



## 3.1: Cotton Genetic Resources

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

#### Nagpur

#### Exploration for perennials

Exploration and collection programmes were

undertaken in Manipur, Nagaland, Meghalaya, Assam, Maharashtra and Gujarat. Thirty five cotton germplasm materials were collected including 25 perennials of *G. barbadense* and 10 traditional cultivars belonging to *G. arboreum* and *G. herbaceum* (Table 3.1.1). All these germplasm were established in pot culture and their seeds were stored in the Gene bank. The collected cotton germplasm were characterized morphologically.

Table 3.1.1: Perennials and landraces of cotton collected from different regions of India

State	Districts	No. of Accessions	Species	Annual/ Perennial/ Landrace
Manipur	Imphal West, Senapati, Thoubal, Tamenglong, Bishnupur, Imphal East & Chandel	16	<i>G. barbadense</i>	Perennial
Maharashtra	Wardha	2	<i>G. barbadense</i>	Perennial
Gujarat	Mehsana, Patan, Banaskantha & Kachchh	5	<i>G. herbaceum</i>	Annual
		4	<i>G. arboreum</i>	Annual
		1	<i>G. barbadense</i>	Perennial
		3	<i>G. barbadense</i>	Perennial
Nagaland	Mon & Tuensang	3	<i>G. barbadense</i>	Perennial
Meghalaya	West Garo Hills	1	<i>G. arboreum</i>	Annual
Assam	Barpeta	3	<i>G. barbadense</i>	Perennial
<b>Total</b>		<b>35</b>		



Traditional Desi cotton *G. herbaceum* of Mehsana Gujarat



Perennial Cotton *Gossypium barbadense* of Bishnupur, Manipur

#### Fiber quality traits of perennial cotton

Fibre quality traits of 19 morphologically distinct germplasm materials were evaluated. Ten unique germplasm were established in perennial species garden. These included important *G. arboreum* land races like Ponduru cotton, Mathio cotton and Karunganni cotton and *G. herbaceum* land races viz. Wagad and Uppam cotton.

#### Molecular characterization of wild species and perennials

A total of 413 Simple Sequence Repeat (SSR) markers were screened using a set of 20 wild species of cotton, 15 races of cultivated species and synthetic polyploids to identify informative markers for genetic diversity assessment. Of the markers tested, 24% (103/413) showed polymorphism (Fig. 3.1.1).

## F<sub>2</sub> mapping population established

S.No	Derivative	Number of F <sub>2</sub> plants
a.	<i>G. herbaceum</i> x <i>G. longicalyx</i>	557
b.	<i>G. arboreum</i> race <i>indicum</i> x <i>G. davidsonii</i>	212
c.	<i>G. arboreum</i> x <i>G. thurberi</i>	53
d.	AK 8401 x <i>G. davidsonii</i>	34

Eleven fibre strength specific SSR primers were found to be polymorphic in the F<sub>2</sub> mapping population of *G. herbaceum* and *G. anomalum*. The F<sub>1</sub> of *G. herbaceum* and *G. anomalum* cross showed only 43% pollen fertility and >50% bolls were deformed in shape resulting in premature shedding but fibre strength was high i.e. 36.7 g/tex.

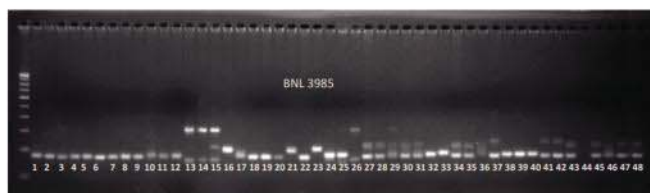


Fig. 3.1.1: Genetic diversity assessment in F<sub>2</sub> mapping population of wild species and races.

