

3. Research Achievements



3.1: Cotton Genetic Resources

Nagpur

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

Collection and maintenance of germplasm

A germplasm collection of 10,597 accessions were maintained in the Gene Bank of the Institute. This included 44 new germplasm and 42 new land races of cultivated varieties and perennial cotton that were added during the season.

Enrichment of Cotton Gene bank

Seeds of wild species, races of cultivated species and exotic germplasms of cotton collected from different states or procured from other countries were added to the Gene bank. Two representatives of *Gossypium australe*, EC 735740 - variety GOS 5014 and EC 735741 - GOS 5001 were procured from CSIRO, Plant Industry Narrabri, Australia. Besides, two commercial varieties of

G. hirsutum viz., IMACD 05-8221 and IMACD- 408, used for cultivation in high density planting system (HDPS) in Brazil were also procured. Later two genotypes possessed compact plant stature with short internode length and could serve as suitable sources for breeding dwarf and compact cultivars, amenable to HDPS.

Unique *Gossypium hirsutum* cultures registered

Four introgressed derivatives with unique traits were registered with NBPGR, New Delhi. (Table 3.1.1). The registered lines belonged to medium staple length category and possessed excellent uniformity ratio. The registered cultures were highly tolerant to jassids. Additionally, Vaidehi 95 – a dark brown introgressed genotype was also tolerant to CLCuV. Besides possessing aesthetic attribute, Vaidehi 95 also showed colour fastness on storage. The three other introgressed derivatives NISC 40, 43 and 44 being compact in nature and relatively pest tolerant are suitable for HDPS and also for breeding genotypes suitable for HDPS.

Table 3.1.1: Introgressed derivatives of cotton registered with NBPGR

S. No.	Name of the line	Unique character	Registration number
1	Vaidehi 95 (MSH- 53)	Dark brown linted introgressed derivative	National ID: IC0584260 Registration No: INGR13032
2	NISC - 40	Jassid tolerant compact introgressed derivative	ID: IC0584261 Registration No: INGR13033
3	NISC - 43	Jassid tolerant compact introgressed derivative	National ID: IC0584262 Registration No: INGR13034
4	NISC - 44	Jassid Tolerant Compact Plant Type Introgressed Derivatives	National ID: IC0584263 Registration No: INGR13035



Jassid tolerant Introgressed *G. hirsutum* genotype NISC-40 (left) and susceptible genotype LRA5166 (right)

Evaluation of germplasm

G. hirsutum

Three thousand four hundred eighty two (3482) germplasm accessions were evaluated for fibre quality traits at CICR Nagpur and CICR Regional Station, Coimbatore. Nineteen long linted accessions (staple length 30-33mm) and 10 high fibre strength accessions (25.2 -26.7 g/tex) were identified and utilized in breeding programme and also distributed among breeders. Five thousand germplasm accessions were evaluated for tolerance against waterlogging and 150 tolerant accessions were identified.

G. arboreum

Fibre quality traits of six *G. arboreum* races including

burmanicum, cernuum, indicum, bengalense, sinense and soudanense were evaluated (Table 3.1.2). Fibre quality of six arboreum races however did not show significant variation. All races possessed medium staple cotton with fibre length ranging from 22.7-24.3 mm, bundle strength 16.5-18.5 g/tex, uniformity ratio 49-54% and fineness ranging from 4.4-5.1 μ .

G. herbaceum

Base collection of *Gossypium herbaceum* consisting 565 accessions were grown at Main Cotton Research Station, Surat and ARS, Bharuch for rejuvenation and evaluation. Some of the promising germplasm lines with good agronomic traits are listed in Table (Table 3.1.2).

Table 3.1.2 : Trait-wise listing of promising *G. herbaceum* germplasm

Early maturing accessions (180 days)	DB- 8 (IC 371095), DB-3-12 (IC 371098), DBSB-4-7 (IC 371107), DCB 423-3-1 (IC 371448), SLG-11 (IC 371524), KAPO 1/36 (IC 371582)
Big boll and high boll weight group (2.5-3.3 g)	Digvijay (Pune) (IC 371116), E-2-12-5 (IC 371120), 27-60 (IC 371326), KAPO 1/20 (IC 371572), KAPO 1/36 (IC 371582)
Pigmented plant body with high yield potential	DB-3-12-8 (IC 371104), 6199-SS (IC 371360), 7182-1-1 (TP-1) (IC 371362)
Complete locule opening and easy to pick	E2-12-5 (IC 371120), DCB 423-3-1 (IC 371448), SLG-11 (IC 371524)

Gossypol estimation

Gossypol was estimated in 100 cotyledonary leaf samples and 670 seed samples. Though, on an average, leaf gossypol content was low, some lines showed leaf gossypol in the range of 0.7-1.4%.

Breeding for genotypes suitable for surgical cotton

A new programme was initiated on development of promising *G. arboreum* genotypes with high micronaire, suitable for surgical industry. For the purpose 20 selected germplasm lines with high micronaire and big bolls, were used in crossing programme. The seeds obtained from the cross were collected for evaluation and selection of suitable plants in next generation.

At Sirsa 48 *G. arboreum* improved lines were evaluated for yield potential and fibre properties. Ten promising cultures were found highly suitable for surgical properties with micronaire >7, absorbance 1.1 second, sinking time 1.7 second, water-holding capacity >23 g and ash content between 0.22-0.45 %.

Naturally coloured cotton

Four single plants were selected from each of the four different crosses developed, involving *G. arboreum* race

cernuum (having high boll weight and GOT) and *G. arboreum* races indicum and bengalense (brown-linted). Cultures in F₄ generation were evaluated for fiber properties, seed cotton yield and yield contributing traits. Each of the 4 single plant selections possessed brown-linted, medium staple cotton. While CNA 405 was characterized by narrow leaf lobes, CNA 406 had broad lobed-leaf. CNA 407 had pigmented plant body and narrow-lobed leaf and CAN 416 –LB had long and elliptical bolls, coupled with high boll weight (5.2g) and high ginning out turn (40.7%).

Exploration for perennial trees and land races of cultivated cotton

Exploratory surveys were conducted in Andhra Pradesh, Maharashtra, Tripura, Gujarat and Tamil Nadu for collection of perennial trees and land races of cotton. Fifty cotton germplasm materials including 14 perennials, 22 landraces and 14 traditional cultivars belonging to *G. arboreum*, *G. herbaceum* and *G. barbadense* were collected (Table 3.1.3). Exploratories were established in the pot culture and their seeds stored in the Gene bank. Ten unique germplasms were established in a newly developed perennial species garden.

Table 3.1.3 : Perennials and landraces of cotton collected from different regions of India

S. No	Districts	State	No. of Accessions	Species	Annual/Perennial/Landrace
1.	Vishakapatnam, Vizianagaram & Srikakulam	Andhra Pradesh	2	<i>G. arboreum</i>	Perennials
			1	<i>G. arboreum</i> race <i>indicum</i>	Landrace
			1	<i>G. barbadense</i>	Perennial
2.	Bhandara, Nagpur, Wardha	Maharashtra	2	<i>G. arboreum</i>	Perennials
			5	<i>G. barbadense</i>	
3.	Gomati, West Tripura, Kowai and Dhalai	Tripura	10	<i>G. arboreum</i>	Primitive traditional cultivar (Annuals)
			4	<i>G. barbadense</i>	Perennials
4.	Bharuch, Bhavnagar, Rajkot, Ahmedabad, Surendranagar & Banaskantha	Gujarat	7	<i>G. herbaceum</i>	Annuals
			4	<i>G. herbaceum</i> (Wagad/Kalyan)	Landraces
			5	<i>G. arboreum</i>	Annuals
			1	<i>G. arboreum</i> (Mathio cotton)	Land race
			6	<i>G. arboreum</i> (Karunganni cotton)	Landraces
5.	Tuticorin	Tamil Nadu	1	<i>G. arboreum</i>	Annual
			1	<i>G. herbaceum</i> (Uppam Cotton)	Landrace
			50	Total	



Ponduru cotton collected from Ponduru mandal, Srikakulam district, Andhra Pradesh as a individual plant, its flower and bolls in the field condition



Uppam cotton (*G. herbaceum*) with flowers and bolls collected from Kovilpatti, Tamil Nadu



Karuganni cotton (*G. arboreum*) with flower and bolls collected from Kovilpatti, Tamil Nadu



Wagad cotton (*G. arboreum*) with closed boll type and round shape collected from Patdi, Gujarat



Wagad cotton with closed boll type and round shape collected from Patdi, Gujarat

Fiber quality traits of perennial and land races of cotton

Fibre quality traits of 19 morphologically distinct *G. arboreum* and perennial *G. barbadense* germplasm materials were evaluated. Staple length of perennial *G. barbadense* cotton ranged from 32.6 – 34.9 mm while that of *G. arboreum* ranged from 17.2-19.1 mm. High micronaire *G. barbadense* (5.8 - 6.4 μ) materials can serve as source for improvement of fibre quality of ELS cotton. Development of absorbent *G. arboreum* cotton suitable for surgical industry is possible with high micronaire *G. arboreum* (> 7.0 μ).

Coimbatore

G. hirsutum

About 450 *G. hirsutum* germplasm lines maintained at the station were rejuvenated and characterized for yield and yield contributing traits. A batch of 1532 germplasm accessions received from CICR were rejuvenated and reevaluated for fiber quality. The 2.5% span length of the germplasm ranged from 18.9 mm to 34.5 mm and bundle strength ranged from 9.2 to 26.4 g/tex.

G. barbadense

Twenty seven *G. barbadense* germplasm were assessed for earliness and seed cotton yield along with the check Suvin. Fourteen genotypes were found to be early maturing (153-159 days) compared to Suvin which matured in 210 days. Besides, 26 long staple genotypes, 18 high strength genotypes and 36 genotypes with high micronaire value were identified.

3.2 : Hybrid Cotton

Nagpur

Development and maintenance of male sterile cotton

One hundred and thirty seven CMS (*harknessii* based),

15 CMS (*G. aridum* based), 19 GMS lines, 57 restorer and one GMS line developed through induced mutation were maintained. Few promising progenies of male sterile plants in M_4 generation were maintained by sibmating

(GMS) while the CMS progenies were maintained by crossing with maintainer lines (CMS). Some mutagenized GMS progenies showed significant variation in morphological characters compared to parental lines. Progenies with big bolls, round and elliptical shape, long pedicel, broad bracts, cluster bearing and plants with two squares at each node were identified.

GMS version of a number of commercial cultivars were developed. Four single plant progenies were raised each for PKV 081, AK 32, Suman, Sharda and L 147. Progenies were tested for segregation of male sterility and fertility in 1:1 ratio followed by maintenance of GMS cotton through sibmating. These GMS lines are now ready for use in hybrid development programme after testing the fibre quality. Two genotypes possessing *ms5ms6* alleles for genetic male sterility were selected. To identify molecular markers for genetic male sterility, fertile and male sterile counterparts of these two genotypes in six replicates were screened using 10 SSR markers available in public domain. None of these primers proved informative in differentiating fertile and male sterile lines. Protein profiling of three fertile lines and their sterile counterparts did not reveal any polymorphism, either.

Sirsa

The local adapted cultivars and parents of promising hybrids viz. CMS LRA 5166, CMS Jhorar, CMS RB 281, CMS LH 1134, CMS Pusa 31, CMS HS 6, CMS K 34007, CMS F 505, CMS F 1183, CMS CSH 25 M, and CMS SH 2379 lines possessing *G. harknessii* cytoplasm were maintained through sibmating. Ten Restorer lines were maintained through selfing. In addition, the GMS lines viz., K 34, J 34, MCU 5 and GMS 13 were maintained through selfing. Crosses were also attempted between GMS lines and good combiner lines.

Maintenance of restorer lines

The new restorer lines viz. CIR 8, CIR 12, CIR 15, CIR 23, CIR 26, CIR 32, CIR 38, CIR 47, CIR 70, CIR 72 were maintained through selfing. Additionally, new restorers CIR97P1, CIR97P3, CIR119P1, CIR119P3, CIR126P1, CIR526P1, CIR526P3, CIR 920 P1, CIR 926 P2, CIR 926 P3, CIR 1169 P1 and CIR 1169 P2 developed through Pedigree method of breeding were maintained through sibmating.

