INTERNATIONAL COTTON RESEARCHERS ASSOCIATION

COTTON *innovations*



VOLUME 5
ISSUE 2

APRIL 2025

www.icra-cotton.org

Egyptian Cotton

The World's Softest Cotton



For More Information
Cotton Egypt Association
www.cottonegyptassociation.com
E-mail: Info@cottonegyptassociation.com

EGYPTIAN COTTON™

Content

Evolution of Introgressed and Highly Cotton Leaf Curl Virus Tolerant Cultivar "CYTO124-" 1
Farzana Ashraf, Khezir Hayat, Hafiz M. Imran, Muhammad Akbar, Mian M. Azam, Zahid Mahmood

Developing SNP-Based Molecular Marker for CLCuV Resistance in Cotton 14
Zarmeen Zafar, Fukhar Zaman, Umar Iqbal1, Shoaib Ur Rehman, Sehrish Ijaz, Furqan Ahamd, Umar Akram, Akhtar Hameed, Zulqurnain Khan

Anatomical breeding for altered leaf parameters in Egyptian Cotton (Gossypium barbadense L.) to impart sucking pest resistance 27
Alagarsamy Manivannan, Thomas Cheeran Amal, and Karuppan Shankarganesh

Development and Optimization of a Synergistic Kairomone Blend (Patent No: 563899) for the Attraction of Cotton Stem Weevil (Pempherulus affinis) 34
K. Senguttuvan, M. Shanthi, M. Murugan

Cotton Innovations Journal

The Cotton Innovations Journal is published twelve times annually. Contributed articles, pictures, cartoons, and feedback are welcome at any time. Please send contributions to the General Editors (see below). The editors reserve the right to edit. .The deadline for contributions is one month before the publication date

Editorial Board

•**Dr. Mohamed Negm, Chairman of ICRA** (mohamed.negm@arc.sci.eg)
Chief Editor, Professor of Cotton fiber and yarn spinning technology, Cotton Research Institute, Giza-Egypt,

•**Dr. Keshav Kranthi Executive Director-ICRA.** (keshav@icac.org).
Chief Scientist, International Cotton Advisory Committee, ICAC.

•**Dr. Eric Hequet, ICRA treasurer.** (Eric.Hequet@ttu.edu)
Horn Distinguished Professor, Fiber and Biopolymer Research Institute, Texas Tech University.

•**Dr. Jodi Scheffler, Vice-Chairman-ICRA.** (jodi.Scheffler@usda.gov)
USDA, ARS, Stoneville, MS -38776USA.

•**Dr. Muhammad Tehseen Azhar** (tehseenazhar@gmail.com)
Associate Professor, Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan. Editor of April2025- Issue.

•**Dr. Hafiz M. Imran, ICRA Secretariat,** icrasecretariatpak@gmail.com
ICRA Secretariat, Pakistan Central Cotton Committee, Multan, Pakistan

Published by ICRA Secretariat, Pakistan Central Cotton Committee, Multan-Pakistan

http://icra-cotton.org

The newsletter is also available at URL: http://www.icra-cotton.org/page/cotton-innovations

ISSN 6611-2788

EVOLUTION OF INTROGRESSED AND HIGHLY COTTON LEAF CURL VIRUS TOLERANT CULTIVAR "CYTO-124"

[a]Farzana Ashraf, [a]Khezir Hayat, [a]Hafiz M. Imran, [a]Muhammad Akbar, [a]Mian M. Azam, [b]Zahid Mahmood*

[a] Scientific Officer Central Cotton Research Institute, Multan.
[b] Director Central Cotton Research Institute, Multan

ABSTRACT:

Central Cotton Research Institute (CCRI), Multan, Pakistan has evolved an upland cotton cultivar "Cyto-124" through introgression for Cotton Leaf Curl Virus (CLCuV) resistance. It was developed through crossing [{2(G. hir. x G. ano.) X 3G. hir.} x {2(G. arbo. x G. ano.) x 2G. hir. It was tested for seed cotton yield in varietal trials, zonal varietal trials at Govt. and farmer fields. Cyto-124 gave 37.1, 19.9 and 22.4% higher yield as compared to three standards MNH-786, CIM-554 and CRSM-38 respectively in varietal trials while Cyto-124 had seed cotton yield 3075 in-contrast to 2919 kg ha-1 of CIM-573 in zonal trials,. Cyto-124 produced more seedcotton yield (SCY) than the standards for two consecutive years (2013 & 2014) in National Coordinated Varietal Trial (NCVT). In NCVT during 2013-14; this strain had maximum yield of 2244 kg ha-1 compared with 1326 kg ha-1 of standard variety CIM-573 in the Punjab. The virus data taken in NCVT indicated that minimum disease incidence (27.9%) was recorded in Cyto-124 making it the more tolerant strain against CLCuV. The approval of this CLCuV resistant cultivar will considerably contribute a lot in revival of cotton.

Keywords: Cotton, interspecific, CLCuV tolerant, fiber quality, Pakistan.

INTRODUCTION

American cotton is considered to be the best for fiber production all over the globe. More than 50 species are found in *Gossypium* genus (Gallagher et al. 2017; Fryxell, 1992). Out of which four are cultivated species while *G. hirsutum* contributes more than 95% production. It has been cultivated in about 80 countries and being the ultimate source of natural fiber for the textile sector (Tan et al. 2014). Upland cotton is the backbone for the livelihood of Pakistan as it contributes 1.8% to GDP and 8.6% to value addition in agriculture (Anjum et al. 2015). Upland

cotton was grown on an area of 2699000 hectare with production of 11.935 million bales which is 8.4% higher than 2016 in Pakistan (Anonumous, 2017). Nonetheless, production was less than expected 10.671 million bales due to CLCuV, scarcity of water. As many biotic and abiotic factors affect the production of cotton but the main threat is CLCuV.

The vector for cotton leaf curl disease is White fly (Bemisia tabici) (Rajagopalan et al. 2012; Briddon et al. 2014) which directly induce cotton leaf curl virus (Sattar et al. 2013); being a menace for cotton production for decades. Iqbal et al. (1997) revealed that Pakistan faced destructive loss to economy of 7.4 billion $ and 4.98 million bales were affected by CLCuV. It has been reported that CLCuV is a menace for lint production as it has reduced (80%) yield production in Pakistan and North India (Varma et al. 2003; Mansoor et al. (2003); Sattar et al. (2013). Morphological traits are severally effected due to CLCuV as on mean basis plant height (40.6%), number of bolls per plant (72.5%), boll weight (33.8%), upper half mean length (3.44%), strength (10%) and about 10% to fiber elongation (Mahmood et al. 1996; Ahmad et al. 2002). Moreover, Ashraf et al. (2013); Hasan et al. (2016) showed that 30% cotton yield is reduced due to CLCuV in Pakistan. Cotton is a crop of prime value for Pakistan's economics and foreign reserves. Nonetheless, Pakistan produces less quantity of seed cotton as compared to other high yielding nations of the world (Amin et al. 2018).

It is a matter of great concern in the cotton community to overcome this disease. CLCuV resistant variety development is need of the day to protect cotton crop from the loss of this devastating disease likewise virus was controlled earlier in Sudan and Egypt (Hussain et al. 1975). The transference of disease resistance from interspecific crosses is the base to tackle this alarming situation. CIM-443, CIM-446 and CIM-448 approved for CLCuV tolerance at Central Cotton Research Institute, Multan (Shah et al. 2006). These cultivars found to be resistant after their approval but with the occurrence of different types of inoculum for of this disease; these genotypes were declared susceptible. The ultimate strategy of this work was to transfer CLCuV resistance to Gossypium hirsutum. Inspite of having resistance for CLCuV to all available approved cultivars; new cultivar Cyto-124 has good yield potential and good fiber traits. Fabric of high quality can be developed using this cultivar. It is thought that registration of this cultivar will substantially contribute to cotton production.

Singh et al. 1973 found statistically significant differences for yield and yield components among the genotypes developed in G. hirsutum. Likewise significant variation was observed for seedcotton yield among genotypes Ahmad et al. (1982). Moreover, statically significant variation was observed among varieties for seedcotton yield, lint percentage and fiber length (Soomro et al. 1986). Wider variation was observed for bolls per plant, boll weight, seedcotton and ginning outturn among the genotypes (Khan et al. 1989). Afzal et al. (2001) revealed that genotypes and their interaction (year x genotypes) were found highly significant for bolls per plant, boll weight and seedcotton yield also interaction year x genotypes. Afzal et al.

(2002) observed statistically significance for plant height, bolls per plant, boll weight and seedcotton yield in the genotypes. Hanif et al. (2001) also observed that genotypes played significant part in seedcotton yield. Khan et al. (2002) observed that varietal differences matters a lot for plant development. Significant differences for different yield-related traits like bolls per plant, boll weight and seed cotton yield etc Arshad et al. 2003a, 2003b) revealed that varieties significantly affect the yield of plant and observed significant difference in cotton crop due to sowing of different varieties. The loss in cotton production to has been minimized by evolution of CLCuV tolerance/ resistant varieties i.e. CIM-1100, CIM-448, CIM-443, CIM-446, CIM-482, CIM-473, CIM-499, CIM-496, CIM-707 and CIM-506 for the first time in the history of Pakistan by the breeders of Central Cotton Research Institute Multan. Later on, Cotton Research Institutes/Stations of Punjab and Federal Government respectively, developed CLCuV tolerance/ resistant varieties i.e. FH-634, FVH-53, FH-900, FH-901 FH-1000, and NIAB-999 up to 2005. With the introduction of this new CLCuV tolerant, early maturing strain Cyto-124 above mentioned problems can be solved (Arshad 2003c, 2003d). Number of studies has been conducted for developing CLCuV tolerant varieties but no durable resistance has been observed yet. So it is need of the day to transfer resistance from wild germplasm sources to upland cotton Baig and Khan (2013). Anjum et al. (2014) found that CIM-608 developed from introgression 2(G. hirsutum × G. anomalum) × 3G. hirsutum showed highest CLCuV tolerance. They also observed that CIM-608 produced highest seedcotton yield as compared to standards MNH-786. With the evolution of this elite CLCuV tolerant, early maturing Cyto-124 aforementioned problems would be solved.

MATERIAL AND METHODS

Parentage/Pedigree:

The new cotton cultivar Cyto-124 was evolved through introgression [{2(G. hir. x G. ano.) x 3G. hir.} x {2(G. arb. x G. ano.) x 2G. hir.}] x 2G. hir. [{2(G.hirs.x G.anom.) x 3G.hirs.}x {2 (G.arbo. x G.anom.) x 2G.hirs.}] x 2G. hirs in 2004-05 at CCRI Multan. The elite strain was bulked in 2009-10 in F6 and then tested in yield comparison and adaptability trials.

Breeding History:

These interspecific hybrids evolved at CCRI, had indeterminate growth habit, better yield and economical fiber traits according to the demands of textile sector. These interspecific hybrids were extremely resistant to the CLCuV, showed earliness and high tolerance to heat (Gill and Bajaj, 1987). Approximately 80 F1 individuals were sown in highly infested field at CCRI, Multan and were selfed to get next generation seed from each individual. CIM-573 was used as a spreader for CLCuV disease in each 3rd row as grown for raising F2 generation at CCRI Multan on 15th June Further, whitefly was not controlled by pesticides till 90 days for the management of whitefly vector. T-shape grafting procedure like petiole grafting was used in F2 plants and following generations for high CLCuV incidence Grafting was done at evening and evaporation from grafted buds was reduced by using polythene strips. CLCuV resistant plants were used for advancement

of generations on daily basis Virus susceptible buds in F3 generation were used for ensuring maximum disease inoculum and white fly population. F4 individuals were developed from F3 plants having good yield and no differences for CLCuV infestation. Same methodology was used till F5. During 2012-13, individuals found to be resistant for CLCuV and good yield were designated as Cyto-124. During 2012-2014, Cyto-124 was screened with standards (CIM-554, CRSM-38 and MNH-786) for yield potential and phenological characters at PSC, Khanewal and CCRI, Multan. RCBD (Randomized complete Block Design) with 4 replications was used at both locations with plot size of 56m2. Standard agronomic practices were followed while pest control measures were done according to insects. Manual picking was done from two mid rows at maturity for assessing the yield potential of the elite strain. Moreover, during 2012-13 & 2013-14, adaptability trials were conducted at 20 different locations and at progressive farmer field's in Punjab following RCBD with 4 replicates using CIM-573 as a check. Recommended agronomic and pest control measures were taken at all locations. Roller gin was done for the determination of lint percentage and fiber traits. Ginning outturn was determined. While about 50g sample was used for fiber quality using HVI like span length, uniformity index, fiber fineness and fiber strength.

RESULTS AND DISCUSSIONS

Varietal Trials: Cyto-124 was screened for yield potential with commercial varieties MNH-786, CIM-554 and CRSM-38 at Central Cotton Research Institute, Multan in varietal trials. Cyto-124 produced significantly higher yield 37.1, 19.9 and 22.4% respectively than all standards (Table 2).

Zonal Varietal Trials were conducted at the progressive growers in different districts of Punjab during 2012-13 and 2013-14 for yield. Table 3 showed that average of 19 locations was 2879 kg ha-1 compared with 2735 kg ha-1 of CIM-573 during 2012-13, During 2013-14; Cyto-124 was further tested in zonal varietal trials at 20 different ecological zones. Cyto-124 gave the highest yield of seed cotton 3075 kg ha-1 compared with 2919 kg ha-1 of CIM-573 as a mean of all locations (Table-3).

Regional Adaptability Trials:

National Coordinated Varietal trials: Yield potential of Cyto-124 was assessed at different locations in National Coordinated Varietal Trials were conducted for two years i.e. 2012-13 and 2013-14. Table 5 showed that Cyto-124 had higher yield (1828 kg ha-1) compared to check CIM-573 with 1790 kg ha-1. Cyto-124 was also included in NCVT during 2013-14. Upon mean basis of all locations in Punjab, Cyto-124 gave higher yield 2244 kg ha-1 in-contrast to control CIM-573 with 1326 kg ha-1 (Table 6).

