

4. Research Achievements

4.1: Cotton Genetic Resources

Nagpur

Biodiversity, characterization, conservation and utilization of cultivated and wild species

Central Institute for Cotton Research (CICR) has a mandate of collection and maintenance of global cotton germplasm. The total cotton germplasm holding is 10375 accessions that include all four cultivated species i.e., *Gossypium hirsutum* (7523), *G. barbadense* (287), *G. arboreum* (1907) and *G. herbaceum* (565), 26 wild species, 15 races and 32 interspecific derivatives and 20 perennials.

Twenty three exotic accessions (*G. hirsutum*-18 and *G. arboreum*-5) were procured from Pakistan under Reciprocal Germplasm Exchange Agreement. Twenty three

morphological variants of *Gossypium arboreum race-cernuum* were collected from Tribal region of West Garo Hills, Meghalaya. The variants possess high boll weight (5 g to 7 g), high GOT (42% to 48%), short medium staple (16 mm to 19 mm) and coarse fiber. Three perennials of *G. arboreum* were also collected from Phulwari, West Garo Hills tribal region of Meghalaya.

Seed of 800 accessions of *G. arboreum* were sent to NBPGR, New Delhi for long term storage and conservation. Another set of 560 accessions of *G. hirsutum* were kept in medium term storage at CICR, Nagpur.

Six genetic stocks with novel & unique traits (*G. hirsutum*-4 *G. arboreum*-2) were identified and registered with NBPGR, New Delhi (Table 1).

Table 1 : The genetic stocks registered with NBPGR during 2010-11.

Name	Species and race	Registration No.	Unique and Novel Traits
SLL - 33	<i>G. hirsutum</i> race - <i>latifolium</i>	INGR 10060	Single leaf lobe
YPLL - 29	<i>G. hirsutum</i> race - <i>latifolium</i>	INGR 10061	Yellow pigmented leaf lobe
CSLL - 59	<i>G. hirsutum</i> race - <i>latifolium</i>	INGR 10062	Cup shaped leaf lobe
ABGMS (CSHN)	<i>G. hirsutum</i> race - <i>latifolium</i>	INGR 10058	Genetic male sterility
CISA - 2	<i>G. arboreum</i> race - <i>bengalense</i>	INGR 10057	Sterile mutant
CINA 333	<i>G. arboreum</i> race - <i>bengalense</i>	INGR 10059	High seed cotton yield potential with shoot claw of petal

Evaluation of germplasm

Five hundred and fifty germplasm accessions of *G. hirsutum* including exotics were evaluated under high density planting system at 30 cm x 20 cm spacing. Eighty-six genotypes were identified and selected on the basis of short internodes, compact fruiting branching habit, early maturity and bolls retentivity. Twenty nine single plant selections and seven segregating progenies were evaluated for seed cotton yield, boll weight, GOT and fiber traits.

Exotic accessions of *G. barbadense* were identified for seed cotton yield EC – 617836 (1024.07 kg/ha) and EC – 617837 (1020.14 kg/ha), ginning outturn EC 617841 (32.3 %), EC – 617844 (31.0 %), staple length EC 617843 (35.5 mm), EC 617854 (34.8 mm), EC 617837 (34.1 mm), EC 617838 (33.4 mm), and fiber strength EC 617841 (32.3 g/tex), EC 617844 (31.0 g/tex), EC 617840 (30.3 g/tex).

Cluster boll bearing accessions

Sixteen accessions of *G. hirsutum* were evaluated for morpho-economic characters and fibre properties. The promising accessions were identified for boll weight CB 214 (5.6 g), CB 204 (4.9 g), CB 206 (4.7 g); for GOT CB 214 (39.3%), CB 216 (37.3%), for staple length CB 215 (26.9 mm) and for fibre strength CB 210 (20.5 g/tex) and CB 215 (20.6 g/tex).

Assessment of gossypol content

Gossypol content of 69 germplasm lines of *G. arboreum* working collection belonging to different character groups such as GOT, MHL, Marker, boll weight, early maturing groups was determined and percent gossypol content was found to be in the range 0.25-0.97%.

Molecular characterization of cotton germplasm

Characterization of core accessions

Molecular characterisation of 94 core accessions using 55 primers (22 SSR, 14 SRAP, 8 TRAP and 11 RAPD) was carried out, of which 48 primers (20 SSR, 13 SRAP, 5 TRAP and 10 RAPD) were polymorphic. Fifty-five primers amplified a total of 277 fragments of which 158 were polymorphic which corresponds to 57.03 per cent polymorphism across 94 accessions. Polymorphism detected by SSR, SRAP, TRAP and RAPD was 26.98, 49.45, 56.75 and 87.20 per cent, respectively. The size of amplified fragments ranged from 300 bp to 3 kb, 170 bp to 3kb, 120 bp to 920 bp and 150 to 400 bp while the similarity coefficient (SC) values ranged from 0.83 to 1, 0.71 to 0.98, 0.56 to 0.94, and 0.31 to 0.94 for RAPD, SRAP, TRAP and SSR, respectively. The combined marker analysis revealed an overall SC ranged from 0.70 to 0.94 with an average of 0.82. The dendrogram analysis using RAPD + SRAP + TRAP + SSR similarity matrix data revealed eight major clusters and three accessions as outgroup members (Cat-3773, Cat-1911, Cat-3056). The lowest SC of 0.70 was found between Cat-299 with Cat-3773, Cat-3846 and Cat-3904 while the highest SC of 0.94 was between Cat-692 and Cat-1034. Distinct genotypes from among the different clusters were identified that can be used in breeding programmes.

Characterisation of working germplasm

Molecular characterization and genetic diversity analysis of 24 working germplasm of *G. hirsutum* (Early group – 10, Bacterial blight resistant group – 8 and Jassid & bollworm resistant group – 6) using PCR based RAPD, ISSR and Microsatellite (SSR) markers was carried out.

Eleven polymorphic RAPD primers produced a total of 98 amplification products of which 54 were polymorphic (54.88%) with 4.9 average number of polymorphic amplicons per primer. The UPGMA clustering of twenty-four working germplasm lines revealed a major cluster consisting of 23 germplasm lines while one line I-81 as distinct outgroup having similarity coefficient of 0.89. The main cluster was grouped into two clusters A and B with similarity coefficient of 0.912. Cluster B consisted of only one germplasm line BAR 1218 from Bacterial blight resistant group. The cluster A was sub-divided into sub-clusters A1 and A2 having similarity coefficient of 0.913. The sub-cluster A1 has twenty germplasm of different character groups whereas sub-cluster A2 has two germplasm KW-61-276 and UPA-57-17, both resistant to Bacterial blight.

Genetic diversity analysis by ISSR markers

Fifteen polymorphic ISSR primers produced 149 amplification products of which 91 were polymorphic (61.94%) with 6.06 average number of polymorphic amplicons per primer. The UPGMA clustering pattern revealed a major cluster consisting a total of 23 germplasm while PEE DEE 0111 A from USA emerged as outgroup and distinct with similarity coefficient of 0.89. Twenty-three lines were grouped into two clusters, cluster A & cluster B having similarity coefficient of 0.901. The cluster B consisted of only one accession, CO2 from Coimbatore, resistant to Bacterial blight. The cluster A was subdivided into sub-clusters A1 and A2 having similarity coefficient of 0.909. The sub-cluster A1 had twenty germplasm whereas sub-cluster A2 had two germplasm lines COKER 413 and KEMP from USA sharing similarity coefficient of 0.921.

Genetic diversity analysis by microsatellite markers

Fifteen polymorphic microsatellite primers produced a total of 75 amplicons of which 61 were polymorphic (85.26%) with 4.26 average number of polymorphic amplicon per microsatellite marker. The UPGMA analysis revealed a major cluster consisting of 23 germplasm while CNH-154 emerged out to be most distinct having similarity coefficient of 0.73. Twenty-three germplasm could be grouped into two clusters, cluster A & B having similarity coefficient of 0.74. The cluster B consisted of two germplasm, CNH-123 and DCI 122 having similarity coefficient of 0.808. The cluster A was further subdivided into two sub-clusters A1 and A2 having similarity coefficient of 0.793. The sub-cluster A1 has eighteen germplasm of different groups whereas sub-cluster A2 has three germplasm i.e. BAR-1218, KW-61-276 and KEMP resistant to Bacterial Blight sharing similarity coefficient ranged from 0.840 to 0.900.

Principle coordinate analysis

Principle Coordinate Analysis (PCA) generated from RAPD, ISSR and microsatellite data of twenty-four cotton working germplasm showed that all the germplasm were relatively spread out on all the three axes. The first, second and third axes explained 15.01 %, 12.70 %, 9.92 % variation for RAPD, 14.75 %, 11.25 %, 9.95 % for ISSR and 22.31 %, 16.49 %, 11.60 % for microsatellite markers, respectively.

Characterization of total working collection using DNA markers

In National Cotton Gene Bank of Cotton at CICR, Nagpur presently a total of 388 working germplasm collection of *G. hirsutum* is maintained that has been divided into various groups depending upon their specific characters viz. yield group (YG), mean halo length group (MHL), boll weight group

(BW), oil content group (OIL), naked seeded group (NS), ginning outturn group (GOT), salt group (SG), marker group (MAR), fibre and strength group (FSG), variety group (VAR), okra group (OK), bacterial blight resistant group (BBR), jassid and bollworm resistant group (JBWR), boll worm resistant group (BWR) and early group (EG). Character groupwise genetic diversity analysis was carried out using PCR based STMS Markers.