Conventional hybrid

Some promising *desi* cottons (*Gossypium arboreum*) are susceptible to Grey mildew caused by *Ramularia areola*. Besides, they possess small sized bolls and are prone to shattering due to low boll retention. *G. arboreum* race 'cernuum' registered earlier by the Institute possessed tolerance against some of these detriments. In order to incorporate resistance against grey mildew in susceptible *desi* cotton cultivars, crosses were effected between six *desi* cotton cultivars (AKA8401, AKA7, CNA1003, PA225, PA402 and Jawahar Tapi) and five *cernuum* cotton (30805, 30814, 30838, 30856 and G135-49). Thirty hybrids generated

involving two groups of cotton in all possible combination showed good combinability with significant heterosis in yield in majority of the crosses (Table 3.2.1). Percent heterosis varied among different hybrids with some showing very high levels of heterobeltiosis. All hybrids possessed big bolls, a trait apparently dominant in *cernuum* cotton and contributed by it. Besides improvement in yield, hybrids showed tolerance against grey mildew, early maturity and improved boll retention - traits conserved in *cernuum*. Heterobeltiosis as high as 106.54% was recorded in combination AKA7 x G135-49. Heterobeltiosis ranged from 50-106.5% in 4 crosses, 25-44.6% in 11 and 1.69-17-44 % in 5, out of 30 combinations.

Table 3.2.1: Heterosis in selected *Gossypium arboreum* hybrids

Parents and hybrids	Yield/plant (g)	Boll wt. (g)	Hetero-beltiosis (%)	Yield/acre (q)	Staple length (mm)	UR (%)	Mic (μ)	Strength (g/ tex)
AKA7 x G 135-49	93.1	3.3	106.5	13.8	22.8	53	6.1	19.9
AKA7	45.1	2.4		6.67	23.9	51	6.0	19.4
G 135-49	40.3	2.7		5.97	22.4	52	6.6	19.1
AKA8401 x 30838	121.0	5.5	86.15	17.9	21.6	52	6.6	17.2
AKA8401	65.0	2.6		9.66	27.7	48	5.3	19.2
30838	26.3	5.9		3.27	20.1	55	7	
J. Tapi x G135-49	66.2	3.3	64.13	9.79	22.4	54	6.8	19.4
J. Tapi	27.8	2.4		7.62	24.9	51	5.5	20.0
G13549	40.3	2.7		5.97	22.4	52	6.6	19.1
J. Tapi x 30856	59.4	4.0	50.0	8.79	20.1	55	6.7	17.8
J. Tapi	39.6	2.4		7.62	24.9	51	5.5	20.0
30856	26.4	4		3.90	17.8	57	7	



AKA 8401 30856 AKA 8401 X 30856
Conventional *Desi* Hybrid

3.3 : Genetic Improvement

Nagpur

G. arboreum (Diploid Cotton)

Identification of promising cultures

Twenty three single plant selections in F₅ generation made from crosses involving parental lines having desirable traits viz; boll weight, high yield, number of bolls per plant, fibre length and fibre strength were

evaluated. Nine segregating progenies were evaluated for seed cotton yield and boll weight.

Two promising cultures viz, CNA 370 and CNA 416 with high yield potential (17-19 q/ha), medium superior staple length (27 mm) and bundle strength (19-20 g/tex) were identified and developed for further evaluation in AICCIP trials.

Similarly, 29 newly developed cultures with improved productivity were evaluated under narrow spacing of 60 x 22.5 cm. Number of cultures with agronomically superior traits like high boll number (50), high yield potential (2000-2500 kg/ha), high boll weight (3.9 g), long staple length (29 mm) and high fibre strength (23.5-23.8 g/tex) were identified. Two promising high yielding cultures viz. CNA2020 and 2024 with 1700 and 1800 kg yield per ha respectively were identified. A promising *G. arboreum* cotton with staple length of 29 mm was improved for fibre strength using Dharwad Extra Long *arboreum* cotton (DLS). Advanced progeny in F₄ generation was identified with staple length of 29 mm and high fibre strength upto 23.8 g/tex.



Identified promising *G. arboreum* culture, CNA 2024

G. hirsutum (Tetraploid Cotton)

Drought tolerance

Three sets of experiment were conducted under rainfed and irrigated conditions to assess the performance and drought tolerance ability of developed cultures and genotypes. In the first set, twelve crosses in F_4 generation along with their parents were tested with drought tolerant check Rajat which recorded seed cotton yield of 1872.65 and 2396.54 kg/ha under rainfed and irrigated conditions. Eight genotypes were at par to check Rajat while three recorded more than 10% increase in seed cotton yield under rainfed conditions. Pusa 56-6 x 29 I topped in performance under rainfed situation, while 29 I x Pusa 56-4 excelled under irrigated conditions. Based on performance under abiotic stress and irrigated conditions, drought tolerant efficiency of genotypes (DTE) was worked out. Four crosses viz. 28 I x Pusa 56-4, 30 I x Pusa 56-6, 30 I x Pusa 56-4 and Pusa 56-4 x 28 I recorded more than 95%, while seven crosses recorded more than 91% DTE.

In the second set, eight cultures identified previously for drought tolerance (DTS 155, 62, 67, 39, 108, 100, 44, 104) and five lines found promising for yield, boll size and fibre quality (NHP1, NHP2, NHP3, NHP4, NHP5) to be utilized as potential candidates in crossing programme were evaluated along with LRA 5166 - moderately drought tolerant cotton. All the identified drought tolerant cultures recorded more than 15% yield over the check while among 5 promising lines, all the genotypes except NHP 5 were at par to the check with respect to seed cotton yield (1379.60 kg/ha) under rainfed condition. DTS 155 recorded ginning outturn of 41.54% with 124% higher lint yield over the check under rainfed condition followed by cultures DTS 62, DTS 44 and DTS 100. Besides, DTS 155 as a summer sown

crop did not show any sign of wilting even after more than 20 days of extremely dry period during the month of May.

Out of 52 drought tolerant selections evaluated in the third set, cultures DTS 78, DTS 76, DTS 75, DTS 70, DTS 72, DTS 92, DTS 91, DTS 71 performed significantly well under rainfed condition. Among these, lines with good fibre length, strength and high GOT were identified.

Besides, an entire set of 17 intercross genotypes were found superior to the check LRA 5166, in terms of yield under rainfed situation. Among them, drought tolerant selection DTS 46-04 performed best with seed cotton yield of 1492.61 kg/ha, boll weight of 3.6 g and GOT of 39.86 per cent. It was followed by DTS 52-02, DTS 1-11 and DTS 46-01 exhibiting more than 1000 kg/ha yield. From amongst eight advanced cultures tested in bigger plots, DTS 95, DTS 147, DTS 121, DTS 123 and DTS 3 were identified for evaluation in AICCIP Br 02(a & b) and Br 06 (a & b) trials.

A new cross was generated involving Moco cotton (*G. hirsutum* race *marie galante*) - known for drought tolerance as male parent and genotypes 28 I and Suraj as female parents. Seeds obtained from the crosses will be advanced for further evaluation and selection.



Response of drought tolerant cultures under extreme summer. a. DTS100, DTS108, LRA5166 and DTS104. b. DTS39, NISC50, 30I and DTS67

Jassid tolerance

In an effort to develop quality cotton with improved yield and jassid tolerance, breeding programme was initiated. Twenty F_6 lines were evaluated for seed cotton yield and fibre properties in randomized block design at a spacing of 45 x 20 cm. CNH 8002-6 (AKH 081 x CIPT 501) recorded highest seed cotton yield of 2183 kg/ha. This was followed by CNH 8005-3 (BM COT 88 x EC 560342) with 1959 and CNH 8003-3 (SR 107 x AKH 081) with 1924 kg/ha seed cotton yield, respectively. CNH 8002-6, besides documenting highest yield recorded staple length of 29.5 mm and fibre strength of 22.5 g/tex. Culture CNH 8004-2 combined promising seed cotton yield (1891 kg/ha) with improved fibre quality traits (staple length 29.8 mm ; bundle strength 23.2 g/tex). Besides high seed cotton yield and good fibre quality

traits, a number of cultures exhibited tolerance to jassids. At least 25 advanced cultures in F₆ generation were identified for jassid tolerance (Grade I, II) and sympodial plant types. Six cultures viz. CNH07-12, CNH 07-2, CNH 08-11, CNH 07-34, CNH 07-10 and CNH 08-55 were identified for earliness in maturity (145-150 days).



An early maturing *G. hirsutum* culture, CNH 2007-10 with Jassid tolerance and increased boll size

Breeding for yield, earliness and fiber quality

Development of *G. hirsutum* culture with big boll

A highly promising *Gossypium hirsutum* culture (Acala-B2 x Ganganagar Ageti) with big bolls ranging from 5.9-7.9 g in F₄ generation was developed. Besides possessing big bolls, it possessed staple length of 25.6 mm, strength 22 g/tex, with S /L ratio of 0.86 and appropriate fineness of micronaire 4.0. The culture has been approved by the Institute's Germplasm Identification Committee for registration as unique culture. Such big boll cultures have the potential to revolutionize productivity of Indian cotton to unprecedented levels especially if deployed under HDPS.



Promising *Gossypium hirsutum* cultures with big boll size

Early maturity

Number of cultures in F₄ generation documented promising seed cotton yields. Some of the high yielding

cultures included CNH 10-10 (EC 277959 × G 67), CNH 2-4 (G. Cot. 16 × EC 277959) and CNH 9-7 (EC 277959 × NH 615) that recorded seed cotton yield of 23, 19 and 15 q/ha respectively. Some cultures generated from crosses involving jassid tolerant wild species (*G. raimondii*) like CNH 8-20 -R (289-4 × EC 277959) and CNH 9-22-R (291 × P-56-6) not only exhibited earliness (140-145 days) but also recorded good yield potential and resistance to jassids.

In addition, 252 single plant selections made in F₄ generation of crosses involving parents showing earliness in maturity and that possessed good fibre properties, showed diverse but agronomically useful and good fibre quality traits like high seed cotton yield per plant, early maturity, high boll weight, high bundle strength, long staple etc. Single plant selections CNH 09-34, CNH-09-29 and CNH 09-1 recorded high seed cotton yield of 155, 137 and 116 g / plant respectively. Similarly, boll weights of selected cultures ranged from 4.0 g (CNH 09-10) to 7.1 g (CNH 09-7). Twenty-four single plant selections documented bundle strength of 24.0 g/tex and above, while five single plant selections recorded bundle strength of 25.0 g/tex and above. Besides, 15 single plant selections generated from the cross G 67 × P 56-4, recorded staple length ranging from 30.2 to 32.8 mm, while highest ginning outturn of 43.1% was recorded in CNH 09-28 (EC 277959 × NH 615). CNH 09-67(CIPT13× P 56-4) combined high fibre strength of 25.4 g/tex with long staple length of 32 mm. CNH 09-47 (G 67 × P-56-4) combined high bundle strength of 25.1 g/tex with long staple length of 31.0 mm and CNH 09-106 (FQ9 × MDR 8) recorded bundle strength of 25.3 g/tex and long staple of 28.8 mm with S/L ratio of 0.8, 0.8 and 0.9, respectively.

Improvement of fiber strength

Breeding of high strength *hirsutum* varieties with wider adaptability

To consolidate and develop high strength cotton, fibre quality of 22 advanced cultures with good fibre properties which were sponsored in AICCIP but were not promoted based on yield competence was revisited for further use in breeding. The strength of the materials tested ranged from 23.1 to 24.7 g/tex. CNH 1114 and CNH 1435 recorded bundle strength of 23.8 and 23.6 g/tex, respectively while CNH 1107 recorded fibre strength of 24.7g/tex and staple length of 29.6 mm with a S/L ratio of 0.83. CSH 3129 recorded staple length of 28.8 mm with bundle strength of 23.1g/tex.

Breeding for compact cotton

Breeding of cotton was initiated to develop compact genotypes suitable for HDPS. A short and compact *G. hirsutum* germplasm N 170 with short height (54 cm) and

short sympodia (9.2 cm) was chosen as female parent. It was crossed individually with three trait specific donors each for fibre strength, boll weight and sucking pest resistance. F₂s have been evolved.

Besides, conscious breeding of compact genotypes amenable to HDPS (45 x 15 cms), 84 genetically enhanced lines were evaluated under HDPS. Few selected lines generated were crosses between RS-875 x MHL-557, RS-810 x MHL-557, RS-B75 x Rex and RS-810 x JBWR-JK 54 were found free from sucking pests. Progeny of H-777 x Rex was found to be high yielding with good staple length and large boll weight.

Cultures SPS-HB-8-1, SPS-6-4 and SPS-6-17 recorded promising yields at 45 x 15 cm spacing. Under narrow spacing of 60 x 12 cm, a few promising backcross populations of PKV-081 x (PKV-081 x PIL-8) in BC3F5 recorded the highest plot yield of 1.9 kg/ 8.3 sq m. area.