Insect-pest Host Resistance: Cyto-124 was evaluated for tolerance against sucking pests (jassid, white fly, thrips) and bollworms at Entomology Section, Central Cotton Research Institute Multan in "Host-plant resistance trial" with standard CIM-573 (Table 7). Cyto-124 had highest tolerance for sucking insects while found about similar to other strains for bollworm damage (Table 8).

Pathological Screening of Cyto-124: NCVT consisting of 15 elite strains conducted at CCRI, Multan for the determination of Cyto-124 response in field conditions for CLCuV resistance, tolerance, stunting and boll rot during 2012-2013. It was observed from CLCuD severity that all the strains found to be highly susceptible but Cyto-124 had least severity (2.98%) (Table 9). While maximum infestation i.e (100%) was found in about all elite strains like (NIA-80, VH300, NIAB-112, CIM-573, PB-38) compared to Cyto-124 with minimum (27.4%) in Cyto-124. During 2013-14, almost same pattern was observed in NCVT as Cyto-124 had least (9.2%) CLCuD compared to standard CIM-573 with 76.0%. Moreover, it was also shown from (Table 10) that TH-112/05 had maximum stunting (2.5%) in-contrast to Cyto-124 (0.5%). It has been reported in the past that disease tolerance/resistance should be included in the screening of genotypes to get outcome from management practices (Weiss, 2000). Anjum et al. (2014) found that elite strain i.e CIM-608 found to highly tolerant to CLCuD and our findings are in accordance to this screening.

Ginning outturn (GOT %) and fiber traits: Cyto-124 had GOT % of 42.0% while other fiber quality traits like fiber length, micronaire and strength were 28.7mm, 4.5 (µg inch–1) and 99.6 thousand pounds per square inch (tppsi) respectively as compared to standards MNH-786 with 39.9%, 26.1, 5.2 and 101.2 alternatively (Table 11). 2208 counts lea strength product (CLSP) at 50 counts was found in CIM-496 which is included in A-grade. It has been observed that Cyto-124 had very good fiber traits than standards.

Morphological characters: Cyto-124 was screened for two years in NCVT and morphological traits were observed which include plant height (124cm), monopodial branches (1.1), sympodia (29.0), bolls (29.5) and boll weight (2.8g/plant) as compared to standard with 153.0cm, 1.15, 24, 18.5 and 2.35g per plant alternatively (Table 12).

Table 1. "Cyto-124" Different stages of selection

Year	Generation/Trial	O p e r a t i o n
2004 -05	F_1	2(*G.hirs.*x *G.anom.*) x ^{3}G.*hirs*. was crossed with 2 (*G.arbo.* x *G.anom.*) x ^{2}G.*hirs.*} in green house when these crosses were at tetraploid level. Selection of CLCuV free plants through petiole grafting and back crossed with *G. hirsutum* to incorporate economic & fibre traits.
2004-2005	BC_1	Selection of CLCuV free plants through petiole grafting and back crossed with *G. hirsutum* to incorporate economic & fibre traits.
2005-06	F_1 BC_2	Selection of CLCuV free plants from F_1 of BC_1 through petiole grafting and back crossed with *G. hirsutum* to incorporate economic & fibre traits.
2006-2009	F_2-F_5	Screening and selection in different segregating generations and finally bulked as strain.

Year	Generation/Trial	O p e r a t i o n
2010-2013	MVT,VT, ZVT, NCVT, PCCT, DUS TRIAL and BIGGER BLOCKS	• Trials at Central Cotton Research Institute, Multan and farmers' Fields. DUS trials by National Seed Certification and Registration Department and bigger blocks at Punjab Seed Corporation Farms, Khanewal. • Spot examination by the Expert Sub-Committee of Punjab Seed Council.
2014-15	Data compilation and preparation of proposal	Submission of proposal for consideration by the Expert Sub-Committee for forwarding it to the Punjab Seed Council for approval.

Table 2. Seedcotton Yield of Cyto-124 inVarietal trials at Central Cotton Research Institute, Multan during 2010-11 to 2013-14

Year	Name of Trial	Location	Varieties / seed cotton (kg ha^{-1})				CD (5%)
			Cyto-124	MNH-786	CIM-554	CRSM-38	
2010-11	MVT-2	Multan	4022	3381	-	-	625.26
2011-12	VT-1	"	3030	-	2621	2498	50.6
2012-13	VT-1	"	2847	1947	2481	-	174.17
2013-14	VT-3	"	2331	1363	-	-	218.2
Average			3058	2230	2551	2498	
Percent increase over				37.1	19.9	22.4	

Table 3. Seedcotton Yield of Cyto-124 in at farmers' fields during 2012-13

Sr. No.	Farmer Particulars	Seedcotton (kg ha^{-1})	
		Cyto-124	CIM-573
1	Mr. Muhammad Tahir, Ali Pur	3150	2950
2	Mr. Muhammad Saleem, Lodhran	3060	2861
3	Haji Tariq Mahmood Bhutta, 6-Faiz, Multan	3092	2924
4	Ch. Ghohar Ali, Makhdum Rasheed	2793	2687
5	Ch. Muhammad Hanif 108/7R, Sahiwal	2831	2587
6	Ch. Muhammad Saddiq, 17/11R, Sahiwal	2744	2459
7	Ch. Muhammad Akbar 70/5L, Sahiwal	2711	2564
8	Mr. Khuda Bux, 19 Kasi, Multan	2692	2647
9	Mian Mehboob Qureshi, Kot Addu	2799	2945
10	Iftikhar Shah D. G. Khan	2792	2764
11	Mr. Shahid Manzoor, Khanpur	2844	2758
12	Ch. Rehmat Ali, 88/10-R, KWL	2906	2675
13	Mr. Aleem Ahmad Khan, Tounsa	2792	2576
14	Sh. Abdul Rasheed, 88/WB Vehari	2844	2864
15	Haji Allah Ditta, Kukar Hatta	3033	2951
16	Ch. Ramzan Ahmad, Hasilpur	2916	2574
17	Mr. Ghulam Mustafa Chatta, Uch Sharif	2863	2681
18	Ch. Zia-ur-Rehman, Liaquat Pur	3111	2875
19	Ch. Hafeez, Rajanpur	2735	2654
	Average	2879	2735

Table 4. Yield performance of Cyto-124 at farmers' fields during 2013-14

Sr. No.	Farmer Particulars	Seedcotton (kg ha^{-1})	
		Cyto-124	CIM-573
1	Haji Zulfiqar Ali, Haroonabad	3099	2790
2	Mr. Muhammad Saleem, Lodhran	2932	2800
3	Haji Tariq Mahmood Bhutta, 6-Faiz, Multan	2709	2730
4	Ch. Ghohar Ali, Makhdum Rasheed, Multan	3222	3040
5	Ch. Muhammad Hanif 108/7R, Sahiwal	2741	2700
6	Ch. Muhammad Saddiq, 17/11R, Sahiwal	3052	2900
7	Ch. Muhammad Akbar 70/5L, Sahiwal	2701	2550
8	Mr. Khuda Bux, 19 Kasi, Multan	2990	2810
9	Mian Mehboob Qureshi, Kot Addu	3333	3100
10	Iftikhar Shah D. G. Khan	3226	3000
11	Mr. Shahid Manzoor, Khanpur	3301	3270
12	Ch. Rehmat Ali, 88/10-R, Khanewal	2943	2830
13	Mr. Aleem Ahmad Khan, Tounsa	2866	2600
14	Sh. Abdul Rasheed, 88/WB Vehari	3121	2920
15	Haji Allah Ditta, Kukar Hatta, Khanewal	3377	3220
16	Ch. Ramzan Ahmad, Hasilpur	3549	3430
17	Mr. Ghulam Mustafa Chatta, Uch Sharif	3267	3220
18	Ch. Zia-ur-Rehman, Liaquat Pur	2990	3100
19	Ch. Hafeez, Rajanpur	2963	2580
20	Mian Muhammad Amjid Zia, Khanewal	3120	2780
	Average	3075	2919

Table 5. National Coordinated Varietal Trial for Adaptibility Testing in Punjab zone during 2012-13

Varieties	Punjab					Punjab Av.
	NIBGE FSD	CCRI Multan	CRS Multan	PSC Khanewal	CRS R Y Khan	
GS-444	1812	1099	1418	3265	2624	2044
PB38	1616	1349	1336	3193	2237	1946
BH-176	1835	1175	1239	2987	2357	1918
CRIS-510	2069	1193	1206	3032	2022	1904
Cyo-124	1359	1507	1581	2673	2021	1828
CIM-573	1888	1310	1483	2189	2081	1790
DNH-105	1706	1418	1190	2260	2283	1771
NIAB-112	1193	1103	1500	2781	2152	1746
VH-300	1284	843	1467	2314	2771	1736
NIA-80	1767	1516	1500	1955	1811	1710
JS-212	1540	992	1060	1812	2134	1508
IUB-11	1268	1106	1108	1668	1766	1383
CIM-612	1133	1213	1108	1740	1563	1351
CIM-591	680	1214	1239	1758	1535	1285
MPS-II	1012	1051	929	1830	1533	1271

Table 6. National Coordinated Varietal Trial for Adaptibility Testing in Punjab zone during 2013-14

Varieties	Punjab							
	Fsd NIBGE FSD	FSD Zone Avg	Multan Zone				Multan zone Avg	Punjab Avg
			CCRI Multan	CRS Multan	PSC Kwal	CRSR Y Khan		
CIM-612	708	708	1712	1437	1506	2484	1785	1569
Cyto-124	1252	1252	2016	2227	3363	2362	2492	2244
DNH-105	842	842	1674	1902	3591	2850	2504	2172
CRIS-533	696	696	1527	1408	3403	2730	2267	1953
MPS-27	682	682	1481	1704	3107	2359	2163	1867
BH-177	1239	1239	1416	1903	4304	2276	2475	2228
TH-112/05	365	365	973	529	1762	2041	1326	1134
PB-896	1005	1005	1644	2054	3699	2410	2451	2162
Sun-2	818	818	1185	1408	3376	2718	2172	1901
CIM-573	812	812	828	781	2098	2113	1455	1326

Table 7. Response of Cyto-124 to sucking pests

Strains	Number of sucking insect pests per leaf								
	Jassid			Whitefly			Thrips		
	June	July	Av.	June	July	Av.	June	July	Av.
Sun-2	3.79	0.40	2.10	3.57	1.96	2.77	0.44	2.09	1.27
BH-177	5.28	0.33	2.81	1.61	1.70	1.66	0.22	5.49	2.86
CIM-612	4.53	0.06	2.30	1.61	0.96	1.29	0.06	11.04	5.55
CRIS-533	5.10	0.25	2.68	1.33	0.93	1.13	0.23	2.40	1.32
TH-112/05	4.28	0.24	2.26	2.07	1.14	1.61	0.17	3.49	1.83
MPS-27	5.40	0.36	2.88	1.38	1.96	1.67	0.06	5.9	2.98
DNH-105	4.00	0.20	2.10	1.96	1.33	1.65	0.11	5.08	2.60
Cyto-124	5.36	0.50	2.93	1.60	0.80	1.20	0.06	9.55	4.81
PB-896	3.34	0.46	1.90	2.03	1.19	1.61	0.10	2.55	1.33
CIM-573 (Std)	4.04	0.04	2.04	3.45	2.06	2.76	1.16	2.44	1.80

Table 8. Bollworm damage of Non-Bt strains at different stges of crop

Strains	Bollworm damage % age				SBW larvae/25 plants		PBW larvae/100 bolls
	September		October				
	Imm	Mat	Imm	Mat	Sep	Oct	
MPS-27	3.5	0.1	6.00	0.29	2.0	3.0	4.17
DNH-105	0.0	0.0	1.82	0.94	0.0	1.0	4.86
Cyto-124	1.0	0.4	2.3	0.73	0.85	1.3	15.28
CRIS-533	2.0	0.5	5.48	1.75	1.0	5.0	6.94
Sun-2	8.0	2.0	14.29	0.78	4.0	3.0	5.56
CIM-612	1.0	0.2	2.52	0.00	1.0	1.0	8.33
TH-112/05	0.0	0.0	1.28	1.90	0.0	1.0	0.00
PB-896	0.0	0.0	1.56	0.98	0.0	1.0	2.08
BH-177	3.2	0.2	5.51	0.61	2.0	2.0	6.25
CIM-573 (Std)	1.0	0.0	6.67	0.45	1.0	1.0	4.17

Table 9. CLCuV Disease Infestation on NCVT Set I 2012-13

NCVT Strain	Disease % age	Disease Severity	Disease Index
Cyto-124	27.49	2.98	20.38
CIM-573	99.68	3.01	75.08
NIAB-112	100.00	3.32	83.08
MPS-II	99.66	3.28	81.60
IUB-11	94.71	3.38	80.43
PB-38	100.00	3.08	77.05
CIM-612	48.26	2.87	34.17
DNH-105	100.00	3.46	86.41
GS-444	100.00	3.60	90.11
BH-176	99.56	3.32	82.58
CIM-591	100.00	3.30	82.62
NIA-80	100.00	3.12	77.88
CRIS-510	100.00	3.45	86.25
VH-300	100.00	3.36	83.95
JS-212	98.00	3.70	90.77
Disease Severity		2 = = Greater parts of veins involved	
*0 = No symptoms		3 Whole veins involved	
1 = Vein thickening and minute scattered		4 = Veins involved completely and severe curling	

Table 10. CLCuV Disease Infestation on NCVT Set-D during 2013-14

NCVT Strain	Stunting (%age)	Cotton Leaf Curl Disease		
		Incidence	Severity	Disease Index
CRIS-533	0.0	100.00	3.17	79.18
MPS-27	0.4	100.00	3.12	77.98
BH-177	0.0	100.00	3.07	76.64
TH-112/05	2.5	100.00	3.33	83.36
PB-896	0.9	100.00	3.04	75.88
Sun-2	0.0	100.00	2.99	74.86
CIM-612	0.0	64.26	2.67	43.94
Cyto-124	0.5	14.95	2.47	9.21
DNH-105	0.8	100.00	2.96	73.91
CIM-573	0.9	100.00	3.04	76.06

Disease Index= Disease percentage x Disease severity/maximum severity value (4)

Table 11. Comparison of fiber quality traits of 'Cyto-124 cotton with the standards, 'MNH-786', CIM554 and 'CRSM-38', recorded from 2013 to 2014.