Twenty eight STMS primers were employed that produced a total of 139 amplicons of which 113 amplicons were polymorphic, resulting in 80.65 per cent polymorphism and 4.03 average number of polymorphic bands per primer. The number of DNA amplified fragment per primer ranges from 3 (JESPR 208) to 8 (BNL 2709) and the average amplicons size between 100 to 1000bp.

Unique amplicons/markers

Unique amplicons were identified for few of the genotypes namely MWR-2 (BNL 1694₁₇₀, BNL 1694₁₉₀), BURI-0-394-Y (JESPR 215₁₀₀, JESPR 360₁₀₀) and IC-356312 (MUCS-422₃₈₀).

STMS cluster analysis

The data obtained by STMS markers were analyzed by NTSYS-pc software Version 2.02. The UPGMA clustering pattern of 388 working germplasm showed two major clusters, Cluster A and B. Cluster A consisted of 379 and cluster B consisted of 9 germplasm. OKRA-LEAF from total 388 working germplasm emerged out to be the most distinct. The similarity coefficient by STMS markers were in the range of 0.74-0.92. Principal Coordinate analysis (PCA) based on genetic similarity matrices were used to visualize the genetic relationships of *G. hirsutum* working accessions.

Groupwise analysis and dendrograms obtained by STMS markers

Group I : Yield Group - Out of 30 working accessions, GRS-60-6-IL-3-G and UPA 57-17 showed 92 % similarity whereas EC-110788 emerged out as distinct and diverse germplasm. The similarity coefficients among STMS markers were in the range of 0.67-0.92.

Group II : Mean Halo Length Group – Out of 10 working germplasm, EL-958 (MHL-690) and G-21-17-619-3-MS showed 92 % similarity. The similarity coefficients were in the range of 0.57-0.92.

Group III : Boll Weight Group - Out of 33 working germplasm, ACALA-8-1 x10-SP and DUNN-56-C-A from USA showed 96% similarity. The similarity coefficients were in the range of 0.73-0.96.

Group IV : Oil Content Group - Out of 13 working germplasm, M-4 and MACHA showed 89% similarity whereas 21 from Siruguppa emerged out as diverse germplasm. The similarity coefficient of STMS markers were in the range of 0.73-0.89.

Group V : Naked Seeded Group - Out of 11 germplasm, AC-520 and Acala-1517-D-64 showed 91% similarity whereas AC-83 and ACALA x HOPE-76-15-5 emerged as most diverse. The similarity coefficient were in the range of 0.76-0.91.

Group VI : Ginning Outturn Group - Out of 21 germplasm, HA-46-124 and 86-AI-1 showed 96% similarity while 7203-14-104 and STAM-42 from USA were most diverse. The similarity coefficients of STMS markers were in the range of 0.75-0.96.

Group VII : SALT Group – Of the 6 germplasm, J-34 and MAHALAXMI showed maximum 85% similarity. The similarity coefficients of STMS markers were in the range of 0.77-0.85.

Group VIII : Marker Group – Of the 66 working germplasm, SA-16 and SA-747 from USA showed 98% highest similarity. The similarity coefficients of STMS markers were in the range of 0.66-0.98.

Group IX : Fibre Strength Group - Out of 16 germplasm, DELTAPINE-45 and DELTAPINE (CJ) from USA showed 91% similarity whereas P-56-6 & MWR-2 emerged as outgroup and diverse among other germplasm. The similarity coefficients of STMS markers were in the range of 0.75-0.91.

Group X : Variety Group – Out of 56 germplasm, J-34 and SS-265 showed 96% highest similarity. The similarity coefficient of STMS markers were in the range of 0.65-0.96.

Group XI : Okra Group - Out of 18 germplasm, AROZONA-SUPER-OKRA-CC-MLL-DH-OKRA & SUPER-OKRA-CC-MLL-DH showed 96% similarity. The similarity coefficients of STMS markers were in the range of 0.72-0.96.

Group XII : Bacterial Blight Resistant Group – Out of 18 germplasm, UPA(62)31 & 101-102-B from Uganda & USSR showed 95% similarity. The similarity coefficients of STMS markers were in the range of 0.72-0.95.

Group XIII : Jassid and Bollworm Resistant Group - Out of 58 working germplasm lines from Coimbatore, Akola, Malwa and Surat showed 96% highest similarity whereas JK-344 & 36-YY-GJHS-53 from Surat and Dharwad emerged out group in cluster as most diverse among other germplasm. The similarity coefficients of STMS markers were in the range of 0.62-0.96.

Group XIV : Boll Worm Resistant Group - Out of 5 germplasm, NDLH-1745 and BW-28 showed 87% highest similarity whereas P-846 from Punjab emerged out as most diverse. The similarity coefficients of STMS markers were in the range of 0.80-0.87.

Group XV : Early Group – Out of 27 germplasm, EC-141679-84 and DCI-108 from USA and Nagpur showed 92% similarity whereas 320-F-CC from Jalandhar emerged out as most diverse. The similarity coefficients of STMS markers were in the range of 0.74-0.92.

Coimbatore

Maintenance and evaluation of *G. barbadense* germplasm

Ninety-two NDGB lines and 14 USAGB lines of *G. barbadense* germplasm were maintained and variability was accessed during 2010-11 crop season. Twenty-two exotic *G. barbadense* accessions were also field evaluated during 2009-10 and 2010-11. EC-617836, EC-617837 and EC-617840 gave higher seed cotton yield than the control Suvin. 2.5% span length of all the EC lines was less than Suvin except the line EC-617843 (36 mm) which was at par with Suvin (36.5 mm). The highest bundle strength was noticed in the accessions EC-617844 and EC-617840. The ginning percentage of EC-617836 (30%) EC-617840 (30.3%) and EC-617844 (31%) was higher than the check Suvin (27.0%). EC-617864 exhibited exceptionally high micronaire of 5.8 against the control Suvin (3.5 μ /inch). EC-617838 and EC-617855 were identified as early maturing germplasm lines, requiring 191 days for uniform bursting while other lines and control matured in 225 days. Accessions EC-617841 and EC-617864 exhibited bigger boll size while EC-617843 with fibre length (35.5 mm), bundle strength (29 g/tex) and micronaire of 3.6 holds promise for commercial exploitation.

Sirsa

Collection, conservation and maintenance of genetic resources

Three hundred and thirty three (333) new germplasm working collection grouped based on superior traits were evaluated for yield contributing parameters, fibre properties, morphological parameters, reaction to pest and diseases etc. These lines were also characterized for 34 DUS parameters. The range for various parameters i.e. for seed cotton yield/ plant 15-153 gm, boll weight 1.3-3.8 g, boll number 5-56, ginning outturn 27-40.3%, seed index 5-11.6 g, lint index 2.2-7, number of monopodia 0-5, number of sympodia 2-14 and for plant height 34-200 cm was observed. The superior accession i.e. for boll weight (g) MEADE 9030 D(3.8), UPA (62) 31-65(3.8), CSH 911 (3.7); yield/plant (g) CNH 151(153), 9-1487(150), DELTAPINE C5 (150); height (cm) LAM Guntur (200), 356587 (170), S4727 (155); number of monopodia GISV -86/58(5), WC 12 NL(4), GALAMA(4); number of sympodia CNH154(14), 356587(13), LAM Guntur (12); boll number DELTAPINE C5 (56), CNH151 (51), 359754 (45); seed index WC 12 NL (11.6), S 344 (11.6), COKER 100 STAPLE(11.5); ginning outturn (%) RED 5-7(40.3), MLL(40), AURBURN OKRA 213 – OBP – SPB 1978 (39.4); lint index B 61 – 2038 (7), L-147 (7), BJR JK-97-16-4 (7) were identified. The germplasm lines were supplied to the breeders of the North Zone by organizing germplasm day.

4.2 : Hybrid Cotton

Nagpur

Maintenance of parental lines (male steriles and restorers)

Fifteen *G. aridum* based CMS, 137 *G. harknessii* based CMS, 57 restorer and 20 GMS lines were maintained by crossing to its fertile counterpart (B-line) by selfing and sibmating. The M2 generation of AK 32 CMS (*G. aridum*), Rajat CMS (*G. harknessii*) and G 67 GMS lines were raised. Individual sterile plants in 356 single plant progenies were identified and maintained through crossing. Eight restorer lines (four each of *G. hirsutum* and *G. barbadense*) received from Israel were evaluated during the crop season. All the *G. hirsutum* restorer lines were susceptible to sucking pest and showed severe leaf reddening with very low boll setting and boll drying.

Comparison of conventional and male sterility based hybrids

CMS and conventional hybrids of the same background were tested for their comparative performance. The CMS hybrid PKV Hy3 recorded higher yield (1358.49 kg/ha) than its conventional counterpart PKV Hy2 (1166.63 kg/ha). The other two CMS hybrids viz., PKV Hy4 and PKV Hy5 recorded poor yield than the conventional hybrids.