Evaluation of genetically enhanced populations

A number of promising genotypes of cotton were subjected to enhancement of specific traits that they lacked. Some of the genotypes that were genetically enhanced drawing the specific trait from selected donor species included LRA 5166, PKV 081, LH 1134, G.Cot 10, LRK 516 etc. (Table 3.3.1).

Table 3.3.1 : Genetically enhanced varieties of cotton (*Gossypium hirsutum*)

S.No	Variety improved	Donor	Trait	Base value For the trait	Improved value
1	LRK-516	Deltapine-66	GOT (%)	36	41.6
2	PKV-081	PIL-8	Boll weight (g)	2.9	3.8
3	H 777	Rex	Boll weight (g)	2.8	3.6
4	LRA-5166	BAR-310	Fibre length (mm)	26	29
5	LRA-5166	CIHS-97-9	Sucking pest	Susceptible	Resistant
6	LH-1134	Reba vt9Lyy	Fibre Strength (g/tex)	18	23.8
7	RS-810	MHL-557	Fibre length (mm)	22	27
8	RS-810	JBWR-JK-54	Sucking pest	Moderately Susceptible	Resistant

Population Improvement

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

The randomly mated population generated through conventional crossing was maintained by bulk harvesting one bursted boll from each plant in both *G. arboreum* and *G. hirsutum*.

Random mating population developed through exploitation of GMS

The sixth cycle of GMS based random mating population was completed in *G. hirsutum*. In the composite population all sterile/fertile plants in the population were tagged at flowering and allowed open pollination. The out crossed bolls from all the sterile plants were bulk harvested to be raised in the next crop season. Similarly, seventh cycle of GMS based random mating was completed in *G. arboreum*.

Single plant selection

Nine hundred and ninety eight single plant selections from randomly mated population were evaluated in plant to row progeny plots. From the composite random mating population 253 plants were selected based on

manual testing for fibre quality traits. About 1200 superior single plants were reselected from the segregating single plant progenies. Several single plant selections were identified for big bolls, better fibre quality and compact plant types. Two progenies superior in fibre quality has been advanced to replicated trial.



Single plant selections for compact plant types (Zero Monopodia) and Big boll size from random mating population of *Gossypium hirsutum*

Development of heterotic pool for superior medium staple

From heterotic population developed based on geographic diversity and specific combinability, 18 breeding lines were identified. Nine lines 14-1-1, 14-6-4, 14-9-2, (PKVR X DHY 286); 7-3-3 (LRK 516 × Acala 1517); 12-2-3, 15-2-1, 15-5-3 (PKVR X Acala 1517) and 3-3-4, 3-3-2 (LRK 516 × DHY 286) were identified from the population of first heterotic group. From the second heterotic group, lines 12-2-3, 20-4-3 (Surabhi × H1252); 14-4-1 (Surabhi × ACCLD 163); 22-5-1 (LH 1948 × MCU 9); 22-4-1, 17-2-2, 17-3-2 (ACCLD 163 × H 1252) and 16-3-1 (Surabhi × LH 1948) were identified with improved fibre length, strength and seed cotton yield. These lines will be utilized for testing their superiority over parents for developing hybrids amongst them.

Several advanced lines in F₇ generation developed from heterotic pool were evaluated in non-replicated yield trial. Breeding line 7-3-3 recorded seed cotton yield of 24 q/ha followed by 15-5-3 (PKV-R × Acala 1517) with 15 q/ha and 19-3-2 (ACCLD 163 × MCU 9) with 13 q/ha.

Amongst 18 breeding lines of heterotic pool evaluated in replicated trial in high density (45 × 20 cm), four lines recorded seed cotton yield of 30 q/ha (CNH7008-1), 29 (CNH 7012-13), 28 (CNH 7012-6) and 27 q/ha (CNH 7012-11). Besides recording high seed cotton yield, CNH 7012-13 also combined desirable staple length (27.9 mm.) with fibre bundle strength (23.7 g/tex).

Evaluation of GMS based hybrids in *G. hirsutum*

To develop the heterotic pools of *G. hirsutum* cotton, 52 GMS hybrids along with parents were tested in RBD with three replications. The highest seed cotton yield was recorded in GMS-20 X MC 88 (1944kg/ha) followed by GMS-26x MC 88 and GMS-20 x SA 1652 (1773 kg/ha) as against 1698 kg/ha of conventional check hybrid LH 2076. Maximum ginning outturn of 36.5 % was recorded in hybrid GMS-17 x MC 88. From this experiment it was found that the male parents SA 1017, SA 1422, MC 88, and SV 413 and female parents GMS-26, GMS-20 were found to be good general combiner for seed cotton yield. For boll weight male parents EC 138572, EC359051, SV 413 and female parents GMS 20, 26 and 27 were found to be good general combiners while parents SA 1652, EC141679, SV 413 and GMS 20 were good general combiners for ginning percentage. Out of 52 cross combinations evaluated 12 crosses viz. GMS 17 x SA 1422, GMS 17 x MC 127, GMS 17 x 358371, GMS 17 x 359051, GMS 20 x SA 1652, GMS 20 x MC 88, GMS 26 x EC 128334, GMS 26 x CSH 3129, GMS 27 x SA 1422, GMS 27 x EC 138572, GMS 27 x 358371 and GMS 27 x SV 413 possessed significant SCA effect for seed cotton yield. However, for boll weight only 3 cross combinations

GMS 17 x SA 1422, GMS 17 x MC 127 and GMS 26 x CSH 3129 showed significant positive SCA effect. The cross combination GMS 26 x CSH 3129 besides yield and boll weight also showed significant SCA effect for ginning out turn, number of sympods, bolls per plant and lint yield.

Coimbatore

Improved *G. hirsutum* culture identified for release

A medium staple culture CCH 2623 was tested in various centers of All India Coordinated Cotton Improvement Project during the past five years in both Central and South Zones. It showed yield superiority in both the zones and recorded a mean seed cotton yield of 1739 kg/ha in Central Zone with 34% yield increase over the zonal check variety LRA 5166 and 1798 kg/ha in South Zone with 17% yield increase over the zonal check variety Surabhi. The culture has undergone trial for agronomic requirements in both the zones.

Performance of medium staple cultures

In station trial 22 medium staple cotton cultures were evaluated along with Sumangala, LRA 5166 and Suraj as check varieties. Analysis of data on seed cotton yield indicated significant difference among the entries and the highest seed cotton yield was recorded in *G. hirsutum* culture MM05-38-2-4 BK (1560 kg/ha).

Performance of long staple cultures

From F₆ population, high yielding compact genotypes were selected and evaluated at closer spacing of 75 cm x 30 cm along with Suraj and Anjali as check varieties. Analysis of data on seed cotton yield indicated significant difference among the entries and the highest seed cotton yield was recorded in PI-36-3-5-2-Bk (1433 kg/ha). Some genotypes had big bolls (as high as 5.3 g in PI-42-2-2-1-Bk) with most genotypes being erect and dwarf, possessing short internode length.

Performance of compact cultures

Data on AICCIP Plant Breeding trials were compiled and high strength cultivars tested during the past eight years (2004-05 to 2011-12) in various trials were identified. Out of 13 genotypes evaluated, genotypes with bundle strength as high as 25.0 g/tex (CCH 4474 and CCH LS 3) were identified. Analysis of data on seed cotton yield indicated significant difference among the high strength genotypes studied with highest yield in GSHV 161. Comparison of fibre quality data indicated consistency in quality of CCH 4474 (SL = 33.5 mm; BS = 25.9 g/tex as against mean value recorded in AICCIP SL = 30.1 mm and BS = 24.7 g/tex). Apart from this culture, the cultures CCH LS 2, CCH 7122 and CCH 820 also showed consistency for fibre quality.

High strength cultures

Besides the high strength varieties selected from the AICCIP trials, number of promising high strength cultures were identified from Station trials for evaluation in TMC trials. In all, 185 plants in F₈ generation were selected for high strength and some plant progenies combined both yield and quality. For instance, the progeny PI 21-1-1-1-2-6 combined promising seed cotton yield (124 g/plant) with very good bundle strength (24.4 g/tex) and 2.5% span length (26.5 mm).

Identification of lintless mutant

In the MCU 5 population, a spontaneous lintless mutant was identified and was found to breed true. The isogenic MCU 5 Lintless mutant is characterized by complete lack of lint and fuzz on the developing seed even after complete bursting of boll. The mutant is highly useful for studying the molecular basis of fibre development in cotton.

G. barbadense genotypes

Eighteen advanced *G. barbadense* cultures generated under TMC MM-I and also under Institute projects were evaluated. Four cultures viz. CCB-5 (11 q/ha), CCB-11 (11.5 q/ha), CCB-33 (9.9 q/ha) and CCB-36 (9.8 q/ha) exhibited promising yield over the check Suvin (5.65 q/ha). Besides, CCB-33 and CCB-36 possessed significantly superior boll weight of 4g and 3.9 g respectively. Cultures CCB-20 and CCB-24 were early maturing (160-165 days) compared to check Suvin (200-210 days).

In Suvin improvement programme, 7 advanced crosses involving Suvin were evaluated for seed cotton yield and earliness. Pooled data of last 3 years revealed cross Suvin x ICB 263-2 had highest yield of 1020 kg/ha with 35mm Span length and 27% g/tex strength. It also had the highest micronaire of 4.2 µ/inch, while the check Suvin under same conditions recorded seed cotton yield of 423 kg/ha, staple length 36 mm span length, strength 27g/tex and micronaire of 2.9 µ/inch.

Sirsa

Improved *G. hirsutum* culture identified for release

A high yielding *G. hirsutum* culture CSH 3129 was recommended for Agronomy trial based on its superior performance over four years in AICCIP trials. Proposal for its identification has been submitted.

Evaluation of CLCuV resistant cultures

Thirty-six *G. hirsutum* cultures were evaluated against CLCuV along with local check RS 921 and LH 2076 in replicated field trial. The highest seed cotton yield was recorded in the advance culture CSH 2931 (2986 kg/ha) followed by that in CSH 2810 (2543 kg/ha), CSH 2811

(2469 kg/ha), CSH 2934 (2393 kg/ha) and CSH 2838 (2345 kg/ha) as against check variety LH 2076 (2222 kg/ha). Highest ginning out turn of 35.1% was recorded in culture CSH 2391 followed by that in CSH 2844 (34.0%) compared to local checks RS 921 and RS 2013 with GOT of 33.5 and 32.6%, respectively. The culture CSH 2931 besides recording highest yield showed lowest incidence of CLCuV (PDI 2.5%) followed by that in cultures CSH 2836 and 2838 which suffered CLCuV incidence of 6.5 and 9.1 % PDI, respectively.

G. hirsutum

To develop improved culture of *G. hirsutum*, crosses were effected between five high strength accessions obtained from CICR Regional Station, Coimbatore and four genotypes developed at Regional Station, Sirsa. Two out of thirteen crosses in F₄ generation viz. CSH-3119-10-28-56 and CSH-3119-10-30-60 were early in maturity with significantly high yield compared to the superior parent CSH 3047 (2469.15 kg/ha) as well as the check variety H-1226(2175.94). Culture CSH-3114-10-14-28 in F₄ generation exhibited high fibre strength > 25 g/tex and staple fibre length >30 mm. Six cultures with promising fibre quality traits including high strength and long staple viz. CSH-3129, 3114, 3047, 3312, 3313 and 3314 were evaluated further under the TMC Project.

GOT of promising *G. hirsutum* cultures

One hundred and sixty five plants in F₅ generation were generated from a cross between a high (SA977) and a low (SA112) GOT culture. Analysis of progeny showed that 2 plants had GOT <30%, 38 possessed GOT between 30-35%, 111 had GOT between 35-40% while 13 plants had GOT >40 %. The promising plants can serve as mapping population for tagging genes for GOT.

G. arboreum

Forty-eight *G. arboreum* improved lines were evaluated for yield potential and fibre properties. Ten promising cultures were found highly suitable for surgical properties with micronaire >7, absorbance 1.1 second, sinking time 1.7 second, water-holding capacity >23 g and ash content between 0.22-0.45 %.