Name of trait	Cyto-124	MNH-786	CIM-554	CRSM-38
Ginning out turn (%)	42.0	39.9	42.4	41.9
Staple length (mm)	28.7	26.1	27.1	28.5
Micronaire value (µg inch^{-1})	4.5	5.2	4.1	4.4
Fiber strength (tppsi)	99.6	101.2	99.1	96.1

Table 12. Comparison of morphological traits of 'Cyto-124' cotton with the standards, 'CIM-554' recorded from 2013 to 2014.

Name of trait	Cyto-124	CIM-554
Plant height (cm)	124	153.0
Monopodial branches plant–1	1.1	1.15
Sympodial branches plant–1	29	24
Avg. boll weight (g)	2.8	2.35
Bolls plant–1	29.5	18.5

DISCUSSIONS

Resistance against biotic e.g CLCuV and abiotic stresses as well as refinement of fiber quality is at slow speed due to confined genetic variability in American cotton. Transference of desirable traits e.g disease and insects resistance from wild species to upland cotton has a considerable potential to overcome such bottlenecks as has been done in the past. Hence, it is highly useful to use such germplasm for the release of cultivars with introgression from wild species.

The ultimate factor for the development of fertile hybrids is the setting of bolls without required traits after hybridization. The researchers have been involved to develop new combinations by using different ploidy level like diploid and tetraploid species (Dhamayanthi et al, 2010). They revealed that it needs tremendous effort to derive such hybrids as it involves hybrid inviability, hybrid break down and self-incompatibility barriers. Nardeli (2018) observed indirect effect of embryo on endosperm in upland cotton accessions in-contrast to desi cotton (G. arboreum). An enormous number of introgressed hybrids were developed via ovule and embryo culture in Gossypium (Louant, 1973F) yet traditional breeding approaches was found to be better for such introgressed materia . Gibrellic acid was utilized for overcoming hybrid development problems like hybrid breakdown, hybrid in viability (Liang and Sun, 1982), 33. The introgressed developed hybrid from G. anomalum and American cotton had exhibited resistance to CLCuV which revealed that biotic stress like disease can be introgressed in upland cotton from G. anomalum using backcrossing and using gibrellic acid and napthelene acetic acid. Hence, it is revealed that autotetraploids can be used for the transference of required characters in American cotton from non-cultivated species of genus via classical breeding especially disease resistance as the developed cultivar Cyto-124 has a tremendous potential to overcome the CLCuV disease. It is also speculated that autotetraploids can be used to transfer required characters in American cotton from G. arboreum while more durable resistance can be produced in the available cotton germplasm for CLCuV via conventional breeding.

ACKNOWLEDGMENT

Cyto-124 was developed through funding by Ministry of National Food Security and Research, Islamabad. We are highly pleased to Vice President (VP), Pakistan Central Cotton Committee

(PCCC), Multan, for his tremendous support and highly obliged to worthy Director CCRI and Heads of different sections for Cyto-124 evaluation.

REFERENCES

Afzal, M., M. Arshad, M. Idrees and N. Illahi. 2002. Yield Response of Indigineously Evolved Upland Cotton Genotypes for Various Traits in National Coordinated Varietal Trails (NCVT) Under Multan Conditions. Asian Journal of Plant Sciences, 1: 119-120.

Afzal, M., M. Arshad, M. Khan, M. Jan, N. Illahi and S. Haider. 2001. Genotypic environmental interaction for yield and its components of newly evolved cotton genotypes under Multan conditions. Pakistan Journal of Biological Science, 4: 440-441.

Ahmad, G., S. A. Malik, Z. Mamood, M. Z. Iqba, S. Ahmad and S. Ahmad. 2002. Effect of Cotton Leaf Curl Virus Disease on Morphology, Yield and Fibre Characteristics of Susceptible Lines/cultivars of Cotton (*Gossypium hirsutum* L.). Asian Journal of Plant Sciences, 1: 705-707.

Ahmad, M., A.M. Memon, and A.H. Baloch. 2018. Effect of site and season on varietal performance in desi cotton. The Pakistan Cottons, 33-35.

Amin, A., W. Nasim, M. Mubeen, S. Sarwar, P. Urich, A. Ahmad, A. Wajid, T. Khaliq, F. Rasul, H. M. Hammad, M. I. A. Rehmani, H. Mubarak, N. Mirza, A. Wahid, S. Ahamd, S. Fahad, A. Ullah, M. N. Khan, A. Ameen, Amanullah, B. Shahzad, S. Saud, H. Alharby, S. T. Ata-Ul-Karim, M. Adnan, F. Islam and Q. S. Ali. 2016. Regional climate assessment of precipitation and temperature in Southern Punjab (Pakistan) using SimCLIM climate model for different temporal scales. Theoretical and Applied Climatology, 131: 121-131.

Anjum, Z. I., Hayat, K., S. Celik., T. M. Azhar, U. Shehzad, F. Ashraf, Tariq M., H., T. Mehmood and M. Azam. 2015. Development of cotton leaf curl virus tolerance varieties through interspecific hybridization. African Journal of Agricultural Research, 10: 1612-1627.

Anjum, Z. I., M. T. Azhar, K. Hayat, F. Ashraf, U. Shahzad and M. Azam. 2014. Development of high yielding and CLCuV resistant ulpand cotton variety “CIM-608”. Pakistan Journal of Phytopathology, 26: 25-34.

Anonymous. 2017. Assessment of damage due to cotton leaf curl virus in the Punjab. A report published by the Govt. of the Punjab.

Arshad, M., C.R. Ali, M.I. Khan, and M. Afzal. 2003c. Indigenous evolution of long 8. staple and high yielding upland cotton variety CIM-707. The Pakistan Cottons, 49: 35-44.

Arshad, M., M. Afzal, M. Khan and R. Mahmood. 2003d. Performances of newly developed cotton strains for economic and fiber traits in national coordinated varietal trials. Pakistan Journal of Scientific and Industrial Research, 46: 373-375.

Arshad, M., M. Afzal, M.I. Khan, and C.R. Ali. 2003b. Evolution of high yielding, heat tolerant and early maturing upland cotton variety CIM-506. . The Pakistan Cottons, 49: 49-62.

Arshad, M., M. Hanif, C.R. Ali, M. Afzal, M.I. Khan, and N. Illahi. 2003a. Evolution of a high yielding with quality fiber upland cotton variety CIM-499. The Pakistan Cottons, 47: 17-23.

Ashraf, M. A., A. A. Shahid, B. B. Mohamed, A. A. Dahab, K. S. Bajwa, A. Q. Rao, M. A. U. Khan, M. Ilyas, M. S. Haider and T. Husnain. 2013. Molecular characterization and phylogenetic analysis of a variant of highly infectious cotton leaf curl Burewala virus associated with CLCuD from Pakistan. Australian Journal of Crop Science, 7: 1113-1122.

Baig, M. S. and J. A. Khan. 2013. Identification of *Gossypium hirsutum* miRNA targets in the genome of Cotton leaf curl Multan virus and beta satellite. Indian Journal of Biotechnology, 12: 336–342.

Baloch, S. A. and Z. A. Soomro. 2002. Varietal Performance for Yield Potentials under Different Row Spacings of *Nicotiana tabacum* L. Journal of Applied Sciences, 2: 1000-1001.

Briddon, R. W., F. Akbar, Z. Iqbal, L. Amrao, I. Amin, M. Saeed and S. Mansoor. 2014. Effects of genetic changes to the begomovirus/betasatellite complex causing cotton leaf curl disease in South Asia post-resistance breaking. Virus Research, 186: 114-119.

Dhamayanthi, K. and V. Gotmare. 2010. Induction of polyploidy in two diploid wild cotton (*G. armourianum* and *G. aridum*) species by colchicine treatment. Electron Journal Plant Breeding, 1: 966-972.

Fryxell, P. 1992. A revised taxonomic interpretation of Gossypium L (Malvaceae). Rheedea, 2: 108-116.

Gallagher, J. P., C. E. Grover, K. Rex, M. Moran and J. F. Wendel. 2017. A New Species of Cotton from Wake Atoll, *Gossypium stephensii* (Malvaceae). Systematic Botany, 42: 115-123.

Hanif, M., M. Arshad, M. Afzal and M. Khan. 2001. Yield response and yield parameters of newly developed cotton varieties of *G. hirsutum* L. Baluchistan Journal of Agricultural Sciences, 2: 9-13.

Hussain, T. and M. Ali. 1975. Review of cotton diseases of Pakistan. The Pakistan cottons, 71-86.

Iqbal, M. J., N. Aziz, N. A. Saeed, Y. Zafar and K. A. Malik. 1997. Genetic diversity evaluation of some elite cotton varieties by RAPD analysis. Theoretical and Applied Genetics, 94: 139-144.

Khan M.I., M. A., N. Illahi, and M.T. Jan. 2002. Genotypes x environmental interactions studies in seven Pakistani upland cotton genotypes. Indus Journal of Plant Science, 1: 325-329.

Khan, W., A. Khan, A. Naz and S. Ali. 1989. Performance of six Punjab commercial varieties of *Gossypium hirsutum* L. under Faisalabad conditions. The Pakistan Cottons, 33: 60-65.

Liang, G.Y. and N. J. Sun. 1982. Chemical studies on active principles of *Veratrum stenophyllum* [J]. Acta Pharmaceutica Sinica, 9.

Louant, B. 1973. Recherches sur les possibilités d'améliorer le cotonnier par l'introgression directe de caractères provenant de *Gossypium Raimondii* ULBR. Ouvrage publié avec le concours du Ministère Belge de L'Education Nationale.

Mahmood, T., M. Tahir, A. Tanveer and M. Mirza. 1996. Effect of cotton leaf curl virus on yield components and fiber properties of four commercial varieties. Pakistan Journal of Phytopathology, 8: 68-70.

Mansoor, S., R. W. Briddon, Y. Zafar and J. Stanley. 2003. Geminivirus disease complexes: an emerging threat. Trends in plant science, 8: 128-134.

Nardeli, S. M., S. Artico, G. M. Aoyagi, S. M. de Moura, T. da Franca Silva, M. F. Grossi-de-Sa, E. Romanel and M. Alves-Ferreira. 2018. Genome-wide analysis of the MADS-box gene family in polyploid cotton (*Gossypium hirsutum*) and in its diploid parental species (*Gossypium arboreum* and *Gossypium raimondii*). Plant Physiology and Biochemistry, 127: 169-184.

Pfister, K. K., P. R. Shah, H. Hummerich, A. Russ, J. Cotton, A. A. Annuar, S. M. King and E. M. Fisher. 2006. Genetic analysis of the cytoplasmic dynein subunit families. PLoS Genet, 2: e1.

Rajagopalan, P. A., A. Naik, P. Katturi, M. Kurulekar, R. S. Kankanallu and R. Anandalakshmi. 2012. Dominance of resistance-breaking cotton leaf curl Burewala virus (CLCuBuV) in northwestern India. Archives of Virology, 157: 855-868.

Sattar, M. N., A. Kvarnheden, M. Saeed and R. W. Briddon. 2013. Cotton leaf curl disease an emerging threat to cotton production worldwide. Journal of General Virology, 94: 695-710.

Singh, H., R. Mital and R. Upadhyay. 1973. Variety x environment interaction in American cotton (*Gossypium-hirsutum* l) in uttar-pradesh. Indian Journal of Agricultural Sciences, 43: 463-466.

Soomro, B., G. Nachnani and G. Memon. 1986. Performance of seven upland cotton varieties at five locations in Sindh. The Pakakistan Cottons, 30: 31-38.

Tan, Z., X. Fang, S. Tang, J. Zhang, D. Liu, Z. Teng, L. Li, H. Ni, F. Zheng, D. Liu, T. Zhang, A. H. Paterson and Z. Zhang. 2014. Genetic map and QTL controlling fiber quality traits in upland cotton (*Gossypium hirsutum* L.). Euphytica, 203: 615-628.

Varma, A. and V. G. Malathi. 2003. Emerging geminivirus problems: A serious threat to crop production. Annals of Applied Biology, 142: 145-164.

DEVELOPING SNP-BASED MOLECULAR MARKER FOR CLCUV RESISTANCE IN COTTON

Zarmeen Zafar[1,2], Fukhar Zaman[3], Umar Iqbal[1], Shoaib Ur Rehman[1,2], Sehrish Ijaz1[1,2], Furqan Ahamd[1,2], Umar Akram[1,2], Akhtar Hameed[4], Zulqurnain Khan[1,2]*

[1] Institute of Plant Breeding and Biotechnology, MNS University of Agriculture, Multan, Pakistan
[2] SINO-PAK Joint Research Laboratory, MNS University of Agriculture, Multan, Pakistan
[3] Institute of Horticultural Sciences, University of Agriculture, Faisalabad.
[4] Institute of Plant Protection, MNS University of Agriculture, Multan, Pakistan
*** Corresponding authors:** zulqurnain.khan@mnsuam.edu.pk

ABSTRACT:

Cotton Leaf Curl Disease (CLCuD) poses a serious challenge to cotton production in Pakistan. Current study aims to screen the Pakistani cotton genotypes against CLCuD by combining genetic and phenotypic evaluation. Heparanase like protein (HSPE) gene is responsible for protein production related to positive regulation of viral infections. This gene was selected based on Genome-Wide Association Study (GWAS) data. HSPE showed potential for Single Nucleotide Polymorphism (SNP) markers, signifying CLCuD resistance. Current study has utilized genomic DNA from forty cotton varieties representing various genetic backgrounds. Disease symptoms were observed in the field. This assessment assisted in understanding the severity of disease and identifying the resistant genotypes. Disease index was calculate for each cotton genotype to categorize in corresponding scale. HSPE was amplified by using PCR technique and the amplification were confirmed by gel-electrophoresis. These results supported the potential of the selected gene as a reliable indicator of CLCuD resistance. By uniting genetic insights with observable traits gained a better understanding of how genetic differences affect disease resistance in cotton. Utilization of molecular marker approach will enhance the development of improved cotton varieties, ultimately leading to the identification of cotton genotypes with natural CLCuD resistance.

