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Molecular, cytological and morphological studies on Jassid resistance in cotton (*Gossypium hirsutum* L.) based on hairiness trait

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Abstract

Background Unravelling the relationship between trichome density and resistance to jassids in upland cotton, nine parental lines, viz. MCU 5, CO 14, CO 17, TCH 1828, KC 2, KC 3, GISV 323, GTHV 15–34, and RHC 1409 were obtained from the Tamilnadu Agricultural University. These genotypes were subjected to molecular analysis using 27 primers, merely the JESPR 154 primer amplifying a 150-bp fragment in genotypes exhibiting the pubescence.

Result This finding validated the association between pubescence and jassid resistance. Further analysis revealed that resistant genotypes (KC 3, GTHV 15–34, GISV 323, and RHC 1409) exhibited significantly higher trichome densities and length compared with susceptible genotypes. These results stalwartly support the hypothesis that trichomes play a pivotal role in conferring resistance to jassids in upland cotton.

Conclusion By breeding cotton varieties with increased trichome density and length, it is possible to reduce jassid infestations, thereby decreasing the reliance on chemical pesticides and promoting a more sustainable agricultural environment.

Keywords Cotton, *Gossypium hirsutum* L., Trichomes, Jassids, Molecular

Introduction

Cotton is the world's top natural fiber crop. It belongs to the Malvaceae family and genus *Gossypium*, which has about 50 species, including 45 diploids (2n = 26) and 5 allotetraploids (2n = 52). *G. hirsutum* L., *G. arboreum* L., *G. herbaceum* L., and *G. barbadense* L. are the four cotton species farmed in India. North and Central America are the geographic centers of the origin for *G. hirsutum*. The trichome hairiness trait confers resistance against Jassids (*Amrasca biguttula*) as reported (Madhu et al. 2024). The important and serious pest that retards the growth of plant was jassid (*Amrasca bigutulla bigutulla* (Ishida) (Homoptera:Cicadellidae)) (Sankeshwar et al. 2016). It also causes yield loss in cotton. The cotton was damaged by both jassid nymphs and adults by sucking the cell sap and laying its eggs on the midrib of the leaves. Hence the sucking causes the leaf to turn pale yellow, curl downwards, and fall off later reported by Belachew et al. (2024).

A familiar way to control the pest is bio-intensive pest management. If the resistant genotypes were identified, it could be transferred to related genotypes through appropriate breeding methods. The epidermis of leaves,

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shoots, and roots have unicellular outgrowths called trichomes. The trichome covering the plant surface is collectively called pubescence. The hairiness acts as non-preference trait against the cotton sucking insect pests. Trichome density exhibits variation among different species and cultivars of *Gossypium* species reported by Mutaviri et al. (2024). The present study aims to identify the polymorphisms in the cultivated species *G. hirsutum* L. related to quality and trichome hairiness traits, and to investigate the traits that may confer resistance to sucking pests, thereby enhancing the yield of cotton by pest population control.

Materials and methods

Parental materials

The *G. hirsutum* varieties, viz. MCU 5, CO 14, CO 17, TCH 1828, KC 2, KC 3, GISV 323, GTHV 15–34, and RHC 1409 were used for the present study. They were obtained from Department of Cotton, Tamilnadu Agricultural University, Coimbatore. They were cultivated in August 2021 with two rows each with the spacing of 90 cm × 45 cm. Agronomical and cultural practices were followed as locally recommended.

Molecular analysis

By generally following the process by Zhang et al. (2000), genomic DNA was extracted from parental cotton leaves, and the quantity and quality of DNA was assessed for polymerase chain reaction (PCR) using a Nanodrop™ 1000 spectrophotometer. After verification the amplified fragments from PCR (using the primers for the fibre quality and trichome hairiness traits) were separated through agarose gel electrophoresis and scoring of banding patterns and parental polymorphism identification.