TGMS system

To determine the suitable stable window period for getting complete sterility, sowing of TGMS line 1-1 was taken up at three different times viz. on 22nd May 2010, 30th June 2010 and 10th February 2011. The crop season in May and June produced flowers which had 10 – 30% pollen fertility mainly due to sudden fall in sunshine hours and cloudy weather and decline in mean minimum temperature. It was observed that the pollen grains were completely round and fully stained in fertile flowers, partially stained, slightly deformed in partially fertile flowers and were completely deformed in fully sterile flowers within the same TGMS line. The February sown plants showed complete male sterility morphologically and through pollen staining. Six hybrids developed through TGMS were sown along with released and popular hybrid, AKDH-7 and popular varieties PA 402 and PA-255 to evaluate their performance. The yield of hybrids was very low due to heavy

rainfall during the crop season.

EGMS System

Eight EGMS lines namely EGMS 145, EGMS 132, EGMS 108, EGMS 18, EGMS 35, EGMS 36, 08093-10R and EGMS 3822266 were maintained at CICR, Nagpur. These lines were sown for recording observations on pollen sterility during summer months i.e. April-June, 2011.

Coimbatore

Nine F₁ hybrids viz; CCHB-2 (2632 kg/ha), CCHB-6 (2776 kg/ha), CCHB-7 (2647 kg/ha), CCHB-8 (2756 kg/ha), CCHB-11 (2649 kg/ha), CCHB-13 (2751 kg/ha), CCHB-20 (2770 kg/ha), CCHB-21 (2645 kg/ha) and CCHB-22 (2799 kg/ha) on par with respect to seed cotton yield and significantly superior over the check hybrids RCHB-708 Bt (2113 kg/ha) and DCH-32 (1927 kg/ha) have been identified. All the best performing hybrids showed better lint index (5.1-5.3) than the check hybrids (4.0). The yield increase of 32.4 % has been recorded in CCHB-22 followed by CCHB-6 (31.4%), CCHB-20 (31%), CCHB-8 (30.4%) and CCHB-13 (30%). The best yielding hybrids CCHB-6, CCHB-8, CCHB-12 and CCHB-13 possessed higher ginning percentage of 36 as compared to the check hybrids DCH-32 (29%) and RCHB-708 Bt (28%).

In another trial, CCHB-33 was identified as the best hybrid with 29.47% increase over RCHB-708 Bt and 33% over DCH 32. A trial was laid out with eight best selections made from various advanced trials conducted during the last five years with RCHB-708Bt and DCH-32 as checks. Out of eight advanced hybrids, CCHB-110 with an average yield of 2801 kg/ha (33%) ranked first over the best check RCHB-708 Bt (Table-2). The hybrids CCHB-110 and CCHB-48 possess highest ginning percentage of 35 as compared to checks RCH 308 Bt (30%) and DCH 32 (32%).

Table 2: Performance of advanced interspecific ELS hybrids

Sr. No.	Hybrids	S C Y (kg/ha)	% increase in yield over RCHB-708	SI	LI	GOT (%)
1	CCHB-56	2615*	24	8.9	4.6	34
2	CCHB-48	2684**	27	9.2	5.1	35
3	CCHB-110	2801**	33	9.5	5.2	35
4	CCHB-215	2612*	24	9.3	4.7	34
5	CCHB-5339	2509	19	8.7	4.3	33
6	CCHB-51074	2236	6	9.5	4.2	31
7	CCHB-2628	2669*	27	9.8	4.0	29
8	CCHB-2630	2544*	21	9.4	4.6	33
	RCHB-708 (c)	2108	-	9.2	4.0	30
	DCH-32 (c)	2005	-	8.9	4.1	32
	S Ed	148.7	-	-	-	-
	CD @ 5%	312.5	-	-	-	-
	CV%	7.30	-	-	-	-

** Highly significant, * Significant

Interspecific ELS hybrids

A new set of crosses were made with seven potential female parents of *G. hirsutum* and five male parents of *G. barbadense*. Thirty-five cross combinations were made in Line x tester mating design.

Sirsa

Diploid hybrid cotton

Identification of parents and general combiners

Thirty GMS hybrids along with parents (CISA 6, CISA-6-214, GBaV 105, JLA 505, JLA 1600, LD 733, PA 532, RG 548, RG 550, Sarvotam 1, DS 5, CISA 2 & GAK 413A) were tested in randomized block design in three replications. Parents CISA 6, JLA 505, PA 532, Sarvotam 1, GAK 413 A were found good general combiner for seed cotton yield, CISA-6-214, JLA 1600, RG 550, GAK 413A for GOT (%), CISA 6, JLA 1600 for boll weight, JLA 505 for 2.5% span length, JLA 505, JLA 1600, LD 733 for fineness and JLA 505, PA 532, RG 548, Sarvotam 1, DS 5 for strength. Based on the significant heterosis and specific combining ability, specific crosses were identified for various traits.

Maintenance and heterosis breeding

Three *arboresum* GMS lines GAK 413A, DS 5 and CISA 2 were maintained through sibmating. Different cross combinations were developed for sponsoring GMS based hybrid in AICCIP trial. Large amount of hybrid seed was produced for CISAA 17 and CISAA 18. Nine GMS lines (CISAG 4, CISAG 7, CISAG 8, CISAG 13, CISAG 14, CISAG 17, CISAG 20, CISAG 27 and CISAG 28) were maintained through sibmating.

In North Zone, the GMS based hybrids CISAA 17 and CISAA 18 gave higher seed cotton of 2072 kg/ha and 2645 kg/ha respectively than the zonal check CICR 2 (2033 kg/ha) (Table 3). Both the hybrids were better with respect to fiber length and strength as compared to check hybrid. The hybrid CISAA 18 has been promoted for large scale testing in North Zone. In Central Zone, CISAA 17 and CISAA 18 gave higher seed cotton of 1859 kg/ha and 1957 kg/ha and ranked 6th and 2nd respectively and both have been promoted in Central Zone. In South Zone, CISAA 17 recorded seed cotton yield of 1385 kg/ha and ranked 4th and it has been promoted for large scale testing. GOT of both the hybrids was consistently high (38 and above) in all the three zones.

Table 3 : Performance of *G. arboresum* hybrid CISAA 17 and 18 (GMS based) in Br-25(a) AICCIP Trial

Entry/ Character	Seed Cotton Yield (kg/ha)	Rank	GOT (%)	2.5% Span Length	Bundle Strength
North Zone					
CISAA-17	2072	7	38.0	24.6	19.2
CISAA-18	2645	2	37.9	23.3	18.5
Zonal Check (CICR 2)	2033	8	39.8	20.3	16.8
Central Zone					
CISAA-17	1859	6	39.1	23.4	19.3
CISAA-18	1957	2	38.2	21.9	18.2
Local Check	1673	9	36.5	26.2	21.1
South Zone					
CISAA-17	1385	4	39.9	24.1	19.4
Local Check	1047	9	34.7	23.9	17.7

Tetraploid Cotton

Maintenance of CMS lines

Eleven CMS lines representing parents of promising hybrids viz. LRA 5166, Jhorar, RB 281, LH 1134, Pusa 31, HS 6, K 34007, F505, F1183, CSH 25 M, and SH 2379 were maintained through sibmating. The restorer lines namely CIR-8, CIR-12,

CIR-23, CIR-26, CIR-32, CIR-38, CIR-47, CIR 70, CIR 97P1, CIR 97P3, CIR 119P1, CIR 119P3, CIR 526P1, CIR 526P3, CIR 926P2, CIR 926 P3, CIR 1169P1 and CIR 1169 P2 were maintained through selfing.

Evaluation of GMS hybrids

Forty-four (44) GMS hybrids along with their parents were tested in Randomized Block Design in three replications. The male parents S -123, Ratna, BN and female parents GMS-20, GMS-21 were found to be good general combiner for seed cotton yield while S-123, LH 900, GMS-17, GMS-21 for ginning percentage. The cross combinations GMS-21xM-45, GMS-21 x LH 900, GMS-21 x CISV-1 were high yielding hybrids with significant SCA effect.

Table 4 : Effect of different row spacing and fertilizer levels on performance of *G. hirsutum* GMS based hybrid (CSHG 1862) in North Zone

Treatments	Faridkot			Bathinda			Sirsa			Sriganganagar		
	SCY (kg/ha)	BN	BW (g)	SCY (kg/ha)	BN	BW (g)	SCY (kg/ha)	A. BN	BW (g)	SCY (kg/ha)	BN	BW (g)
Row spacing (cm)												
67.5 X 60	1656	39	4.05	1875	31.5	-	1662	23	3.27	2544	83	3.39
67.5 X 75	1278	43	4.13	1955	33.0	-	-	-	-	2540	84	3.49
67.5 X 90	-	-	-	1660	29.7	-	-	-	-	2377	85	3.64
100 X 45	-	-	-	-	-	-	1514	26	3.54	-	-	-
100 X 60	-	-	-	-	-	-	1423	25	3.60	-	-	-
CD at 5%	-	-	-	230	NS	-	NS	NS	NS	NS	NS	NS
Fertilizer levels (kg/ha)												
75% RDF	1349	36	3.87	1750	29.1	-	1638	25	3.28	2233	82	3.32
100% RDF	1484	43	3.99	1977	34.7	-	1540	24	3.53	2520	84	3.58
125% RDF	1568	45	4.42	1772	30.6	-	1423	23	3.63	2708	86	3.61
CD at 5%	-	-	-	175	2.37	-	NS	NS	NS	274	2.3	0.24

Performance of intra-hirsutum hybrid CSHH 3008 in Br 05 (a)

An intra-hirsutum hybrid CSHH 3008 recorded mean seed cotton yield of 2184 kg/ha with 32.7 percent ginning outturn and ranked at 4th position as compared to zonal check CSHH 198 (Table-5). The hybrid also recorded higher 2.5 % span length (29.1 mm) and bundle strength (23.8 g/tex). The hybrid has been promoted for 2nd year of testing in North zone.