Keywords: Keywords: *CLCuV*, Phenotyping, Genotyping, Molecular markers, SNP-based marker

1. Introduction

Cotton (Gossypium spp.) is an important cash crop and well known natural fibre and widely grown around the world (Thyssen et al., 2019). In Pakistan, cotton.is the most extensively produced cash crop. Punjab has several warm and arid areas that are important for producing cotton, including Bahawalpur, Bahawalnager, Multan, Rahim Yar Khan, D.G. Khan, Khanewal, Rajanpur, Lodhran, and Muzafargar. Biotic stress poses a significant threat to cotton cultivation, encompassing various diseases and pests that can lead to reduced yields and crop losses. Cotton plants are vulnerable to a range of biotic stressors such as fungal infections, insect infestations, and viral diseases. Biological causes, such as pests and diseases, which can significantly reduce yield (by up to 10–30%), can have a major impact on the yield of cotton in addition to abiotic stresses (Tarazi et al., 2019). The majority of the viruses that affect cotton crops are members of the begomovirus family. Begomoviruses, which result in leaf damage (leaf curl, swollen veins, stunting and enation), are the symptoms of CLCuD. There is a considerable decrease in yield when the attack is in the early phases of development. The stage of plant growth is exaggerated by the extent of the infestation (Chohan et al., 2020).

CLCuD was originally recognized in Pakistan in 1967, the outbreak of CLCuD started in the Punjab region close to the city of Multan. Early in the 1990s, CLCuD was the main factor limiting cotton production of Pakistan. It has now spread to include India and, more recently, Pakistan's south and west. The symptoms of disease comprise vein enlargement, darkening, enations and leaf curling either upward or downward. Enations are the structures resembling leaves which are formed on the undersides of leaves when disease is severe. Early-infected plants continue to have restricted growth and small leaves. The outbreak has been greatly influenced by the types of cotton grown in Pakistan. It is now understood that the variety S12 is extremely vulnerable to CLCuD. This type, which was initially made available in 1988, enjoyed the greatest popularity in the Punjab during the early stages of the pandemic, accounting for up to 46% of the region's total cotton acreage. In the years between 1980 and 1990, CIM-70, another sensitive cultivar, was cultivated on 2-8% of the land. NIAB-78 (5 and 70% for 1980 and 1990, respectively) and CIM-240 (growing since 1990) were two of the most popular types that demonstrated varying levels of tolerance.

CLCuD.is a threat.to all cotton-growing areas where whiteflies are a frequent, significant, or major pest. A variety of short-term (mostly management practices) and long-term (creating resistant cotton cultivars) methods were created to battle this damaging disease. Long-term plans are responsible for the production of resistant cotton cultivars using both traditional and unconventional methods. Short term strategies included treating the seeds to prevent the early establishment of whitefly populations, using pesticides to control whiteflies on cotton crops, eliminating weeds which serve as alternative hosts for the virus, and ensuring the health of the plants by providing a

balanced dose of fertilizers and biological agents. (Bleekar et al., 2011; Powell et al., 2012; Yuan et al., 2012). The long term strategies are the use of DNA markers in producing cotton cultivars resistant to CLCuD and associated viral causative agents is crucial for a number of reasons. Due to changes in the surrounding environment, it is very challenging to impose uniform disease stress in greenhouses, which might affect the screening process. Therefore, it is preferable to breed for really resistant cotton cultivars using DNA markers (Rahman et al., 2017).

Due to their high amount of polymorphism, SNP markers are crucial tools for marker assisted selection, map-based cloning, and linkage mapping. SNPs can discriminate between heterozygous and homozygous alleles due to their co-dominant character (Shaheen et al., 2009). Due to their high polymorphism, SNPs were employed in cotton (Michael et al., 2014; Hulse-Kemp et al., 2015) to observe variety, characterize, map, and conduct QTL analysis. This high-throughput genotyping test is expected to be utilized by researchers, breeders, geneticists, and other professionals in both public and private sectors globally. Its application will significantly contribute to the advancement of cotton genetic studies, breeding programs, genome sequence assembly, and various other research applications.

For breeding programs to be successful, plant breeders must be able to recognize the parental sources that will provide a range of populations for selection and have an understanding of the genetic diversity within and among the genetic resources of the available germplasm. In order to preserve genetic variety in a breeding program, it is important to characterize the genetic similarity across genotypes (Becelaere et al., 2005). Understanding the genetic links between plant genotypes aids in understanding the complexity of the available germplasm, identifying the variations in the genotypes, and developing practical conservation strategies (Dahab et al., 2013). Therefore, molecular marker-based analysis can offer crucial insight into the genetic make-up of a plant community, assisting in the development of new species. Many genetic diversity studies have been conducted on cotton using several marker techniques, such as random amplified polymorphic DNA (RAPD) (J. Wendel and Cronn, 2003), amplified fragment length polymorphism (AFLP) (Rana et al., 2005), and simple sequence repeats (Qayyum et al., 2009).

2. Material and Methods

2.1. Cotton material and Experimental design

Cotton material is available at MNSUAM and a sub-set of 40 diverse genotypes were selected to evaluate the CLCuD disease. Sub-set of cotton genotypes were selected on the basis of contrasting traits. The experiments were conducted using a randomized complete block design (RCBD) with three replicates. Standard agronomic and cultural practices, such as irrigation, weeding, hoeing, and fertilizer applications, were implemented as necessary throughout the study. A sub-set of forty cotton genotypes were screened for CLCuD response during the growing

season 2021-22- Fifteen plants were selected from each genotype to collect disease scoring data. The survey was carried out at experimental field of MNSUAM, Multan (71º2636.8"E, 30º**0851.7"**N) is the favorable location where the disease has been noted often and is thought to be a CLCuV hotspot. Scoring of CLCuV disease along with disease reaction for each plant is described in (Supplementary Table 1).

Table 1: Scoring of CLCuV disease along with disease reaction for each plant.

Symptoms	Disease Response	Disease Scoring
Complete absence or no symptoms.	Immune	0
Enlargemento f few small scattered veins.	Highly resistant	1
Enlargement of small groups of veins.	Resistant	2
Enlargement of all present veins.	Moderately resistant	3
Severe vein enlargement, leaf curling at the top of plant.	Moderately susceptible	4
Severe vein enlargement, leaf curling on the half of the plant.	Susceptible	5
Severe vein enlargement, reduced fruit production along with stunted growth, leaf Curling	Highly susceptible	6

2.2. CLCuV disease index

Using the disease scale proposed by Farooq *et al*. (2011) and reported by Akhtar *et al*. (2010), the Index of CLCuV disease (%) and response of the genotypes were calculated. Following formula was used to calculate % age of CLCuV disease Index

CLCuV disease Index (%) =

$$\frac{Sum\ of\ all\ disease}{ratings} x16.16$$

Table 2.3: Modified disease scale for rating of cotton leaf curl virus (CLCuV)

Rating	Syptoms	Disease reaction	% disease index
0	Complete absence or no symptoms.	Field Immune	0
1	Enlargement of few small dispersed veins.	Highly resistant	0.1-5
2	Enlargement of small groups of veins.	Resistant	5.1-10
3	Enlargement of all present veins.	Moderately resistant	10.1-20
4	Severe vein enlargement, leaf curling at the top of plant.	Moderately susceptible	20.1-30
5	Severe vein enlargement, leaf curling on the half of the plant.	Susceptible	30.1-50

Rating	Syptoms	Disease reaction	% disease index
6	Severe vein enlargement, reduced fruit production along with stunted growth, leaf Curling	Highly susceptible	50.1-100

2.3. Retrieval of resistant gene CDS sequences and genomic sequence

Potential SNPs associated with disease resistance have been identified by the Gossypium hirusutum Genome Wide Association Study (GWAS). Only SNPs found in the coding section of genes were selected, even though both coding and non-coding sequences contained many SNPs. For the purpose of genotyping, the candidate gene hepranase like protein was selected. The cotton genome sequence in the NCBI database was BLAST against the candidate gene CDS sequence. Based on the candidate gene's most significant similarity index, the genomic sequence was identified. From the NCBI data base, the genomic sequence was retrieved in FASTA format. The potential genomic sequence was 3244 nucleotides in length.

2.4. Development of novel gel-based molecular marker

SNP (C/A) was detected at 1528 bp on the 5th exon of gene. This polymorphic site were producing non-synonymous mutation (CTA-leucine to ATA-Isoleucine). First nucleotide of codon was changing the amino acid type. Considering basic information of gene and on the basis of non-synonymous mutation novel SNP based molecular marker was developed. Cleaved Amplified Polymorphic Sequence (CAPS) was utilized to develop marker. There were following steps to develop molecular marker given in sub-headings. Restriction enzymes have the ability to digest at specific recognition site of DNA. For the selection of restriction enzyme dCAPS software was used. Based on SNP-1528 nt (C/A) restriction enzyme was naturally present polymorphic site. Restriction enzyme SpeI was selected and its specific recognition sites was (A^CTAGT) complementary DNA sequence.

Table 3.1: Primers information of Gh-HSPE

Marker	Primers	Sequence	Fragment size
Gh-HSPE	Forward	GCTTGGAACTACACCAATGC	400 bp
	Reverse	GGTGAGTGACCACATCTAAGG	

2.5. Genotyping of Cotton genotypes for Candidate gene

Fort cotton genotypes were used for genotyping (Supplementary Table). CAPS marker name as Gh-HSPE were used for genotyping of Pakistani diverse cotton germplasm for the identification of potentially linked gene against CLCuD. To perform genotyping followed steps such as DNA extraction, Polymerase Chain Reaction (PCR), Restriction enzyme digestion and Gel-electrophoresis. Recipe for restriction enzyme and PCR are given supplementary table.

3. Results

3.1. Response of Cotton genotypes against CLCuV

The scoring of CLCuV was done by using the above scale mentioned in the material and methods in Table. Cotton genotypes MNH-875, Okra X hir, MAC-07 and MNH-6070 exhibited highly resistance against CLCuV. While MNH-1016, MNH-1035, MNH-1045, MNH-1050, MNH-1016, MNH-1050, FH-Super Cotton, CIM-473, Bt.CIM-598, IR-8, IR-13, CKC-3, IUB-2013, N-121, FH-Lalazar and UAM-21 showed resistance against CLCuV. RH-Super and CYTO-124 (Non Bt) were moderately resistant. Moderately susceptible response showed by genotypes were MNH-886, FH-900, FH-901 and colored cotton brown. MNH-1045, RH-Afnan II, VH-259, VH-305, VH-327, VH-427, VH-430, SLH-55, CIM-443 and MNH-886 were susceptible genotypes of cotton. While highly susceptible genotypes were Z-33, S-12, CIM-496, CIM-1100, CIM-598 and UAM-20 (Table 2.4). On the basis disease index genotypes were categorized accordingly.

Table 2.5: Summary statement of cotton varieties evaluated against cotton leaf curl virus (CLCuV) under field condition.

Sr.no	Highly Resistant	Resistant	Moderately resistant	Moderately susceptible	Susceptible	Highly susceptible
1	MNH-875	MNH-1016	RH-Super	MNH-886,	MNH-1045	Z-33
2	Okra X hir, MAC-07	MNH-1035	CYTO-124 (Non Bt)	FH-900,	RH-Afnan II	S-12
3	MNH-6070	UAM-21		FH-901	VH-259	CIM-496
4		FH-Lalazar		colored cotton brown	VH-305	CIM-1100
5		N-121			VH-327	CIM-598
6		IUB-2013			VH-427	UAM-20
7		CKC-3			VH-430	
8		IR-13			SLH-55	
9		IR-8			CIM-443	
10		Bt.CIM-598			MNH-886	
11		CIM-473				

Sr.no	Highly Resistant	Resistant	Moderately resistant	Moderately susceptible	Susceptible	Highly susceptible
12		FH-Super Cotton				
13		MNH-1045,				
14		MNH-1050,				

Total 40 cotton were screened in which four genotypes exhibited the high resistance (HR) response, 14 genotypes showed resistance (R), two genotypes showed moderately resistance (MR), four exhibited moderately susceptible (MS), 10 showed the susceptible (S) reaction and six genotypes showed high susceptible (HS) response.

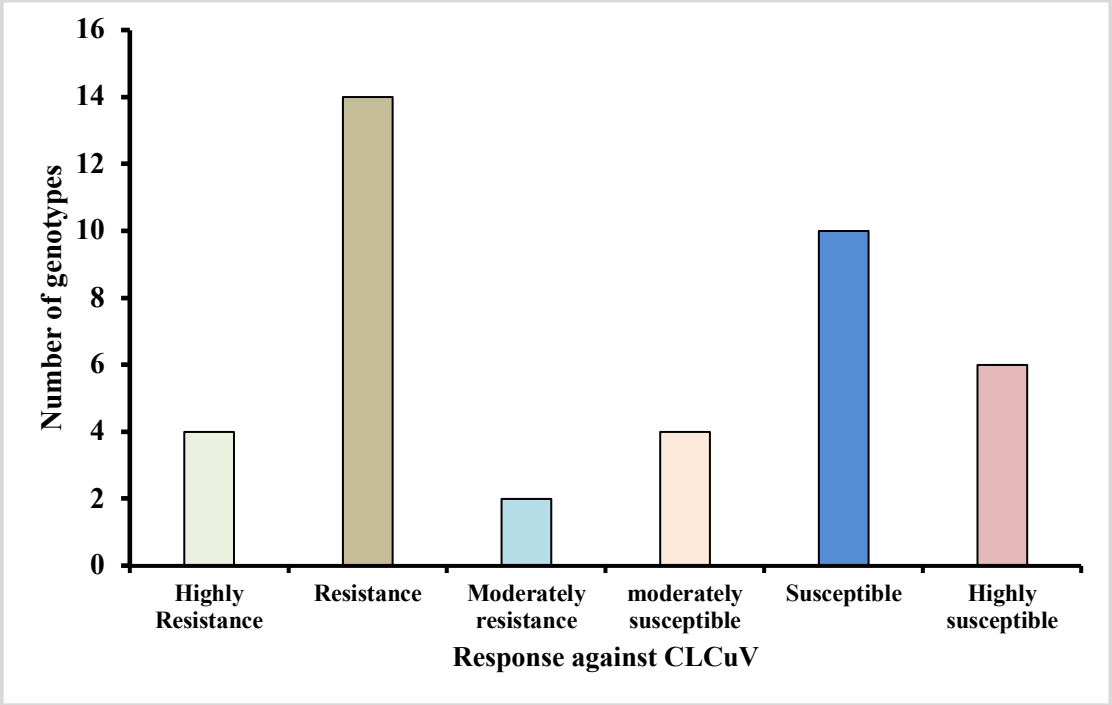


Figure 2.4: Response of Pakistani cotton diverse panel against CLCuV

3.3. Results of gel-based SNP molecular marker

The amplification of our candidate gene in all forty cotton genotypes signifies a successful and consistent outcome of our PCR efforts. The observed fragment length of 400 bp aligns precisely with the anticipated size, substantiating the accuracy of our amplification process. Through a meticulous comparison with the 2 kb DNA ladder, we further validated our results, effectively confirming the targeted fragment's size. The ladder's distinct bands at 100 bp intervals provided a reliable reference, and our amplified band notably positioned itself within the vicinity of the

500 bp marker, aligning coherently with our expectations. This congruence in size emphasizes the fidelity of our PCR technique and the reliability of our candidate gene amplification across the diverse genotypes. These findings underscore the potential significance of the amplified gene in cotton genotypes and lay a solid foundation for subsequent analyses aimed at unraveling its functional implications and potential agricultural applications.