1.Africanum 2.Herbaceum 3.Digvijay 4.Indicum 5.Burmanicum 6.Bengalense 7.Cernuum 8.Sinense 9.Soudanense 10.Anomalum 11.Triphyllum 12.Capitis virides 13.Barbosanum 14.Sturtianum 15.Australe 16.Thurberi 17.Armourianum 18.Davidsonii 19.Klotzchianum 20.Aridum 21.Raimondi 22.Lobatum 23.Trilobum 24.Stocksii 25.Somalense 26.Bickii 27.Latifolium 28.Palmeri 29.Marie galente 30.Richmondii 31.Mexicanum 32.AK8401 33.Thespesia 34.Barbadense type Kuhu mandhal 35.Barbadense type Bhandara 36.IC 3981 pigmented arboreum 37.RHCW-1 kidney cotton 38.G. Barbadense collected from West Bengal 39.IC 3912 arb 40.IC 3914 arb 41.Exotic-3 42.Diu 90 43.Diu 91 44.Moco 45.Diu 92 46.Diu 93 47.Serido 48.Diu 4494

Molecular characterization was carried out for 31 perennials using 19 SSR markers. Out of nineteen, five SSR markers were polymorphic (Fig. 3.1.2)

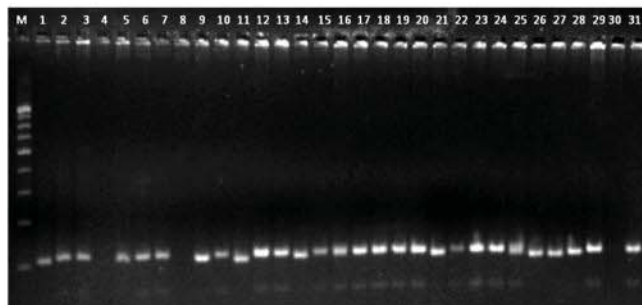


Fig. 3.1.2: SSR profile of 31 collected landraces of desi cotton and perennials from Tamil Nadu, Tripura and Gujarat with primer NAU 396

1-PLC124, 2-PLC125, 3-PLC126, 4-PLC127, 5-PLC128, 6-PLC129, 7-PLC130, 8-PLC131, 9-PLC132, 10-PLC133, 11-PLC134, 12-PLC135, 13-PLC136, 14-PLC137, 15-PLC110, 16-PLC111, 17-PLC112, 18-PLC113, 19-PLC114, 20-PLC115, 21-PLC116, 22-PLC117, 23-PLC118, 24-PLC119, 25-PLC120, 26-PLC139, 27-PLC140, 28-PLC141, 29-PLC142, 30-PLC143, 31-PLC144

### Genetic stocks identified for registration

Five genetic stocks viz. three of *G. arboreum* and two of *G. hirsutum* were identified for registration with NBPGR, New Delhi (Table 3.1.2).

CNH 219 - Pigmented plant body



Table 3.1.2 : Genetic stocks identified for registration

Designated material	Species	Traits
CNA-405	<i>Gossypium arboreum</i>	Narrow leaf lobe and brown linted
CNA-406	<i>Gossypium arboreum</i>	Broad leaf lobe and brown linted
CNA-407	<i>Gossypium arboreum</i>	Narrow leaf lobe, Pigmented plant body, brown linted
CNH -219	<i>Gossypium hirsutum</i>	Pigmented plant body, deeply palmate leaf lobe and brown linted
CNH CB-228	<i>Gossypium hirsutum</i>	Densely hairy bolls and cluster bolls bearing habit

### Enrichment of cotton gene bank and maintenance of germplasm

Seven hundred and twenty three (723) exotic accessions of *hirsutum* were procured through NBPGR, New Delhi for enrichment of the cotton gene bank. In addition to the above, trait specific 349 germplasm lines (*G. barbadense* - 209, *G. hirsutum* - 75, *G. arboreum* - 2,

*G. herbaceum* - 43 and wild species - 20) were selected from Germplasm Resource Information Network (GRIN). Nine import permits were issued by the NBPGR for procurement of above exotic germplasm.

### Maintenance of germplasm

The following were maintained in the wild species garden:

- 26 wild species
- 15 races cultivated species
- 40 synthetic polyploids
- One new species (EC 669583) was established in pot

### Germplasm evaluation

Two thousand and eighty-five accessions of *G. hirsutum* were evaluated for fibre quality traits. Fifteen long linted accessions (staple length, 30.8-32.9 mm) and 38 accessions for high fibre strength (23.0 – 24.8 g/tex) were identified and documented.

### Long fibre accessions

IC 292705 (32.9 mm), EC 1044 (32.9 mm), IC 292566 (32.7 mm), IC 359044 (32.7 mm), EC 344109 (32.6 mm), IC 291381 (32.4 mm), EC 344048 (32.3 mm), EC 344049 (32 mm), EC 344201 (31.4 mm), EC 1072 (31.3 mm), EC 1035 (31.2mm), IC 158943 (31.1 mm), EC 1015 (31.1 mm), IC 292478 (30.8 mm) and EC 343702 (30.8 mm).