Table 5 : Performance intra-hirsutum hybrid CSHH 3008 in Br 05 (a) CHT trial

Name of the hybrid	Seed Cotton Yield (kg/ha)	Lint Yield (kg/ha)	Boll Weight (g)	GOT (%)	2.5% Span Length (mm)	Mic. value	Tenacity (g/tex)
CSHH 3008	2184(4)	716	4.6	32.7	29.1	4.9	23.8
Local Checks	2105(5)	702	4.6	33.3	27.3	4.8	22.7
CSHH 198 (ZC)	1929(8)	636	4.3	33.1	27.6	5.1	23.0
CD	321.2	-	-	-	-	-	-
CV (%)	10.2	-	-	-	-	-	-

Performance of GMS based hybrid CSHG 1862

The GMS based hybrid CSHG 1862 recorded more than 12 percent seed cotton yield over the zonal check hybrid CSHH 198 in three years of testing. An agronomy trial was conducted in North Zone during 2010-11. The different row spacing were statistically at par at Faridkot and Sirsa, whereas 67.5 x 75 and 67.5 x 60 cm spacing gave highest seed cotton yield at Bathinda and Sriganganagar, respectively. Among the fertilizer levels, 100% RDF seems to be better at Sriganganagar and Bathinda, whereas 75% RDF & 125 % RDF was optimum at Sirsa and Faridkot. The detailed performance of the GMS hybrid CSHG 1862 in agronomy trial is given in Table-4.

4.3 : Genetic Improvement

Nagpur

A. *G. arboreum* (diploid cotton)

Three trials, viz. Common, Zonal and Introgression trial, were conducted in three replications involving two arboreum checks viz., AKA 8401 and PA-255 and a hirsutum check LRK-516 in all three trials. LD-1010 recorded the highest seed cotton yield (2156 kg/ha) and GOT of 39.4% in common trial and was superior to all the three checks followed by CISA-6-214 (1719 kg/ha). PA-720 recorded a superior staple length of 29.1 mm with a fibre strength of 19.0 g/tex. In the introgressed trial PAIG-39 exhibited staple length of 28.5 mm, strength of 22 g/tex and a micronaire of 4.8 followed by PAIG-12 with staple length of 28.8 mm, fibre strength of 21.7 g/tex and micronaire of 4.9. PAIG-62 recorded maximum yield of 666 kg/ha in the introgressed material trial of *G. arboreum*.

G. arboreum variety CNA 1003 (ROJA) identified

G. arboreum culture, CNA 1003 (ROJA) with medium fibre properties performed extremely well in south zone for three consecutive years and emerged as top ranking entry. The entry underwent agronomical trial in South Zone and the seed cotton yield of 1463 kg/ha was obtained at Dharwad. It gives better yield at spacing of 60x30 cm and 75 x 30 cm and showed higher yield at higher doses of fertilizers (Table 6). CNA 1003 (ROJA) was identified for rainfed situations of South Zone by Central Variety Identification Committee during AICCIP workshop at CCSHAU, Hisar on April 6-8, 2011.

Table 6 : Agronomic performance of CNA 1003 at Dharwad in South Zone

Treatments	Seed Cotton Yield (kg/ha)	No. of Bolls /Plant	Boll Weight (g)	No. of Bolls/ Plant	Yield /Plant (g)
CNA 1003	1463	19.5	2.64	19.5	44.2
AKA 0110	1417	20.1	2.77	20.1	49.7
CD 5%	26.0	NS	NS	NS	NS
Spacing (cm)	CNA 1003	AKA 0110			
60 x 30	1464	1407			
75 x 20	1445	1487			
75 x 30	1480	1427			
CD 5%	NS				
Fertility levels	CNA 1003	AKA 0110			
NPK (kg/ha)	CNA 1003	AKA 0110			
40:25:25	1439	1389			
60:30:30	1442	1432			
80:40:40	1507	1500			
CD 5%	83				

Entries promoted

G. arboreum culture CNA 1007 has been evaluated in Zonal trial Br. 24(b) in South Zone. It ranked 3rd in seed cotton yield and first in lint yield (Table-7). CNA 1007 was retained for second year trial in South Zone.

Table 7: Lint yield of CNA 1007 in Zonal trial Br. 24(b) in South Zone

Genotype	Dharwad	Nandyal	Kovilpatti	Mean(SZ)	Rank	GOT
CNA1007	1064	288	472	608	1	39.1
ARBa08-49	927	370	425	574	3	35.8
DLSa-17 (SZ)	798	285	486	523	5	35.9

Three *G. arboreum* cultures CNA 1008, CNA 1009 and CNA 1010 were evaluated in IET of the AICCIP in 2010-11. *G. arboreum* culture CNA 1008 performed well in Central and South zone and ranked at 6th and 5th position, respectively for seed cotton yield. CNA 1008 has been promoted to Br.24(a/b) in Central and South Zone for the crop season 2011-12.

Entries sponsored for AICCIP trials

Four *G. arboreum* entries namely CNA 397, CNA 398, CCA 1014 and CCA 1015 were sponsored for Initial Evaluation Trial Br. 22 (a/b) of AICCIP for crop season 2011-12.

Promotion in non-traditional areas

During 2010-11 crop season, 46 kg seed of *G. arboreum* (AKA-5, AKA 7, AKA-8, CINA- 385 , PA (402) and *G. hirsutum* (PKV-O81, LRK-516, NH-615 and Suraj) were distributed to 26 farmers through NGO, CARD, Anjangaon Surji for plantings in Melghat area of Vidarbha. Only three farmers grew *G. arboreum* AKA-5 in their fields. One of the farmer based at foothills of Melghat region with supportive irrigation harvested 2000 kg/ha on medium type of soil. Some farmers have been adopting arboreums, despite the spread of Bt cotton in Melghat region.

Sirsa

Notification of variety CISA 614 for commercial cultivation in North Zone

The variety CISA 614 was tested in 32 locations in the North Zone (Punjab, Haryana and Rajasthan) during 2004-2007 and



recorded an overall mean seed cotton yield of 2204 kg/ha as against 1834 kg/ha of HD 123 (zonal check) and 1990 kg/ha of local check. The increase in seed cotton yield of the new variety CISA 614 over the common (Zonal check) check was of the order 20.17 per cent. In agronomy trial the variety CISA 614 recorded the highest seed cotton yield of 3792 kg/ha at 67.5 x 30cm spacing. CISA 614 recorded slightly superior fibre characteristics in comparison to zonal, local checks and qualifying varieties. It was identified by Variety Identification Committee (AICCIP) held at ANGRAU, Hyderabad 6-8 April, 2009 and notified vide Gazette of India No.608 dated April 1, 2010.

Registration new genetic male sterile line CISA 2

CISA-2 – a spontaneous sterile mutant of *G. arboreum* having yellow flower with red petal and green plant has been registered with unique germplasm by the plant Germplasm registration Committee of the ICAR vide Registration No. INGR10057 and National Identity No. IC 0538548. The new GMS line has open flowers which facilitates easy crossing whereas the earlier reported lines have closed flowers that do not allow for easy crossing.

Evaluation of promising varieties

Under station trial, 8 genotypes were evaluated, none of the genotypes could out yield the check CISA 614 (2997 kg/ha). However the genotype CISA-6-256 (2465 kg/ha) gave numerically higher yield than the local check LD 694 (2329 kg/ha). The entries with better yield are CISA-6-256 (2465 kg/ha), followed by CISA-6-214 (2130 kg/ha), CISA -6-209 (2106 kg/ha), CISA-6-295 (1784 kg/ha) & CISA-6-350 (1578 kg/ha). The highest GOT (41.5%) was recorded by CISA-6-350 and highest boll weight by CISA-6-123 (3.4 gm). Forty four single plants were selected based on their yield performance.

Seven genotypes were evaluated in RBD and identified CISA 8 (3203 kg/ha), CISA 294 (3150 kg/ha) and CISA 405 (3098 kg/ha) as high yielder than the checks (CISA 310, LD 733, HD 123). CISA 405 was sponsored in Br22a/b trial of AICCIP.

Development of mapping population for fibre length and strength

A mapping population is being developed by effecting crosses between parents with distinct 2.5% span length and strength (g/tex). In Cross I, 251 F₄ plants of cross HD 123 x Arbha 35 were evaluated and seed cotton yield ranged from 20 g to 295 g/plant, GOT (%) from 21.8 to 52.2% and mean halo length from 12 to 26.6 mm. In Cross II, 297 F₄ plants of cross RG 8 x

Arbha 35 were evaluated. The seed cotton yield ranged from 21.5 to 242.2 g/plant, GOT from 24.4 to 52.4% and mean halo length from 13 to 28.0 mm.

