGGCGGTTTTAGAGC	A07	55°	M
52	NAU870	F: GGCTTACTTTGTTTCCGTTT R: GGAGCTAAAACCCATCACC	A07	55°	M
53	STV174	F: TTGCAGTACCTCAATCTGCTGTTC R: TCCCAAACTGTGTTTTTGTTCCTT	A.04	55°	M
54	BNL1902	F: TTGGTAGGCTTTGAAATTGTAACA R: GACCAAAAGATTTCCTTCTTAGCA	AD_ch06_At.06 AD_ch25_Dt.06	55°	M
55	NAU3052	F: CGCAGCCTTTTCCTTTTT R: ACAAGCAAGCGATTCATACA	AD_ch09_At.09	55°	M
56	MUCS291	F: TGATTCCAAGGAACACCACC R: TGTGGGACACCTCTAGGAGC	A.12	55°	M
57	BNL244	F: AGATTGAAATGCAGCTTCGG R: TTTGGAAGAGCACAAAACCC	AD_ch03_At.03 AD_ch14_Dt.02	55°	M
58	NAU5212	F: GCAAAAAGAGACAACCTTCC R: TAAAGAACCGCCTCTACACC	AD_ch21_Dt.11	55°	M

59	BNL4059	F: GAGTTACGCCTGGCAATCAT R: CCATCCCCAGTGGTGTTATC	AD_ch12_At.12	55°	M
60	NAU5351	F: AAGTGTTGTGGGGAAATGTT R: CATTCTTGGACACCACAAAA	AD_ch05_At.05	55°	M
61	CIR131	F: AAGAGAATATCAAAGCCC R: TCAGCTTGACCCTCC	A.07	55°	M
62	NAU3139	F: CGTCATCATAAACCAACGTG R: CAAGCAATTGTTTCTCATCCT	AD_ch19_Dt.05	55°	M
63	MUCS048	F: GCTTTTCCTTGATGAAATCGG R: TTAATAATGGCAGCAGCATCC	A.08	55°	M
64	MUCS496	F: ATTCGGCACGAGGATTGC R: AATCTCTACCGTCGGTTTCG	A.08	55°	M
65	NAU2252	F: CCCCCTCCCTTGTATTTTT R: TACCTGGGTATCCAACCATC	AD_ch05_At.05	55°	M
66	NAU836	F: GAGGCAATTGAAGCAAGAAC R: AGGTCGACAGAGACAAGAGG	A.05	55°	M
67	BNL1671	F: CAAAAATAGTTAATAAAACAAAGTAGAACG R: ATAACATCAGGGGGAAAGGG	CH19_05	55°	P
68	NAU810	F: TCACCGTATCACCACCATTA R: AATGTTCTCAACACCCTTGA	A.05	55°	M
69	NAU5352	F: CAAGGCTGCTGTGTGATAAT R: AATAATGCCACAGGAGAAGG	AD_ch05_At.05	55°	M
70	NAU903	F: CCTCTTCTTCACCACTGCTT R: GAGTGATGCAGACGAAATGA	A.08	55°	M
71	BNL1080	F: AGCTAAAGCACACACCCCTC R: AATGGAGGGTTGGTCCCTAC	AD_ch03_At.03	55°	M
72	MUCS474	F: GAAAGCAATGGCCAAAAGG R: TCTGTTGGTGTCTCCCAAGG	A.08	55°	M
73	NAU1135	F: GGAAAACCAAATTCAAATAACC R: TTTTCCCTATGCTTGCTTTC	AD_ch02_At.02	55°	M
74	NAU3889	F: TGATGCCTTTAGGGTTGAAT R: TTTGCTGAGTTTGCATTTGT	AD_ch21_Dt.11	55°	M
75	MUCS499	F: GCTTTGAAAGAGGAATCAACG R: GCCTTGAATGGCATAAAACC	A.11	55°	M
76	BNL3449	F: AAGCTGTGGCTATGATGCCT R: AGAGCAAAAAACAATTACAAAA	AD_ch21_Dt.11	55°	M