AICCIP Trials

Nagpur

Status of cultures sponsored in AICCIP trials

Fourteen promising cultures were sponsored in various AICCIP trials during 2012-13. This included four *G. hirsutum* entries CNH 15, 16, 315 and 1110 sponsored in Initial Evaluation Trial (IET) in irrigated or rainfed conditions. Two cultures CNH 14 and CCH 1111 were sponsored in preliminary varietal trial in Central zone under rainfed conditions. Among *G. arboreum*

cultures, two entries namely CCA 390 and CNA 1020 were sponsored each in national trials under rainfed and irrigated conditions. One *G. arboreum* entry viz., CNA 398 was sponsored in coordinated varietal trial for evaluation under rainfed conditions in Central zone while two entries, CNA 1007 and CNA 1016 were sponsored in South zone. Besides, three compact *G. hirsutum* cultures including CNH 1105 and 1109 were sponsored in south zone while a drought tolerant genotype 28 I was promoted for second year in both central and south zones for evaluation of their suitability under AICCIP HDPS trial (Table 3.3.2).

Table 3.3.2: Status of cultures sponsored in AICCIP trials

Name of culture	Species	Promoted to
CNA 1016	<i>G. arboreum</i>	Br 24 b – Central Zone
CNA 1016	<i>G. arboreum</i>	Br 24 b – South zone
CSH 1110	<i>G. hirsutum</i>	Br 03 b – South zone
CNH 28 I	<i>G. hirsutum</i>	Br 06 b – Central zone
CNH 28 I	<i>G. hirsutum</i>	Br 06 b – South zone
CNH 1109	<i>G. hirsutum</i>	Br 06 b – South zone

Evaluation of advanced cultures

Sixteen advanced cultures of *G. arboreum* and *G. hirsutum* evaluated and found promising in the Institute trials were identified for sponsorship in AICCIP trials during 2013-14. This included six *G. hirsutum* entries viz. CNH 19, 1116, 2001, 7008, CSH 95 and CSH 1115 and four *G. arboreum* cultures viz. CNA 375, 1013, 1021 and CCA 3 that were sponsored for Initial Evaluation trial in rainfed and irrigated conditions. Besides, 6 *G. hirsutum* cultures promising for yield and / or drought tolerance were also identified for sponsorship in AICCIP compact HDPS trials in irrigated and rainfed conditions.

Coimbatore

Eight cultures from Coimbatore were tested in AICCIP multi-location testing for yield and other characters during 2012-13. In the initial evaluation trial of *G. hirsutum* varieties under irrigated condition, the culture CCH 12-2 recorded 1854 kg/ha of seed cotton yield in south zone and ranked 5th overall. The culture CCH 12-6 showed superiority in both central and south zone locations in evaluation trial of compact genotypes under irrigated condition. In the preliminary varietal trial in Central Zone, culture CCH 11-2 showed superiority in seed cotton yield (1969 kg/ha), besides documenting superior fibre quality and was promoted to coordinated varietal trail in irrigated condition.

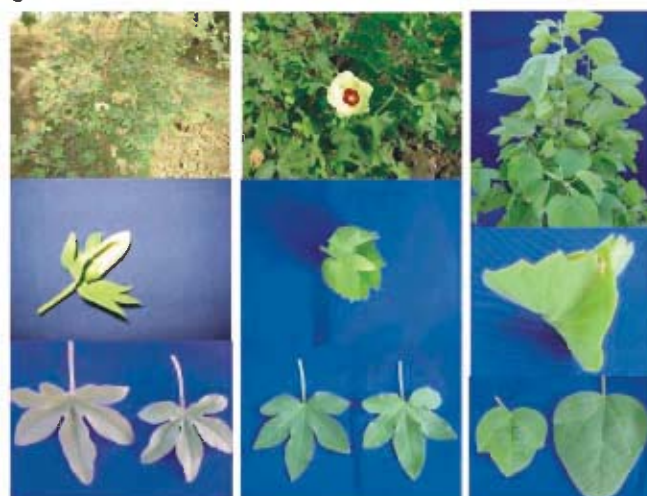
Sirsa

Two Promising cultures each of *G. hirsutum* (CSH 3114 and 2982) and *G. arboreum* (CISA 8 and 111) were sponsored in IET of AICCIP in irrigated and rainfed zones. Similarly, GMS based hybrid one each for *G. hirsutum* (CSHG 2118) and *G. arboreum* (CISAA 22) were also sponsored in AICCIP preliminary hybrid trials under irrigated and rainfed conditions. Based on their superior performance in national trials, genotype CSH 3088 was promoted to zonal trial (north & south zone) in irrigated condition, while GMS based hybrid CISAA 20 was promoted to coordinated hybrid trial (central zone) under irrigated and rainfed conditions.

3.4: Genetic Diversity through Introgression

Nagpur

Four hundred and twenty six introgressed derivatives were evaluated for yield and yield contributing traits coupled with biotic and abiotic stress tolerance. Fifty single plant selections were made for high yield and compact plant types under unprotected condition. The introgressed derivatives were apparently tolerant to jassids. An introgressed derivative generated from a cross between LH1134 and *G. hirsutum* race *palmeri* showed the highest fibre strength of 29.0 g/tex in F₄ generation.



G. arboreum x *G. somalense* (E_j) *G. somalense* (E_j) x *G. arboreum* *G. somalense* (E_j)

Promising introgressed derivatives developed

3.5: State Multi-Varietal Trial (SMVT)

Nagpur

A State Multi-Location Varietal Trial consisting of 17 genotypes of *G. arboreum* and 13 of *G. hirsutum* was conducted at CICR, Nagpur. Among *G. arboreum* cultures, few cultures out-yielded even upland

genotypes. Seed cotton yield in *G. arboreum* ranged from 879-1806 Q/ha. All *G. a*

rboreum genotypes showed seed cotton yield of more than 1100 Q/ha except JLA-0807 with seed cotton yield of 879 Q/ha. The highest seed cotton yield of 1806 Q/ha was recorded in CNA1003 followed by that in PA-741 (1720 Q/ha) and PA-08 (1690 Q/ha). AKH 2006-2, besides supporting highest yield among *G. hirsutum* cultures, also combined promising fibre length (30.8 mm) and bundle strength (22.1 g/tex). CNH 1110, however, recorded highest fibre strength of 23.0 g/tex and fibre length of 29.8 mm.

3.6: Molecular Breeding

Nagpur

i. Mapping QTLs for fibre quality traits

In an effort to develop linkage map of diploid A genome cotton, an interspecific F₂ mapping population (*G. arboreum* cv. KWAN-3 x *G. herbaceum* cv. Jaydhar) was developed. During 2012-13, parental polymorphism was surveyed using 1098 SSR markers based on which 77 polymorphic markers were identified (Fig.3.6.1). Genotyping of mapping population with additional 15 informative markers has been completed (Fig. 3.6.2). In *G. hirsutum*, survey for parental polymorphism (for the parental lines used for development of RIL population) was carried out using 1098 SSR markers based on which 92 informative markers were identified.



Fig. 3.6.1: Survey for parental polymorphism using SSR markers in cotton 1-EL-958, 2-UPA-5717 (*G. hirsutum*), 3-Jaydhar (*G. herbaceum*), 4-KWAN-3 (*G. arboreum*)

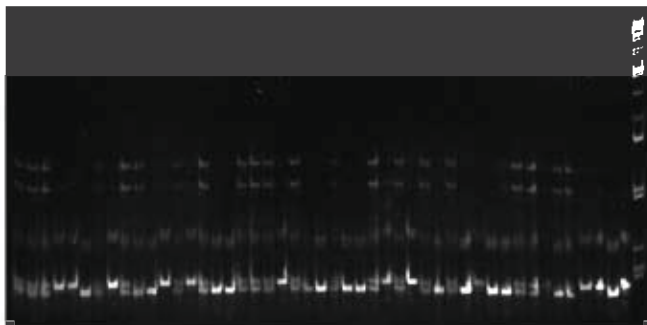


Fig. 3.6.2: Genotyping of F₂ mapping population with SSR marker NAU 905 in diploid cotton

Development of mapping populations (RIL's) for fibre quality traits in diploid and tetraploid cotton

For diploid cotton, 193 F₂ plant progenies were raised. One random plant from each progeny was selfed for maintaining RIL progenies. Few progenies yet showed segregation for morphological characters; hence selfed bolls were selected for advancing to further generation. For the progenies that showed uniformity, all plants in 5 dibble rows were bulk harvested, ginned and the lint was subjected to quality evaluation. Significant variability was observed for the quality attributes.

In *G. hirsutum*, 273 F₇ boll to row progenies were planted, selfed bolls were obtained from single random plants from each progeny and the seed cotton of remaining plants was bulk harvested to be used for evaluation in replicated trial.

ii. Molecular markers for disease resistance

CLCuV resistance

CLCuV resistant (CP 15/2) and susceptible (F 846) parental cotton were identified and crossed at CICR, Sirsa to obtain F₂ mapping population. The F₁ was advanced at CICR, Coimbatore for advancement of generation. An additional cross was generated between LRA 5166 (R) x HS 6 (S) to develop mapping population. Besides, 5000 germplasm lines were screened against CLCuV and 30 lines were observed to be free from the virus under field conditions, for two consecutive years at Sirsa. The lines which remained free of CLCuV will be screened under epiphytotic conditions in polyhouse for further use in marker assisted breeding.

Bacterial blight resistance

For Bacterial Leaf Blight (BLB), four parents including three BBR genotypes (101-102B, IM216, S295) and one susceptible genotype (Ganganagar Ageti) involved in developing four mapping populations were screened for parental polymorphism using 400 SSR markers. Four SSR markers were found to be polymorphic with the parental genotypes, Ganganagar Ageti and S295. F₂ mapping populations of three independent crosses involving three bacterial blight resistant and one susceptible parents were grown in the field. The entire populations of 259 (Ganganagar Ageti x 101-102B), 216 (Ganganagar Ageti x IM216) and 450 (Ganganagar Ageti x S295) individual F₂ plants were phenotyped for their reaction against bacterial blight disease under natural epiphytotic conditions. Genomic DNA was extracted from few individual plants for genotyping. The three populations have been retained as ratoon crop and the phenotyping will be completed during crop season 2013-14.

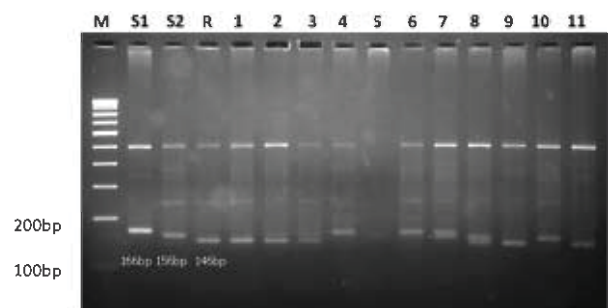
Validation of published markers for Bacterial blight resistance (BBR)

Thirty-nine genotypes were screened against race 18 of *Xanthomonas campestris* pv. *malvacearum* by artificial inoculation. Based on reaction of genotypes, 13 were found to be immune while 26 were susceptible to the bacterium.



Screening of germplasm lines for bacterial blight resistance by artificial inoculation - Host differentials and germplasm lines covered with poly bag after *Xam* inoculation

Three SSR markers linked to bacterial blight resistance (BBR) viz. CIR 246, BNL 3545 and BNL 3644 were used to screen resistant and susceptible lines. SSR marker CIR 246 (146 bp) was found to co-segregate with bacterial blight resistance in 9 out of 13 genotypes while in susceptible genotypes the same set of primers amplified amplicons of 156 and 166 bp (Fig 3.6.3). The marker however, remained uninformative in remaining 4 resistant cotton.



M- 100 bp ladder; S1- Susceptible-166bp (LRA5166); S2-Susceptible-156bp (Acala44); R-Resistant-146 bp (101-102B); 1, 2, 3, 9 and 11 – Resistant lines (146bp); 5 and 10- Susceptible lines (156 bp); 4 and 6 –Susceptible lines (166 bp); 7-Susceptible (156+166bp); 8- Heterozygote (146 +156 bp)

Fig.3.6.3: Validation of CIR 246 Market with germplasm lines for bacterial blight resistance

Nematode resistance

Parental lines for nematode (Reniform and Root-knot nematodes) resistance and susceptibility were identified. These included Bikaneri Nerma for Root Knot Nematode (RKN) resistance; G. Cot 10 and American nectariless for Reniform Nematode (RN) resistance and Suraj as susceptible parent to both RKN and RN. Survey of parental polymorphism using SSR primers has already been initiated.

Coimbatore

To develop a Nested Association Mapping population, selected promising varieties of cotton viz., Suraj and Surabhi (Long staple), Sumangala (Medium staple) and Arogya (Short staple) varieties were crossed with germplasm lines like IC 359856 (Low Strength line with BS 14.5 g/tex) and IC 358438 (High Strength line with BS 26.4 g/tex) and also with Suvin.