Mapping population for GOT

The F₂ populations consisting of 185 plants from a cross involving parents with High x Low GOT [SA-977(HG) x SA-112(LG)] was obtained.

Development of heterotic genepool

Twenty one multiple crosses were made for development of heterotic genepool. Sirsa cultures: CISA-101p-2, CISA-101p-3, CISA-101p7, CISA11p10, CISA101P3, CISA111p1, CISA111p2, CISA112p5, CISA130, CISA104 and single plant selections were crossed with VNWA-1 a culture from Nagpur.

Tetraploid cotton improvement

Nagpur

Drought tolerance

Drought tolerant efficiency (DTE) was used as a criteria for identification of genotypes based on their performance under rainfed and irrigated condition. Three crosses Pusa 56-4 x 30 I, Pusa 56-4 x 29I and Pusa 56-4 x 28 I recorded DTE of 76.86, 95.42 and 70.34 per cent, respectively. Pusa 56-4 x 30I and Pusa 56-4 x 29 I had shown high DTE for two consecutive

years. Cross Pusa 56-4 x 29 I recorded highest yield of 506.34 under rainfed condition while Pusa 56-6 x 28 I recorded higher yield under irrigated condition. Eleven lines (DTS 155-09, DTS 100-09, DTS 104-09, DTS 39-09, DTS 62-09, DTS 108-09, DTS 67-09, DTS 44-09, 28I, 29 I and 30 I) were found to be tolerant based on chlorophyll value, membrane stability, reducing sugar, amino acid content and phenols. One culture (DTS 121) has been sponsored for testing in AICCP national trial as CSH 121. Application for registration of culture CNH 30I tested under AICCP trial and identified as drought tolerant (Seed cotton yield: 1515.08 kg/ha, boll weight of 3.5–4.0 g, 2.5 % span length of 24.5 mm, uniformity ratio of 49 per cent, micronaire of 3.8, fibre strength of 19.5 g/tex and ginning outturn of 35 per cent) has been submitted to NBPGR. Seeds from individual F₂ plants of three crosses Pusa 56-4 x 30I, 30I x Pusa 56-4 and Pusa 56-4 x 29I have separately been harvested to carry forward to F₃ and development of RILs for drought tolerance.

Biotic stress resistance

To develop genotypes and hybrids with tolerance / resistance to boll worm, efforts were made to transfer approved BN Bt event into elite cotton genotypes and parents of hybrids from different agro-climatic zones. The following genotypes are under conversion and are in different back cross generation:

BC generation	Genotypes (61)
BC4 (20)	NZ : F 1861, H1242, CSH 198 ?, CSH 198 ?, CSH 238 ?, CSH 238 ?, CZ : KH 138, Surat Dwarf SZ : CPD 731, CPD 758, SCS 9, LRA 5166, SCS 101, Surabhi, Abadhita, RAH 30, PSS 2, TCH 1002, CCH 5104, SCS 37
BC 3 (5)	NZ : RK 4145 SZ : DS 28, MS x 72-1358, CPD 787, L 761
BC 2 (12)	NZ : Pusa 56-1, Pusa 56-2, Pusa 56-4, Pusa 56-6, RS 810, RS 875, NH 452, CZ : Rajat, PKV 081, AK 32, AKH 8828, SZ : MCU 12
BC 1 (15)	NZ : H 1117, HHH 287(m), HD 324, HD 123, F 2236, F2036, F 2086, F 2188, LH 2076, F 2164 CZ : PH 93, NH 152, NH 615, PH 1009 SZ : NARSIMHA
BC 2 (4)	GMS LINE : LRA 5166, KH 2, B 59-1684, G 67
F ₁ (9)	NH 452, DHY 286, Laxmi, CSHH 198 (M), CSHH 243 (F), CSHH 243(M), Khandwa 2, G Cot 16

Nine GMS based Bt hybrids were evaluated along with RCH 2 Bt, Bunny Bt and BN Bt. All the GMS Bt hybrids were superior to RCH 2 Bt hybrid (801.71 kg/ha). The heterosis over RCH 2 Bt ranged from 3.27 to 46.65 per cent. FP 14 x BN followed by FP 5 x BN and FP 17 x BN were some of the promising hybrids recording more than 20 per cent heterosis.

Jassid tolerance

Three hundred and ninety single plants were selected based on tolerance to jassid, higher number of bolls per plant, earliness, compact plant type and tolerance to jassid from crosses PKV Rajat x Acala 1517, AKH 081 x ND 63, Daet -S-SL x G Cot 16, NH 545 x ND 63. Crosses viz. H 1252 x LH 1948 and PKV Rajat x LH 1948 were identified for compact plant type. Seventeen F₃ progenies of these crosses were carried forward based on their consistent performance for plant type and tolerance to jassid. From 25 F₂ populations (interspecific Rai - derivatives crossed with cultivated genotypes), 90 single plants were selected based on earliness, tolerance to jassid and yield potential. Promising segregating material was identified from NISC 261 x P 56-4, NISC 261 x AKH 081, NISC 261 x PKV Rajat, NISC 63 x LRK 516, NISC 64

x Pusa 56-6, NISC 64 x EC 277959, NISC 65 x Pusa 56-6 crosses.

CISH-16, a *G. hirsutum* culture was found highly tolerant to sucking pests particularly Jassids and white fly based on one year field testing (2009-10) and one year controlled condition evaluation (2010-11) under AICCP trials at LAM, Guntur.

Genetic enhancement

Fourteen selections were evaluated using NH-615 as check variety in two replications. Three selections showing significantly higher seed cotton yield namely Sps-7-5 (1818 kg/ha), Sps-7-7 (1402 kg/ha) and Sps-7-1(1396 kg/ha) were identified. Selections with staple length of 30 mm namely, SPS-7-6, SPS-7-7, SPS-7-10 and SPS-7-13 were identified for further evaluation.

In 78 F₃ populations, few single plant selections with superior traits such as SPS 3-29 – an erect naked seeded type and SPS 3-42 - a very short staple fibre type (<13 mm approx) were identified. In F₆ Populations, SPS-6-2 (Pedigree- RS 875 x Rex) and SPS 6-10 (Pedigree – Deviraj x CAT 685) two compact plant progenies were phenotypically confirmed. SPS 7-12

(Pedigree – LRA-5166 x CIHS-97-9) was sucking pest free in 3 years testing. SPS 7-3 was found resistant to sucking pests, medium early and hairy in phenotypic expression. SPS IM- 4-16 was determinate, medium boll uniform bursting and free from sucking pests. JBWR-JK 54 which was highly tolerant to sucking pests, hairy and had determinate growth contributed this character.

Development of heterotic pool for superior medium staple

One hundred and forty single plant selections were made for yield potential and superior medium staple from crosses involving PKV Rajat × NH 545, LRK 516 × Acala 1517, PKV Rajat × Acala 1517 in one group and ACCLD 163 × H 1252, H 1252 × LH 1948, Surabhi × ACCLD 163, H 1252 × MCU 9 in other group. Twelve genotypes selected from working collection based on molecular diversity were crossed in a 12 × 12 diallel mating design. The selected parents include IC - 356581, BAR 7-2-P-5 (IC -356819), Sima-1, BM COT (95 BIL), AV- 3670, Stonevile-213(EC 137805), BJR-JK-97-16-4(IC-359059), NH-545, CIPT-1080(A), IC-357218, LH- 2076, IC-358429, COTOM-9, NH 630 and IC 358280. Crossed seeds were collected from diallel crosses for further evaluation.

Notification of CNHO 12 (Saraswati)

A new variety CNHO 12 (Saraswati) developed by CICR, Nagpur, identified in 2009 for the irrigated, timely sown condition of Central zone was notified in the Gazette of India in the year 2010-11 vide Notification No. SO -733 (E), dt. 01.04.2010.

New entries sponsored under AICCIP

Six new advance selection of *G. hirsutum* namely, CNH 14, CNH 44, CNH 50, CNH 1107, CSH 121 and CSH 1111 were sponsored for Initial Evaluation Trials of AICCIP Br. 02(a/b). Three entries namely, CNHO 12, CNH 1108 and CNH 1109, were also sponsored for compact plant type trial Br. 06(a/b).

Population improvement

Random mating population in *G. arboreum* and *G. hirsutum*

After fifth cycle of random-mating through conventional crossing, the population is being constituted and maintained by bulk harvesting one busted boll from each plant. A composite population was constituted and grown on large size plots. Gain in economic yield was compared with the base population. Gain in seed cotton yield was realized to the extent of 57% in *G. arboreum* despite bad crop conditions during 2010-11. In *G. hirsutum*, the corresponding gain in seed cotton yield was 42%.

GMS based random mating population

A composite population has been constituted from the seeds obtained from out-crossed bolls and a large population (each in an area of 2000 sq.m.) was grown in 2010-11. At flowering, the individual plant in the population was checked for sterility/fertility at anthesis at an interval of a week and tagged all sterile plants. The segregation for fertile and sterile plant was monitored throughout the crop season. All the out-crossed bolls from the sterile plants in the population were bulk harvested and ginned to constitute a next cycle of GMS based random mating population. A 5th cycle of GMS based random mating has been completed in *G. arboreum* while 4th cycle has been completed in *G. hirsutum*.

