

Identification of contrasting parents and development of mapping population for sucking pest resistance in cotton

Abstract

The current study was conducted to identify the best parents with good agronomical features and contrasting characteristics for sucking pest resistance to develop a population that can be used for Quantitative Trait Loci mapping of resistance component traits. Initial attempts at this university have enabled to identify of 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 Simple Sequence Repeats 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, to develop 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 an F₂ mapping population. This population is believed to be useful for QTL mapping of anatomical features that confer sucking pest resistance such as trichome length, density, breadth, and sharpness besides fiber yield and quality traits.

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 the 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 resulting in a 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 a 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 that 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)(Collard and Mackill, 2008), 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 (Boopathi, 2020). Such mapping population is established by crossing resistant parents with a susceptible but high-yielding parent. The resulting hybrids are verified using molecular markers like SSRs. This study employed a diverse range of SSR markers to detect genetic variations between the parents, aiding in the validation of hybrids and their progression to subsequent generations. This process offers substantial advantages in understanding and isolating traits related to tolerance against sucking pests (Ulloa *et al.*, 2013). It also paves the way for expediting future molecular breeding programs. This strategy not only accelerates the breeding process but also provides a more precise insight into the genetic foundations of resistance traits. This knowledge can be harnessed in upcoming molecular breeding endeavors, potentially leading to the development of even more resilient and productive crop varieties. Overall, this method signifies a notable progression in agricultural breeding techniques, highlighting the effectiveness of molecular tools in enhancing crop quality and sustainability. Hence, this study was conducted with the 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*, and *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 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 F₁ seeds from each cross combination were sown in (Feb) 2023 by following the 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 (<i>G. hirsutum</i>)	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 (<i>G. hirsutum</i>)	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 (<i>G. hirsutum</i>)	-	-	Regional Agricultural Research Station,	High yielder, Resistant to Leafhoppers and

				Nandyal.	other sucking pests. Suitable for high-density planting systems.
CO18 (<i>G. barbadense</i>)	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 (<i>G. arboreum</i>)	-	1986	Rajasthan Agricultural University, Sriganganagar.		Moderately resistant to bollworms, and aphids, Resistant to root rot, and tolerant to drought. Suitable for low-input cultivation practices.
K12 (<i>G. arboretum</i>)	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 (General R-shiny based Analysis Platform Empowered by Statistics) version 1.0.0 software. The analysis of variance was performed for assessed traits, and the Latin Square Design test was performed to reveal the statistical differences among the treatments.

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

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

(Treatments with the same letters are not significantly different).

Trichome analysis

Data on leaf trichome density were recorded in parental lines and individual plants of the F₁ 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 plant'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 being examined 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-Marooof *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), 1 µL Taq buffer 10 X, 1 µL dNPTs, 0.5 µL Taq DNA polymerase, 5 µL autoclaved water and 1 µL each of forward (0.5 µM) and reverse primer (0.5 µM). The 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. Concerning fiber strength and fiber length, CO18 had the maximum fiber strength (36.2 g/tex) and fiber length (31.02 mm) followed by *hirsutum* species viz., CO17, KC3 and NDLH1938. Similarly, Kumaret al. (2009) noted a fiber strength of 33.4 g/tex in recombinant lines of upland cotton and Hampannavaret al. (2015) reported a fiber length of approximately 29.67 mm and a fiber strength of 39.35 g/tex in *G. hirsutum* germplasm, whereas RG-8 and KC3 had the lowest fiber length and strength. Similar results were also observed in *G. arboretum*, they noted a fiber length ranging from 19 to 21 g/tex. 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 the case of spinnability to a higher count (which suggests finer and more desirable cotton for spinning) CO17, KC3, and NDLH 1938 were rated with a 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 had 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
Mean	1563.83	25.72	25.07	33.08	35.83

Trichome analysis

Trichome characters of the investigated parents were measured, compared, and presented in Table 4. Analysis of the variance 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 trichomes per milli meter square. It is believed that trichomes provide a robust physical barrier against herbivores and potentially make them 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. CO17 had the lowest trichome density of 2 per milli meter square. Notably, CO18