PCR amplification

About 27 primers with high polymorphism information content (PIC) values were obtained from the cotton marker database (CMD) (http://www.cottonmarker.org/) developed by Main Lab at Washington State University. These primers were commercially synthesized and procured from Eurofins Genomics Pvt. Ltd. The 2X SMART PRIME PCR Master mixes were used for amplification. The primers used in this study were shown in Table 1.

Separation of amplified fragments using agarose gel electrophoresis

A 3% (w/v) agarose gel was casted in a chamber filled with 0.5 X TBE buffer, and the amplified products, together with a ladder of 100 base pairs, were loaded into the wells to determine the size of the amplified fragment of the product. For 2 hours and 30 minutes, the PCR products were operated at 90 volts. After electrophoresis, the gel was taken from the tank, inspected under UV light, and photographed using a gel documentation equipment (Bio-rad Gel Doc XR imaging system).

Analysis and scoring of banding patterns

A total of 27 SSR primer pairs were used to study polymorphism among 9 parents. All 27 primers produced clear, scorable, and unambiguous bands and they were chosen for parental polymorphism analysis. DNA bands were scored which was present in the agarose gel for their presence and absence across the genotypes in the form of binary codes (presence – 1; absence – 0).

Number of jassids per plant and injury grade

Hopper burn injury was assessed as per the Indian Central Cotton Committee (ICCC 1960) methods and based on resultant symptoms of infestation. The hopper burn grades consist of following grades (Table 2).

To assess jassid populations in cotton field, a group of 10 plants were selected by random sampling per genotype. Carefully examine each plant, focusing on the undersides of the leaves where jassids often hide. Count the number of adult jassids and nymphs present on each plant was counted to estimate the overall population level on the 15th, 30th, 45th, 60th and 75th days after sowing, and means injury index (grade index) was calculated (ICCC 1960).

A leafhopper resistance index was calculated as proposed by Rao (1973), which is

$$\text{Leafhopper resistance index} = \frac{G_1 \times P_1 + G_2 \times P_2 + G_3 \times P_3 + G_4 \times P_4}{P_1 + P_2 + P_3 + P_4}$$

Where G represented the number of the grade of ICCC and P represented the number of plants under the each entry. Grouping of injury index to categories of resistance was as follows (Table 3).

Trichome length and density analysis

The pubescence traits, involved in jassid resistance, were observed on the plants based on qualitative grading (pubescence rating) and quantitative measurement (trichome density and trichome length) as detailed (Bourland et al. 2003; Bourland et al. 2007).

The trichome length was analyzed through mounting the plant material on a slide and positioning it under a microscope. The microscope was carefully adjusted until the pointer aligned precisely with base of the trichome, and the measurement was directly taken from the micrometre scale.

The number of trichomes/unit area were recorded in each genotype for abaxial leaf pubescent count