		GC			
77	NAU805	F: TAAGAAACAGGCATCCAATG R: TCCTCAGGAGAAAGATTTGG	AD_ch17_Dt.03	55°	M
78	MUCS229	F: CTTAAAACCCAACAGCTCCG R: TTGTACGGTCTTCCACCAGC	A.04	55°	M
79	NAU1225	F: CAGCAAATTCGCAAGAGTTA R: CTAACAGGGGTGACATAGGG	AD_ch05_At.05	55°	M
80	JESPR82	NA	AD_ch21_Dt.11	55°	M
81	MUCS262	F: CTAGCTTGAAATCGGGTTCG R: AGTAATCGGATGATGGCTGG	A.11	55°	M
82	MUCS198	F: AGAGAGGCAATCAGGAATCG R: AATTACCCTCCAATGGTGCC	A.13	55°	M
83	NAU2887	F: CACCATGAGCCACTAATTCA R: ACACATTTTTCCCTTTTTGG	AD_ch07_At.07	55°	M
84	STV190	F: GTTGAAGAAGCAGAGACCGTGAAT R: CCTACAGATATGGGAGCCAACAAA	A.03	55°	M
85	JESPR274	F: GCCCACTCTTTCTTCAACAC R: TGATGTCATGTGCCTTGC	AD_ch23_Dt.09	55°	M
86	NAU1167	F: CTGACTTGGACCGAGAACTT R: AAGAGCCCTGGACAATGATA	AD_ch17_Dt.03	55°	M
87	NAU2700	F: TTTACGACAGCCTCATTTCA R: AATTTGCACCCTGCTTTTAG	AD_ch25_Dt.06	55°	M
88	NAU2181	F: AGCAAGCTTCCACAGCTTAT R: GGGCTCGAAAATGTAGTTTG	AD_ch03_At.03	55°	M
89	BNL3347	F: AGACTGACATGCAGCTTCCA R: ATCTTAATTTTGAGTATAGGATAGGGG	AD_ch19_Dt.05	55°	M
90	CIR141	F: CGCACAAGGAATAGAAG R: ACCCAACATAAGGACTAAA	AD_ch07_At.07	55°	M
91	NAU5152	F: TCCTTTCTACCCATGCCTAC R: GTCACGAGAAGCAGAGGACT	AD_ch07_At.07	55°	M
92	NAU414	F: TCTCTCAAAATCTCAAACCCAGA	AD_ch09_At.09	55°	M

		R: GCTTAGGGCAAACCACTGAA			
93	NAU758	F: CATTTCGGAACAAGAAAACC R: GAGCTCATTGCAGACATTTG	A.02	55°	M
94	NAU1035	F: CTCATCGGAAATCCACTCAT R: TGAAGCCTTGGTTGTAGATG	AD_ch23_Dt.09	55°	M
95	CIR003	F: AAACCAACAGGAATATGAG R: GGGGAAGATAACACGA	AD_ch11_At.11	55°	M
96	CIR009	F: GTGGGTATGGAGTTGTTT R: CCGTTAGGTGTCTTTCTC	AD_ch15_Dt.01	55°	M
97	CIR13	F: TCATTGCCATTTGACC R: TCCACCTTCCACACC	AD_ch21_Dt.11	55°	M
98	CIR17	F: CATTTCGGGTTCATTC R: GATTTCATCCATTCCAAC	AD_ch24_Dt.08	55°	M
99	CIR18	F: TCAACTATCAGTCCAAT R: AAAGAGACCCACAAG	AD_ch10_At.10 AD_ch01_At.01	55°	M
100	CIR20	F: ATGCTGGGAAGCTGTA R: TTCTTGTGGTGGATTTG	AD_ch18_Dt.13 AD_ch13_At.13	55°	M
101	CIR24	F: TGTAGACAGTCATGTCCTTAT R: TGAGTCGGATACATTGC	AD_ch19_Dt.05	55°	M
102	CIR27	F: CGTTAGAATACCAAGCTG R: AGGAGGATTTGTTAAAGG	AD_ch04_At.04 AD_ch13_At.13	55°	M
103	CIR28	F: CACGAATCTCCAATC R: ATAGATGCCTTCTCTTT	AD_ch07_At.07	55°	M
104	CIR30	F: CAATATCTCACTTGGACCT R: TGCTACACATCATAGTTGG	AD_ch03_At.03	55°	M
105	CIR32	F: CAAGTTTCCTTGTAAGTTC R: GATCTGCTTCTTTAGCTC	AD_ch26_Dt.12	55°	M
106	CIR33	F: CTATCCAGCCTTCGATT R: TGCATGACACAACATGA	AD_ch03_At.03	55°	M
107	CIR34	F: ACCCTTGACAGTTACCAC R: TGCCCATTTAGGTATGA	AD_ch05_At.05	55°	M
108	CIR36	F: GTGAGACTTGAACCCAA R: GACTCACAACCTGATTCTAC	AD_ch22_Dt.04	55°	M
109	CIR38	F: AGAGCTTCAGGTAAGACAA	AD_ch17_D	55°	M