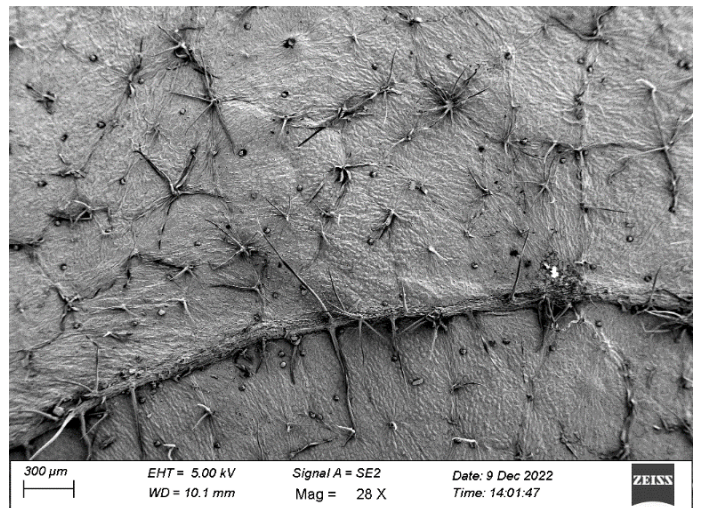
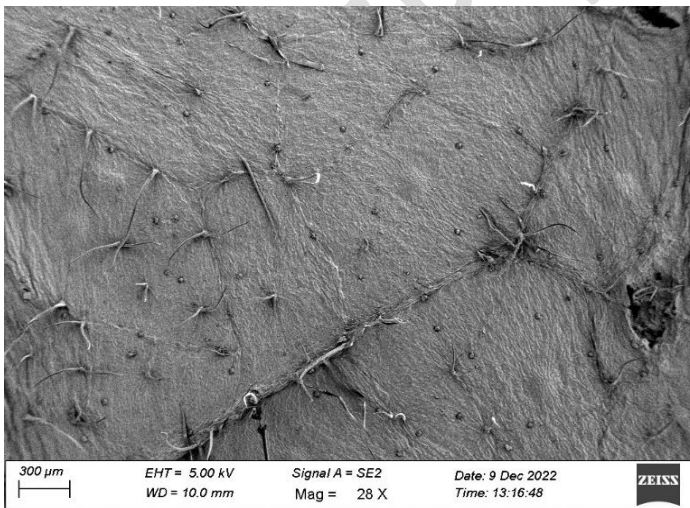
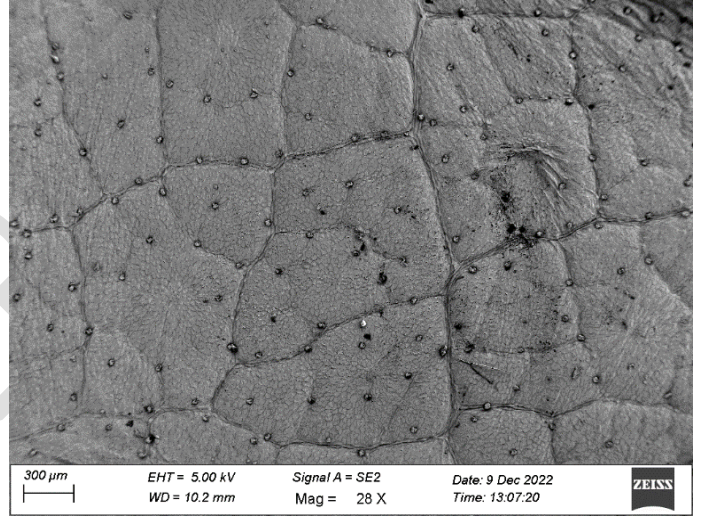
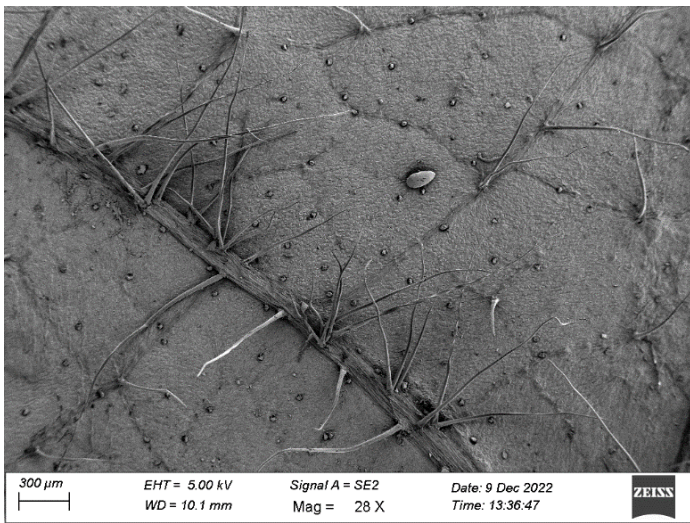
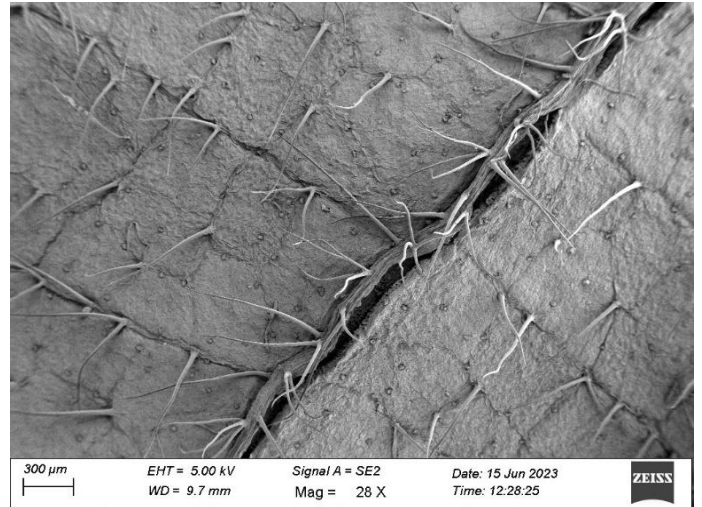
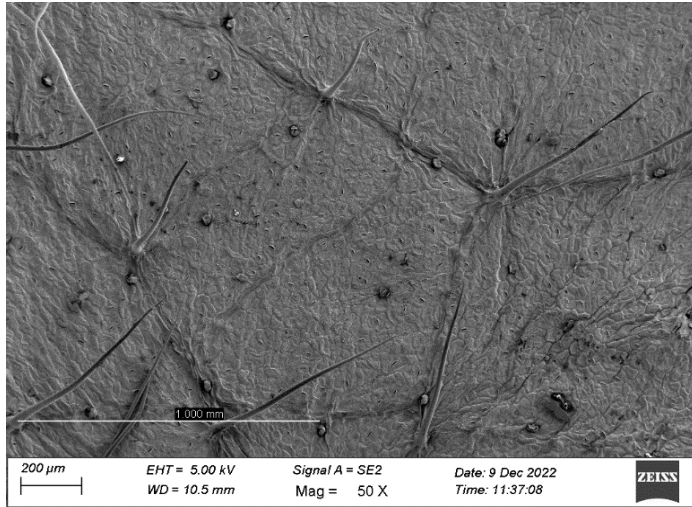


Fig. 1 - Scanning electron microscope pictures of abaxial leaf surface showing trichomes density of six parents A) CO17, B) KC3, C) NDLH1938, D) CO18, E) RG-8, F) K-12