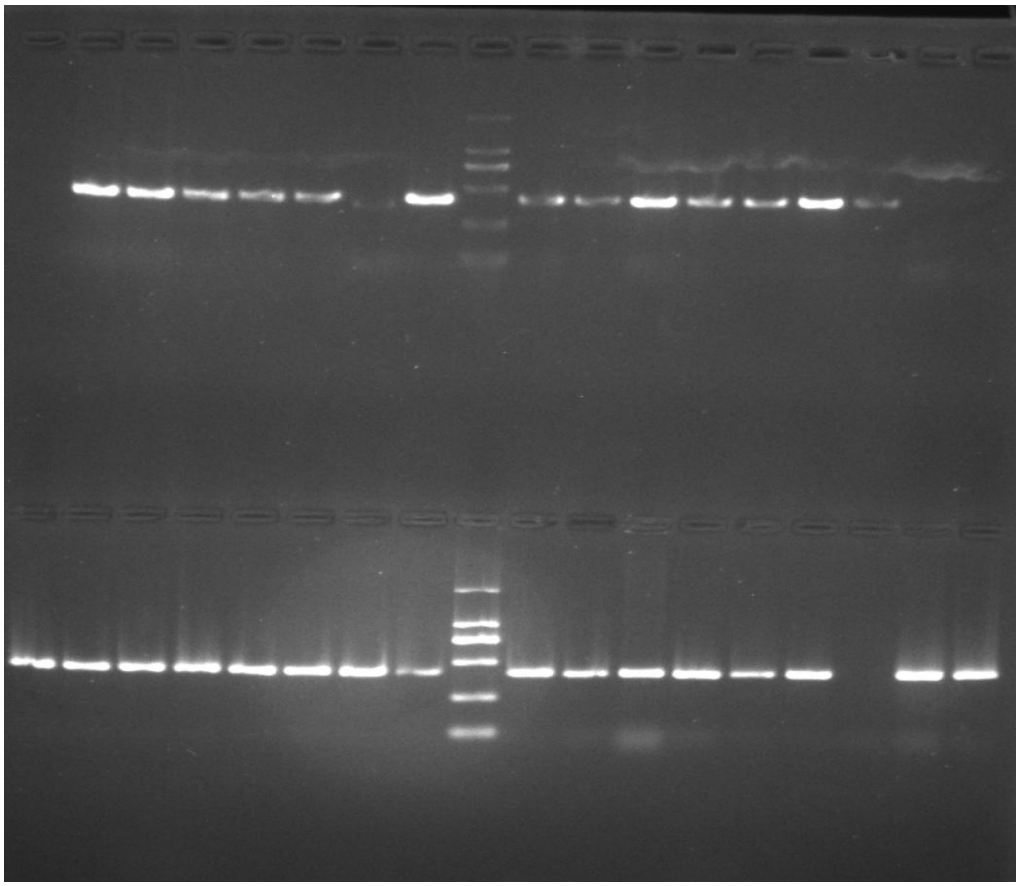


Figure 3: PCR results of studied gene. 2kb ladder was used to confirm the actual amplification of gene. Fragment length was 400bp and bands are present slightly below the 500bp band of ladder.

The CAPS marker Gh-HSPE was derived from a SNP at position 1525 nt (C/A) in the target gene, using SpeI enzyme recognizing A^CTAGT. Optimal digestion occurred at 37°C for 20 minutes. Gel electrophoresis displayed distinct bands: one at 280 bp, another at 120 bp, alongside a 2 kb ladder reference. These outcomes signify genetic variations related to the SNP, offering insights into gene characteristics and potential implications.

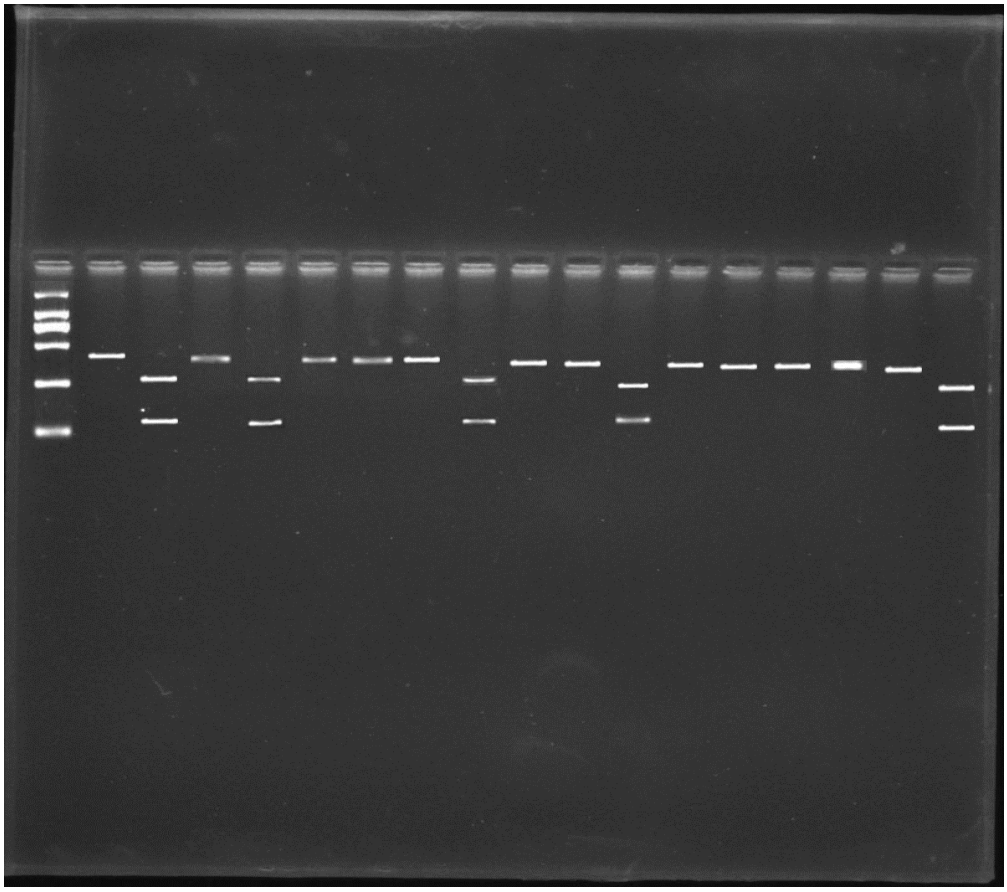


Figure 3.8: Restriction digestion of amplified gene by. *Spe*I restriction enzyme.2kb ladder was used to confirm the actual digestion location of gene. After restriction two bands vualized having 280 and 120 bp size, respectively

4. Discussion

The CLCuV is spread by the Bemisia tabaci, a whitefly that is challenging to manage due to the occurrence of several virulent viral strains or similar species (Briddon, 2003). The CLCuD is not spread via seeds. It can also live on plants including ageratum, hibiscus, okra, tomato, and tobacco. numerous herbaceous and woody plants, including cotton, okra, hibiscus, hemp, sunflower, tobacco, and numerous weeds, have been seen in the field to exhibit symptoms resembling leaf curling (Nour and Nour, 1964). Similar to this, avoiding growing other crops in cotton-producing regions during the off-season may aid in disrupting the lifecycle of whiteflies (Knight et al., 2017). In current study cotton genotypes were categorized on the basis of disease index scale and three kind of susceptible responses were revealed. Moderately susceptible (MS) response showed by genotypes were MNH-886, FH-900, FH-901 and colored cotton brown and Severe vein thickening, leaf curling at the top of plant were observed. Susceptible (S) response was revealed in MNH-1045, RH-Afnan II, VH-259, VH-305, VH-327, VH-427, VH-430, SLH-55, CIM-443 and MNH-886 and in these cotton genotypes severe vein thickening, leaf curling on the half of the plant were noticed. And cotton genotypes such as Z-33, S-12, CIM-496, CIM-1100, CIM-598 and UAM-20

exhibited severe symptoms of CLCuD such as severe vein thickening, reduced fruit production along with stunted growth, leaf Curling,Vein darkening, vein enlargement, and either upward or downward leaf curling are all symptoms of the condition. In Pakistan, most of the cotton genetic resources are susceptible to CLCuD especially cultivated varieties. However, it has been observed that the susceptibility level varies among genotypes (Akhtar et al., 2002; Mahmood, 2004). The level of susceptibility varies in terms of infection, symptoms development, delay in symptoms development, and plant recovery.

The candidate gene sequence have polymorphism and polymorphic sites information provided from the GWAS and might be linked with CLCuD. Studied gene encodes heparanase like protein which positively play a significantly role in viral infections (Koganti et al., 2020). The SNP-induced mutation at position 1528 bp in the 5th exon of the candidate gene replaces a leucine codon (CTA) with an isoleucine codon (ATA). This change alters the amino acid sequence from leucine to isoleucine. The substitution involves hydrophobic amino acids, potentially impacting local protein folding and interactions. Consequently, the mutation may affect binding, stability, enzymatic activity, and cellular localization, contingent upon the protein's function and context. Experimental analysis is crucial to ascertain the precise implications for the protein's structure and function. Molecular marker has been developed to perform genotyping diverse panel of Pakistani cotton genotypes. Extracted DNA from forty cotton genotypes and amplified gene of interest by utilizing PCR technique. Next step is to application of restriction enzymes to determine sequence difference among cotton genotypes.

A 70 K SNP chip has just been created through an international joint effort using the Illumina Infinium genotyping assay. Public and private breeders, geneticists, and other scientists from across the world will be capable to utilize this high-throughput genotyping test to improve cotton genetic studies, breeding, genome sequence assembly, and a variety of other purposes (Malik et al., 2014). Advancements in next generation sequencing (NGS) and RNA-seq technologies have led to the identification of single nucleotide polymorphisms (SNPs) as valuable and highly informative markers for genetic studies in cotton. These technologies have enabled the generation of large-scale sequence data with high throughput, allowing for the identification and analysis of SNPs across the cotton genome. This has greatly enhanced the ability to investigate the genetic diversity, population structure, and trait associations in cotton, providing valuable insights into the genetic basis of important traits and facilitating breeding efforts in this economically significant crop. The combination of SNP marker insights into breeding programs holds the potential to accelerate the creation of robust cotton cultivars, marking a significant stride toward sustainable and effective disease management strategies.

Conclusion

In conclusion, the challenges posed by Cotton Leaf Curl Disease (CLCuD) in cotton production of Pakistan have

prompted rigorous efforts to develop resilient cotton genotypes. The development of molecular markers to identify CLCuD-resistant genotypes represents a pivotal advancement in our ability to combat this disease. Genetic diversity emerges as a cornerstone solution, offering a multi-faceted approach to enhance cotton resistance. By melding precise phenotypic assessments with genotyping techniques, researchers can discern and propagate cotton lines exhibiting robust genetic diversity and resistance traits.

Furthermore, the integration of Next Generation Sequencing (NGS) and high-throughput approaches introduces a new dimension to the battle against CLCuD. NGS provides unprecedented insight into the cotton genome, enabling comprehensive analysis of genetic variations linked to resistance. High-throughput methodologies expedite the evaluation of numerous cotton genotypes, accelerating the identification of potential resistant lines. These cutting-edge tools not only underscore our commitment to addressing CLCuD but also hold promise for a more resilient and sustainable future of cotton production in Pakistan. Through strategic amalgamation of these approaches, we are poised to usher in a new era of disease-resistant cotton cultivation, safeguarding the agricultural backbone of the nation

CRediT authorship contribution statement

Conceptualization by Z.Z and Z. K; Data curation by Z.Z; Analysis by Z.Z, Z.K, Original draft written by Z.Z; Reviewed by Z.K, S.I, U.R.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

The authors are thankful to the Director of University Farms, Dr. Abdul Ghaffar and Deputy Director of University Farms, Mr. Mahmood Alam Khan, MNS University of Agriculture, Multan, for assisting in field experimentation.

Funding

This experiment was financially assisted by the “LO21002838-National Crop Genomics and Speed Breeding center For Agriculture Sustainability - ADP-2021-22 to 2023-24”.

Reference

Briddon, R. W., S. Mansoor, I. D. Bedford, M. S. Pinner, K. Saunders, J. Stanley, Y. Zafar, A. K. Malik, and P. G. Markham. 2001. Identification of DNA Components Required for Induction of Cotton Leaf Curl Disease. Virol. 285(2): 234-243.

Bleeker, P. M., P. J. Diergaarde, K. Ament, S. Schütz, B. Johne, J. Dijkink, and R. C. Schuurink. 2011. Tomato-produced 7-epizingiberene and R-curcumene act as repellents to whiteflies. Phytochemistry. 72(1): 68-73.

Chohan, S., R. Perveen, M. Abid, M. N. Tahir, and M. Sajid. 2020. Cotton diseases and their management. Cotton Production and Uses: Agronomy, Crop Protection, and Postharvest Technologies. 239-270.