Table 1 Particulars of primer pairs used for molecular study

S. No.	Marker name	Primer sequence (5′—3′)		Annealing temperature/˚C
1	BNL1153	F	CTTTATCCGGAGACGGAACA	55
		R	CTAACTTTTGCTCACCCCCA	
2	CIR316	F	TTACAGGCACTACCACC	60
		R	CCTTTCTGGCGACTT	
3	BNL1122	F	TCGATAACGGCTATAGTAATCTCTC	55
		R	CAACAAATAAGCAGCCAAGAAA	
4	BNL3599	F	TTTAGCCCCAGTAACATGCC	55
		R	ACTGCAAGCTCTGCCCTAAA	
5	BNL3147	F	ATGGCTCTCTCTGAGCGTGT	55
		R	CGGTTCAGAGGCTTTGTTGT	
6	BNL1513	F	TTTCACAAGCACAACCATAGG	55
		R	AATACAGGTTCAAAGTTGATAGGG	
7	BNL1440	F	CCGAAATATACTTGTCATCTAAACG	55
		R	CCCCCGGACTAATTTTTCAA	
8	BNL1964	F	AGGGAGGGGGAGGTTCTC	55
		R	CGGTAGTCCTCCACCATGTT	
9	BNL1417	F	TTATTCTAACCACCGCCTCC	55
		R	TGAGTGGATATGCTTGGCCT	
10	CIR014	F	AGCTTGCCTCTTTCTG	60
		R	ACATTAGAACTCCCTGCT	
11	BNL1878	F	TGCTTCAACTGCTCTTGCAT	55
		R	TCGATATCTGGAACACCCAC	
12	BNL2568	F	GGGAAGAGAGGGAGACTAACG	55
		R	ATTTTGATAGGTTGGTTTTGTCC	
13	BNL1059	F	CCTTCTCTGACACTCTGCCC	55
		R	TGTATTCTCTTCTTTTCCTTATACTTTT	
14	BNL1693	F	CCCTTGGGAATAGC	55
		R	CATGTGTCTCCGTGTGTGTGTG	
15	BNL2884	F	TCAACTCATACCAAATCAATTCC	55
		R	CCCTGTTTTGTTCAATGGGT	
16	NAU1215	F	GAGTGAGACTGGAGCTGGTT	55
		R	CAAATCATTGTTTGCAGCAG	
17	CIR199	F	CAGAATTTGACCGTTTC	50
		R	GCCATGATATTTCGGT	
18	BNL3580	F	CTTGTTTACATTCCCTTCTTTATACC	55
		R	CAAAGGCGAACTCTTCCAAA	
19	MUSB1035	F	TCATGATAGCAACGAGTGGTGC	58
		R	GTCGAGTTGTATTAGTGTGCCCG	
20	CIR0407	F	GCACAGAACATCCATACA	50
		R	TCTCTCTCTCTTTCACACAC	
21	BNL 1059	F	CCTTCTCTGACACTCTGCCC	55
		R	TGTATTCTCTTCTTTTCCTTATACTTTT	
22	BNL 3255	F	GACAGTCAAACAGAACAGATATGC	60
		R	TGTATTCTCTTCTTTTCCTTATACTTTT	
23	CIR 246	F	TTAGGGTTTAGTTGAATGG	55
		R	ATGAACACACGCACG	
24	CIR 307	F	GACTTGAAAAGATTACACAC	55
		R	GAATTTGCTGGCTCT	

Table 1 (continued)

S. No.	Marker name	Primer sequence (5′—3′)		Annealing temperature/˚C
25	JESPR 153	F	GATTACCTTCATAGGCCACTG	55
		R	GAAAACATGAGCATCCTGTG	
26	JESPR 127	F	GATTTGGGTAACATTGGCTC	55
		R	CTGCAGTGTTGTGTTGGGTAGA	
27	JESPR 154	F	GTTCCCTCAGTTGCTCAGAAG	55
		R	GGAGGAGTTGGCAGAAAATAGC	

Table 2 Jassid grade index

Grades (G)	Symptoms
G1	Leaves free from crinkling or with no yellowing, bronzing and drying
G2	Few leaves on lower portions of the plant curling, crinkling and slight yellowing
G3	Crinkling and curling all over, yellowing, bronzing and browning in the middle and lower portion, plant growth hampered
G4	Extreme curling, yellowing, bronzing and browning, drying of leaves and defoliation, stunted growth

Table 3 Grouping of injury index to categories of resistance

Grade index	Category
0– 1.0	Resistant
1.1– 2.0	Moderately resistant
2.1 – 3.0	Susceptible
3.1 – 4.0	Highly susceptible

(ALPC), leaf midrib pubescent count (LMC), and leaf vein pubescent count (LVC). The trichome density was recorded on leaves based on the two criteria devised (Bourland et al. 2003). Qualitative grading system for trichomes: Upper, middle, and lower portion of the selected plant leaves were assessed for the trichome density rating in random (Table 4).

Quantitative measure of leaf trichomes: The same leaves taken for the study of qualitative grading were used for the quantitative measure of trichomes. Index cards with an area of 0.1 cm^2 were used for the observation. Data was recorded over the abaxial side, leaf veins, and leaf midrib of each leaf from three different positions and averaged. Trichomes in the 0.1 cm^2 area were counted with a high magnifying power microscope (Stereo Zoom Microscope). The mean for trichomes per cm^2 was determined separately for each trichome count taken on three different positions of the plant.