		R: TGAACTAGCAAATCAGACA	t.03		
110	CIR39	F: GGAGCAGAAACAACCA R: TTCCCATCTTCACTTCTC	AD_ch26_Dt.12	55°	M
111	CIR40	F: CCCACCAAGAGCATT R: TTCTTATATTTGCTACCCAC	AD_ch13_At.13	55°	M
112	CIR42	F: GGGTAGTTCTCAGCTTT R: CTATTTCTCTATACCCAAGA	AD_ch12_At.12	55°	M
113	CIR43	F: CAGGGCTTCTTTATTATGT R: GGTGAAGTGGTTTCTCTC	AD_ch20_Dt.10	55°	M
114	CIR47	F: ATTCTACCAAACCTCTACC R: CCCGTGTGAGTGAAA	AD_ch14_Dt.02	55°	M
115	CIR48	F: CCCACCAAGA R: TTCTTATATTTGCTACCCACGCAT	AD_ch22_Dt.04	55°	M
116	CIR51	F: AGAGATTAGTTGCTGGAGA R: TCACAGACGAAGGCA	AD_ch11_At.11	55°	M
117	CIR54	F: TTTCCCTGGTATGCTG R: CAATTTCTTCCTCTCGTT	AD_ch13_At.13	55°	M
118	CIR55	F: AACCATTGTCGAGTAAGTAA R: CCGAGTAGGTCATTGTCT	AD_ch01_At.01	55°	M
119	CIR57	F: TTTCGTTCCATTGCTT R: GTGTCTTTGATTCGGTTT	AD_ch13_At.13	55°	M
120	CIR58	F: CCATCTTCCTTTCATACC R: AGCTGAAGAACTATACCCA	AD_ch03_At.03	55°	M
121	CIR60	F: CTTGCTTCCTCACCC R: GAATGCTACTTTCATCCTAC	AD_ch23_Dt.09	55°	M
122	CIR61	F: TTAGTCCTCTACATACCGAA R: TCATAATAAAGGCGTGG	AD_ch24_Dt.08	55°	M
123	CIR62	F: TTTAGAGGAGAAGTTTAGG R: CAGTCTCTTGTAGTTTCATT	AD_ch05_At.05	55°	M
124	CIR63	F: CACTATAAACCCAAGCAGT R: GTGTGTTGTTGTTGAGGA	AD_ch20_Dt.10	55°	M
125	CIR67	F: AAATGCAAAGCATGGA R: TCTGGAAGCAAACTGAA	AD_ch05_At.05	55°	M
126	CIR68	F: TAGCCATCCAAATCATC R: TGTACCTTGGTTAATTCCT	AD_ch21_Dt.11	55°	M
127	CIR69	F: GTCACTGCTATACACTTTCC R: TATTGGGCTTTGATTTG	AD_ch21_Dt.11	55°	M
128	CIR70	F: AACCACCAACCATTCA	AD_ch24_D	55°	M