3.7: Development of Transgenic Cotton

Bollworm resistant transgenic cotton

Molecular characterization of *cry1Ac* transgenic *G. hirsutum* cultivar Anjali (CICR-Bt-LRK-7) was done. Integration of gene was ascertained by PCR and Southern hybridization while expression of Bt protein was estimated by ELISA. Characterization of 5-new putative Bt transformants of cv Suraj showed the presence of *cry1Ac* gene. Cultivar Suraj was also transformed with *cry1F* gene through pollen-tube transformation method. Bolls set on transgenic plants were harvested and plants raised from boll to row progeny were screened for the presence of transgene. All 18 plants raised from a single boll showed the presence of *cry1F* gene (Fig. 3.7.1).

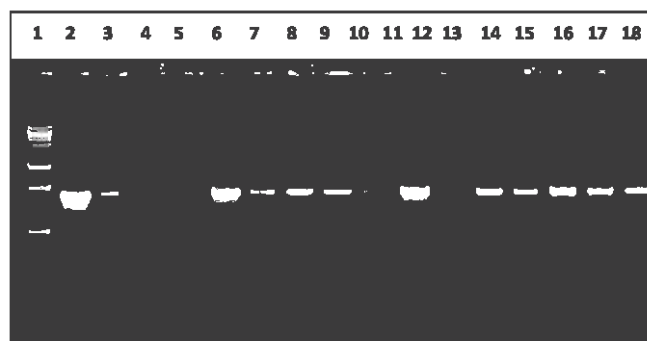


Fig. 3.7.1: PCR amplification of *cry1F* gene in putative transformants of *G. hirsutum* cv Suraj. Lane 1, 1kb ladder; 2, plasmid with cloned gene; 3, positive control; 4, negative control; 5, wild type Suraj; 6-18, *cry1F* transgenic cotton.

Development of Multi-gene Constructs and Bt Cotton Varieties for Sustainable Pest Management

A new gene cassette comprising *G. hirsutum* rubisco small subunit chloroplast transit peptide and codon optimized fusion *cry* gene was generated. Fusion *cry* gene comprising of modified *cry2* and truncated *cry1Ac* were individually cloned in pET28 expression vector (Fig. 3.7.2) and introduced in *E. coli* (BL21 codon plus). Expression of gene and efficacy of the fusion protein was confirmed through insect bioassay. Entire gene cassette was sub-cloned in plant transformation vector under double 35S promoter with NOS as terminator.

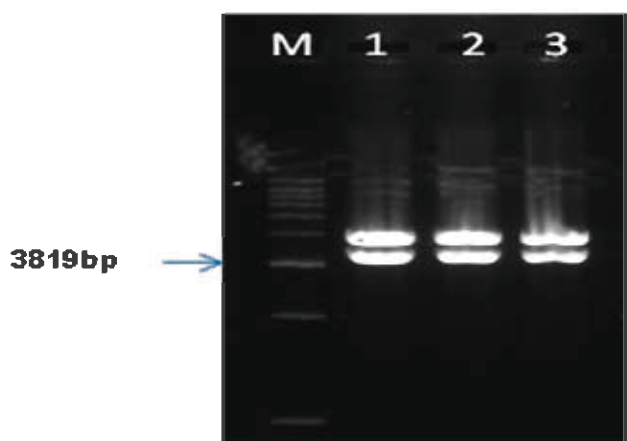


Fig. 3.7.2: Fusion cry gene (*cry2+* *cry1Ac*) in plasmid pET28. M, 1.0 kb ladder, lanes 1-3, fusion plasmid digested with specific restriction enzymes to release the gene.

RNAi construct

Cotton leaf curl virus isolates collected from the strategic locations of Northwestern India was obtained from the CICR Regional Station, Sirsa. The genome of a recombinant, Mohanpura (Rajasthan) isolate of the virus, was characterized for the purpose of designing dsRNAi construct for RNAi-mediated transgenic cotton. The single stranded, circular genome, comprising of Gemini viral and beta satellite DNA were subjected to rolling circle amplification using ϕ 29 DNA polymerase (GE, USA) following manufacturer's protocol. The amplified DNA was subjected to restriction digestion using unique restriction endonuclease viz., *Aat*II for geminiviral DNA and *Bcl*I for β satellite DNA (Fig. 3.7.3). The linearized DNA was cloned and sequenced (KC412251). Once a number of recombinant isolates obtained from the strategic locations of North India are characterized, their nucleotide sequences will be subjected to alignment and the conserved regions in different ORFs will be used to design RNAi constructs.

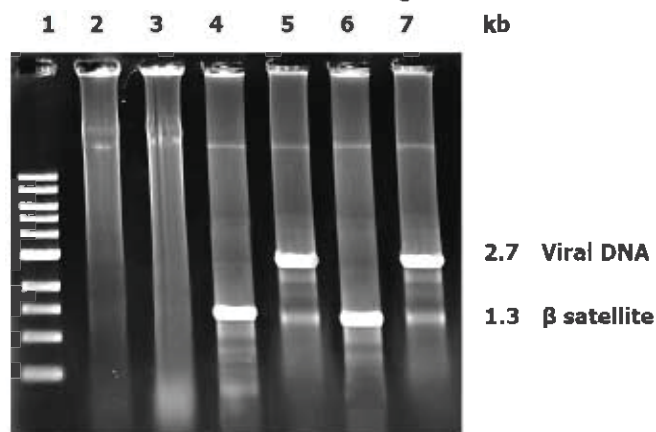


Fig. 3.7.3: Multiple displacement amplification of ss DNA genome of CLCuV. Lanes 2&3, rolling circle amplified viral DNA; 4&6, β satellite linearised using *Bcl*I and 5&7, geminiviral DNA linearised with *Aat*II

RNAi - mediated leaf curl virus resistance

Five dsRNAi constructs were used to transform two popular *G. hirsutum* cultivars, HS6 and LH 2076. Successful transformation was achieved with 4 inverted repeat constructs, pBin-AC-S-int-A and pBin-CP-S-int-A, pBin- β C1-S-int-A and pBin- β V4-S-int-A by agro-inoculation (Fig.). Frequency of *in planta* transformation and regeneration in HS6 ranged from 4.1 to 7.7 % (Table 3.7.1).

Table 3.7.1: Transformation of cultivar HS6 with RNAi constructs

Inverted repeat construct	No. of explants co-cultivated	No. of explants selected on kanamycin	No. of explants regenerating shoot	Transform frequency (%)
AC2	310	12	15	7.7
CP	780	22	25	5.6
β C4	240	05	09	4.1
β V4	448	15	13	6.6

During 2012-13, a new RNAi event of dsRNAi-CP gene construct in *G. hirsutum* cultivar LH 2076 was developed. The T0 plant was found positive for the transgene (Fig. 3.7.4).

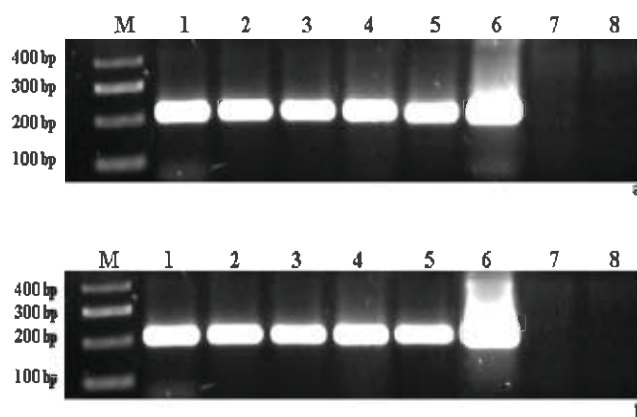


Fig. 3.7.4: Characterization of transgenic LH2076-CP transgenic cotton: amplification of sense (a) and antisense strands (b) each of 162 bp. Lanes 1-5, LH2076-CP transgenic plant; lane 6, pBSK-CP-SA plasmid (+ve control); 7, wild type HS6 (-ve control); lane 8, PCR -ve control.

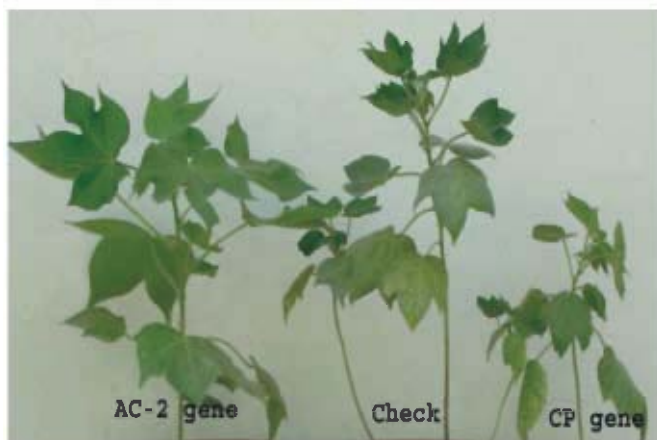
Molecular characterization of transgenic events

Putative transformants with pBin-AC2-S-int-A and pBin-CP-S-int-A were documented by PCR for presence of viral sequence, both in sense and antisense orientations. Twelve PCR positive events of CICR-HS6-AC2 and three events of CICR-HS6-CP were established. Contained greenhouse trial with 215 transgenic cotton (T1) belonging to 5 transgenic events (T0)- comprising of 3, CICR-HS6-AC2 events and 2,

CICR-HS6-CP events, were conducted at CICR regional Station Sirsa. Entire progeny of 5 transgenic (T0) plants that were found positive for presence of inverted repeat constructs by PCR and Southern hybridization were screened against viruliferous whitefly in glass house along with the wild type cotton HS6.



Contained green house trial at Sirsa with RCGM approval



CLCuV tolerant transgenic cotton challenged with viruliferous whitefly in contained glass house at CICR Regional station, Sirsa. Transgenic plants showed delayed pathogenesis

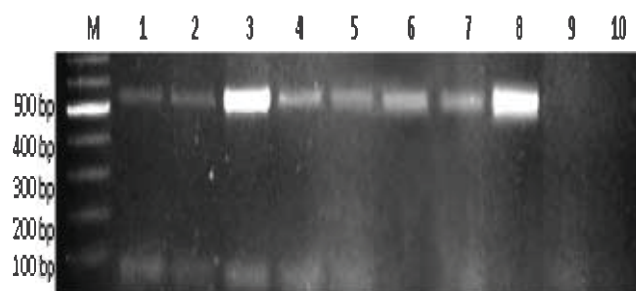


Fig. 3.7.5: Detection of inverted repeat construct in transgenic cotton with *nptII* primer. M-100 bp ladder, 1, HS6-AC2-, 2, HS6-CP-, 3, HS6-CP-, 4, HS6-CP-, 5, HS6-CP-, 6, HS6-CP-, 7, HS6-CP-, 8, pBin plasmid (positive control); 9, HS6 (wild type); negative control.

Transgenic plants appeared to vary in degree of tolerance against CLCuV, with some plants showing delayed initiation of disease symptoms. By the end of the season at least six transgenic plants possessing HS6-CP-SA construct remained free from CLCuV symptoms. Seven transgenic plants including 2 that showed delayed pathogenesis and 5 plants that remained symptomless (Table 3.7.2), were analyzed for the presence of inverted repeat sequence as well as the *nptII* reporter (Fig. 3.7.5).

Rt-PCR analysis of two transgenic plants that showed delayed pathogenesis revealed 5 (HS6-AC2-SA) and 9 fold (HS6-CP-SA) reduction in virus titre compared to wild type control plants. While analysis of 5 RNAi-mediated transgenic plants that remained symptomless till the end of the season showed more than 12 fold reduction in viral load (Fig. 3.7.6). Nevertheless, all transgenic plants showed latent infection of virus when diagnosed with PCR.