Single plant selection

Based on manual testing of fibre length and strength 371 plant

in *G. arboreum* and 87 plants in *G. hirsutum* composite random mating population were selected. About 201 single plant selections from GMS based plant progenies and 121 from conventional crossing based random mating plant progenies were re-selected for further evaluation that include plants with big boll size, better fibre quality and compact plant type. Selections are in the early segregating stage and need to be further evaluated in advance generations.

Coimbatore

Evaluation of advance selections

Two superior cultures viz., CCH 820 (Long Staple Culture) and CCH 10-1 (Medium Staple Culture) were sponsored for IET of AICCIP under irrigated conditions during 2010-11. Performance of both the cultures was not in top five and have not been promoted. Three other superior cultures viz., CCH 10-2, CCH 10-3 and CCH 807 were tested under rainfed situation in IET of AICCIP. Though the fibre quality of these cultures were superior to check varieties they could not be promoted as there was no yield advantage.

Culture CCH 2623 was tested in the Coordinated Varietal Trial under irrigated conditions in both central and South Zone. In Central Zone, CCH 2623 recorded a mean seed cotton yield of 1910 kg/ha and was superior to Zonal check LRA 5166 (1489 kg/ha). It recorded a mean ginning out turn of 34%. In South Zone, the culture CCH 2623 recorded a mean seed cotton yield of 1515 kg/ha and was superior to Zonal Check LRA 5166 (1277 kg/ha) and ranked at 5th position (Table 8). With a mean ginning out turn of 38% and lint yield 541 kg/ha, it ranked third among the entries. The culture has been retained in Coordinated Varietal Trial for one more year of testing in both Central and South Zones.

Table 8 : Performance of CCH 2623 for Seed Cotton Yield (kg/ha) in AICCIP Trials under irrigated conditions.

Culture	IET (2008-09)	PVT (2009-10)	CVT (2010-11)	Mean	% inc over ZC
Central Zone					
CCH 2623	1995 (1)	1932 (1)	1910 (9)	1945	43
Zonal Check	1226 (17)	1360 (8)	1489 (11)	1358	
Local Check	1610 (16)	1745 (6)	2022 (4)	1792	
South Zone					
CCH 2623	1911 (2)	1834 (2)	1515 (5)	1753	27
Zonal Check	1537 (31)	1338 (8)	1277 (11)	1384	
Local Check	1758 (10)	1289 (10)	1584 (3)	1543	

Figures in parenthesis indicate Ranking

Sirsa

Evaluation of CLCuV resistant cultures

Forty-seven *G. hirsutum* hybrids were evaluated for seed cotton yield, ginning outturn, fibre traits and resistance/susceptibility to CLCuV against the check variety RS 2013 and CLCuV susceptible variety RS 921. The highest seed cotton yield was recorded in the hybrid CSH 2936 (2723 kg/ha) followed by CSH 2938 (2603 kg/ha) as against the check variety RS 2013 (2135 kg/ha). Maximum ginning outturn of 35.7 per cent was recorded in the hybrids CSH 2816 and CSH 2916 as compared to local check varieties RS 2013 (31.8%) and RS 921 (32.2%). The culture CSH 2939 recorded the highest 2.5 % span length (30.1 mm), whereas the highest bundle strength of 24.9 g/tex was observed in CSH 2939 and

4.7: Development of Transgenics

Nagpur

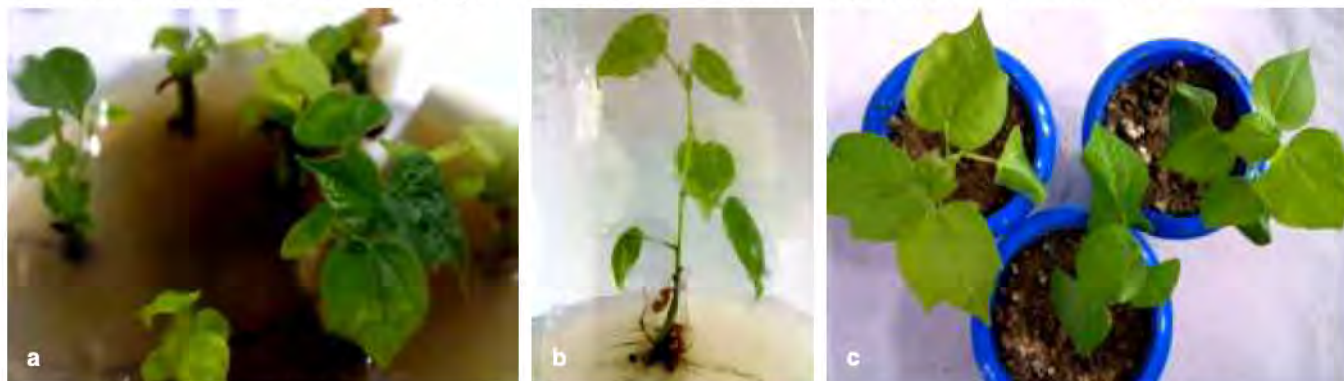
Bollworm resistant transgenic cotton in tetraploid and diploid cotton

Permission was obtained from RCGM/GEAC to conduct event selection trial of 7 T₅ events of *G. hirsutum* variety LRK 516 (ILK Bt 77-1 to 7), 3-events of T₂ generation (Anjali AcBt-1) having Bt *cry1Ac* and 2 T₁ events of Anjali (FBT-1 & 2) having Bt *cry1F*. As per RCGM / GEAC guidelines, confined field trials were conducted and data were recorded at 40 and 90 days of plant growth. The protein expression ranged from 1.05 to 2.65 ppm at 40 days after sowing (DAS) and 1.04 to 3.50 ppm at 90 DAS. The occurrence of beneficial insects i.e. ladybird beetle, spider

etc. and non-target insects especially sucking pests (jassids, thrips, aphids and whitefly) on Bt and non-Bt plants were recorded. Off season sowing of LRK 516 *cry1Ac* Bt (ILK-Bt-77) and Anjali FBT was undertaken for multiplication of seeds and to test Cry protein expression.

New transformed events

New transformation events were generated using *G. hirsutum* cultivars viz., Suraj and LRA 5166 with *cry1Ac* gene and *cry1F* genes. Total 3864 embryonic explants were subjected to Agrobacterium mediated transformation and 33 primary putative transformants were selected in the kanamycin medium. Six independent transformants of LRA 5166 carrying Bt *cry1Ac* and 8 transformants with Bt *cry1F* showed transgene integration by gene specific PCR amplification.



a) LRA 5166 plants under selection media

b) Rooted complete plant

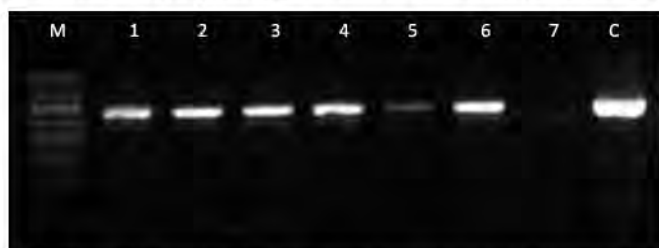
c) Established plants

G. hirsutum variety viz. LRA 5166 was also transformed with *cryIAa3*, *cryIIa5* and *cryIF*. A total of 70 putative transformants with *cryIF* construct, 35 with *cryIIa5*, 35 with *cryIAa3* were obtained.

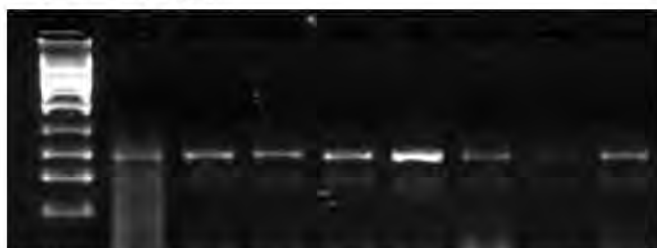
Pollen tube pathway transformation

A new method of gene transfer was standardized to overcome

genotype dependent somatic embryogenesis in cotton. *G. hirsutum* variety Suraj was subjected to pollen tube pathway transformation using Bt *cry1Ac* and *cry1F* genes. Total of 1340 flowers were effected for transformation, out of which 96 bolls were set and harvested. Transformed plants were confirmed for gene integration using gene specific primers for PCR amplification (Fig.4).



M: Marker, C: control, 1-7: putative transformants carrying Bt *cry1Ac* gene



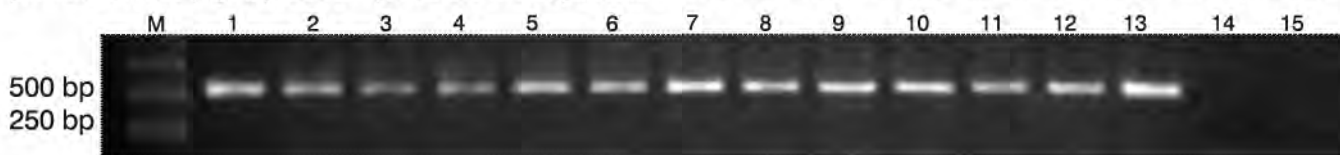
M: Marker, C: control, 1-7: putative transformants carrying Bt *cry1F* gene

Fig. 4 : Transformed plants through pollen tube pathway

Molecular Characterization of transgenic events

Four events of RG 8, six events of PA 255 and four events of PA 402 of *G. arboreum* containing *cryIAc*, *cryIAa3* and *cryIF* genes were raised in contained facility. One hundred sixty three plants of Bt RG8 (*cryIAc*) were subjected to PCR amplification

using gene specific primers of which 38 plants were found to be positive. In PA 255 Bt, 25 plants were PCR tested of which 7 plants were PCR positive (Fig.5). The PCR positive plants of Bt RG 8 and Bt PA 255 were selfed and individual bolls were harvested separately for further evaluation and multiplication.