Table 4 Trichome density scale and its corresponding categories

Trichome density scale	Category
1	Smooth
3	Lightly hairy
5	Hairy
7	Very hairy
9	Pilose

A compound microscope equipped with an Aptina MT9M001 image sensor was used to capture high-quality images of leaf samples. The images exhibited excellent contrast and color fidelity. Utilizing Scopephoto software, the leaf samples, freshly collected at 50 days after sowing and cut into one-centimeter squares, were directly viewed on the computer screen. Still photographs were then captured and saved for subsequent trichome studies.

Statistical analysis

The statistical analysis of the data was performed using TNAUSTAT-Statistical package, which was retrived from https://sites.google.com/site/tnaustat https://sites.google.com/site/tnaustat.

Result

PCR amplification

Among the 27 primers used, only the JESPR 154 primer showed an amplified fragment at 150 bp, which validated the corresponding parents with hairiness trait conferring resistance to jassid given in Fig. 1. In order to phenotypically and cytologically determine the trichome length and density, the Aptina MT9M001 image sensor was used with compound microscopes, and the results were shown in Fig. 2. From the images of Fig. 1, correlating the primer amplified at 150 bp in KC 3(Fig. 2F), GISV 323 (Fig. 2G),

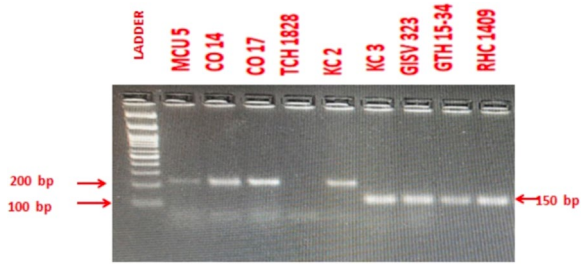

Fig. 1 Primer JESPR 154 shows amplified fragment at 150 bp which validated the corresponding parents with hairiness trait conferring resistance to jassid

GTHV 15–34 (Fig. 2H), and RHC 1409 (Fig. 2I) had trichomes with count of three to five with the same initiating point and other parents MCU 5 (Fig. 2A), CO 14 (Fig. 2B), CO 17 (Fig. 2C), TCH 1828 (Fig. 2D), and KC 2 (Fig. 2E) were amplified at 200 bp.

Comparison of genotypic and phenotypic results

Higher than 150 bp correlating with the images in Fig. 2A-E could be apparently visualized the development of only one or two hairs, which exhibited lower trichome density compared with the Fig. 2F-I.

The JESPR-154 primer amplified a 150-bp DNA fragment associated with the hairiness trait in a cotton population. SSR markers analysis revealed the inheritance pattern of this trait, suggesting potential improvements in insect resistance through targeted breeding.