Table 3.7.2: Analysis of Transgenic plants for CLCuV infection, Inverted repeat construct, disease reaction and viral load by qualitative and Q-PCR

Sr. No.	Event	PCR result		Down regulation of virus	Disease reaction
		<i>nptII</i> Prim	CP Primer		
1	HS6-54-AC2-10-8	Positive	Positive	5 Folds	Delayed expression
2	HS6-4-CP-2-1-A	Positive	Positive	10 Folds	Delayed expression
3	HS6-11-CP-2-8-B	Positive	Positive	12 Folds	Free
4	HS6-11-CP-2-8-C	Positive	Positive	12 Folds	Free
5	HS6-6-CP-2-3-D	Positive	Positive	12 Folds	Free
6	HS6-5-CP-2-2-E	Positive	Positive	12 Folds	Free
7	HS6-9-CP-2-6-F	Positive	Positive	12 Folds	Free

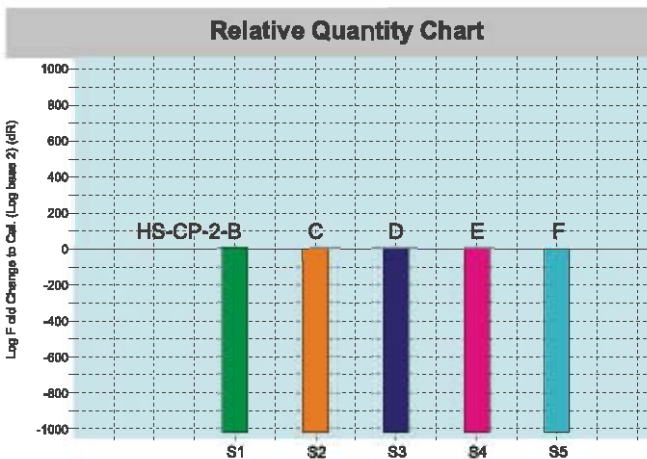
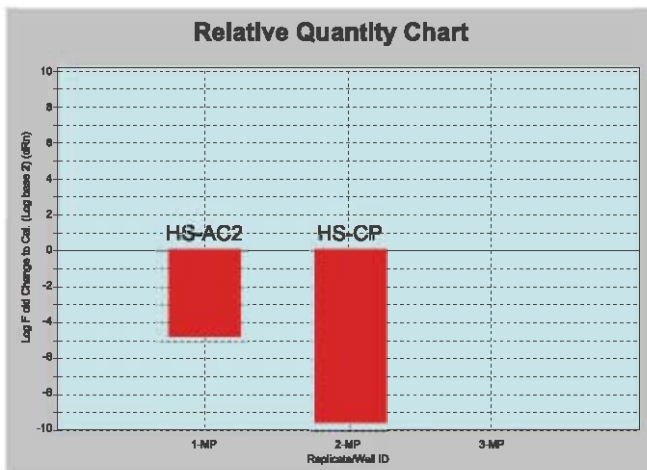


Fig. 3.7.6 : Reduction in CLCuV titer in RNAi-mediated transgenic cotton: a, plants showing delayed pathogenesis; b, symptomless plant.

Antisense resistance against CLCuV

Transgenic plants for Cotton leaf curl virus with Sense coat protein (AV1), anti-sense coat (AV1) protein and antisense replicase protein (AC2) in the elite genotypes RS 875 were developed. T₁ Transgenic events namely, HS6 (ARep)- 1 event, (ACP)- 1 event, (SCP)- 1 event; H777(ACP)-1 event, F 846 (ARep) -1event, (ACP)-1 event, (SCP)- 1 event were selfed and the bolls were harvested. Quantitative RTPCR of the transgenic events was carried to determine the relative quantity and copy number which revealed that HS 6 with antisense coat protein and HS 6 with sense coat protein had one copy of the gene.

New events of RS 875 (SCP)-6, RS 875 (ACP)-7, RS 875 (ARep)-5 were developed and characterized for the presence of the reporter gene *npt II* besides, specific genes (Antisense Coat protein, Sense Coat Protein and Antisense Replicase gene). The transgenic plants were screened against viruliferous whiteflies at CICR RS Sirsa. Three plants of RS 875 (SCP) and one plant of RS 875 (ARep) remained symptomless.



Transgenic plants *G. hirsutum* cultivar RS 875 with sense coat protein gene

Progenies of T₃ transgenic events namely, HS6 (ARep)- 1 event, (ACP)- 1 event, (SCP)- 1 event; H777(ACP)-1 event, F 846 (ARep) -1 event, (ACP)-1 event, (SCP)- 1 event were selfed and the bolls were harvested. The events were subjected to qPCR analysis for quantitative expression of transgene and assessment of copy number. Rt-PCR analysis revealed single copy integration in one of the HS₆-ACP events.

Fungal resistance

Inheritance of *ch1* gene in progenies of transgenic cotton

Southern hybridization of T₁ progeny of transgenic *Gossypium arboreum* cv PA255 raised from independently transformed T₀ shoot by each of three *in planta* protocols viz., meristem bisections, seed inoculations with *Agrobacterium* without injury and with injury was done. Four T₁ plants raised from 4 seeds in one boll on three independent T₀ plants generated by three methods of regeneration was subjected to Southern hybridization. All 4 plants generated by each of the methods 1 and 2 showed presence of single copy of transgene (Fig. 3.7.7). However, the plants generated by two methods possessed independent events as evident from the variation in size of the hybridization signal. In progeny of the T₀ plant generated by third method, 3 out of 4 T₁ plants showed integration of single copy of gene while one T₁ had two copies of transgene. This shows that transgene were present in majority of the populations thereby having the possibilities of the transgene segregating in 3:1 ration if adequate number of populations are analysed.

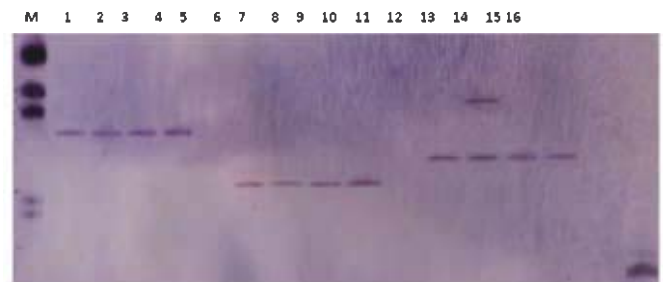


Fig. 3.7.7: Southern blot of T₁ progeny of transgenic *Gossypium arboreum* cv PA255 raised from independently transformed T₀ shoot meristem bisections

(Lanes 1-5) and seed inoculations without (Lanes 6-10) and with (Lanes 11-15) injury. Southern blot was hybridized with DIG labelled *Gossypium hirsutum* Chitinase gene as DNA probe. Lane M, λ -Hind marker; Lane 5, 10, 15 PCR -ve plants, Lane 16, uncut probe DNA. DNA digested with *EcoRI*.

Each of the T₁ plants generated by 3 methods and analysed above were planted and allowed to set bolls by selfing. One T₂ seed from one of the bolls on each plant were again subjected to Southern hybridization. All 4 plants generated by each method showed uniform sized signals characteristic of the original event (Fig. 3.7.8)



Fig. 3.7.8 : Southern blot of T₂ progeny of transgenic *Gossypium arboreum* cv PA255 raised from T₁ positive events independently sown, shoot meristem bisections (Lanes 1-5) and seed inoculations without (Lanes 6-10) and with (Lanes 11-15) injury. Southern blot was hybridized with DIG labelled *Gossypium hirsutum* Chitinase gene as DNA probe. Lane M, λ -Hind marker; Lane 5, 10, 15 PCR -ve plants; Lane 16, uncut probe DNA. DNA digested with *EcoRI*.

Expression of chitinase gene in Cisgenic cotton

Gene expression analysis was also carried out in selected T₁ progeny using a chitinase activity assay. The transgenic plants showed chitinase enzyme activity that was 44.48% - 90.15% higher than that in untransformed plant (Fig. 3.7.9). The chitinase activity was highest in T₁ plants-A1 (4.87 mg/ml) and A2 (4.76 mg/ml). In remaining 4 out of 6 transgenic events it remained more or less similar in range between 3.67 - 4.02 mg/ml. Although the activity of chitinase gene appeared to vary with different transformation events, it was not apparently affected with copy number of genes integrated in the plants.

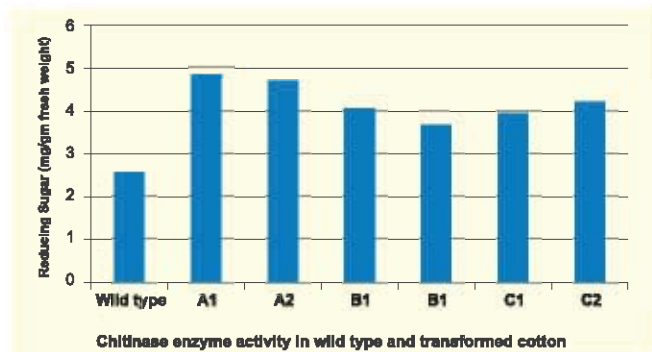
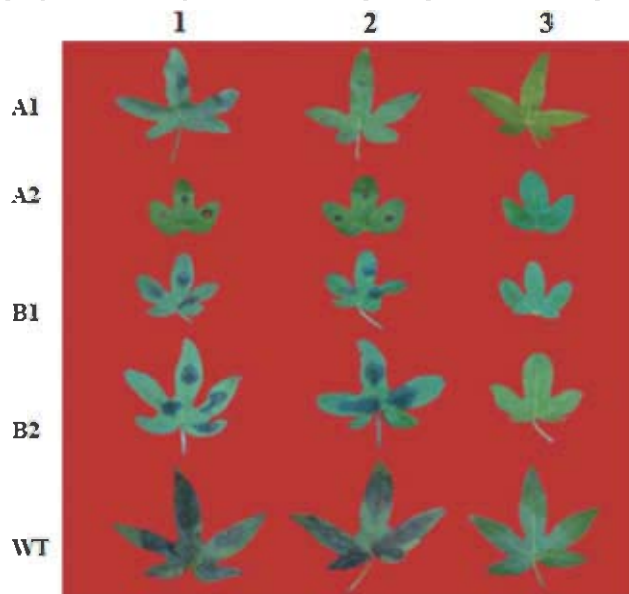


Fig. 3.7.9: Chitinase activity of T₁ progeny plants of

transgenic cotton (*Gossypium arboreum*) cv PA255 raised from independently transformed T₀ cotton by shoot meristem bisection (A) and seed inoculation with (B) or without (C) injury

Cisgenic T₁ cottons and the wild type PA255 plant were bioassayed against *Myrothecium roridum*, a saprophytic leaf spotting disease of many plant species, and *Ramularia areola*, a semi-obligate areolate mildew of cotton. In case of *R. areola* the plants were spray inoculated with aqueous spore suspension. The spores were scraped off the leaves of freshly infected cotton and a final suspension was made in water with 0.2 % Tween 20. The concentration of spores was adjusted to 10⁸ spores/ ml. The plants were maintained under glass house conditions at a temp of 30 +/- 2°C and >RH 95% for 24 h. Plants were examined for the development of disease. In case of *M. roridum* leaves of the plants were spot inoculated with aqueous spore suspension. The spores/ sporodochia formed in one week old culture growing on PDA at 27°C were used for inoculation. Inoculated plants were subjected to temp and humidity as above and observed for the development of disease. The cisgenic plants showed reduced lesion size and symptom development was delayed by at least 10 days.



Evaluation of resistance of chitinase cisgenic *G. arboreum* PA255 against *M. roridum*. T₁ progeny plants raised from independently transformed T₀ cotton by shoot meristem bisection (A1 & A2) and seed inoculation (B1 & B2). 1, 2 Inoculated; 3, water without spores (control); WT, wild type.

Transgenic cotton for drought tolerance

Two events of DREB1A each, in LRA 5166 (Fig. 3.7.10) and LRK 516 (Fig. 3.7.11 a & 3.7.11 b) and one event of ZF1 each in LRA 5166 and LRK 516 were selected earlier in contained field trial. T₃ generations of these six

events were grown in boll to row progeny in pot house. The transgenic plants which were positive for specific genes as ascertained by gene specific and reporter *npfl* specific genes were selfed (Table 3.7.3).