M, 1 Kb Ladder, 1-12, +ve transgenic plants, 13, Bunny Bt +ve sample, 14, Non-transgenic RG8 sample, 15-PCR -ve.

Fig. 5 : PCR amplification of *cryIAc* in Bt RG 8-16 progenies

Bt expression in Bt RG8 and Bt PA 255

Leaf samples from PCR positive Bt RG 8 and Bt PA 255 were collected and subjected to ELISA test. The Bt protein expression was estimated in the range of 3.05 to 4.3 ppm in Bt RG 8-515 progenies. In Bt RG8-16 progenies, protein expression was estimated in the range of 3.05 to 5.6 ppm. These PCR positive plants hold promise for further

advancement (Table 9). The Bt protein expression of Bt RG8-109 was recorded in the range of 3.0 to 4.6 ppm, however, none of the plant was PCR positive. In Bt PA255 progenies containing cryIAc gene, protein expression was observed in the range of 2.0 to 3.0 ppm. Seven plants were found PCR positive (Fig. 6).



M, 1 Kb Ladder, 1-9, +ve transgenic plants, 10, Bunny Bt +ve sample, 11, Non-transgenic RG 8 sample, 12-PCR -ve.

Fig. 6: PCR amplification of *cryIAc* in Bt PA 255

Insect Bioassay

Bioassay studies were conducted in 35 Bt RG 8 plants. Insects were allowed to feed for three days continuously with Bunny Bt as a positive control and non Bt RG 8 as negative control. Progenies of plants no. 515 appeared to be resistant as evident from less feeding by insect.

Table-9: Protein expression in different events

Genotype	Gene	Range of Bt protein (ppm)
RG8-515	<i>cryIAc</i>	3.05-4.3
RG8-16	<i>cryIAc</i>	3.07-5.6
RG8-109	<i>cryIAc</i>	3.0-4.6
PA 255	<i>cryIAc</i>	2.0-3.0



Bt RG8-515



Bunny Bt (+ve control)



-ve control (non Bt RG 8)

Insect bioassay of Bt and non-Bt RG 8.

Transformation with Chitinase

Transformation was carried in *G. arboreum* cv PA 255 by *Agrobacterium* containing chitinase gene. In all, 142 T₁ progenies from selfed bolls were raised. Twenty-three of the 47 T₁ plants obtained from plant no.1(T₀) were PCR positive while 17 of the 45 plants obtained from plant No.2 (T₀) were PCR positive (Fig. 7). Few of the plants tested for Southern analysis showed single gene insertion (Fig. 8). Assay of Chitinase activity carried out in Bt and non-Bt plants showed significant difference for Chitinase activity (Fig. 9).

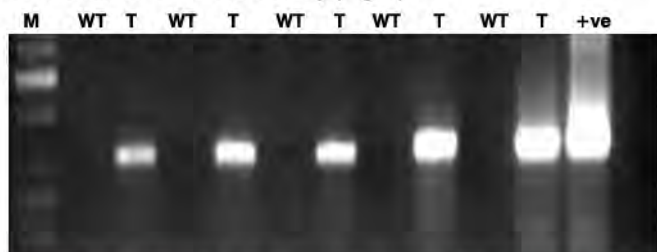


Fig. 7: PCR confirmation of chitinase gene in putative transformants

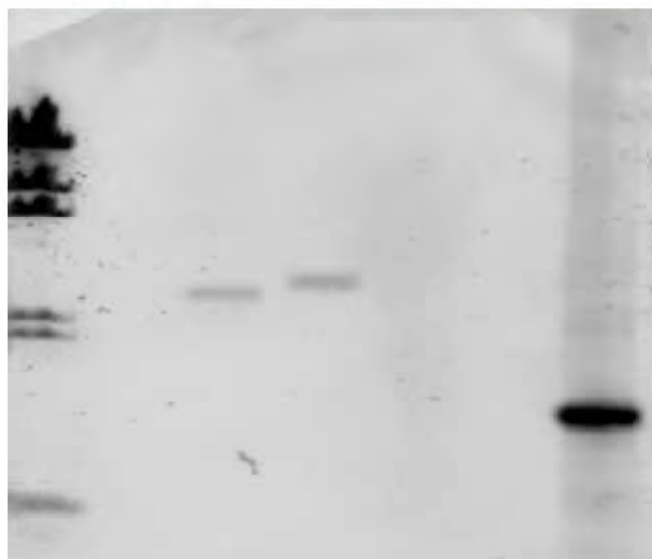


Fig. 8: Southern analysis of chitinase gene in putative transformants

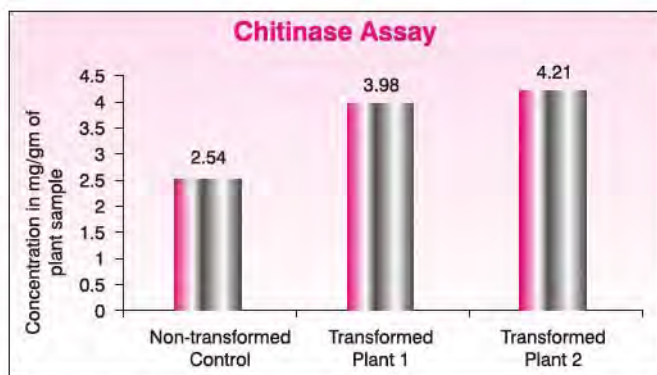


Fig. 9 : Chitinase activity assay



Evaluation of resistance of chitinase transgenic *G. arboreum* PA 255 against *R. areola*

New *in-planta* transformation technique

One hundred twenty five new events by new *in-planta* transformation technique were established containing *cry1Ac*, leaf curl virus and chitinase gene. Thirty-nine plants were tested PCR positive. Fifty three boll to row progenies containing *cry1Ac* and *cry1F* gene have been sown in the field during summer of 2011.

Transgenics for CLCuV resistance : Antisense approach

Transgenic plants of the three genotypes H 777, HS 6 and F 846 with Sense coat protein, Antisense coat protein and Antisense replicase protein genes were grown in the contained field trial of RCGM for event selection. Twenty-eight (28) transgenic events viz., HS6 (ARep)- 3, HS6 (ACP)- 4, HS6 (SCP)-3, H777(ARep)-2, H777(SCP)-2, F 846 (ARep)-5, F846(ACP)-4, F846(SCP)-5 were grown in the screening nursery and 62 plants were found to be free of disease till harvesting stage. The bolls from these plants were collected separately for further evaluation. The transgenic plants were reconfirmed for the presence of the gene by PCR and RT-PCR using gene specific primers.

RNA interference approach

Four gene constructs AC2, CP, β C1 and β V4 were used through *Agrobacterium* mediated transformation and the transformation frequency in different gene construct was found in the range of 7.4 to 11.7% (Table-10). The transformed plants were confirmed by PCR for the presence of gene (Fig.10).



M-100 bp ladder, 1-12 HS6-AC2-SA transgenic plant, 13- pBSK-AC2-SA plasmid +ve, 14-HS6 non-transgenic -ve, 15- PCR -ve sample.

Fig. 10 : PCR Confirmation of putative transgenic HS6-AC2 plants by AC2-Sense primer.

Inverted repeat constructs	No. of explants co-cultivated	No. of explants selected in kanamycin	No. of regenerating shoots	Transformation frequency on kanamycin (%)
AC2	155	18	09	11.6
CP	390	29	25	7.4
β C1	120	10	09	8.3
β V4	224	25	13	11.7

Table 10 : Frequency of transformation of *Gossypium hirsutum* cv HS6

Genetic engineering for abiotic stress tolerance

Transgenics were developed in the elite genotypes LRA 5166 and LRK 516 with *DREB 1A* and *PLEA1:BcZF1* gene. The transgenics were confirmed for the presence of the gene by PCR using specific primers for *DREB 1A* and *npt II*. Sixteen transgenic plants of LRA 5166 (5 of *DREB 1A* and 3 of *BcZF1*) and LRK 516 (6 of *DREB 1A* and 2 of *BcZF1*) with *DREB 1A* and *BcZF1* were raised in pots along with one control plant of LRA5166 and LRK 516. The plants were grown under optimum growth conditions till flowering. Moisture was withheld at 75 days after sowing and plants were allowed to grow under receding moisture. The leaves of both non-transformed and transformed plant started drooping after seven days after stress induction. The transformed plants showed recovery during night and leaves were turgid in early part of the day. This helped the transformed plants to recover and grow normally.