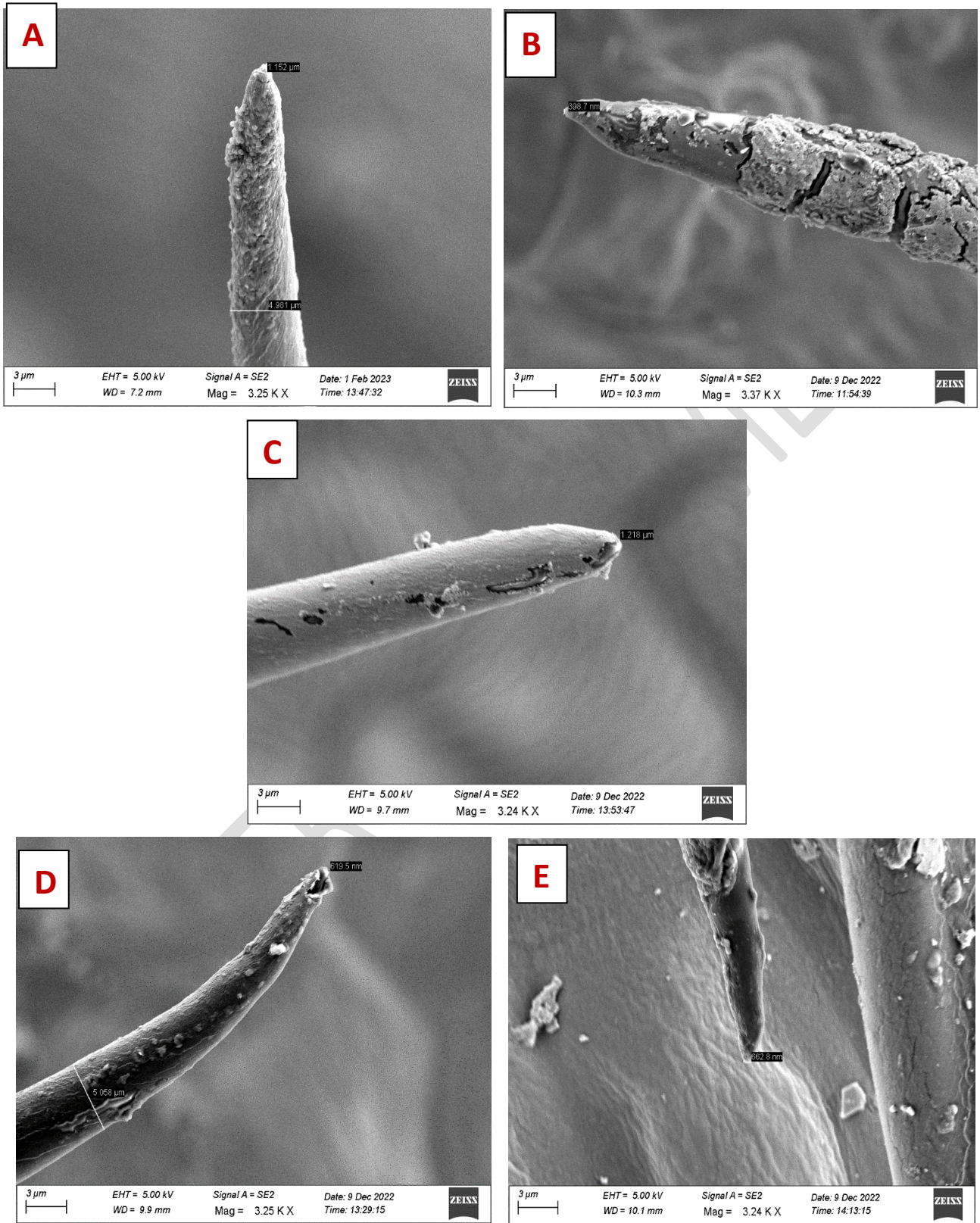


Fig 2. - Scanning electron microscope pictures of abaxial leaf surface showing trichomes sharpness of 6 parents A) CO17, B) KC3, C) ND LH1938, D) RG-8, E) K-12

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 were 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 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 to its pest-resistance behavior and make 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 the mapping population derived from these two parents would segregate for sucking pest resistance. The F_1 plants that 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

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

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

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

Parents	Trichomes Density (1mm)	No. of Glandular Trichomes (1mm)	Trichomes Length (μm)	Trichomes Width (μm)	Trichomes Sharpness (μm)
CO17	2c	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
Mean	3.50	19.67	436.06	18.16	0.67

SSR Assay to Fix True Hybrids

The F_1 plants derived from CO17 and KC3 were found to be anatomically (trichomes characteristics) better performing and they were analyzed for 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 a polymorphic marker as it permitted to differentiation and identification of 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 the 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 F₁s which were not shown to have the two alleles of these two parents were discarded (F₁ lines *viz.*, 19- which has only one band and 29- which has no band) and not used further in generation advancement. As a result, 38 of the 40 F₁ plants were tagged as true hybrids and they were advanced to generate an F₂ mapping population. This population is believed to be useful for QTL mapping of anatomical features that confer 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_D t.02	51°	M
2	BNL28 84	F: TCAACTCATAACAAATCAATTCC R: CCCTGTTTTGTTCAATGGGT	AD_ch06_A t.06	51°	M
3	BNL16 93	F: CCCTTGGGAATAGCAGGTG R: CATGTGTCTCCGTGTGTGTGTG	AD_ch15_D t.01	51°	M
4	CIR014	F: AGCTTGCCTCTTTCTG R: ACATTAGAACTCCCTGCT	NA	51°	M
5	BNL11 53	F: CTTTATCCGGAGACGGAACA R: CTAAC TTTTGCTCACCCCA	Chr25	51°	M
6	CIR316	F: TTACAGGCACTACCACC R: CCTTCTGGCGACTT	AD_ch11_A t.11	51°	M
7	BNL10 59	F: CCTTCTCTGACTCTGCCC R: TGTATTCTCTTCTTTTCCTTATACT TTT	AD_ch14_D t.02 AD_ch03_A t.03	51°	M
8	BNL25 68	F: GGG AAGAGAGGGAGACTAACG R: ATTTTGATAGGTTGGTTTTGTCC	AD_ch24_D t.08	51°	M
9	BNL31 47	F: ATGGCTCTCTGAGCGTGT R: CGGTT CAGAGGCTTTGTTGT	AD_ch24_D t.08	51°	M

10	BNL19 64	F: AGGGAGGGGGAGGTTCTC R: CGGTAGTCCTCCACCATGTT	AD_ch16_D t.07	51°	M
11	BNL11 22	F: TCGATAACGGCTATAGTAATCTCT C R: CAACAAATAAGCAGCCAAGAAA	AD_ch16_D t.07	51°	M
12	BNL14 40	F: CCGAAATATACTTGTCATCTAAAC G R: CCCCCGGACTAATTTTTCAA	AD_ch25_D t.06	51°	M
13	BNL14 17	F: TTATTCTAACCACCGCCTCC R: TGAGTGGATATGCTTGGCCT	AD_ch25_D t.06	51°	M
14	BNL18 78	F: TGCTTCAACTGCTCTTGCAT R: TCGATATCTGGAACACCCAC	AD_ch19_D t.05	51°	M
15	BNL15 13	F: TTTCAACAAGCACAACCATAGG R: AATACAGGTTCAAAGTTGATAGG G	AD_ch18_D t.13	51°	M
16	BNL35 99	F: TTTAGCCCCAGTAACATGCC R: ACTGCAAGCTCTGCCCTAAA	NA	51°	M
17	JESPE R 153	F: GATTACCTTCATAGGCCACTG R: GAAAACATGAGCATCCTGTG	AD_ch18_D t.13	51°	M
18	JESPE R 127	F: GATTTGGGTAACATTGGCTC R: CTGCAGTGTTGTGTTGGGTAGA	AD_ch24_D t.08	51°	M
19	CIR307	F: GACTTGAAAAGATTACACAC R: GAATTTGCTGGCTCT	AD_ch15_D t.01	51°	M
20	BNL32 55	F: GACAGTCAAACAGAACAGATAT GC R: TTACACGACTTGTTCCCACG	AD_ch04_A t.04	51°	M
21	CIR316	F: TTACAGGCACTACCACC R: CCTTTCTGGCGACTT	AD_ch11_A t.11	51°	M
22	MUCS 277	F: TATACCTCCACTCCCTCCCC R: CCAATACCCTCTTCTCACCG	AD_ch21_D t.11	55°	M
23	NAU34 85	F: GTTCAAAGTCGGGTTATTGG R: AGTGCAACGGCTTAGGATAC	NA	55°	M
24	MUCS 459	F: TAAGACACGCAAGCCATTTTC R: CGCAAGCCACTCCCTTTAC	AD_ch02_A t.02	55°	M