		R: TGGGACTCGGTCATC	t.08		
129	CIR71	F: TGAGCTTTACCGCTTT R: CCCGTATTCTCCCTTT	AD_ch25_Dt.06	55°	M
130	CIR78	F: TGCATGATGAAGTTAGA R: ACATAAATCCCAAGAAC	AD_ch26_Dt.12	55°	M
131	CIR79	F: TCCTCGTAGCTCGGT R: GCTGAGCTGAACCATT	AD_ch09_At.09	55°	M
132	CIR80	F: TGAAACAAACAGAGCC R: CATGGAAGAAAGATTGA	AD_ch20_Dt.10	55°	M
133	CIR81	F: AAAGAACCCATGAGAAGA R: GCTGTCTATGTTGGTGG	AD_ch12_At.12	55°	M
134	CIR82	F: AAAGAACCCATGAGAAGA R: GCTGTCTATGTTGGTGG	AD_ch10_At.10	55°	M
135	CIR85	F: ATTCCGATGTCTCCCT R: TTTAGCCTGATAAGTTCGT	AD_ch26_Dt.12	55°	M
136	CIR89	F: CTCCATTCCTCGTTTG R: AGATTTCGTTTCCCATT	AD_ch01_At.01	55°	M
137	CIR94	F: ATACCTCCTTTGGCATC R: ATTCAGCAACTTCACACA	AD_ch20_Dt.10	55°	M
138	CIR96	R: GATCTCATATTTGGCTCTG F: ACCCATCACCGTATCTT	AD_ch13_At.13	55°	M
139	CIR97	F: ACTGGATGATGAAGCC R: GACTTTCCCTTTACCTCA	AD_ch14_Dt.02	55°	M
140	CIR99	F: ATGATTCAAGTCGCGT R: TTCAAGGCTGAGTCAAA	AD_ch18_Dt.13	55°	M
141	CIR100	F: GAGAGGCGATGCTAAA R: GGGATACAAATGGAGAAA	AD_ch16_Dt.07	55°	M
142	CIR102	F: TAACAACTGGATGAGATGA R: CATAACTGCAAAGGAGAA	AD_ch05_At.05	55°	M
143	CIR104	F: GAGAGCATTTGATTCCTT R: GAACTGCTAACACCACCT	AD_ch10_At.10	55°	M
144	CIR105	F: GTCTCTTGTCTTTCTTTCTTAC R: AACCAAACTGAACCCA	AD_ch15_Dt.01	55°	M
145	CIR107	F: AATCACTATCCTCCTCCC R: TGGATTGCTTCTTCTTCT	AD_ch16_Dt.07	55°	M
146	CIR109	F: GAGGAAATCTTCAATTAGGT R: ATCCACAAGAAACTGAAAC	AD_ch25_Dt.06	55°	M
147	CIR110	F: GCTTTCTTCTTCGGTTT R: CAGTGATGCTTGAGTTTC	AD_ch15_Dt.01	55°	M

148	CIR112	NA		55°	M
149	CIR114	F: TTGTAATGGAACTTTGGTC R: GGTGAGTAAATAAACGGG	AD_ch01_At.01	55°	M
150	CIR119	F: GGACCATGCA R: AAAGGCTGTTTCTACCCAAGAAG	AD_ch24_Dt.08	55°	M
151	CIR121	F: GGTGAATCCGACAAAC R: AATTGTAAGAGCCGAAA	AD_ch20_Dt.10	55°	M
152	CIR122	F: AATGTGGGCTGATACG R: CAGACACAATCCACAAAG	AD_ch04_At.04	55°	M
153	CIR128	F: AACAAACATGCGTGC R: TGATGAGTGTAAATGGGA	AD_ch06_At.06	55°	M
154	CIR133	F: TAGCCATTCTCACCCA R: AGGCAGTCAGAGTCAAAG	AD_ch03_At.03	55°	M
155	CIR135	F: AAGCAAAGCAAACAAAG R: GCTTGCCAGTATGATGT	AD_ch13_At.13	55°	M
156	**CIR139**	**F: AAACAAATGGAGAGGGT R: ACCTGTGGTCTGCAAT**	**AD_ch19_Dt.05**	55°	**P**
157	CIR141	F: CGCACAAGGAATAGAAG R: ACCCAACATAAGGACTAAA	AD_ch07_At.07	55°	M
158	CIR142	F: ACCCTGCTCTGTTTCTC R: GAAGTCTATCATTGTTGGC	AD_ch04_At.04	55°	M
159	CIR143	F: AAGAAAGAAGAAACTTCCC R: GCCATTAAGAAGGACAAA	AD_ch15_Dt.01	55°	M
160	CIR148	F: CTAATCTTTGGATTCTACCC R: TCCAAGCCCAGATAAGT	AD_ch12_At.12	55°	M
161	CIR150	F: TTTACAACTCAATCCCATC R: TCCCTTCTTTCACTTCC	AD_ch25_Dt.06	55°	M
162	CIR156	F: CATTGCCCAAGGAGA R: GTAACCAATGAATCCCAG	AD_ch21_Dt.11	55°	M
163	CIR158	F: TGTTGCTCCTTAATTGG R: GGGAGATTGTTGGAGTT	AD_ch15_Dt.01	55°	M
164	CIR165	F: ATAAGTGGAGACAGGCA R: GACCAGCACAGGAAAC	AD_ch19_Dt.05	55°	M
165	CIR166	F: CTACTCTTTCTTCTTTGTGG R: ACTTGTGGAAACTATTCTTG	AD_ch20_Dt.10	55°	M
166	CIR167	R: ACTTGTGGAAACTATTCTTG R: AAATTCCAGCTCATGGT	AD_ch26_Dt.12	55°	M