Table 3.7.3: Summary of transgenic events generated with DREB1A and ZF-1 genes

Transgenic Event	No. of plants germinated	<i>npfl</i> Positive plants	Transgene positive plants	Total number of transgenic plants
LRK 516-DREB 1A-1	22	17	17	17
LRK 516-DREB 1A-2	14	12	12	12
LRA 5166 -ZF1	17	10	10	10

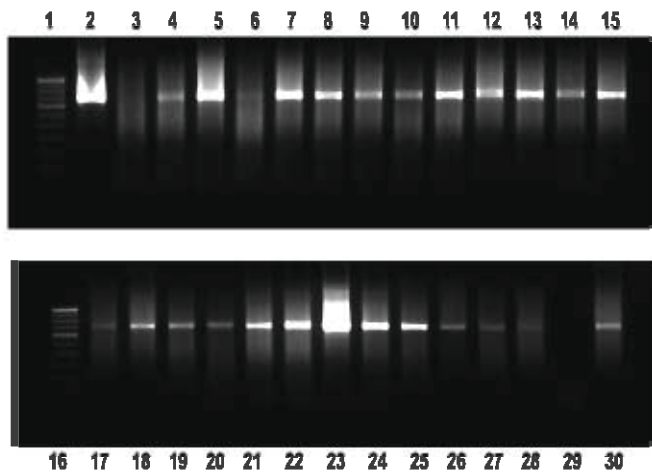


Fig. 3.7.10: Transgenic cotton expressing DREB1A and ZF1 genes. Transgenicity was ascertained by amplification of *npfl* sequence. Lanes 1 & 16, 100 bp ladder; lane 2 positive control ; Lane 3 negative control; lanes 4-7, LRK 516, DREB 1A-1 plants E19-22; lanes 8-15 and 17-20, DREB 1A-2 plants F3-F14 ; lanes 21-30, LRA 5166, ZF1- plants G1-G11.

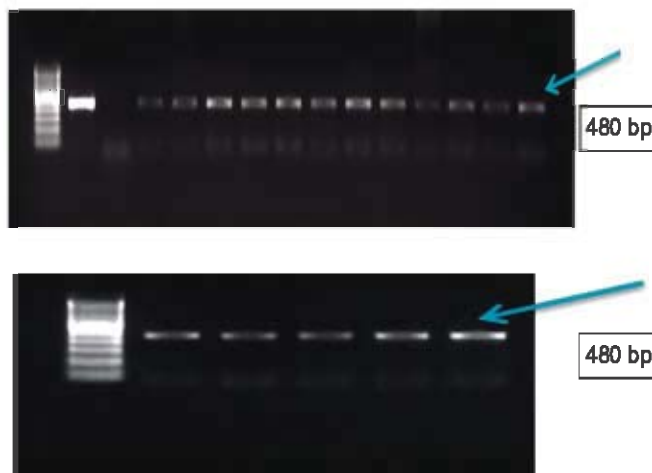


Fig. 3.7.11 a: Transgenic cotton expressing DREB 1A gene. Transgenicity was determined by amplification of

DREB 1A gene. Lane 1, 100 bp ladder ; lane 2, positive control; lane 3, negative control; lanes 4-22, LRK 516-DREB1A-1 plants, E1-E22.



Fig. 3.7.11 b: Cotton expressing DREB1A gene. Lane 1, 100 bp ladder; lane 2, positive control; lane 3, negative control; lanes 4-15, LRK 516-DREB1A-2 plants, F2-F14.

Gene expression analysis in drought resistance related transgenic cotton

Total RNA was isolated from leaves of transgenic LRA 5166-DREB 1A and LRK 516- ZF1 plants. Reverse transcription was performed using Oligo-dT primer to get first strand cDNA. PCR was performed using DREB 1A and ZF-1 primers. Rt-PCR analysis revealed over expression of gene in transgenic cotton compared to wild type control.

Molecular characterization and validation of fiber strength genes

Genes expressed during secondary wall formation (16-35 DPA) play vital role in cotton fiber strength. Under this project efforts have been taken to identify, characterize, clone and validate genes associated with secondary cell wall formation in cotton fiber. Few genes such as *GhcesA4*, *GhcesA7* and *GhcesA8*, TC59194 (*GhFLA2*), TC59405 (*GhMT1*) and *AtCob14* have been targeted to study the transcript level during 16-35DPA. Presently the identified candidate genes would be cloned and validated with fiber specific promoter for its defined function. Experiments were carried out with selection of high and low fiber strength genotypes of *G. hirsutum* and *G. barbadense*. Total RNA was isolated at different stages of fibre development and cDNA synthesis was carried out. qRTPCR analysis was harmonized with expression profile of selected genes viz. *cesA4*, *cesA7* and *cesA8*, *GhFLA2*, *GhMT1*, *AtCob14*, *AGP4* and *WDL1* at different stages of secondary wall synthesis viz., 16, 18, 21, 24, 27, 33 and 36 DPA. From expression analysis, it was clear that above mentioned genes maintained relatively high expression at secondary wall synthesis phase in both cotton species. Among these genes, *GhcesA2* and *GhcesA7* showed relatively higher level of expression during secondary wall synthesis at 27, 33, and 36 DPA. The selected candidate genes were amplified directly from cDNA using gene specific primers (Fig. 3.7.12). Cellulose contents were analysed from 16 to 33 DPA for all the four species. *G. barbadense* genotypes showed higher deposition of cellulose during secondary wall synthesis compared to *G. hirsutum*.

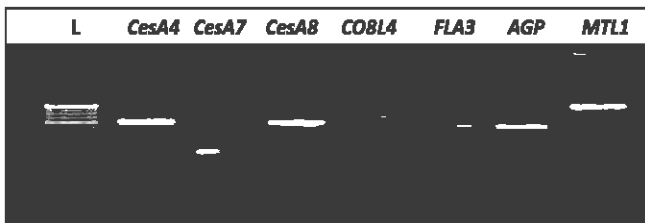
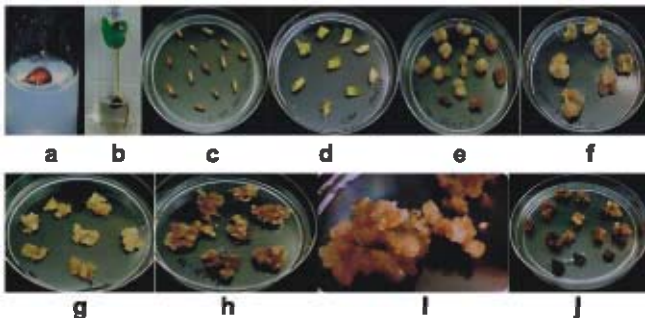


Fig. 3.7.12: PCR Amplification of candidate genes with gene specific primers

Genomics of cotton boll and fibre development

As an additional partner in NAIP Programme on "Genomics of cotton boll and fibre development", CICR has been entrusted with responsibility on transformation and validation of functional roles of genes associated with high fibre length and strength documented during the fibre extension phase. ICGEB provided three gene constructs viz. *Expansin*, *Aquaporin*, and *Arabinogalactan* in *Agrobacterium tumefaciens*. For effecting transformation by somatic embryogenesis, calli were induced using Coker 310 genotype in MS medium containing auxin (IAA 1.0 mg/l) followed with *Agrobacterium* mediated transformation with the above three genes. The transformed calli were subjected to selection in high concentration of kanamycin (100 mg/l). Agro-inoculated calli were sub-cultured to induce somatic embryos.



Callus Induction and *Agrobacterium*-mediated transformation with Coker 310 genotype. Different stages of callus induction and transformation. (a) seed inoculated on half strength MS medium (b) seven day old seedling (c) callus induction from hypocotyls explants (d) callus induction from cotyledonary leaf explants (e-i) callus proliferation and somatic embryogenesis (j) transformed callus on selection medium

Development of Inverted repeat construct to silence gossypol biosynthesis genes

To develop RNAi construct for silencing gossypol biosynthesis in cotton seed (Fig. 3.7.13, 3.7.14 & 3.7.15), the target genes coding for δ Cadinene synthase and δ Cadinene hydroxylase were isolated, sequenced and cloned in inverted repeat generating 3.1 kb plasmid pBSK-int (HQ343203). For expression of the dsRNAi construct in seed, seed-specific Beta Globulin

Promoter sequence was isolated and cloned upstream of the inverted repeat sequence. Additionally, alpha globulin promoter (AGP) along with Intron A of the alpha globulin gene was also cloned to drive the inverted repeat construct, expectedly at a greater efficiency (Fig. 3.7.15).

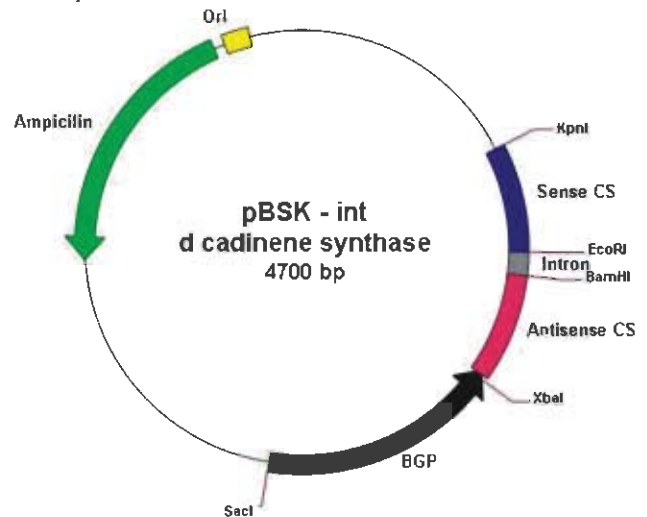


Fig. 3.7.13: Schematic representation of pBSK-Int- δ Cadinene synthase plasmid (4.58 kb)

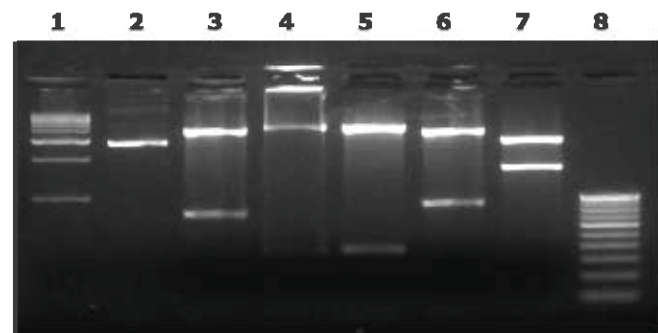


Fig. 3.7.14: Restriction digestion of Inverted repeat (IR) plasmid pBSK-int- δ Cadinene synthase to reveal components of the constructs. Lanes 1 & 8, 1kb and 100bp ladder; 2, uncut plasmid pBSK-int (3.1 kb); 3, Beta-Globulin promoter (XbaI+SacI= 756bp); 4, sense strand of δ Cadinene synthase (KpnI + EcoRI = 375bp); 5, antisense strand of δ Cadinene synthase (XbaI + BamHI=375bp); 6, IR construct (XbaI + KpnI = 873 bp); 7, IR with promoter (KpnI+SacI=1629bp).

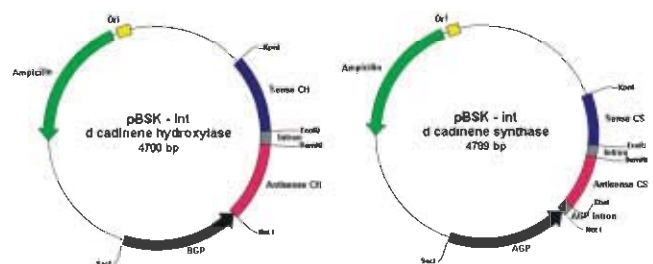


Fig. 3.7.15: Schematic representation of pBSK-Int- δ -cadinene hydroxylase and δ -cadinene synthase inverted repeat construct.

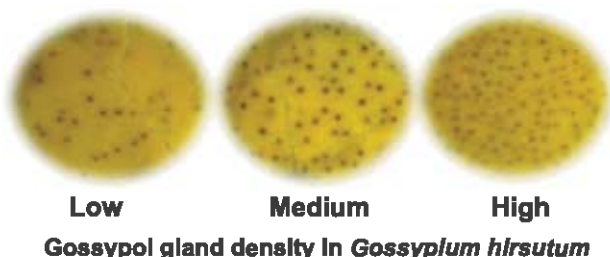
3.8: Seed Production and Seed Quality Improvement

Nagpur

DUS characterization

During 2012-13, 69 genotypes (39 candidate and 30 reference) were characterized for DUS traits. Besides, 28 diploid cotton (*G. arboreum* and *G. herbaceum*) varieties were cultivated for maintenance breeding for use as reference genotypes for characterization of DUS traits.

In order to determine feasibility of using gossypol gland density as special DUS trait, 25 *G. hirsutum* and 16 *G. arboreum* varieties were examined for variation in gossypol gland density / 2 sq.mm area under high resolution stereo-microscope. The observations were recorded on two cotyledonary leaves of three seedlings per variety. Gossypol gland density was determined in seven days old cotyledonary leaves of cotton germinated under controlled condition. Based on gland density, varieties were categorized into three groups viz. high (130 and above), medium (100-130) and low (less than 100) gossypol groups. Sixteen varieties were tested for inheritance of the trait over generation. Gossypol gland density was stably inherited in 14 out of 16 varieties tested (Table 3.7.4). The results showed that gossypol gland density could serve as an additional trait for DUS testing of cotton. Based on gossypol gland density PLC 62, a perennial cotton could be distinguished from a set of morphologically similar cotton.