### High fibre strength accessions

- **24.1 to 24.8 g/tex:** EC 344131 (24.8 g/tex), EC 1044 (24.7 g/tex), EC 1035 (24.6 g/tex), EC 344158 (24.4 g/tex), EC 344150 (24.4 g/tex), EC 1072 (24.4 g/tex), EC344049 (24.3 g/tex), EC 344048 (24.2 g/tex), EC 1084 (24.1 g/tex)
- **23.1 to 23.9 g/tex:** IC 358317 (23.9 g/tex), IC 359893 (23.9 g/tex), EC 343737 (23.9 g/tex), EC 1015 (23.8 g/tex), IC 291617 (23.7 g/tex), IC 150047 (23.7 g/tex), EC 343750 (23.7 g/tex), IC 158943 (23.6 g/tex), EC 1087 (23.6 g/tex), IC 292478 (23.6 g/tex), IC 358410 (23.6 g/tex), IC 358479 (23.5 g/tex), IC 358571 (23.5 g/tex), IC 359914 (23.5 g/tex), IC 358473 (23.4 g/tex), IC 357906 (23.4 g/tex), IC 358278 (23.4 g/tex), EC 343662 (23.4 g/tex), IC 357845 (23.2 g/tex), IC 359889 (23.2 g/tex), IC 291375 (23.2 g/tex), EC 344053 (23.2 g/tex), EC 343952 (23.2 g/tex), IC 292063 (23.1 g/tex), EC 343794 (23.1 g/tex), EC 1085 (23.1 g/tex)
- **23.0 g/tex:** IC 360036 (23.0 g/tex), EC 343609 (23.0 g/tex) and EC 343702 (23.0 g/tex).

### Evaluation of germplasm for salinity

Twenty-five germplasm lines including promising advanced cultures (*G. hirsutum* – 10, *G. herbaceum* – 10 and *G. arboreum* – 5) were evaluated in saline Vertisols (9.2 dS/m) at Central Soil Salinity Research Institute, Regional Research Station, Bharuch, Gujarat. The highest seed cotton yield was recorded in germplasm accessions; *G. herbaceum* : IC 371126 and IC 371099, *G. arboreum* : CNA 443, CNA 442 and CNA 447 and *G. hirsutum* : DTS 301, CNH 19 and 29 I.

### Gossypol estimation

Seed gossypol was estimated in 1994 germplasm accessions. Gossypol content ranged from 0.03 to 1.17 % and 1750 accessions showed gossypol content between 0.1 to 0.5 %.

### Distribution and utilization of germplasm in crop improvement programmes

Six thousand two hundred thirty two (6232) germplasm accessions (*G. hirsutum* - 6194, *G. barbadense* - 3, *G. arboreum* - 27, *G. herbaceum* - 1 and wild species - 7) were distributed to scientists of different State Agricultural Universities.

### Barcoding of germplasm

Barcoding of conserved germplasm accessions was undertaken and 5000 accessions of *G. hirsutum* were barcoded for easy retrieval and identification in gene bank.

### Coimbatore

In common trials of germplasm lines, 28 select *G. hirsutum* germplasm lines and 16 *G. arboreum* germplasm lines were evaluated for yield and other characteristics. Among the *G. hirsutum* germplasm lines evaluated, IC 359780 recorded the highest seed cotton yield (1805 kg/ha) followed by Sumangala (1453 kg/ha). Zero branching habit was noted in IC 291509, IC 360026, IC 356618 and IC 359992. Among *G. arboreum* germplasm lines evaluated, the highest seed cotton yield was recorded in IC 439854.

Three hundred and five *G. barbadense* germplasm lines were maintained and catalogued. Eighteen new *G. barbadense*, perennials were morphologically characterized.

### Sirsa

Out of 260 compact lines identified and evaluated at 67.5 x 10 cm, 10 lines with maximum 120 cm height and 20 cm width, with superior yield and tolerance to sucking pest and CLCuV were identified. DCI-48 (3120 kg/ ha), H-1091/99 (3000 kg ha) and GJHV-47(2970 kg/ ha) were the highest yielders. In addition, 3 lines viz. SP-3897 (2900 kg/ha), PAKISTAN-2 (3020 kg/ha) and N-78 (2900 kg/ha) with ultra narrow characteristics and earliness were observed against check RS 875 (2103 kg/ha).