167	CIR169	F: GAAGCACAATAAGGCAA R: CAAACAAGCGATGAAAC	AD_ch07_At.07	55°	M
168	CIR170	F: TCGGTAAAGATGGGTG R: ATTGGTGCTGGTTGAG	AD_ch26_Dt.12	55°	M
169	CIR171	F: GAAATCCAAATCCAACC R: AACCACCCGACTCTTT	AD_ch20_Dt.10	55°	M
170	CIR172	F: GGTCCATTCCTTCTTGT R: CCGAAATCCTCTTCTTC	AD_ch22_Dt.04	55°	M
171	CIR175	F: TGCAACAAACTTCAACTC R: TCCAAAGAAGAATATGGTC	AD_ch16_Dt.07	55°	M
172	CIR176	F: TTTACCTCACAAGACTCTCA R: ATTTGAGCAATGAACACTAC	AD_ch19_Dt.05	55°	M
173	CIR179	F: ATGGGTCCAAAGTAAGAG R: GATTTGGGAAGCAGAA	AD_ch19_Dt.05	55°	M
174	CIR180	F: TTGAAGAACGTAGGTGG R: TCCGACCTGTTCAAAT	AD_ch17_Dt.03	55°	M
175	CIR181	R: TCCGACCTGTTCAAAT R: TCTTGAAGGGAAACGA	AD_ch14_Dt.02	55°	M
176	CIR183	F: CCAGATTAGAACCTATGAAAC R: TAGCCCATTTCTTACCAC	AD_ch22_Dt.04	55°	M
177	CIR184	F: TACACGGAAGACAACAAG R: CTTTGTTACTGAAATGGGT	AD_ch02_At.02	55°	M
178	CIR185	F: GCATTTGTATTTCCCTGT R: GATCAAGTCCAGAGTCCA	AD_ch05_At.05	55°	M
179	CIR187	F: TGGTTCTTCTTGATTGG R: TTGGGAAACTTGTAGGA	AD_ch20_Dt.10	55°	M
180	CIR194	F: ATTGTCTTGGACTTTGGT R: TCAGTTGAAAGTATTCCCT	AD_ch23_Dt.09	55°	M
181	CIR196	F: TGAGTGGTTGTTTGTTTC R: TTAGACAGAGGGAATGCT	AD_ch11_At.11	55°	M
182	CIR199	F: CAGAATTTGACCGTTTC R: GCCATGATATTTCGGT	AD_ch01_At.01	55°	M
183	CIR200	F: CCAGGATTCAGTTAGTTT R: TTCTTGAACACATTTGG	AD_ch23_Dt.09	55°	M
184	CIR202	F: TGAAAGTTGAAAGTGTGG R: ACGCTTTAGTTGCAGAG	AD_ch03_At.03	55°	M
185	CIR207	F: AACAAGAGAAGGTGAAGG R: ATCTCAAAGGGCACAA	AD_ch11_At.11	55°	M
186	CIR209	F: TGCATGGATTCCTTATT R: TTCCAGTCAAACCAAAC	AD_ch08_At.08	55°	M