25	MUCS 359	F: GAGCTTGGTTGGTTTCAAGC R: CCCCCTGTGATCTTTTTACG	AD_ch14_D t.02	55°	M
26	BNL39 71	F: CACATATTTTTGCCTCACGC R: TGTGGACCCAAAAAGGAAGA	AD_ch14_D t.02	55°	M
27	NAU29 51	F: CGATGCATCCATATCCATTA R: ATTGGCTTCAATTTGTGATG	A02	55°	M
28	TMF17	F: TTTGCGCCCTTACTCAATC R: CAATGTATGAATCTTTTCCACAA	AD_ch02_A t.02	55°	M
29	NAU13 00	F: CAGGAGAATTAAGGCAAGG R: TTACGGTGCAAATCATCAAC	A.09	55°	M
30	NAU32 84	F: CGAACACTATGATTGCTGGA R: CCCACCCCTACCACATTT	AD_ch09_A t.09	55°	M
31	NAU34 78	F: ATGGCACTTCCTCATCATCT R: TGTTGGTTGACTGGAATGAC	A.10	55°	M
32	STV13 8	F: AACTACCAACCGTTATCATGTC A R: TTGGGGTGATGAAGAGAAAGGT AA	AD_ch11_A t.11	55°	M
33	BNL16 07	F: TCCAAAAAGGAAGGGTTGTG R: ATGTGGCACCTCGAGATCAC	AD_ch11_A t.11	55°	M
34	TME20	F: CGCAAACGAACCAGTACAGA R: GCGTCTACATTAGCGCCATA	A.12	55°	M
35	MUCS 329	F: CTGATTTTCTTTGCGTGTGG R: TGACAATCAAAGCCAAAACC	AD_ch14_D t.02	55°	M
36	JESPR5 8	NA	AD_ch19_D t.05	55°	M
37	NAU34 82	F: GTGGGTGCAACAAAATGTA R: TTCTTCAAGCTCCCATGAAT	NA	55°	M
38	MUCS 379	F: CAGGACTAGCCGATTGAACC R: AACAGACACCATTGTGATTCG	NA	55°	M
39	NAU45 3	F: CCACCACCACTCTCCTTCTC R: TCTTCTGCTGGTGCAATGAC	AD_ch08_A t.08	55°	M
40	BNL21 8	F: AGGAAGGCATGTCATGGGTA R: TTCGCTTTGCAGGAAGACT	AD_ch05_A t.05	55°	M
41	NAU27 30	F: TATAAGCAAAGGGTCAAGG R: GTACCATGGAACAGGGAAAG	AD_ch13_A t.13	55°	M
42	NAU34	F: CCTTTGACTTCGAGAAGAGG	A.05	55°	M

	75	R: CCTTCTTTTCCCCCTTTTAC			
43	NAU31 66	F: AACTGGTTTTTCTGGCTTTG R: GTATTGCAGGCCTCATTTTG	AD_ch25_D t.06	55°	M
44	NAU49 43	F: TCCTCTAAGAACCCAACCTCG R: TTTCTGTGCCTTCTTCATCA	AD_ch16_D t.07	55°	M
45	NAU50 68	F: CCAGATTTTGAAGTCCAACC R: ATAGCAGCTAACGACGCTCT	A.07	55°	M
46	BNL31 47	F: ATGGCTCTCTCTGAGCGTGT R: CGGTTCAGAGGCTTTGTTGT	AD_ch11_A t.11	55°	M
47	MUCS 452	F: CCAACAGGAGGAGCATTG R: CAATCACGGCTTTCACGAG	A.11	55°	M
48	BNL40 53	F: TGAAGGCTTTGAAGCAAACA R: AAGCAAGCACCAAGTTAGCC	AD_ch09_A t.09	55°	M
49	NAU32 75	F: CTCGTGATCCGTTCTCTTCT R: TTTTGTTCCTCCAAAAGGTA	A.04	55°	M
50	TMC05	F: ATCAGCCAATCACCGAGAAC R: CTCAATGGCTGCATGAACAG	AD_ch19_D t.05 AD_ch10_D t.10	55°	M
51	MUCS 240	F: TCCCTAGGATAAGCACTGCC R: TTACACGGCGGTTTTAGAGC	A07	55°	M
52	NAU 870	F: GGCTTACTTTGTTTCCGTTT R: GGAGCTAAAACCCATCACC	A07	55°	M
53	STV17 4	F: TTGCAGTACCTCAATCTGCTGTT C R: TCCCAAAGTGTGTTTTTGTTCCT T	A.04	55°	M
54	BNL19 02	F: TTGGTAGGCTTTGAAATTGTAAC A R: GACCAAAAGATTTCTTCTTAGC A	AD_ch06_A t.06 AD_ch25_D t.06	55°	M
55	NAU30 52	F: CGCAGCCTTTTCCTTTTT R: ACAAGCAAGCGATTCATACA	AD_ch09_A t.09	55°	M
56	MUCS 291	F: TGATTCCAAGGAACACCACC R: TGTGGGACACCTCTAGGAGC	A.12	55°	M
57	BNL24	F: AGATTGAAATGCAGCTTCGG	AD_ch03_A	55°	M

	4	R: TTTGGAAGAGCACAAAACCC	t.03 AD_ch14_D t.02		
58	NAU52 12	F: GCAAAAAGAGACAACCTTCC R: TAAAGAACCGCCTCTACACC	AD_ch21_D t.11	55°	M
59	BNL40 59	F: GAGTTACGCCTGGCAATCAT R: CCATCCCCAGTGGTGTATC	AD_ch12_A t.12	55°	M
60	NAU53 51	F: AAGTGTTGTGGGGAAATGTT R: CATTCTTGACACCACAAA	AD_ch05_A t.05	55°	M
61	CIR131	F: AAGAGAATATCAAAGCCC R: TCAGCTTGACCCTCC	A.07	55°	M
62	NAU31 39	F: CGTCATCATAAACCAACGTG R: CAAGCAATTGTTTCTCATCCT	AD_ch19_D t.05	55°	M
63	MUCS 048	F: GCTTTTCCTTGATGAAATCGG R: TTAATAATGGCAGCAGCATCC	A.08	55°	M
64	MUCS 496	F: ATTCGGCACGAGGATTGC R: AATCTCTACCGTCGGTTTCG	A.08	55°	M
65	NAU22 52	F: CCCCTCCCTTGTATTTTT R: TACCTGGGTATCCAACCATC	AD_ch05_A t.05	55°	M
66	NAU83 6	F: GAGGCAATTGAAGCAAGAAC R: AGGTCGACAGAGACAAGAGG	A.05	55°	M
67	BNL16 71	F: CAAAAATAGTTAATAAAACAAAG TAGAACG R: ATAACATCAGGGGGAAAGGG	CH19_05	55°	P
68	NAU81 0	F: TCACCGTATCACCACCATTA R: AATGTTCTCAACACCCTTGA	A.05	55°	M
69	NAU53 52	F: CAAGGCTGCTGTGTGATAAT R: AATAATGCCACAGGAGAAGG	AD_ch05_A t.05	55°	M
70	NAU90 3	F: CCTCTTCTTCACTGCTT R: GAGTGATGCAGACGAAATGA	A.08	55°	M
71	BNL10 80	F: AGCTAAAGCACACACCCTC R: AATGGAGGGTTGGTCCCTAC	AD_ch03_A t.03	55°	M
72	MUCS 474	F: GAAAGCAATGGCCAAAAGG R: TCTGTTGGTGTCTCCCAAGG	A.08	55°	M
73	NAU11 35	F: GGAAAACCAAATTCAAATAACC R: TTTCCCTATGCTTGCTTTC	AD_ch02_A t.02	55°	M
74	NAU 3889	F: TGATGCCTTTAGGGTTGAAT R: TTTGCTGAGTTTGCATTTGT	AD_ch21_D t.11	55°	M