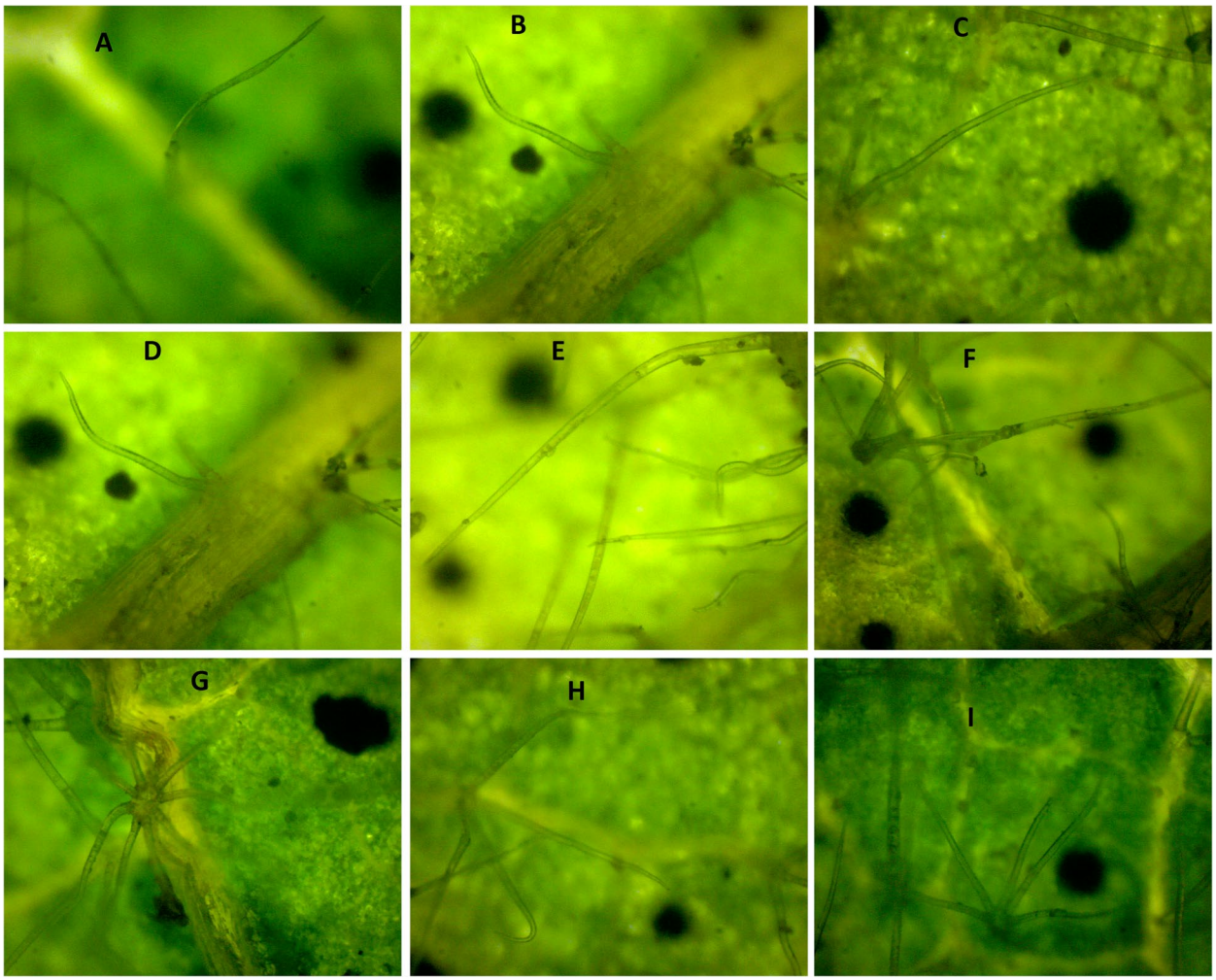

Fig. 2 Aptina MT9M001 image sensor used with compound microscopes, producing clear images of A) MCU 5, B) CO 14, C) CO17, D) TCH 1828, E) KC 2, F) KC 3, G) GISV 323, H) GTHV 15–34, and I) RHC 1409. The images A, B, C, D, and E depicts the point where trichome arises and divides into two or remains only one, whereas in images F, G, H, and I had four or five trichome hairs arising at each point showing more hairiness