187	CIR210	F: CCTGATAGTGAGTTTCTTCTT R: TGAAATGTGAGTGTTTGTG	AD_ch14_Dt.02	55°	M
188	CIR212	F: ATGAACGCTACTGGGA R: ACAAGCAAACAACCTGA	AD_ch19_Dt.05	55°	M
189	CIR216	F: ATCTGAACCATCATCCTC R: TTCTGATTGGCACTTTC	AD_ch18_Dt.13	55°	M
190	CIR218	F: GCGAAGCAAAGGAAG R: CTCCAACATCGTCTCAA	AD_ch04_At.04 AD_ch22_Dt.04	55°	M
191	CIR219	F: TTGCCTTGGTCTTTGT R: CGAAACCATTAACTTCCT	chr19	55°	M
192	CIR222	F: TCATCAACAATCCTTCC R: TACTGTCCCTCTTGCAT	AD_ch19_Dt.05	55°	M
193	CIR223	F: AGAAACATCTGCAAACCT R: ATCTCTCTAATCCTTGCTTC	AD_ch04_At.04	55°	M
194	CIR224	F: AGGTTTGCTGTTTCTACC R: AACAGAGGGTGACAGTTT	AD_ch19_Dt.05	55°	M
195	CIR227	F: ATCAGCCATCCAGAAA R: TAAGATTGAAGTGAAGGTGT	AD_ch09_At.09	55°	M
196	CIR228	F: TCCAGGTAAACTCAACAA R: TCATCAGTTCAATCACAAG	AD_ch03_At.03 AD_ch14_Dt.02	55°	M
197	CIR229	F: AATAACTGGACTCAACGAC R: ACCTGTGTTCTTATCCTAAA	AD_ch19_Dt.05	55°	M
198	CIR233	F: AGGCAGTAGCATTTATCAG R: GTGTTGGTTGTTTATGGTT	AD_ch26_Dt.12 AD_ch06_At.06	55°	M
199	CIR234	F: AGCACTCATCCATCACA R: GCACCCTTTAGAAACAAG	AD_ch15_Dt.01	55°	M
200	CIR235	F: GCTAGTGCCTGACGAC R: CCCACACCCGTATTT	AD_ch13_At.13 AD_ch05_At.05	55°	M

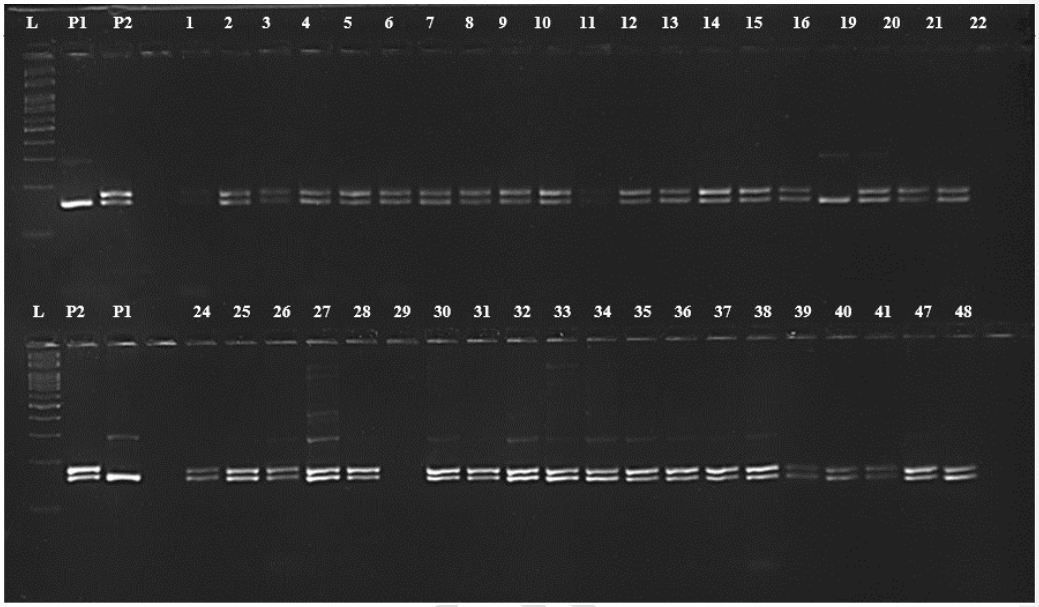

Fig. 3 - The SSR profile of CIR 139 that enable to fix the true to type F_1 plants. Lane 'L' denotes 100 bp ladder, lane 'P1' denotes female parent CO17, 'P2' denotes male parent KC3 and numeric from 1 – 48 denotes the F1 hybrids of cross CO17 X KC3.

Conclusion

This study was undertaken to identify contrasting parents for yield, quality attributes, and sucking pest tolerant features, and it was effective in identifying two cultivars *viz*., CO17 (with high yield and better fiber quality parameters but having poor sucking pest resistance) and KC3 (having anatomical properties that confer sucking pest resistance but having poor fiber yield and quality traits). Both parents were successfully crossed, and the success of this cross combination was determined using SSR markers. These F_1s were advanced to F_2 generation to establish a mapping population suitable for QTL mapping of genes conferring resistance to sucking pest.

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