Dahab, A. A., M. Saeed, B. B. Mohamed, M. A. Ashraf, A. N. Puspito, K. S. B. A. A. Shahid, and T. Husnain. 2013. Genetic diversity assessment of cotton (Gossypium hirsutum L.) genotypes from Pakistan using simple sequence repeat markers. Aust J Crop Sci. 7(2): 261-267.

Farooq, A., J. Farooq, A. Mahmood, A. Shakeel, K. A. Rehman, A. Batool, and S. Mehboob. 2011. An overview of cotton leaf curl virus disease (CLCuD) a serious threat to cotton productivity. Aust J Crop Sci. 5(13): 1823-1831.

Hulse-Kemp, A. M., J. Lemm, J. Plieske, H. Ashrafi, R. Buyyarapu, D. D. Fang, and D. M. Stelly. 2015. Development of a 63K SNP array for cotton and high-density mapping of intraspecific and interspecific populations of Gossypium spp. G3-GENES GENOM GENET. 5(6): 1187-1209.

Knight, I. A., G. C. Rains, A. K. Culbreath, and M. D. Toews. 2017. Thrips counts and disease incidence in response to reflective particle films and conservation tillage in cotton and peanut cropping systems. Entomol. Exp. Appl. 162(1): 19-29.

Koganti, R., R. Suryawanshi, and D. Shukla. 2020. Heparanase, cell signaling, and viral infections. Cell. Mol. Life Sci. 77: 5059-5077.

Malik, W., J. Ashraf, M. Z. Iqbal, A. Ali Khan, A. Qayyum, M. Ali Abid and G. Hasan Abbasi. 2014. Molecular markers and cotton genetic improvement: current status and future prospects. Sci. World J. 3: 14-20.

Nour, M. A., and J. J. Nour. 1964. Identification, transmission and host range of leaf curl viruses infecting cotton in the Sudan. Emp. Cott. Grow. Rev. 41: 27–37.

Powell, M. E., A. G. Cuthbertson, H. A. Bell, N. Boonham, J. Morris, and P. Northing. 2012. First record of the Q Biotype of the sweetpotato whitefly, Bemisia tabaci, intercepted in the UK. Eur. J. Plant Pathol. 133: 797-801.

Qayyum, A. 2009. Diffusion-weighted imaging in the abdomen and pelvis: concepts and applications. Radiographics. 29(6): 1797-1810.

Shahin, A., P. Arens, S. Van Heusden, and J. M. Van Tuyl. 2009. Conversion of molecular markers linked to fusarium and virus resistance in asiatic lily hybrids. Acta Hortic. 836: 131–136.

Tarazi, R., J. L. S. Jimenez, and M. F. Vaslin. 2019. Biotechnological solutions for major cotton (Gossypium hirsutum) pathogens and pests. J. innov. res. 3: 19-26.

Thyssen, G. N., J. N. Jenkins, J. C. McCarty, L. Zeng, B. T. Campbell, C. D. Delhom, M. S. Islam, P. Li, D. C. Jones and B. D. Condon. 2019. Whole genome sequencing of a MAGIC population identified

genomic loci and candidate genes for major fiber quality traits in upland cotton (Gossypium hirsutum L.). Theor. Appl. Genet. 132: 989-999.

Wendel, J., and R. Cronn. 2003. Polyploidy and the evolutionary history of cotton.

Yuan, L., S. Wang, J. Zhou, Y. Du, Y. Zhang, and J. Wang. 2012. Status of insecticide resistance and associated mutations in Q-biotype of whitefly, Bemisia tabaci, from eastern China. Crop Prot. 31(1): 67-71.

ANATOMICAL BREEDING FOR ALTERED LEAF PARAMETERS IN EGYPTIAN COTTON (*Gossypium barbadense* L.) TO IMPART SUCKING PEST RESISTANCE

Alagarsamy Manivannan*, Thomas Cheeran Amal, and Karuppan Shankarganesh

ICAR-Central Institute for Cotton Research, Regional Station, Coimbatore, India 641003

***Corresponding authors email: manivannan461@gmail.com**

ABSTRACT:

The significance of morphological and anatomical resistance from wild species into cultivated cotton for host plant resistance is highly important. This experiment was conducted to introgress the leaf anatomical feature from wild species (*G. armourianum*) to cultivated Egyptian cotton (*G. babadense*). The interspecific hybrid F_1 exhibited intermediate expression in terms of plant growth habit, leaf size, petal colour and petal spot. Comparing the leaf sizes, *G. armourianum* was exhibited a thicker leaf (256.5 µm) compared to *G. barbadense* (178.2 µm), while the hybrid showed significant expansion in leaf thickness (198.4 µm). The phloem bundle in *G. barbadense* appeared loosely packed (215.1µm), whereas it was more compact in *G. armourianum* (95.6µm). The F_1 hybrid exhibited an intermediate range for phloem thickness (154.1µm). This study elucidated the role of leaf anatomical features for improving sucking pest resistance in *G. barbadense* using *G. armourianum* by introgression.

Keywords: *Gossypium barbadense*, interspecific hybrid, anatomical breeding, leaf traits, introgression.

Introduction

Among the new world tetraploid cotton *Gossypium barbadense* commonly known as Egyptian cotton or Sea Island cotton plays a key role for its hallmark fibre properties. Extraordinary fibre properties of *G. barbadense* which fetched the term Extra Long Staple (ELS) cotton, as per industry standard it should possess the minimum fibre length of is 1-3/8" or 34.925 mm. *G. barbadense* is grown in about 10% of the cotton area and supports about 3% of the world production

(Alagarsamy 2023). The major ELS producing countries are USA, Egypt and Sudan. The quantum jump in cotton production has helped the textile industry to get cotton at a competitive price and also the farming community to get higher income. ELS production is primarily concentrated in 7 countries namely USSR, India, Egypt, Sudan, the United States, Peru, and Israel. These countries produce more than 95 per cent of the world's ELS cotton.

G. barbadense is sensitive plant that produces yellow flowers with thick maroon petal spot at the base of the petal and has black seeds. Heliotrophic response is weak or almost absent in *G. barbadense*, likewise, the day neutral flowering response is simply inherited trait in *G. barbadense* (Lewis and Richmond 1960). All the intrinsic traits of *G. barbadense* limited its spread to a few environmental niches all over the world. Long duration of growth, high level of sensitivity to suboptimum growth conditions, lower yield all these factors are major impediments for high quality of *G. barbadense* cotton. Since increase in demand and premium prices of ELS cotton warrants need to breed for high yielding *G. barbadense* genotypes. Grand luxurious vegetative phase of the *G. barbadense* attracts lots of pest and disease problems. After introduction of *Bt* cotton in India; incidences of sucking pests were being recorded at regular intervals in spite of the use of insecticides (Nagrare *et al*. 2014; Rajendran *et al*. 2018; Alagarsamy *et al*. 2024; Manivannan *et al.* 2025). Breeding for sucking pest tolerant cultivars in *G. barbadense* is in higher priority to increase the yield.

Among the wild species, *G. armourianum* is known for its sucking pest tolerance due to unique leaf anatomy features and cellular arrangement. However, in *G. barbadense* has thin and broad leaf which makes susceptible to sap feeding insects. Leaf anatomical features were found to be stable over the seasons in cotton (Rajendran 2004). Ansingkar *et al*. (2004) improved the anatomical features in derivatives of trispecies hybrid (*G. hirsutum* × *G. barbadense* × *G. arboreum*) and observed compact and long parenchyma, as they transferred the compactness of phloem elements from *G. arboreum* to H × B hybrid.

An attempt has been made to introgress these anatomical parameters into tetraploid *G. barbadense* to impart resistance to sucking pest from diploid *G. armourianum*. This study was made to compare gross leaf anatomy between *G. barbadense*, *G. armourianum* and interspecific hybrids (F_1).

Materials and Methods

Morphometric measurement of plant characters

The interspecific hybrid between *G. babrbadense* (CCB 129) x *G. armourianum* was developed during 2022 using CCB 129 as the female parent whereas *G. armourianum* was male parent (Fig. 1). No fertilization barriers between these *Gossypium* species were encountered in this direction and the F_1 seeds were obtained with ease through direct crossing in field at ICAR- Central Institute for Cotton Research, Regional Station, Coimbatore, India. F_1 hybrid along with parents was used for recording data on various morphological parameters and molecular analysis. The morphological

characters studied namely plant growth habit, stem hairiness, stem color, leaf shape, leaf lobe, leaf pubescence, bract, leaf size (average) (maximum width) (cm), sepals, petal color, petal spot, filament color, pollen color, boll shape, pollen size and pollen shape. Z-test was performed to test the significance in average pollen size (µm) and fertility (%) (Panse and Sukhatme 1995). Same way leaf size for each parent and hybrid were subjected to Student's t-test to analyze the differences among them.

Anatomical Measurement of leaf features

Five fully expanded uniformly sized leaves per accession of all lines were collected from peak blooming stage from field. Anatomical measurements namely leaf area, palisade cell height, thickness of phloem and distance of phloem from lower epidermis observations were recorded (10X) (Fig. 2) and group mean was analyzed using "t" test.

Results and Discussion

Cotton belongs to C_3 photosynthesis system which has typical leaf development pattern and anatomical structure as of mesophyte (Esau 1965). Mesophyll layer of cells are differentially packed inside the leaf, in adaxial side (upper) is densely packed with palisade mesophyll cells and in abaxial side is loosely packed with spongy mesophyll cells. These structural differences attribute for the main difference physiological and anatomical role (Vogelmann *et al*. 1996). Upper side palisade parenchyma has more of chlorophyll than the lower spongy parenchyma.

Plant morphological characters of parents *G. barbadense* (CCB 129), *G. armourianum* and their hybrid F_1 were compared (Table 1). Interspecific F_1 hybrid showed either dominance or intermediate expression for various morphological traits are in agreement with Pushpam and Raveendran (2006). The growth habit, leaf shape and size, petal color and petal spot of hybrid were found to be intermediate. Leaf hairiness and stem hairiness was absent in hybrid which is considered to be transferred from male parent (*G. armourianum*). Different traits showed different level of dominance in the hybrid compared with their parents. Same trend was reported in wide cross studies in cotton (Tahir *et al*. 2011; Liu *et al*. 2015). Pollen fertility rate was low in F_1 (2.01%) as compared to the parents because of triploid (2n=4x=39) nature of the cross as *G. barbadense* is a tetraploid (2n=4x=52) and *G. armourianum* is diploid (2n=2x=26). Size of the pollen grain was shriveled in F_1 (64.82 µm) compared to parents.

Physiological and anatomical traits contribute to the limitation of productivity in *G. barbadense*. In general, *G. barbadense* leaves are thinner compared to other gossypium species. Leaf anatomical parameters namely distance of phloem from lower epidermis, palisade cell height, thickness of phloem and leaf thickness, which are the major indicator of tolerance against leaf hopper was measured (Table 2). As of in our studies, *G. armourianum* was thicker (256.5 µm) compared to *G. barbadense* (178.2 µm). But the cross between both species showed considerable improvement in leaf thickness (198.4 µm). Palisade cell was thin in *G. barbadense* (74.3 µm) and thicker in *G. armourianum*

(89.1 µm). The size of palisade got increased in the F_1 (81.2 µm) which would be desirable traits for sucking pest tolerance. Bhatt and Andal (1979) reported the spongy parenchyma of *G. barbadense* was thicker than the palisade; they were the undesirable attribute of *G. barbadense* which makes them susceptible for pests as well as moisture deficit.

Sucking pests have wide range of feeding mechanism as per their mouth part types. Leafhoppers employs different pattern to feed the saps, either by making contiguous, repeated, short duration probes in vascular bundles or long duration of fewer probes in mesophyl parenchyma cells (Backus *et al*. 2005; Alagarsamy *et al.* 2024). Phloem is the vascular tissue which is responsible for transport and distribution of sugars produced by the photosynthesis. Majority of the sucking pest complexes are sap phloem feeders. With help of stylet, insects pierce the mesophyll tissues and reach the phloem to suck the sap. When the phloem elements are compact the chances of reaching stylet to the phloem cells are less. Large size of phloem elements recorded in *G. barbadense* (215.1 µm). In contrast the phloem was compact in *G. armourianum* (95.6 µm) and the F_1 hybrid had shown an intermediate range (154.1 µm) between both parents. Distance between lower epidermis and phloem is considered as critical for imparting resistance to sucking pests. Since, the stylet of the phloem feeders has to deeply enter to reach the phloem to feed the sap. More the distance, stylet penetration to reach the phloem is considered as difficult, this genotype generally shows resistance for insects. As in *G. armourianum* possess longer distance (261.1 µm) compared to *G. barbadense* (144.5 µm), however the hybrid was intermediate (201.7 µm). Sucking pest tolerance lines shown higher distance of lower epidermis to the phloem when compare to susceptible parental line (Harijan *et al*. 2017).

This study elucidated the importance of leaf anatomical features for sucking pest resistance. Thickness of leaf, length of palisade, phloem compactness and phloem element size were play key role in resistance. Hybrid (F_1) showed considerable improvement in anatomical features in comparison with *G. barbadense*. Derivatives of these hybrids in future could be screened for improved leaf traits. This study will enable breeders to use *G. armourianum* as source for sucking pest resistance and insight for breeding anatomical traits.

Acknowledgments

The authors acknowledge the support from, ICAR-Central Institute for Cotton Research (ICAR-CICR), RS, Coimabtore for conducting this research work.