Gossypol gland density in *Gossypium hirsutum*

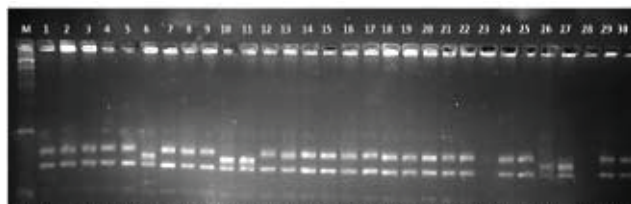
Table 3.7.4: Grouping of varieties of *Gossypium hirsutum* based on gossypol gland density

High (130 and above)	Medium (100-130)	Low (less than 100)
JLH 168	MCU10	NH545
AKH 8828	F1054	Abadhita
Surabhi	F1378	Sumangala
MCU 12	MCU 10	Sahana
	G Cot 16	Narasimha

DNA fingerprinting of released varieties

Fifty released (public sector) varieties of cotton were characterized using 25 SSR markers that were earlier

found informative based on their ability to delineate genetic diversity among large number of genotypes fingerprinted. Four SSR markers (BNL 3371, NAU4485, DPL398 and DPL468) were found polymorphic within the tested varieties. Variety HS-6 could be distinctly identified using two markers, BNL 3371 and DPL468 (Fig. 3.7.16). Further characterization of candidate varieties with more informative markers are in progress.



M: 100 bp Ladder, 1- KC3, 2-JK4, 3-Kandwa2, 4-Kandwa3, 5-F11225, 6-H36, 7-H117, 8-R5816, 9-R52013, 10-Gagad, 11-R5T9, 12-R5873, 13-BN1, 14-F1078, 15-F1054, 16-F1061, 17-Surabhi, 18-Sumangala, 19-Suraj, 20-Anjali, 21-Supriya, 22-LRA5168, 23-MCUBVT, 24-Gcot10, 25-Surat dwarf, 26-G67, 27-Gcot16, 28-F346, 29-Gcot18, 30-Devraj

Fig. 3.7.16: DNA Fingerprinting of *Gossypium hirsutum* varieties with SSR marker, BNL 3371

Seed treatment for quality Improvement

The effect of seed treatment with H_2O_2 was compared with KCl, hydration with water and untreated controls at germination in 48 hours old seedlings. Preliminary observations indicated that H_2O_2 treatment supported comparatively higher germination besides, seedling fresh and dry weight. Concomitantly, improvement of cell membrane integrity as measured by reduction in release of volatile aldehydes and reduction in electrical conductivity of seed leachates was observed in H_2O_2 treated seeds compared to other treatments (Fig. 3.7.17 & 3.7.18). The activities of major antioxidant enzymes such as peroxidase and catalase were also found to be high in the H_2O_2 treated seeds.

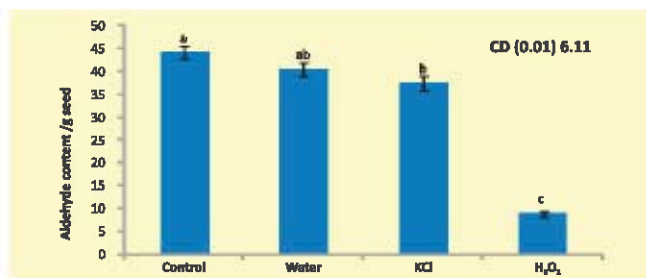


Fig. 3.7.17: Effect of seed treatment on release of volatile aldehydes

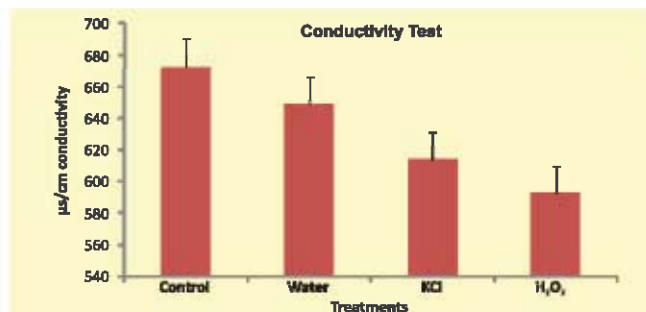


Fig. 3.7.18: Effect of seed treatment on elevated electrical conductivity of seed leachates

Effect of Nursery substrates and transplanting on plant stand

Sixteen different combinations of nursery substrates were evaluated in commercial seedling trays for their efficacy to raise cotton seedlings for transplanting. Kelbrick was found to support good seedling stand besides being the most inexpensive substrate. Root growth observed at 20 DAS was superior in commercial (Kelbrick, Kelpeat etc.) compared to conventional substrates (Soil, FYM, Sand etc.). Vigour of the seedlings was superior in seeds sown in large slotted trays compared to trays with smaller slots.

One and 2 months old cotton seedlings were transplanted following treatments with microbial consortia, H₂O₂ and *Pseudomonas fluorescens* individually as well as in combinations compared to directly sown crop. Preliminary observations indicated that one and two months old cotton transplanted during normal season (9.7.12) yielded better than early season (5.6.12) direct sown cotton. Besides, yield advantage transplanted seedlings supported comparatively higher number of bolls, produced fewer monopodia and were comparatively short in height.

Coimbatore

Effect of Seed Coating with Synthetic Polymers and Additives

An experiment was initiated during 2009 to improve storability and sustainability of viable cotton seeds with polymer coating along with insecticides or fungicides.

After treatment, seeds were stored in cloth bag under ambient condition. Viability of seeds was evaluated at quarterly interval till August 2012. It was observed that seeds of NHH 44 Bt coated with Polymer (Polykote @ 3 ml/kg of seed), Polykote with Imidacloprid @ 6 ml / kg of seed and Polykote with Vitavax @ 2 g/kg of seeds were efficient in maintaining the viability. In case of coating seeds with polymer-polyloc alone or with carbendazim (Bavistin) @ 2 g/kg was also found effective in maintenance of viability of seeds on storage.

Effect of seed treatment on productivity of cotton

Effect of seed treatment on plant growth, promotion and enhancement and productivity was studied. Seeds were subjected to simple hydration, or treated individually with inorganic chemicals like CaCl₂, KNO₃, H₂O₂, KH₂PO₄, KCl; growth hormone GA₃; amino acid and succinic acid; or fungicide Thiram at specified concentration and sown. Significant enhancement in productivity in terms of increased plant height, number of sympodia, number of bolls / plant and boll weight was achieved with hydration for 10-12 h. followed by drying the seeds at room temperature and dressing with 0.25% Thiram. Seed

soaking in H₂O₂ @ 40 mM for 6 hrs. or in aqueous solution of succinic acid @ 0.2% followed by drying at room temperature before sowing were equally effective.

Effect of magnetic pulse treatment on seed cotton productivity

Seed subjected to magnetic pulse treatment showed significant enhancement in viability in all the genotypes tested especially at magnetic field intensity of 750nT followed by that in 1500nT and 500nT. Pulse field treated seeds however did not show significant enhancement in cotton productivity.

Implementation of PVP legislation, 2001

During 2012-13, 104 genotypes (56 candidate and 38 reference and 10 essentially derived varieties) were characterized for DUS under Implementation for Protection of Plant Varieties and Farmers' Rights Act, 2001. Based on filing of application for registration of extant and new cotton varieties, Plant Variety Registration Certificate for 29 extant cotton varieties have been received. The complete database on 160 *G. hirsutum*, 3 *G. barbadense*, 60 *G. arboreum*, 15 *G. herbaceum*, 50 intra-*hirsutum*, 19 interspecific, 9 intra-*arboreum* and 4 *h x a* hybrids were documented alongwith 91 varieties of common knowledge.

Under varietal maintenance, 145 *G. hirsutum*, 6 *G. barbadense*, 28 *G. arboreum* and 8 *G. herbaceum* were maintained and multiplied. Monitoring of DUS trials were conducted at Nagpur, Coimbatore, HAU Hisar, PAU Ludhiana and UAS Dharwad under the chairmanship of Dr. M.S.Kairon, Former Director, CICR Nagpur.

Sirsa

Seed Setting efficiency in *G. hirsutum* cotton

Cotton genotypes were found to vary in their seed setting efficiency. Nature of variability in seed setting efficiency was studied in two sets of cotton genotypes at CICR regional stations at Coimbatore and Sirsa. Seed setting efficiency in a set of 100 germplasm lines was analysed each at Sirsa and Coimbatore by estimation of number of ovules per ovary and corresponding number of seeds set per boll. At Sirsa, number of ovules per ovary ranged between 22-35 and corresponding conversion into seeds/boll ranged from 19-34, with seed setting efficiency of 72.2-97.1%, while at Coimbatore number of ovules per ovary ranged from 28.1-35.3 and corresponding number of seeds per boll ranged from 20.0-33.3, with a setting efficiency of 65.9-99.6%. If seed setting efficiency is an inherent trait, efforts can be made to enhance seed setting efficiency and consequentially the productivity, by seed technological interventions.

Seed Production

Nagpur

Under Mega Seed Project, TFL seed production of 24 straight varieties of cotton, *G. arboreum* race *cernuum*, Red Gram cv. BSMR-736, Bengal Gram, certified seed of Gram cv. Jaki 9218 and Digvijay was taken up. Nearly 170 Q quality seeds of cotton including breeder seed of Suraj and DS-5 - female parent of CICR 2; TFL seeds of *G. arboreum* race *cernuum* and 20 varieties of cotton), pigeon pea and chick pea were produced and resource

worth Rs. 16 lakhs was generated.

Seed production of *G. arboreum* race *cernuum* was taken up. Wide genetic diversity was observed among the populations. Morphological variants within *G. arboreum* race *cernuum* such as those with different shades of lint, number of locules/ bolls and bolls/plant, erect plants with zero monopodia, etc., were documented. Morphological variants were also evaluated for stability of different traits and trait-wise purification of the material was done.



Four loculed cotton

Variation in boll size

Coloured lint

Variability in *Gossypium arboreum* race *cernuum* cotton

Coimbatore

Breeder seed of cotton varieties viz., Suraj, LRA 5166, Anjali, Surabhi, Suraj, MCU 5 VT and Suvin was produced at Coimbatore. In all, 239 kg breeder seeds of varieties were supplied to indenters including private seed producers.

Sirsa

At Sirsa, 190 kg breeder seed of *desi* cotton (*G. arboreum*) including parents of CICR-2 and varieties CISA 614 and CISA 310, was produced and distributed to stakeholders and indenters.

3.9: Nutrient Management

Nagpur

Organic nutrient sources with release rates compatible to crop demand is one of the key principles of nutrient management. Therefore, field studies were conducted with four organic components viz. FYM, vermicompost, bio-enriched cotton compost and mulching of green manure (sunhemp) besides a control treatment. All organic sources were applied in equal quantities (5t/ha). Two varieties, namely, Suraj (*G. hirsutum*) and JLA 794 (*G. arboreum*) were taken up under organic cotton cultivation. Three sprays of neem oil (@300 ppm) were given for controlling insect pests. Seed cotton yield of 13.6 q/ha was recorded in JLA 794 followed by Suraj (9.8

q/ha). Soil analysis data indicated that application of bio-enriched compost resulted in a buildup of available soil-N and soil organic carbon (SOC). SOC content in the organic manure amended plots ranged from 0.64 to 0.76% which was substantially greater than the SOC content in the farmers' practice plot (0.54%).

Cotton stalk compost was prepared using improved composting technique and the nutrients enriched compost was evaluated as a substitute to FYM. Field experiment was conducted in a split plot design with two main treatments [seed treatment with microbial consortia (MC) and without MC] and 6 sub treatments [recommended NPK (90:45:45 kg NPK/ha), INM Practice (60:30:30 kg NPK + 5 t FYM/ha), modified INM Practice (60:30:30 + 5 t cotton compost), FYM, cotton compost and control]. Bunny Bt (NCS-145Bt) was used as a test hybrid with a spacing of 60 x 60 cm. All the treatments were replicated 5 times. Results showed significant differences between treatments. Incorporation of cotton compost (the modified INM) was found to be as good as the INM practice comprising FYM. Seed treatment with microbial consortia improved seed cotton yield by 2-5 q/ha compared to the un-treated seeds.

Coimbatore

Recommended NPK with cotton stalk compost @ 2.5 t/ha had seed cotton yield and boll numbers similar to the