### Identification of germplasm sources of resistance to insect pests

One hundred and sixty eight compact accessions were evaluated for reaction towards sucking pest. For whitefly, 36 entries were found to be moderately resistant (1-10 whitefly/3 leaves) and 29 entries susceptible (> 30whitefly/3 leaves). For thrips, only one entry was susceptible (> 45 thrips/3 leaves) and 116 entries were moderately resistant (1-15 thrips/3 leaves).

For leafhopper, 151 and 17 entries fall under highly resistant (0-3 leafhopper/3 leaves) and moderately resistant (4-10 leafhopper/3 leaves) category, respectively.

### 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), 20 GMS lines and 57 restorers were maintained through crossing with their counter B-lines, sib mating and selfing respectively. Seed multiplication for four new GMS lines developed through backcross breeding was taken up and crosses attempted to study the combining ability of these lines. Fourteen GMS *arboreum* lines were sib multiplication mated for seed and maintenance. A maximum of 26.1 g/tex fibre strength was recorded for GMS line GAK-8615(A) followed by 25.7 g/tex for GMS-4-1. These lines could be used to improve fibre quality of existing *arboreum* cultures.

Fifteen crosses were attempted to develop *Desi* hybrids (both conventional and GMS based). Two *Desi* GMS lines were crossed with five grey mildew immune lines and five promising genotypes were crossed with five grey mildew immune lines to develop grey mildew resistant *arboreum* hybrids with big bolls and high boll retentivity.

#### Sirsa

##### Tetraploid cotton

##### Release of intra-*hirsutum* GMS based hybrid CSHG 1862



Intra-*hirsutum* GMS based hybrid CSHG 1862 was released by Central Varietal Release Committee for irrigated conditions of Haryana, Punjab and Rajasthan vide Gazette Notification No S.O. 952(E) dated 10 April 2013. This hybrid recorded an overall mean seed cotton yield of 2102 kg/ha as against 1882 kg/ha of CSHH 198 (Common Check) and 1980 kg/ha of local checks. Increase in seed cotton yield over the common (Zonal) check was 11.6 % and over that of local checks 5.8 %. Besides easy seed production, the GMS based hybrid CSHG 1862 is also capable of spinning at 40s counts.

##### Evaluation of intra-*hirsutum* crosses

To develop segregating population of *G. hirsutum* cotton, 16 crosses were attempted among CLCuV tolerant germplasm lines in a Line x Tester fashion. This year, crosses were evaluated against two checks CSHH 198 and CSHH 238 and the highest seed cotton yield was recorded in RS 2013 x CSH 2811 (2278 kg/ha), followed by RS 2013 x CSH 2937 (2074 kg/ha) as against 2167 kg/ha of conventional check hybrid CSHH 238. Maximum ginning out-turn of 34.1 % was recorded by the hybrid PIL8 x CSH 2937. The highest 2.5 % span length (27.5 mm) and bundle strength (23.3 g/tex) was recorded by the hybrid CB-33 x AKH 9620 respectively. The cross combination Jhorar x LRA 5166 showed the minimum CLCuV incidence of 19.5 PDI but gave the seed cotton yield of 1481 kg/ha only as compared to 2167 kg/ha of CSHH 238 with 33.3 PDI CLCuV.

##### Diploid cotton

Fifteen new crosses were attempted in line x tester fashion for hybrid development in *G. arboreum*. Four crosses using 4 GMS lines were done for large scale testing to ascertain their yield potential. All the GMS lines are maintained through sibmating. GMS based hybrid CISAA27 was sponsored in Br 25a AICCIP national trial.

### 3.3 : Genetic Improvement

#### Nagpur

##### *G. arboreum* (Diploid cotton)

##### New cultures for surgical cotton

From the existing progenies, 12 short stapled cultures with high micronaire were identified. Among these, the lint samples of CNA 441, CNA 443, CNA 444, CNA 445 and CNA 447 were found suitable for absorbent and surgical purpose as per the IP 1996 standards. From another set of 13 cultures, CNA 418 and CNA 423 were found to be good in terms of liquid absorbency (1.1-1.2 second) and sulphate ash content (0.30-31%).