Table 1 Morphological characters among parents and F_1 hybrid

Plant character	*G. barbadense*	*G. armourianum*	F_1
Plat growth habit	Errect	Spreading	Errect and Semi-spreading
Stem hairiness	Present	Absent	Absent
Stem color	Red	Reddish green	Reddish green
Leaf shape	Palmate	Cordate	Palmate
Leaf lobe	5	-	2-3

Plant character	*G. barbadense*	*G. armourianum*	F_1
Leaf pubescence	Present	Absent	Absent
Bract	Cordate with serrations	Caducous bracts	Cordate with serrations
Leaf size (average) (cm)	18.5 (6.5-8.4)	4.1 (6.5-9.7)	9.3 (8.5-12.2)
Sepals	5	5	5
Petal color	Yellow	Yellow	Yellow
Petal spot	Dark maroon spot	Medium red spot	Variable intensity of spot
Filament color	Yellow	Colorless	Variable expression
Pollen color	Yellow	Yellow	Yellow
Boll shape	Elliptical with pointed tip (thorny)	Oval with pointed tip	Oval with pointed tip
Pollen fertility (%)	82.3	94.2	2.01
Pollen size (µm)	135.10 (101.2-140.5)	105.2 (71.3-110.5)	64.8 (22.2-105.1)

Table 2 Leaf anatomical parameters among parents and F_1 hybrid

Leaf anatomical traits	*G. barbadense*	*G. armourianum*	F_1	P
Thickness of leaf (mm)	178.2 ± 50.2	256.5 ± 54.8	198.4 ± 45.5	<0.01
Palisade cell length (µm)	74.3 ± 21.3	89.1 ± 16.8	81.2 ± 12.5	<0.01
Thickness of phloem (µm)	215.1 ± 51.2	95.6 ± 28.2	154.1 ± 84.2	<0.01
Distance of phloem from lower epidermis (µm)	144.5 ± 25.1	261.1 ± 49.5	201.7 ±39.6	<0.01

Mean ± S.D. Statistical differences between means (P) were determined using a Student's t-test.



Fig. 1. Plant stature of (**a**) *Gossypium barbadense* (**b**) *Gossypium armourianum* (**c**) Interspecific hybrid F_1 (*Gossypium barbadense* × *Gossypium armourianum*)

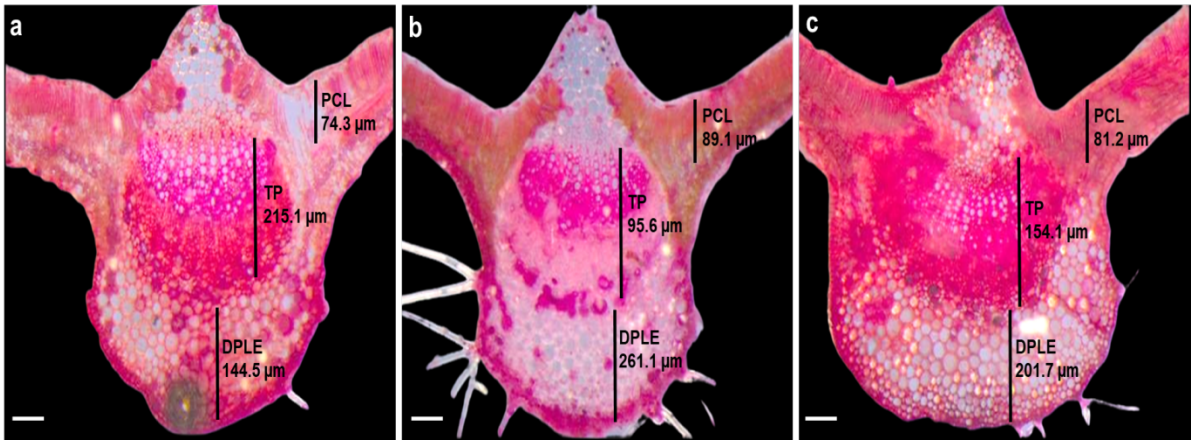


Fig. 2. Leaf anatomical cross section of (**a**) *Gossypium barbadense* (**b**) *Gossypium armourianum* (**c**) Interspecific hybrid F_1 (*Gossypium barbadense* × *Gossypium armourianum*) PCL: Palisade cell length; TP: Thickness of phloem; DPLE: Distance of phloem from lower epidermis

References

Alagarsamy, M., 2023. Assessing genetic variation in *Gossypium barbadense* L. germplasm based on fibre characters. *Journal of Cotton Research*, **6**: 15.

Alagarsamy, M., Amal, T.C., Karuppan, S., Adhimoolam, K., 2024. Comparative proteomic analysis of resistant and susceptible cotton genotypes in response to leaf hopper infestation. *Journal of Proteomics*, **305**: 105258.

Alagarsamy, M., Karuppan, S., Amal, T.C., Markkandan, K., 2024. Sequencing and analysis of the complete mitochondrial genome of *Amrasca biguttula biguttula* Ishida, 1913 (Hemiptera: Cicadellidae: Typhlocybinae : mpoascini). *Mitochondrial DNA Part B*, **9**: 725-728.

Ansingkar, A.S., Khade, P.P., Borikar, S.T., and Bhosale, S.S. 2004. Altering *G. hirsutum* cotton at cellular level to impart multiple sucking pest resistance through interspecific hybridization. In: Proceedings of International Symposium on Strategies for Sustainable Cotton Production - A Global Vision, Nov. 23-25, 2004, University of Agricultural Sciences, Dharwad.

Backus, E.A., Serrano, M.S., and Ranger, C.M. 2005. Mechanisms of hopperburn: an overview of insect taxonomy, behavior and physiology. *Annual Review of Entomology*, **50**: 125-151.

Bhatt, J.G. and Andal, R. 1979. Variation in foliar anatomy of cotton. *Proceedings of the Indian Academy of Sciences*, **8**: 451-453.

Esau, K. (1965). Plant anatomy, John Wiley and Sons, New York.

Harijan, Y., Nishanth, G.K., Katageri, I.S., Khadi, B.M. and Patil, B.R. 2017. Biochemical and Anatomical Factors Imparting Resistance against Sucking Pests in Cotton. *International Journal of Current Microbiology and Applied* Sciences, **6**: 2542-2553.

Lewis, C.F. and Richmond, T.R .1960. The genetics of flowering response in cotton. II. Inheritance of flowering response in a *Gossypium barbadense* cross. *Genetics*, **45**: 79.

Liu, Q., Chen, Y., Chen, Y., Wang, W., Chen, J., Zhang, T., and Zhou, B. 2015. A new synthetic allotetraploid (A1A1G1G1) between *Gossypium herbaceum* and *Gossypium australe*: Bridging for simultaneously transferring favourable genes from these two diploid species into upland cotton. *Plos One*, **10**: e0123209.

Manivannan, A., Amal, T.C., and Shanmugam, A.2025. Evaluation of genetic diversity of cotton genotypes using morphological and molecular markers and evaluation for leaf hopper resistance, *South African Journal of Botany*, **179:**206-215.

Nagrare, V., Deshmukh, A., and Bisane, K. 2014. Relative performance of Bt-Cotton hybrids against sucking pests and leaf reddening under rainfed farming. *Entomology, Ornithology & Herpetology: Current Research*, **3**:1-6.

Panse, V.G. and Sukhatme, P.V. 1995. Statistical Methods for Agricultural Workers. ICAR, New Delhi.

Pushpam, R. and Raveendran, T.S. 2006. Production of interspecific hybrids between *Gossypium hirsutum* and jassid resistant wild species *G. raimondii* and *G. armourianum*. *Cytologia*, **71**: 407-418.

Rajendran, T., Birah, A., and Burange, P.S. 2018. Insect pests of cotton. In: Omkar (Eds.), Pests and Their Management. Springer Publication, Singapore. Pp 361–411.

Rajendran, T.P. 2004. Challenges for management of sap sucking pests in cotton. In: Proceedings of International Symposium on Strategies for Sustainable Cotton Production - A Global Vision, Nov. 23-25, 2004, University of Agricultural Sciences, Dharwad.

Tahir, M.S., Khan, N.U.I., and Rehman, S.U. 2011. Development of an interspecific hybrid (triploid) by crossing *Gossypium hirsutum* and *G. arboreum*. *Cytologia*, **76**:193-199.

Vogelmann, T.C., Nishio, J., and Smith, W.K. 1996. Leaves and light capture: light propagation and gradients of carbon fixation within leaves. *Trends in Plant Science*, **1**: 65-70.

DEVELOPMENT AND OPTIMIZATION OF A SYNERGISTIC KAIROMONE BLEND (Patent No: 563899) FOR THE ATTRACTION OF COTTON STEM WEEVIL (*Pempherulus affinis*)

K. Senguttuvan*[1], M. Shanthi[2], M. Murugan[3]

[1] **Assistant Professor (Entomology), Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, India**
[2] **Director, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore, India**
[3] **Professor and Head, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, India**
* corresponding author : senguttuvan.k@tnau.ac.in

ABSTRACT:

The cotton stem weevil (*Pempherulus affinis*) is a major pest in cotton cultivation, causing significant yield loss by boring into plant stems. Traditional chemical-based control strategies pose environmental risks and often lead to pesticide resistance. In this study, a novel synergistic kairomone blend (Patent No: 563899) was developed and optimized to attract adult weevils using a mixture of three key compounds: Compound A (60%), Compound B (32%), and Compound C (8%). The blend was tested in liquid formulation for its efficacy under laboratory (olfactometer assays) and field conditions. Laboratory results demonstrated significantly higher attraction of adults toward the blend compared to control, indicating a synergistic effect. Field trials using the liquid formulation confirmed strong attraction during the first three days, although declining thereafter, suggesting the need for formulation enhancements for prolonged activity. The study supports the use of kairomone-based strategies for eco-friendly monitoring and potential mass-trapping of *P. affinis*, contributing to sustainable cotton pest management.

Keywords: *Kairomone blend, Pempherulus affinis, cotton stem weevil, pest management, olfactometer bioassay.*

Introduction

The cotton stem weevil (*Pempherulus affinis*) has become a serious pest in Indian cotton cultivation, particularly in Tamil Nadu and other southern states. This insect infests cotton plants by boring into the stem, disrupting vascular tissues, and inducing gall formation, which severely

hampers plant growth and yield. Since its initial detection in Tamil Nadu, the pest has spread across multiple states and neighboring regions, prompting extensive surveys between 2015 and 2023 to assess its distribution, biology, and host range.

Conventional control measures, largely reliant on chemical insecticides, face several challenges, including environmental concerns, non-target effects, and the potential for resistance development. As a sustainable alternative, kairomone-based strategies have gained prominence. Kairomones—semiochemicals that attract insects by mimicking host-related cues—offer promise for targeted pest monitoring and mass trapping. Recent advancements in kairomone blend formulations, particularly when combined with trap systems such as Delta traps, present an effective and environmentally friendly approach to managing *P. affinis* populations.

Aim of the Study

This study aims to develop a novel, synergistic kairomone blend formulated in liquid forms to attraction of adult *Pempherulus affinis*. The goal is to improve field-level efficacy of pest monitoring and control, contributing to the development of an eco-friendly, biorational management strategy for the cotton stem weevil.

Review of Literature

The cotton stem weevil (*Pempherulus affinis*) has emerged as a significant pest in cotton-growing regions, causing substantial yield losses by boring into plant stems and disrupting vascular function. Traditional chemical control measures, though initially effective, have led to growing concerns regarding environmental pollution, non-target effects, and the evolution of pesticide-resistant weevil populations (Singh et al., 2017). These limitations have encouraged researchers to explore more sustainable and ecologically compatible pest management strategies.

One promising avenue involves the use of kairomones, a class of semiochemicals that mediate interspecific communication and benefit the receiving organism—in this case, the pest. Kairomones mimic natural plant volatiles that signal the presence of a suitable host, making them ideal candidates for pest attraction and monitoring tools (Bruce et al., 2005; Pickett & Khan, 2016).

Previous studies have shown that volatiles such as green leaf volatiles (GLVs) and floral compounds emitted by stressed or damaged cotton plants can strongly attract *P. affinis* and related stem-boring weevils. These compounds, typically comprising alcohols, aldehydes, and esters, have been identified through GC-MS (Gas Chromatography-Mass Spectrometry) and GC-EAD (Gas Chromatography-Electroantennographic Detection) analyses (Gupta & Lal, 2020). Synthetic analogs of these volatiles have also been evaluated for their potential in monitoring systems, though individual compounds often produce limited responses due to lack of specificity or volatility mismatch.

The concept of synergy among kairomones has gained traction in recent years, with multiple studies demonstrating that blends of specific volatiles can elicit

stronger behavioral responses than single compounds (Tasin et al., 2011). This synergistic phenomenon is attributed to the complex olfactory coding in insects, where combinations of volatiles more accurately mimic natural host signals, thus triggering heightened behavioral attraction.

Despite this knowledge, few studies have specifically addressed *P. affinis*, and no commercially available kairomone blend exists for its control. Most related research has focused on major cotton pests like *Helicoverpa armigera* or *Spodoptera litura*, leaving a critical knowledge gap for stem weevil management. Furthermore, the development of liquid formulations for kairomone delivery is still at a nascent stage, though recent advances in polymer matrices and slow-release technologies suggest considerable potential (Ramani et al., 2021).

This study builds upon these foundations by identifying key volatiles attractive to *P. affinis*, optimizing their synergistic ratios through dose-response and combinatorial bioassays, and incorporating them into formulated delivery systems. This research not only contributes to the eco-friendly management of *P. affinis* but also serves as a model for future semiochemical-based pest control programs.

Materials and Methods

1. Insect Rearing

Adult *Pempherulus affinis* specimens were collected from infested cotton fields in [Location, e.g., Tamil Nadu, India] and reared under controlled laboratory conditions (27 ± 2°C, 70 ± 5% RH, 14:10 L:D photoperiod). The insects were maintained on excised cotton stems placed in ventilated containers. Only healthy, 5–7-day-old unmated adults were used for olfactory and field experiments.

2. Kairomone Selection and Procurement

Three key kairomone components (Compound A, B, and C) were selected based on prior electrophysiological and behavioural studies. Compound A is a green leaf volatile mimicking plant stress cues, Compound B is a floral volatile enhancing oviposition attraction, and Compound C is a synthetic analog of a weevil-attracting pheromone. These compounds (with ≥97% purity) were procured from a certified semiochemical supplier and verified using Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

3. Blend Formulation (Liquid)

Initial blend ratios were determined through literature-supported combinations and previous studies on insect olfactory responses (Tasin et al., 2011; Bruce et al., 2005). The final optimized ratio was determined as:

- **Compound A** – 60% (Range: 48–72%)
- **Compound B** – 32% (Range: 25.6–38.4%)
- **Compound C** – 8% (Range: 6.4–9.6%)

4. Laboratory Olfactometer Assays

Dual-choice Y-tube olfactometer assays were used to evaluate behavioural responses of adult *Pempherulus affinis* to different blend ratios. In this study, a Y-tube olfactometer was used to evaluate the efficacy of a kairomone blend in attracting adult cotton stem weevils. The olfactometer consisted of a Y-shaped glass

tube with two arms, one containing a kairomone-impregnated septa and the other a blank control septum. Airflow through both arms was regulated at a constant rate (200–500 mL/min) via an air pump, with activated charcoal filters and humidifiers ensuring clean, humidified air. Adult weevils, starved for 2 days prior to the experiment, were introduced individually at the base of the Y-tube and given 1 minute to choose between the kairomone or control arm. Data were analyzed using a chi-square test to assess the significance of weevil attraction to the kairomone.