75	MUCS 499	F: GCTTTGAAAGAGGAATCAACG R: GCCTTGAATGGCATAAAACC	A.11	55°	M
76	BNL34 49	F: AAGCTGTGGCTATGATGCCT R: AGAGCAAAAAACAATTACAAAA GC	AD_ch21_D t.11	55°	M
77	NAU80 5	F: TAAGAAACAGGCATCCAATG R: TCCTCAGGAGAAAGATTTGG	AD_ch17_D t.03	55°	M
78	MUCS 229	F: CTAAAACCCAACAGCTCCG R: TTGTACGGTCTTCCACCAGC	A.04	55°	M
79	NAU12 25	F: CAGCAAATTCGCAAGAGTTA R: CTAACAGGGGTGACATAGGG	AD_ch05_A t.05	55°	M
80	JESPR8 2	NA	AD_ch21_D t.11	55°	M
81	MUCS 262	F: CTAGCTTGAAATCGGGTTCG R: AGTAATCGGATGATGGCTGG	A.11	55°	M
82	MUCS 198	F: AGAGAGGCAATCAGGAATCG R: AATTACCCTCCAATGGTGCC	A.13	55°	M
83	NAU28 87	F: CACCATGAGCCACTAATTCA R: ACACATTTTTCCCTTTTTGG	AD_ch07_A t.07	55°	M
84	STV19 0	F: GTTGAAGAAGCAGAGACCGTGA AT R: CCTACAGATATGGGAGCCAACAA A	A.03	55°	M
85	JESPR2 74	F: GCCCACTCTTCTTCAACAC R: TGATGTCATGTGCCTTGC	AD_ch23_D t.09	55°	M
86	NAU11 67	F: CTGACTTGACCGAGAACTT R: AAGAGCCCTGGACAATGATA	AD_ch17_D t.03	55°	M
87	NAU27 00	F: TTTACGACAGCCTCATTCA R: AATTTGCACCCTGCTTTTAG	AD_ch25_D t.06	55°	M
88	NAU21 81	F: AGCAAGCTTCCACAGCTTAT R: GGGCTCGAAAATGTAGTTTG	AD_ch03_A t.03	55°	M
89	BNL33 47	F: ARACTGACATGCAGCTTCCA R: ATCTTAATTTTGAGTATAGGATAG GGG	AD_ch19_D t.05	55°	M
90	CIR141	F: CGCACAAGGAATAGAAG	AD_ch07_A	55°	M

		R: ACCCAACATAAGGACTAAA	t.07		
91	NAU51 52	F: TCCTTTCTACCCATGCCTAC R: GTCACGAGAAGCAGAGGACT	AD_ch07_A t.07	55°	M
92	NAU41 4	F: TCTCTCAAATCTCAAACCCAGA R: GCTTAGGGCAAACCACTGAA	AD_ch09_A t.09	55°	M
93	NAU75 8	F: CATTTCGGAACAAGAAAACC R: GAGCTCATTGCAGACATTTG	A.02	55°	M
94	NAU10 35	F: CTCATCGGAAATCCACTCAT R: TGAAGCCTTGGTTGTAGATG	AD_ch23_D t.09	55°	M
95	CIR003	F: AAACCAACAGGAATATGAG R: GGGGAAGATAACACGA	AD_ch11_A t.11	55°	M
96	CIR009	F: GTGGGTATGGAGTTGTTT R: CCGTTAGGTGTCTTTCTC	AD_ch15_D t.01	55°	M
97	CIR13	F: TCATTGCCATTTGACC R: TCCACCTTCCACACC	AD_ch21_D t.11	55°	M
98	CIR17	F: CATTTCGGGTTCATTC R: GATTTCATCCATTCCAAC	AD_ch24_D t.08	55°	M
99	CIR18	F: TCAACTATCAGTCCAAT R: AAAGAGACCCACAAG	AD_ch10_A t.10 AD_ch01_A t.01	55°	M
100	CIR20	F: ATGCTGGGAAGCTGTA R: TTCTTGTGGTGGATTG	AD_ch18_D t.13 AD_ch13_A t.13	55°	M
101	CIR24	F: TGTAGACAGTCATGTCCTTAT R: TGAGTCGGATACATTGC	AD_ch19_D t.05	55°	M
102	CIR27	F: CGTTAGAATACCAAGCTG R: AGGAGGATTTGTAAAGG	AD_ch04_A t.04 AD_ch13_A t.13	55°	M
103	CIR28	F: CACGAATCTCCAATC R: ATAGATGCCTTCTCTTT	AD_ch07_A t.07	55°	M
104	CIR30	F: CAATATCTCACTTGGACCT R: TGCTACACATCATAGTTGG	AD_ch03_A t.03	55°	M
105	CIR32	F: CAAGTTTCCTTGTAAGTTC R: GATCTGCTTCTTTAGCTC	AD_ch26_D t.12	55°	M
106	CIR33	F: CTATCCAGCCTTCGATT R: TGCATGACACAACATGA	AD_ch03_A t.03	55°	M

107	CIR34	F: ACCCTTGACAGTTACCAC R: TGCCCATTTAGGTATGA	AD_ch05_A t.05	55°	M
108	CIR36	F: GTGAGACTTGAACCCAA R: GACTCACAACCTGATTCTAC	AD_ch22_D t.04	55°	M
109	CIR38	F: AGAGCTTCAGGTAAGACAA R: TGAACTAGCAAATCAGACA	AD_ch17_D t.03	55°	M
110	CIR39	F: GGAGCAGAAACAACCA R: TTCCCATCTTCACTTCTC	AD_ch26_D t.12	55°	M
111	CIR40	F: CCCACCAAGAGCATT R: TTCTTATATTTGCTACCCAC	AD_ch13_A t.13	55°	M
112	CIR42	F: GGGTAGTTCTCAGCTTT R: CTATTTCTCTATACCCAAGA	AD_ch12_A t.12	55°	M
113	CIR43	F: CAGGGCTTCTTTATTATGT R: GGTGAAGTGGTTTCTCTC	AD_ch20_D t.10	55°	M
114	CIR47	F: ATTCTACCAAACCTCTACC R: CCCGTGTGAGTGAAA	AD_ch14_D t.02	55°	M
115	CIR48	F: CCCACCAAGA R: TTCTTATATTTGCTACCCACGCAT	AD_ch22_D t.04	55°	M
116	CIR51	F: AGAGATTAGTTGCTGGAGA R: TCACAGACGAAGGCA	AD_ch11_A t.11	55°	M
117	CIR54	F: TTTCCCTGGTATGCTG R: CAATTTCTTCTCTCGTT	AD_ch13_A t.13	55°	M
118	CIR55	F: AACCATTGTCGAGTAAGTAA R: CCGAGTAGGTCATTGTCT	AD_ch01_A t.01	55°	M
119	CIR57	F: TTTTCGTTCCATTGCTT R: GTGTCTTTGATTTCGGTTT	AD_ch13_A t.13	55°	M
120	CIR58	F: CCATCTTCCTTTCATACC R: AGCTGAAGAACTATACCCA	AD_ch03_A t.03	55°	M
121	CIR60	F: CTTGCTTCCTCACCC R: GAATGCTACTTTCATCCTAC	AD_ch23_D t.09	55°	M
122	CIR61	F: TTAGTCCTCTACATACCGAA R: TCATAATAAAGGCGTGG	AD_ch24_D t.08	55°	M
123	CIR62	F: TTTAGAGGAGAAGTTTAGG R: CAGTCTCTTGATGTTTCATT	AD_ch05_A t.05	55°	M
124	CIR63	F: CACTATAAACCCAAGCAGT R: GTGTGTTGTTGTTGAGGA	AD_ch20_D t.10	55°	M
125	CIR67	F: AAATGCAAAGCATGGA R: TCTGGAAGCAAACCTGAA	AD_ch05_A t.05	55°	M