##### Identification of promising cultures

Twenty nine new *arboreum* cultures were evaluated under narrow spacing of 60 x 22.5 cm. Sixteen cultures

yielded more than 893 kg/ha. Eight cultures recorded boll weight > 3 g. CNA 2017 (4.1 g) recorded the highest boll weight which was superior to check AKA 8401 (3.7 g). Two entries viz., CNA 2006 and CNA 2009 have been entered in institute trial. Culture CNA 2023 was identified for high productivity with good boll number.



High yielding *G. arboreum* culture CNA 2023

Sixty six *G. arboreum* cultures identified previously for yield and fiber quality, were evaluated in replicated trial. The seed cotton yield ranged from 302 kg/ha to 1160 kg/ha. Cultures CNA 1013 and CNA 1021 were identified for sponsoring into AICCIP trials.

### *G. hirsutum* (Tetraploid cotton)

#### Breeding for compactness

Three  $F_1$ s viz., N 170 ( Early maturing dwarf compact) x IC 356750, N 170 x introgressed LRA 5166 and N 170 x IC 358358 were evaluated for boll weight, boll number and plant type. Sixty three  $F_1$  plants recorded variation for number of bolls per plant (3 to 25) and boll weight ( 1 to 3.2 g) and plant height ranged from 27 to 100 cm. New  $F_1$  cross viz., N 170 x IC 356771 has been generated which is trait specific for big boll size. Fifteen dwarf  $F_1$ s were identified with plant height ranging from 25 cm to 56 cm.

Five trials were conducted with a spacing of 60 x 15 cm. The cross RS 875 x Rex recorded optimum yield levels with plant height of 77 cm, 18 cm sympodial length, 63 bolls/sq metre, small leaf size and medium boll weight (3 g). The checks grown were NH 615 and PKV 081. RS 810 x MHL 557 has been identified as having fibre length upto 29 mm.

#### Evaluation of genetically enhanced populations

Fifty-nine ( $F_8$ ), 29  $BC_3F_6$  and 22  $BC_2F_5$  genetically advanced lines were evaluated at 60 x 45 cm spacing. In  $F_9$ , the cross PKV 081 x Deltapine 66 recorded boll

number improvement (upto 36 bolls) as compared to parent (with 26 bolls) while cross G.cot 10 x Deltapine 66 recorded 45 bolls/plant as compared to parent G.cot 10 with 27 bolls/plant. In  $BC_2F_5$ , the backcross PKV 081 x (PKV 081 x PIL 8) gave the highest plot yield of 880 kg/ha with a spacing of 60 x 45 cm. However, in  $BC_3F_6$ , the same cross PKV 081 x (PKV 081 x PIL 8) had recorded 1407 kg/ha at a spacing of 60 x 45 cm. Fibre strength upto 23.8 g/tex was achieved in a unreplicated trial of advanced material. A medium duration high yielding culture, CNH 2015 was also developed.

In 29 inter-mating lines derived from 5 parental crosses (sixth cycle), 10 best lines were identified based on seed cotton yield and boll number (Table 3.3.1). Line IM 11 had the highest yield while line IM 26 had the highest boll weight (4.6 g). Many single plant selections for semi-compact and compact types were made which can serve as supportive useful material in breeding for compact types.



Table 3.3.1: Yield and yield attributes of superior inter-mating lines derived from five parental crosses

S.No.	Name of the entry	Seed cotton yield(kg/ha)	Boll wt.(g)	Boll no./plants
1	IM-1	1677.2	2.0	29
2	IM-11	1851.8	3.0	25
3	IM-12	1481.4	3.0	18
4	IM-13	1058.2	2.8	24
5	IM-14	1322.7	3.6	21
6	IM-16	1296.3	3.6	20
7	IM-19	1419.7	3.4	20
8	IM-21	1543.2	3.6	26
9	IM-22	1586.4	3.8	22
10	IM-26	1080.2	4.6	23
11	Suraj (check)	851.8	3	12

## Abiotic stress tolerance

### Nagpur

#### Drought

Four sets of experiments were conducted under rainfed and irrigated conditions to assess drought tolerance efficiency of the genotypes which are under various stages of development. Trials under irrigated conditions were vitiated due to heavy rains that persisted till October end. In the first set, eight identified drought tolerant cultures were tested along with five new genotypes for second year. All the identified cultures recorded more than 15 % increase over the check (LRA 5166). DTS 155, DTS 62, DTS 104 and DTS 44 were better performers for the second year of testing. DTS 155 recorded highest yield of 610 kg/ha with 67 % increase over the check LRA5166.