5. Field Evaluation

Field trials were conducted in a randomized block design (RBD) in cotton-growing areas of Cotton Farm, Tamil Nadu Agricultural University, Coimbatore with 3 replications per treatment. Treatments included: Optimized kairomone blend (liquid), Individual kairomones and Untreated controls

Sticky traps (delta) with liquid blend formulation were installed at 1.2 m above ground level. Traps were monitored weekly, and the number of *P. affinis* adults captured was recorded.

6. Data Analysis

All behavioral data were analyzed using Friedman Test (Non-parametric repeated measures Test) for mean separation at $p < 0.05$. Synergistic effects were calculated by comparing observed attraction in the blend versus the additive response of individual kairomones. Field data were transformed where necessary to meet assumptions of normality.

Result and Conclusion

Lab Study

To evaluate the behavioural attraction of *Pempherulus affinis* to the liquid formulation of the newly developed synergistic kairomone blend, a Y-tube olfactometer assay was conducted. The results from ten replicates (Table 1) revealed a consistent and significant preference for the test chemical over the control.

Table 1. Olfactometer study with Liquid formulation for Cotton Stem Weevil (*Pempherulus affinis*)

S.No.	Test	No. of Adults Released	Test Chemical		Control (♂)	
			(♂)	(♀)	(♂)	(♀)
1	Test 1	10♂ + 10♀	6	7	3	3
2	Test 2	10♂ + 10♀	8	9	2	0
3	Test 3	10♂ + 10♀	9	7	0	3
4	Test 4	10♂ + 10♀	6	8	2	2
5	Test 5	10♂ + 10♀	7	7	0	3
6	Test 6	10♂ + 10♀	8	6	2	0
7	Test 7	10♂ + 10♀	6	7	1	2
8	Test 8	10♂ + 10♀	9	9	1	0
9	Test 9	10♂ + 10♀	8	8	1	0
10	Test 10	10♂ + 10♀	7	6	0	1

Across the ten olfactometer assays: The average number of males responding to the test chemical was 7.4, while only 1.2 males responded to the control. The average number of females attracted to the test chemical was 7.4, compared to 1.4 females for the control.

These results demonstrate a significantly higher attraction of both male and female weevils to the kairomone blend compared to the control arm (solvent only). The consistent preference indicates the effectiveness of the kairomone blend in mimicking host plant volatiles and inducing behavioural attraction in *P. affinis*.

Moreover, the response pattern also suggests no sex-specific bias, as both male and female weevils were equally responsive to the blend. This broad-spectrum attraction is advantageous in pest management strategies, especially for mass trapping and population monitoring.

The observed synergistic response supports the hypothesis that the optimized kairomone blend has higher efficacy than individual components, validating its potential as a reliable tool for integrated pest management (IPM) in cotton ecosystems.

Field study

This study assessed the effectiveness of liquid formulation a blended kairomone attractant on cotton stem weevils in field conditions. Field studies revealed that initially attracted comparable numbers of weevils (8–13 males, 7–14 females). the liquid formulation's effectiveness declined rapidly within five days. Results indicate that the liquid formulation not only attracted more weevils but also maintained attraction over a longer duration, making it superior for field applications against cotton stem weevils (Table 2).

Table 2. Cotton Stem Weevil (*Pempherulus affinis*) attraction by Liquid formulation

S.No.	Trap	1st Day		3rd Day		5th Day		7th Day	
		(♂)	(♀)	(♂)	(♀)	(♂)	(♀)	(♂)	(♀)
1	Trap 1	9	13	7	6	0	1	0	0
2	Trap 2	12	8	8	5	1	1	0	0
3	Trap 3	13	12	12	6	0	1	0	0
4	Trap 4	9	11	7	7	0	0	0	0
5	Trap 5	8	10	6	7	0	1	0	0
6	Trap 6	12	7	8	5	1	2	0	0
7	Trap 7	13	9	8	6	0	0	0	0
8	Trap 8	9	14	6	7	0	0	0	0
9	Trap 9	12	10	9	7	2	0	0	0
10	**Trap 10**	**8**	**11**	**7**	**7**	**1**	**0**	**0**	**0**

The field results demonstrate a strong and immediate attraction of *P. affinis* to the liquid kairomone formulation: Peak activity was observed on Day 1 and Day 3, with capture numbers declining rapidly by the 5th and 7th days. On Day 1, the average catch per trap was: 10.5 males and 10.5 females, suggesting high responsiveness across sexes. On Day 3, the average catch dropped to: 7.8 males and 6.3 females, still indicating significant attraction. By Day 5, catches declined sharply to: 0.6 males and 0.7 females. On Day 7, no insects were caught in any trap, suggesting exhaustion of the volatile release or field saturation.

This temporal pattern indicates that the liquid formulation is highly effective during the initial 72 hours of deployment, after which its efficacy diminishes. This aligns with the expected volatility and release dynamics of the kairomone blend under field conditions.

- **Males:** χ^2=29.23, **p-value** = **1.99×10^{-6}**
- **Females:** χ^2=29.21, **p-value** =**2.02×10^{-6}**

Since the p-values are highly significant ($p<0.05p < 0.05p<0.05$), we conclude that the number of captured weevils varies significantly across different days.

The results also reinforce earlier olfactometer findings—no sex-specific preference was noted, as both males and females responded comparably to the kairomone stimulus (Fig 1).

Prior studies, such as those by Bruce et al. (2005), Dickens (2000), and Germinara et al. (2009), emphasized the utility of kairomones and host-derived volatiles in insect behavioral manipulation. However, most of these studies were limited to individual components or binary combinations. The current study goes a step further by demonstrating a synergistic three-component kairomone blend that not only increased attraction in laboratory assays but also performed well under real-world field conditions.

Furthermore, the ratio of 60% Compound A, 32% Compound B, and 8% Compound C falls within optimized thresholds reported for maximal insect response (Tasin et al., 2011; El-Sayed, 2020), affirming the blend's formulation based on semiochemical principles.

Conclusion:

The **synergistic kairomone blend (Patent No: 563899)** has demonstrated strong potential as an effective attractant for adult *Pempherulus affinis* in both laboratory and field environments. It offers a **non-toxic and eco-friendly alternative** to conventional insecticides, making it highly suitable for **monitoring and mass trapping** applications in cotton pest management. Although the liquid formulation showed reduced efficacy over time in field trials, this limitation underscores the need for **formulation improvements**, such as incorporating **slow-release additives or gel-based carriers** to prolong field activity.

References

Bruce, T. J. A., Wadhams, L. J., & Woodcock, C. M. (2005). Insect host location: A volatile situation. *Trends in Plant Science*, 10(6), 269–274. https://doi.org/10.1016/j.tplants.2005.04.003

Dickens, J. C. (2000). Orientation of Colorado potato beetle to natural and synthetic blends of volatiles emitted by potato plants. *Agricultural and Forest Entomology*, 2(3), 167–172.

El-Sayed, A. M. (2020). The Pherobase: Database of pheromones and semiochemicals. Retrieved from http://www.pherobase.com

Germinara, G. S., De Cristofaro, A., & Rotundo, G. (2009). Behavioral responses of *Sitophilus granarius* to individual cereal volatiles. *Journal of Chemical Ecology*, 35(3), 306–315.

Gupta, A., & Lal, R. (2020). Identification of host plant volatiles for management of cotton pests using GC-MS and EAG techniques. *Journal of Insect Science*, 30(2), 189–196.

Pickett, J. A., & Khan, Z. R. (2016). Plant volatile-mediated signalling and its application in agriculture: successes and challenges. *New Phytologist*, 212(4), 856–870. https://doi.org/10.1111/nph.14274

Ramani, S., Murthy, K. S., & Patel, D. (2021). Advances in slow-release formulations for pest semiochemicals: Techniques and applications. *Pest Management Science*, 77(3), 1042–1050. https://doi.org/10.1002/ps.6091

Singh, A., Verma, R. K., & Sharma, R. (2017). Challenges in cotton pest management: The rise of secondary pests and resistance development. *Indian Journal of Entomology*, 79(3), 326–331.

Tasin, M., Knudsen, G. K., Pertot, I., & Witzgall, P. (2011). Synergism and redundancy in a plant volatile blend attracting grapevine moths. *Phytochemistry*, 72(9), 897–904. https://doi.org/10.1016/j.phytochem.2011.02.011

Tasin, M., Lucchi, A., Ioriatti, C., Mraihi, M., De Cristofaro, A., & Germinara, G. S. (2011). Electrophysiological responses of *Lobesia botrana* females to plant volatiles. *Bulletin of Insectology*, 64(1), 33–38.

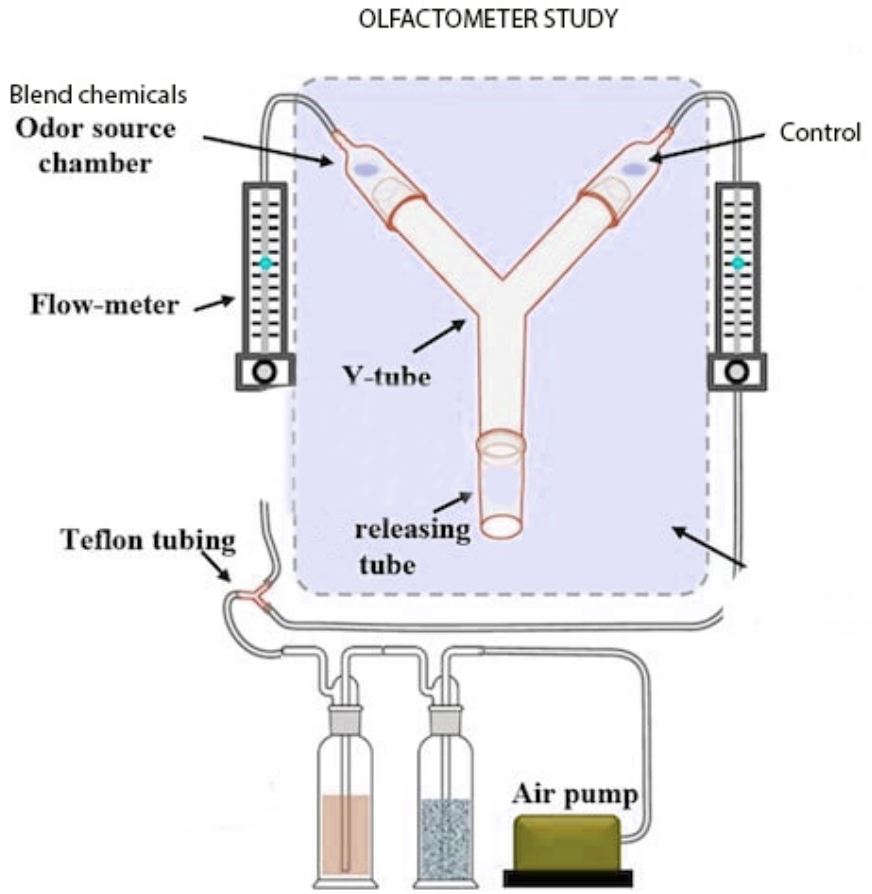


Plate 1. Efficacy test for cotton stem weevil for liquid blend kairomone



Plate 2. Cotton Stem Weevil adults – Trap catches in field efficacy



Plate 3. Cotton Stem Weevil adult

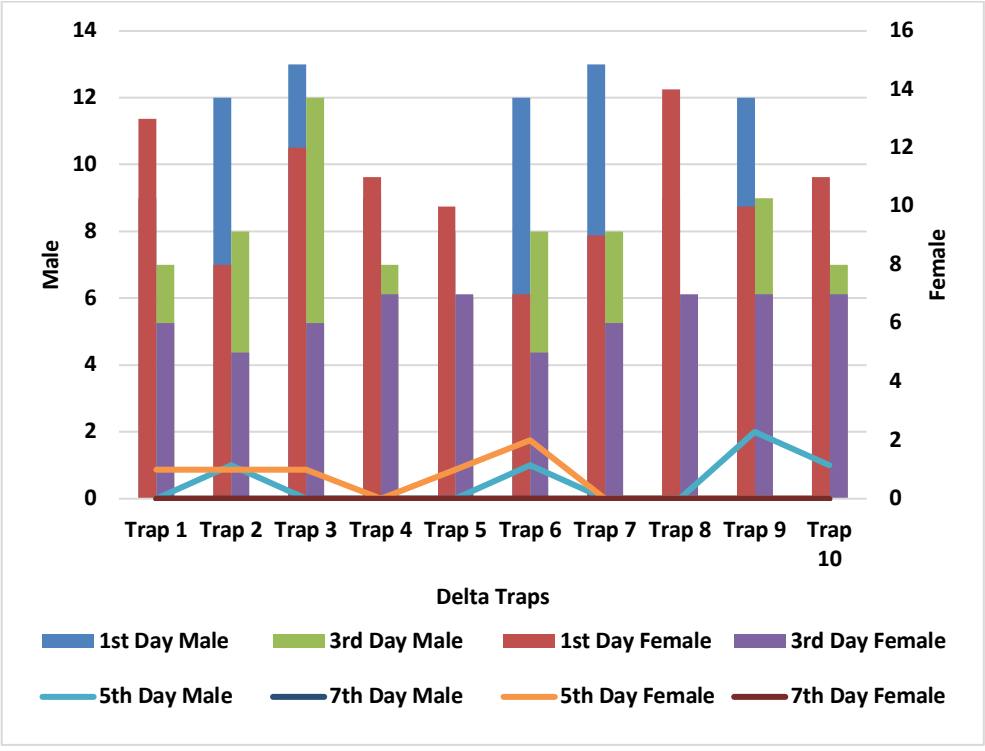


Fig 1. Liquid formulation Filed-efficacy test in Cotton stem weevil (Male & Female