126	CIR68	F: TAGCCATCCAAATCATC R: TGTACCTTGGTTAATTCCT	AD_ch21_D t.11	55°	M
127	CIR69	F: GTCACTGCTATACACTTCC R: TATTGGGCTTTGATTTG	AD_ch21_D t.11	55°	M
128	CIR70	F: AACCACCAACCATTCA R: TGGGACTCGGTCATC	AD_ch24_D t.08	55°	M
129	CIR71	F: TGAGCTTTACCGCTTT R: CCCGTATTCTCCCTTT	AD_ch25_D t.06	55°	M
130	CIR78	F: TGCATGATGAAGTTAGA R: ACATAAATCCCAAGAAC	AD_ch26_D t.12	55°	M
131	CIR79	F: TCCTCGTAGCTCGGT R: GCTGAGCTGAACCATT	AD_ch09_A t.09	55°	M
132	CIR80	F: TGAAACAAACAGAGCC R: CATGGAAGAAAGATTGA	AD_ch20_D t.10	55°	M
133	CIR81	F: AAAGAACCCATGAGAAGA R: GCTGTCTATGTTGGTGG	AD_ch12_A t.12	55°	M
134	CIR82	F: AAAGAACCCATGAGAAGA R: GCTGTCTATGTTGGTGG	AD_ch10_A t.10	55°	M
135	CIR85	F: ATTCCGATGTCTCCCT R: TTTAGCCTGATAAGTTCGT	AD_ch26_D t.12	55°	M
136	CIR89	F: CTCCATTCTCGTTTG R: AGATTTGTTTCCCATT	AD_ch01_A t.01	55°	M
137	CIR94	F: ATACCTCCTTTGGCATC R: ATTCAGCAACTTCACACA	AD_ch20_D t.10	55°	M
138	CIR96	R: GATCTCATATTTGGCTCTG F: ACCCATCACCGTATCTT	AD_ch13_A t.13	55°	M
139	CIR97	F: ACTGGATGATGAAGCC R: GACTTCCCTTTACCTCA	AD_ch14_D t.02	55°	M
140	CIR99	F: ATGATTCAAGTCGCGT R: TTCAAGGCTGAGTCAAA	AD_ch18_D t.13	55°	M
141	CIR100	F: GAGAGGCGATGCTAAA R: GGGATACAAATGGAGAAA	AD_ch16_D t.07	55°	M
142	CIR102	F: TAACAACCTGGATGAGATGA R: CATAACTGCAAAGGAGAA	AD_ch05_A t.05	55°	M
143	CIR104	F: GAGAGCATTGATTCCTT R: GAACTGCTAACACCACCT	AD_ch10_A t.10	55°	M
144	CIR105	F: GTCTCTTGTCTTTCTTTCTTAC R: AACCAAACCTGAACCCA	AD_ch15_D t.01	55°	M
145	CIR107	F: AATCACTATCCTCCTCCC	AD_ch16_D	55°	M

		R: TGGATTGCTTCTTCTTCT	t.07		
146	CIR109	F: GAGGAAATCTTCAATTAGGT R: ATCCACAAGAACTGAAAC	AD_ch25_D t.06	55°	M
147	CIR110	F: GCTTTCTTCTTCGGTTT R: CAGTGATGCTTGAGTTTC	AD_ch15_D t.01	55°	M
148	CIR112	NA		55°	M
149	CIR114	F: TTGTAATGGAAC TTTGGTC R: GGTGAGTAAATAAACGGG	AD_ch01_A t.01	55°	M
150	CIR119	F: GGACCATGCA R: AAAGGCTGTTTCTACCCAAGAA G	AD_ch24_D t.08	55°	M
151	CIR121	F: GGTGAATCCGACAAAC R: AATTGTAAGAGCCGAAA	AD_ch20_D t.10	55°	M
152	CIR122	F: AATGTGGGCTGATACG R: CAGACACAATCCACAAAG	AD_ch04_A t.04	55°	M
153	CIR128	F: AACAAACATGCGTGC R: TGATGAGTGTAATGGGA	AD_ch06_A t.06	55°	M
154	CIR133	F: TAGCCATTCTCACCCA R: AGGCAGTCAGAGTCAAAG	AD_ch03_A t.03	55°	M
155	CIR135	F: AAGCAAAGCAAACAAAG R: GCTTGCCAGTATGATGT	AD_ch13_A t.13	55°	M
156	CIR139	F: AAACAAATGGAGAGGGT R: ACCTGTGGTCTGCAAT	AD_ch19_D t.05	55°	P
157	CIR141	F: CGCACAAGGAATAGAAG R: ACCCAACATAAGGACTAAA	AD_ch07_A t.07	55°	M
158	CIR142	F: ACCCTGCTCTGTTTCTC R: GAAGTCTATCATTGTTGGC	AD_ch04_A t.04	55°	M
159	CIR143	F: AAGAAAGAAGAACTTCCC R: GCCATTAAGAAGGACAAA	AD_ch15_D t.01	55°	M
160	CIR148	F: CTAATCTTTGGATTCTACCC R: TCCAAGCCCAGATAAGT	AD_ch12_A t.12	55°	M
161	CIR150	F: TTTACAAC TCAATCCCATC R: TCCCTTCTT TCACTTCC	AD_ch25_D t.06	55°	M
162	CIR156	F: CATTGCCCAAGGAGA R: GTAACCAATGAATCCCAG	AD_ch21_D t.11	55°	M
163	CIR158	F: TGTTGCTCCTTAATTGG R: GGGAGATTGTTGGAGTT	AD_ch15_D t.01	55°	M
164	CIR165	F: ATAAGTGGAGACAGGCA	AD_ch19_D	55°	M