Table 5 Parental mean value assessment of jassid resistance related traits

Parents	Mean number of jassid nymphs per plant	Jassid injury grade	Resistance rating scale	Mean trichome length /mm	TD rating	Yield /g	ALPC	LMC	LVC	GG
MCU 5	8.40	1.80	MR	0.72	3.00	142.47	76.81	34.56	34.76	54.00
CO 14	8.20	1.77	MR	0.74	3.00	150.35	71.21	40.90	55.60	46.80
TCH 1828	9.89	1.72	MR	0.75	1.00	119.23	56.70	24.35	30.90	58.90
CO 17	9.55	1.51	MR	0.76	3.00	138.84	55.68	40.90	31.20	57.60
KC 2	9.12	1.38	MR	0.56	1.00	140.77	45.67	42.45	34.50	56.80
KC 3	**3.11**	**0.12**	**R**	1.21	**5.00**	**115.50**	112.30	60.54	58.10	80.23
GTHV 15–34	3.25	0.88	R	1.01	5.00	103.96	100.10	50.20	55.60	75.65
GISV 323	4.12	0.87	R	1.11	5.00	130.84	90.10	36.50	42.30	70.90
RHC 1409	6.34	0.84	R	1.06	5.00	96.72	67.80	32.43	45.56	69.87
DCH 32	**13.25**	**4.00**	**HS**	**0.19**	**1.00**	**80.78**	20.90	15.60	10.90	10.00
MIN	3.11	0.12		0.19	1.00	80.78	20.90	15.60	10.90	10.00
MAX	13.25	4.00		1.21	5.00	150.35	112.30	60.54	58.10	80.23
SE	0.99	0.31		0.09	0.53	6.85	8.13	3.80	4.40	6.00
CD at 5%	0.44	0.69		0.37	0.55	0.19	0.39	0.33	0.37	0.34

ALPC Abaxial leaf pubescent count, *LMC* Leaf midrib pubescent count, *LVC* Leaf vein pubescent count, *GG* Gossypol gland

Comparison of resistance with yield performance

According to the Table 5, the resistant parent KC 3 exhibited the lowest jassid population size (3.11), followed by GTHV 15–34 (3.25). The maximum number of jassid population was observed in the susceptible control DCH 32 (13.25). Based on the jassid injury grade, the total parental genotypes were divided into moderately resistant (MCU 5, CO 14, TCH 1828, CO 17, and KC 2) and resistant genotypes (KC 3, GTHV 15–34, GISV 323, and RHC 1409). The mean trichome length was found to be higher in KC 3 (1.21 mm), followed by GISV 323 (1.11 mm), and the highly susceptible control DCH 32 had the lowest mean trichome length of 0.19 mm. The trichome density was observed to be smooth, light hairy or hairy genotypes. Among the moderately resistant genotypes, either smooth or light hairy nature of trichome density was observed. Hairy nature of trichome density was observed among the resistant genotypes. The maximum seed cotton yield per plant was observed in the parent CO 14 (150.35 g), and the lowest yield was obtained in the parent DCH 32 (80.78 g). The highest mean values of abaxial leaf pubescent count (112.30), leaf midrib pubescent count (60.54), leaf vein pubescent count (58.10), and gossypol glands (80.23) were observed in KC 3. The lowest mean values of abaxial leaf pubescent count (20.90), leaf midrib pubescent count (15.60), leaf vein pubescent count (10.90), and gossypol glands (10.00) were observed in DCH 32.

Correlation studies

To know the association between the traits taken under study, correlation studies was carried out as illustrated in Table 6. The number of jassids per plant showed a highly significant positive correlation with injury grade (0.878) and a non-significant negative correlation with seed cotton yield per plant (−0.053), while all the remaining traits under study exhibited highly significant negative correlations with the number of jassids per plant.

Discussion

Among the 96 species of insects, a major destructive pest to the crop is the jassid, *Amrasca devastans* (Dist.), and it is considered the most noxious pest of the cotton. A total of 5%−45% of yield losses are caused by sucking pest complex as reported (Ahuja et al. 2009). The improper and non-selective usage of insecticides causes insects resistance(Midega et al. 2012). This also poses problems like environmental pollution and health hazards for human beings as suggested (Ruba et al. 2023). The host plant resistance methodology is the only hope to minimize pest pressure on cotton crops. Physio-morphological changes against herbivore insects increase the host plant resistance. Jassid, *A. devastans* is a major pest of cotton, which feeds on seedling, vegetative growth upto the maturity of the crop. While sucking the cell sap from plants it injects toxic material (Tayyab et al. 2024). The plants get dry, their leaves turn down, and finally die during heavy infestations of jassid which is responsible for reduced yield losses (Madar et al. 2010). The trichome density on cotton leaves decides the host plant's resistance/susceptibility. The jassid avoids reproducing and feeding on highly dense and elongated trichomes at the lower side of the leaf as reported (Kanher et al. 2016).