Alternately, the leaf discs from non-stressed and both transformed and non-transformed plants were placed on PEG medium with varying degrees of stress (0, 0.4, 0.6, and 0.8 MPa). The biochemical changes induced due to stress was quantified at 0, 7 and 15 days after inoculation. The control plants showed an immediate burst in synthesis of reducing sugars, amino acids and proline, but declined by seven days, while the transformed plants showed a gradual increase in solute accumulation and was high even after 7 days. The non-transformed discs produced very high phenolics which leads to death of the tissues with stress.

RT-PCR study

The mRNA from transgenic plants were isolated and cDNA was synthesized using the transcriptor high fidelity cDNA synthesis Roche kit. The cDNA of the transgenic plants were used for amplification with *npt II* primer and *DREB 1A* gene specific primer. The amplified product was electrophoresed by agarose gel electrophoresis (Fig.11).



Fig.11 : Transformants with *DREB 1A* gene. L-100bp ladder, 1:P-plasmid, Lane 2-3 : Transgenic plants (LRA 5166) with *DREB 1A* gene. Lane 4:Non transformed plant, Lane 5-7: cDNA samples transgenic plants (LRK 516) with *DREB 1A* gene.

Isolation of seed specific promoter

Using known available sequences, primers were designed for Alpha globulin gene promoter (AGP) and PCR amplified from all four cultivated cotton species. The sequence analysis showed no variation in the amplified promoter sequence

across the species except for few bases. Genome Walker method (Clontech Palo Alto, CA, USA) was performed to obtain sequences of the promoter upstream of the published sequence. Genomic DNA was isolated from seed sample and genome walker library was constructed. Library was used for nested PCR using adopter and gene specific primers. The desired band (Fig,12) from the gel was eluted, cloned in to pGEM-T easy vector (Promega, Madison, WI, USA) and sequenced. Further, the unknown upstream sequence was PCR amplified using forward primer from the unknown sequence and reverse primer from the known sequence that resulted in expected fragment of (650bp) (Fig.13).

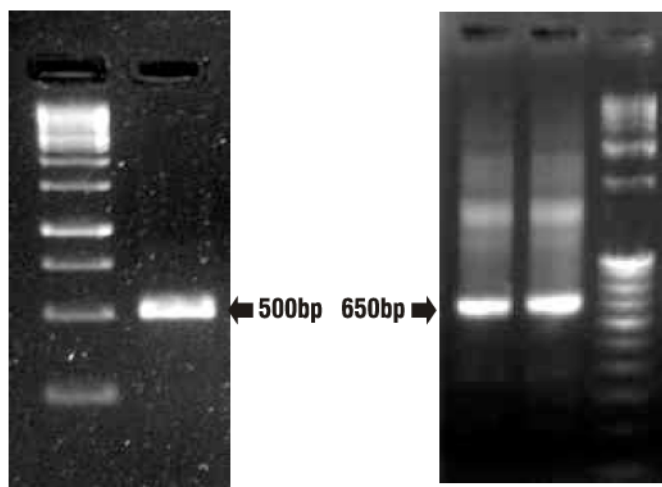


Fig.12 : Secondary PCR eluted product from Genome walking

Fig.13 : PCR confirmation of unknown upstream sequence

Isolation of genes involved in gossypol biosynthesis

Primers were designed to amplify 375 bp conserved target sequences of δ cadinene synthase gene. The selected portion of the clone showed 78– 99 % homology to several other published sequences of δ cadinene synthase genes. Total RNA from matured embryo was reverse transcribed to obtain cDNA pool. δ cadinene synthase gene (375bp) was PCR amplified, sequenced and sequence was confirmed through homology search.

RNAi vector construction

pBSK vector with intron sequence was used to construct Intron hairpin loop RNAi vector. The δ cadinene synthase gene sequence was analyzed and specific enzymes were employed to clone sense and antisense strands of δ cadinene synthase individually on either side of the intron sequence in plasmid pBSK-int creating inverted repeat construct. The gene was PCR amplified in both sense and antisense orientation and cloned in to the pBSK-int vector. Also, the target sequence from delta-cadinene hydroxylase was PCR amplified and sequenced.

Impact of Bt Cotton on poultry

Performance of White Leghorn layers under feeding of Bt and Non Bt cotton seed

Results of the experiment revealed that there was no significant difference in live body weight, egg weight, egg length and egg width between the layer which were fed with concentrate comprising Bt cotton seed cake, non Bt cotton seed cake and no cotton seed cake. Bt no. of eggs laid was significantly high in the group fed with concentrate comprising non Bt cotton

seed cake. Similarly shell weight was also high in this group when compared with other groups. It was also found that crude protein content was comparatively high in Bt cotton seed cake than non Bt cotton seed cake.

4.8 : Seed Production and Seed Quality Improvement

Nagpur

Agro-techniques for enhancing productivity and seed quality

Four growth hormones (Primary messengers, growth retardant, secondary messenger and antioxidant) were tested on high yielding early maturing variety of *G. hirsutum*, NH 615. Foliar application of Salicylic acid @ 0.25% at flower initiation (58 DAS) and 15 days after flowering (73 DAS) gave significantly high seed yield over control. Spray of gibberillic acid @ 50 ppm at square initiation (40 DAS) and at flower initiation (58 DAS) as well as foliar application of secondary messenger at flower initiation (58 DAS), 15 days after flowering (73 DAS) and 30 days after flowering (88 DAS) produced seeds with significantly higher seed index (7.86 g) compared to control (6.22 g). In another experiment conducted on late variety Narasimha, foliar application of primary messenger (Cytokinin) @25 ppm at square initiation (55 DAS) and at flower initiation (72 DAS) along with foliar application of nutrients (P, K,Mg) at 15 (87 DAS) and 30 days after flowering (102 DAS) and foliar application of secondary messenger (CaCl₂) @ 0.75% at square initiation (55 DAS), 10 days after flowering (82 DAS) and 20 days after flowering (92 DAS) gave significantly higher seed yield over control. Foliar application of secondary messenger application at flower initiation (72 DAS), 15 days after flowering (87 DAS) and 30 days after flowering (102 DAS) gave significantly higher seed index over control. In the third experiment, foliar spray of triconanol @0.01% along with CaCl₂ @ 0.75% gave highest seed cotton yield as well as highest seed index compared to control.

DUS Characterization of working and core germplasm collection

A total of 352 germplasm lines belonging to eleven working groups as well as 530 lines of core collection have been characterized based on DUS traits. The working collection lines were observed for 27 DUS characters including seedling, flower, leaf and boll characters as well as on variation in number of leaf gossypol glands. Significant variation in gossypol glands density on leaves hold promise for consideration as a special character in DUS testing.



Testing & documentation of extant varieties, hybrids and their parents for DUS

Thirty-six candidate varieties/hybrids along with twenty-eight reference varieties including those of *G. hirsutum* and *G. arboreum* were raised. Complete thirty-seven total characters were recorded as per the DUS guidelines.

Coimbatore

Implementation of PVP legislation, 2001

DUS testing of tetraploid cotton genotypes were taken up in two trials comprising 23 and 19 candidate varieties. Similar trial was conducted for diploid cotton with one candidate and two reference varieties.

Registration of extant and new cotton varieties under PPV&FR Act, 2001 was initiated. In total, 87 application forms comprising of new and extant cotton varieties were submitted to PPV&FRA through NBPGR. Seeds of three varieties was deposited with national gene bank and the process of augmentation of seed for other 37 varieties from different cotton breeding stations is in progress.

Maintenance of reference collection assumes importance for the successful conduct of DUS test. One hundred and twenty two *G. hirsutum*, nine *G. barbadense*, three *G. arboreum* genotypes were sown during winter 2010 season. In all the varieties, off type plants were removed at flowering and selfed bolls were harvested. Harvested bolls were ginned and seeds were stored as reference varieties. A database on varieties released by State Varietal Release Committee, varieties in common knowledge, farmer's varieties, etc., was updated.

Establishment of genetic purity of hybrids using seed protein profile

Electrophoretic analysis of seed proteins was performed using Tris soluble proteins, salt soluble Globulins and Methanol precipitated fraction; separated by SDS-PAGE method for a hybrid CSHH-243 and its parents. Among the three methods, electrophoretic estimation using Salt Soluble Globulins gave promising results.

Sirsa

Table-13 : Maintenance of Nucleus and Breeder seeds

S.No	Variety	Quantity (kg)
1	CSHH 198 (F)	25
2	(M)	16
3	CSHH 243 (F)	15
4	(M)	10
5	CSHH 238 (F)	20
6	(M)	15
7	CICR 2 (F)	37
8	(M)	20
9	CISA 310	60
10	CISA 614	50

Seed Production

Nagpur

Table 11: Seed Production during 2010-11 under Mega Seed Project of ICAR

Crop	Stage	Production (in quintals)
Cotton -20 varieties (<i>G.hirsutum</i> & <i>G.arboreum</i>)	TFL	32.225
Soyabean cv. JS-335	CS	4.41
Red Gram-BSMR	TFL	15.49
Gram-Jali 9218	FS	13.51

Coimbatore

Breeder Seed Production

Table-12 : Breeder seeds produced and distributed during the year 2010-11.

Variety	Seed Production (kg)
LRA 5166	99
Supriya	9
MCU 5 VT	48
Surabhi	114
Suraj	42
Supriya	9