		R: GACCAGCACAGGAAAC	t.05		
165	CIR166	F: CTACTCTTTCTTCTTTGTGG R: ACTTGTGGAAACTATTCTTG	AD_ch20_D t.10	55°	M
166	CIR167	R: ACTTGTGGAAACTATTCTTG R: AAATTCCAGCTCATGGT	AD_ch26_D t.12	55°	M
167	CIR169	F: GAAGCACAATAAGGCAA R: CAAACAAGCGATGAAAC	AD_ch07_A t.07	55°	M
168	CIR170	F: TCGGTAAAGATGGGTG R: ATTGGTGCTGGTTGAG	AD_ch26_D t.12	55°	M
169	CIR171	F: GAAATCCAAATCCAACC R: AACCACCCGACTCTTT	AD_ch20_D t.10	55°	M
170	CIR172	F: GGTCCATTCTTCTTGT R: CCGAAATCCTCTTCTTC	AD_ch22_D t.04	55°	M
171	CIR175	F: TGCAACAAACTTCAACTC R: TCCAAAGAAGAATATGGTC	AD_ch16_D t.07	55°	M
172	CIR176	F: TTTACCTCACAAGACTCTCA R: ATTTGAGCAATGAACACTAC	AD_ch19_D t.05	55°	M
173	CIR179	F: ATGGGTCCAAAGTAAGAG R: GATTTGGGAAGCAGAA	AD_ch19_D t.05	55°	M
174	CIR180	F: TTGAAGAACGTAGGTGG R: TCCGACCTGTTCAAAT	AD_ch17_D t.03	55°	M
175	CIR181	R: TCCGACCTGTTCAAAT R: TCTTGAAGGGAAACGA	AD_ch14_D t.02	55°	M
176	CIR183	F: CCAGATTAGAACCTATGAAAC R: TAGCCCATTCTTACCAC	AD_ch22_D t.04	55°	M
177	CIR184	F: TACACGGAAGACAACAAG R: CTTTGTTACTGAAATGGGT	AD_ch02_A t.02	55°	M
178	CIR185	F: GCATTTGTATTTCCCTGT R: GATCAAGTCCAGAGTCCA	AD_ch05_A t.05	55°	M
179	CIR187	F: TGGTTCTTCTTGATTGG R: TTGGGAAACTTGTAGGA	AD_ch20_D t.10	55°	M
180	CIR194	F: ATTGTCTTGGACTTTGGT R: TCAGTTGAAAGTATTCCCT	AD_ch23_D t.09	55°	M
181	CIR196	F: TGAGTGGTTGTTTGTTC R: TTAGACAGAGGGAATGCT	AD_ch11_A t.11	55°	M
182	CIR199	F: CAGAATTTGACCGTTTC R: GCCATGATATTCGGT	AD_ch01_A t.01	55°	M
183	CIR200	F: CCAGGATTCAGTTAGTTT R: TTCTTGAACACATTTGG	AD_ch23_D t.09	55°	M
184	CIR202	F: TGAAAGTTGAAAGTGTGG	AD_ch03_A	55°	M

		R: ACGCTTTAGTTGCAGAG	t.03		
185	CIR207	F: AACAAGAGAAGGTGAAGG R: ATCTCAAAGGGCACAA	AD_ch11_A t.11	55°	M
186	CIR209	F: TGCATGGATTCCTTATT R: TTCCAGTCAAACCAAAC	AD_ch08_A t.08	55°	M
187	CIR210	F: CCTGATAGTGAGTTTCTTCTT R: TGAAATGTGAGTGTTTGTG	AD_ch14_D t.02	55°	M
188	CIR212	F: ATGAACGCTACTGGGA R: ACAAGCAAACAACCTGA	AD_ch19_D t.05	55°	M
189	CIR216	F: ATCTGAACCATCATCCTC R: TTCTGATTGGCACTTTC	AD_ch18_D t.13	55°	M
190	CIR218	F: GCGAAGCAAAGGAAG R: CTCCAACATCGTCTCAA	AD_ch04_A t.04 AD_ch22_D t.04	55°	M
191	CIR219	F: TTGCCTTGGTCTTTGT R: CGAAACCATTAACCTCCT	chr19	55°	M
192	CIR222	F: TCATCAACAATCCTTCC R: TACTGTCCCTCTTGCAT	AD_ch19_D t.05	55°	M
193	CIR223	F: AGAAACATCTGCAAACCT R: ATCTCTCTAATCCTTGCTTC	AD_ch04_A t.04	55°	M
194	CIR224	F: AGGTTTGCTGTTTCTACC R: AACAGAGGGTGACAGTTT	AD_ch19_D t.05	55°	M
195	CIR227	F: ATCAGCCATCCAGAAA R: TAAGATTGAAGTGAAGGTGT	AD_ch09_A t.09	55°	M
196	CIR228	F: TCCAGGTAAACTCAACAA R: TCATCAGTTCAATCACAAG	AD_ch03_A t.03 AD_ch14_D t.02	55°	M
197	CIR229	F: AATAACTGGACTCAACGAC R: ACCTGTGTTCTTATCCTAAA	AD_ch19_D t.05	55°	M
198	CIR233	F: AGGCAGTAGCATTTATCAG R: GTGTTGGTTGTTTATGGTT	AD_ch26_D t.12 AD_ch06_A t.06	55°	M
199	CIR234	F: AGCACTCATCCATCACA R: GCACCCTTTAGAAACAAG	AD_ch15_D t.01	55°	M
200	CIR235	F: GCTAGTGCCTGACGAC R: CCCACACCCGTATT	AD_ch13_A t.13	55°	M

			AD_ch05_A t.05		
--	--	--	-------------------	--	--

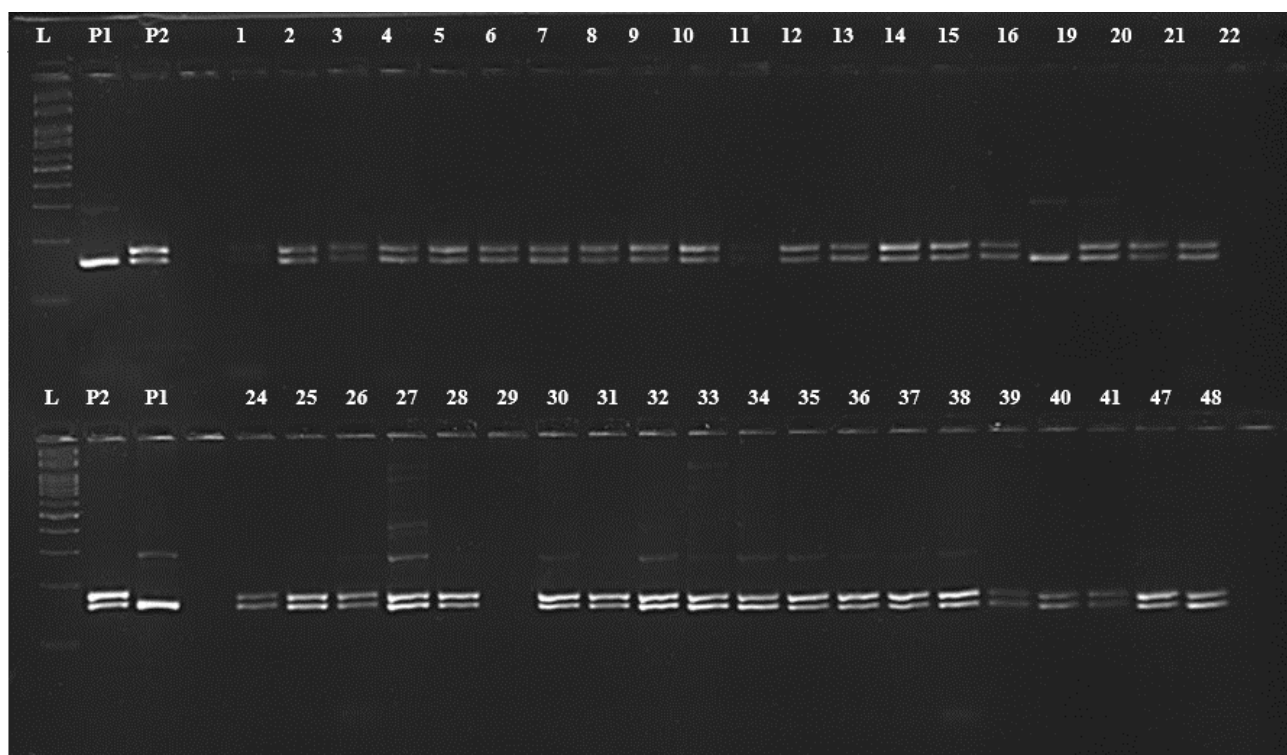


Fig. 3 - The SSR profile of CIR 139 that enables fixing the true to type F_1 plants. Lane 'L' denotes 100 bp ladder, lane 'P1' denotes female parent CO17, 'P2' denotes male parent KC3, and numeric from 1 – 48 denotes the F_1 hybrids of cross CO17 X KC3.