Table 6 Correlation of traits for jassid resistance studies in parental genotypes

Traits	NJ/P	IG	TL	TD	Y	AL	LM	LV	GG
NJ/P	1								
IG	0.878**	1							
TL	−0.927**	−0.931**	1						
TD	−0.888**	−0.722*	0.875**	1					
Y	−0.053	−0.287	0.105	−0.088	1				
AL	−0.948**	−0.834**	0.899**	0.835**	0.173	1			
LM	−0.776**	−0.802**	0.678*	0.599	0.336	0.791**	1		
LV	−0.858**	−0.821**	0.814**	0.742*	0.264	0.865**	0.819**	1	
GG	−0.881**	−0.982**	0.934**	0.720*	0.221	0.838**	0.740*	0.773**	1

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

NJ/P The number of jassids per plant, *IG* Injury grade, *TL* Trichome length (mm), *TD* Trichome density, *AL* Abaxial leaf pubescent count, *LM* Leaf midrib count, *LV* Leaf vein count, *GG* Gossypol gland

Genotypic and cytological studies

The genetic linkage map of leaf hairiness in upland cotton using molecular markers by using 400 random amplified polymorphic DNA (RAPD) and 54 simple sequence repeats (SSR) primers (Zafar et al. 2009). JESPR-154 primer amplified at a 150-bp DNA fragment in hairiness population. Insect resistance in cotton could be improved through the construction of genetic linkage map using RAPD and SSR markers which revealed the inheritance for leaf hairiness exists (Zhang et al. 2000).

Correlation studies

Correlation studies with the jassid population per leaf and the hair density on midrib and vein had a significantly negative correlation (Bhatti et al. 2015). A negative correlation of the number of jassids with trichome density was also reported (Ashfaq et al. 2010; Rustamani et al. 2014; Gonde et al. 2015; Sankeshwar et al. 2016; Khurshid et al. 2023). The trichome length had significantly positive correlations with trichome density (0.875), abaxial leaf pubescent count (0.899), leaf midrib pubescent count (0.678), leaf vein pubescent count (0.814), and gossypol glands (0.934). The trichome density had significantly positive correlations with abaxial leaf pubescent count (0.835), leaf vein pubescent count (0.742), and gossypol glands (0.720). Abaxial leaf pubescent count had highly significantly positive correlations with leaf midrib pubescent count (0.791), leaf vein pubescent count (0.865), and gossypol glands (0.838). Leaf vein pubescent count had highly significantly positive correlations with leaf midrib pubescent count (0.819) and gossypol glands (0.773).

Trichomes conferring resistance

The trichomes were found to confer resistance to sucking pests (Grover et al. 2016). This henceforth reduce the pesticide consumption in cotton plants thereby paving ways for the safety environment for ecological species without harming them. Pesticide usage would be lowered with the use of tolerant genotypes. It also paves ways for the improvement of future integrated pest management (IPM) programme (Madhu et al. 2024).

Conclusion

This study demonstrates that incorporating jassid resistance traits into cotton breeding programs is crucial for improving crop productivity and sustainability. Resistant varieties offer a significant advantage by increasing yields while minimizing the reliance on chemical pesticides. This integrated approach promotes a healthier agricultural ecosystem and a more environmentally responsible cotton production practice. The genotypic, cytological, and performance analyses consistently show that resistant cotton phenotypes exhibit lower jassid populations and injury levels. These resistant varieties also displayed higher trichome length, density, and pubescence counts on abaxial surfaces, midribs, and veins. These results strongly suggest that hairiness plays a vital role in conferring jassid resistance. These findings provide valuable insights for cotton breeders to develop high-resistance genotypes, ultimately reducing jassid infestations and promoting more sustainable cotton production.

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Authors' contributions
All the authors contributed to the study conception and design. Subhashini S executed the experiment and analysed the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final manuscript.

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Data availability
All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate
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Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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