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_1 s were advanced to F_2 generation to establish a mapping population suitable for QTL mapping of genes conferring resistance to sucking pests.

References

Ahmad, M., Arif, M. I., Ahmad, Z. and Denholm, I. 2002. Cotton whitefly (*Bemisia tabaci*) resistance to organophosphate and pyrethroid insecticides in Pakistan. *Pest Management Science: formerly Pesticide Science*, **58(2)**: 203-208.

Ahmad, M. and Khan, M. R. 1991. Insecticide resistance management strategies in cotton pests in Pakistan. *Pak. Entomol*, **13(1-2)**: 99-103.

Amjad, M., Bashir, M. H. and Afzal, M. 2009. Comparative resistance of some cotton cultivars against sucking insect pests. *Pak. J. Life Soc. Sci*, **7(2)**: 144-147.

Attique, M. R. and Ahmad, Z. 1990. Investigation of Thrips *tabaci* Lind. as a cotton pest and the development of strategies for its control in Punjab. *Crop Protection*, **9(6)**: 469-473.

Bhat, M. G., Joshi, A. B. and SINGH, M. 1984. Relative loss of seed cotton yield by jassid and bollworms in some cotton genotypes (*Gossypium hirsutum* L.). *Indian journal of entomology*, **46(2)**: 169-173.

Blenda, A., Fang, D.D., Rami, J.F., Garsmeur, O., Luo, F. and Lacape, J.M., 2012. A high density consensus genetic map of tetraploid cotton that integrates multiple component maps through molecular marker redundancy check.

Collard, B.C. and Mackill, D.J., 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **363(1491)**, pp.557-572.

Dhawan, A. K. and Sidhu, A. S. 1986. Assessment of losses due to attack of cotton jassid on *hirsutum* cotton. *Indian Journal of Plant Protection*, **14(1)**: 45-50.

DOAK, C. C. 1934. A new technique in cotton hybridizing: Suggested changes in existing methods of emasculating and bagging cotton flowers. *Journal of Heredity*, **25(5)**: 201-204.

Dongre, A.B., Raut, M.P., Bhandarkar, M.R. and Meshram, K.J., 2011. Identification and genetic purity testing of cotton F₁ hybrid using molecular markers. *IJBT*, **10(3)**: 301-306.

Hamburg, H.V. and Guest, P.J., 1997. The impact of insecticide arthropods in cotton agroecosystem in South Africa. *Arch. environ. Contam. Toxicol.*, **32**: 63-68.

Hampannavar, M.R., Patil, B.R., Katageri, I.S., Kumar, B.A. and Janagoudar, B.S., 2020. Genetic variability and correlation analysis for agronomic and fibre quality traits in intra-specific cotton (*G. hirsutum* × *G. hirsutum*) recombinant inbred lines (RILs). *Int. J. Curr. Microbiol. Appl. Sci*, **9**, pp.493-503.

Kamburova, V., Salakhutdinov, I. and Y. Abdurakhmonov, I. 2022. Cotton Breeding in the View of Abiotic and Biotic Stresses: Challenges and Perspectives. *IntechOpen*. doi: 10.5772/intechopen.104761

- Kannan, M., Uthamasamy, S. and Mohan, S., 2004. The impact of insecticides on sucking insect pests and natural enemy complex of transgenic cotton. *Curr. Sci.*, **86**: 726-729
- Khadi, B. M., Kulkarni, V. N. and Narjji, S. S. 1998. Achieving multiple pest tolerance through manipulation of morphological features in cotton. In *Proceedings of the World Cotton Research Conference -2*: 201-203
- Kumar, K.J.Y., Patil, R.S., Patil, S.S. and Katageri, I.S., 2009. Genetic studies of yield components, seed oil and fibre quality traits in cotton (*Gossypium hirsutum* L.). *Karnataka Journal of Agricultural Sciences*, **22(2)**, pp.428-432.
- Boopathi, N. M., 2020. Marker-assisted selection (MAS). *Genetic Mapping and Marker Assisted Selection: Basics, Practice and Benefits*, 343-388
- Ma, D., Hu, Y., Yang, C., Liu, B., Fang, L., Wan, Q., Liang, W., Mei, G., Wang, L., Wang, H. and Ding, L., 2016. Genetic basis for glandular trichome formation in cotton. *Nature Communications*, **7(1)**: p.10456.
- Ma, L., & Li, C. 2000. A study on the bollworm resistance of CRI 29 and the target to control the F3 bollworms. *China Cottons*, **27(3)**, 20-22.
- Men, X., Ge, F., Edwards, C. A. and Yardim, E. N. 2005. The influence of pesticide applications on *Helicoverpa armigera* Hübner and sucking pests in transgenic Bt cotton and non-transgenic cotton in China. *Crop Protection*, **24(4)**: 319-324.
- Muthuraj, M., Mahalingam, L., Premalatha, N., Senguttuvan, K., and Kumar, M. 2019. F1 Interspecific hybridity confirmation in cotton through morphological, cytological, and molecular analysis. *Electronic Journal of Plant Breeding*, **10(2)**: 862-873.
- Naeem-Ullah, U., Ramzan, M., Bokhari, S.H.M., Saleem, A., Qayyum, M.A., Iqbal, N., Habib ur Rahman, M., Fahad, S. and Saeed, S., 2020. Insect pests of cotton crop and management under climate change scenarios. *Environment, climate, plant and vegetation growth*, **15**:367-396.
- Parnell, F. R., King, H. E., and Ruston, D. F. 1949. Jassid resistance and hairiness of the cotton plant. *Bulletin of Entomological Research*, **39(4)**: 539-575.
- Saghai-Marouf, M. A., Soliman, K. M., Jorgensen, R. A., and Allard, R. 1984. Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences*, **81(24)**: 8014-8018.
- Sorejani, M., 1998. Current trend in pesticide usage in some Asian countries. *Rev. Appl. Ent.*, **77**: 219–234.

Ulloa M., Hutmacher R. B., Roberts P. A., Wright S. D., Nichols R. L., Davis R. M., 2013. Inheritance and QTL mapping of Fusarium wilt race 4 resistance in cotton. *Theor. Appl. Genet.* 126 1405–1418. 10.1007/s00122-013-2061-5.

Wannamaker, W. K. 1957. The effect of plant hairiness of cotton strains on boll weevil attack. *Journal of Economic Entomology*, **50(4)**: 418-423.

Webster, J.A., 1975. *Association of plant hairs and insect resistance: an annotated bibliography*. Agricultural Research Service, US Department of Agriculture.

Yadava, H. N., Mital, R. K., & Singh, H. G. (1967). Correlation studies between leaf mid-rib structure and resistance to Jassids (*Empoasca Devastans Dist*) In Cotton. *Indian Journal of Agricultural Sciences*, **37(6)**: 495.

UNDER PEER REVIEW