The second set comprised of 12 genotypes in F<sub>5</sub> generation, their parents and check Rajat. The seed cotton yield ranged from 401-646 kg/ha and ten genotypes were on par to the check Rajat (477 kg/ha). Pusa 56-6 x 30 I was the highest yielder followed by 29 I, 29 I x Pusa 56-4 and Pusa 56-4 x 29 I. These crosses recorded more than 20 % increase over the check.

The third set consisted of 49 single plant selections which during their preliminary evaluation had recorded high seed cotton yield, good fibre length (>28 mm) and fibre strength (> 22 g/tex). The highest yield was recorded by DTS 79 (625 kg/ha) followed by DTS 116, DTS 141, DTS 123, DTS 69 and DTS 75. Some of these selections recorded boll weight upto 4.0 g. Twelve F<sub>5</sub>s and six parents were tested in the fourth set under replicated trial. Cross PKV 081 x Suraj, 28 I x Suraj and NH 615 x Rex were some of the good performers recording 30% increase over the check LRA5166. Cross CCH 510-4 x Moco was completely fertile and showed good boll bearing with 12% increase over the check LRA 5166 (763 kg/ha). Cross LRA 5166 x N 170 was very compact and early, while 28 I x Suraj was early as well as resistant to jassid.

Eighteen intercross material were tested along with a moderately tolerant check LRA 5166 under laboratory,

pot culture and field condition. The lines were tested for germination using PEG 6000 from -0.2 to -0.9 MPa stress. Only one line 41(55)-02 could germinate at above -0.6 MPa stress. In pot culture studies, none of the lines could survive more than three days of moisture stress. In field studies, all these lines were found similar to LRA 5166 for seed cotton yield.

Five cultures were tested for salt tolerance at Central Soil Salinity Research Institute, Regional Station, Bharuch. Two of these cultures (DTS 29 I and DTS 30 I) recorded good yield (1252 and 1280 kg/ha) at salinity level as high as 9.2 dS/m. Culture CNH 28 I was promoted to Br 06 (b) trial of AICCIP in both central and south zone.

#### Screening for drought tolerance

Earliness (drought avoidance) is a phenological trait which increases the relative amount of moisture that is vital during reproductive stage to cotton grown on shallow and medium soil. Of the 2000 cotton germplasm accessions grown, 84 short duration lines were identified based on days to 50 % boll opening.

Fifty one cotton germplasm lines were identified to have high epicuticular wax content in leaves. Presence of epicuticular wax content is a water conservation strategy for drought tolerance traits. Epicuticular wax content in the okra type leaf of cotton germplasm accessions was in the range 47.1 to 473.7 µg/ cm<sup>2</sup> values for early maturing accessions with normal leaf ranged from 46.4 to 132.9 µg/ cm<sup>2</sup>.

#### Waterlogging

##### Nagpur

Two thousand - *G. hirsutum* germplasm accessions were screened against waterlogging during the cropping season 2013-14 and of these 200 accessions tolerant to waterlogging were selected. The 45 days old saplings of 150 germplasm accessions selected during 2012-13 were kept waterlogged continuously for 70 days. The maturity of the plants in waterlogged field was delayed as compared to that of the plots in normal field i.e. control field by 25 -30 days. Selected single plants (200 number) showed tolerance to waterlogging.



Field artificially waterlogged for 70 days during crop growth

## Coimbatore

Lenticel formation was found to be a good index in screening waterlogging tolerance. Out of the 125 cultures treated Culture 2186, 2605, 2709, 2728, 2853, 3164, 3341, 4481 initiated lenticel formation within 3 days after waterlogging while cultures 193, 891, 1093, 1422, 2648, 2698, 2711, 4261, initiated lenticel formation in response to water logging only after 6 days.

## Biotic stress tolerance

### Jassid

A breeding programme was initiated to develop *hirsutum* variety with improved yield, quality and tolerance to jassids. Of the 86 progenies evaluated, 19 were early (145-160 days) and tolerant to jassid. Lines CNH 09-7, CNH 09-9, CNH-09-5, CNH 09-62, CNH 09-74, CNH 09-13, CNH 09-15, CNH 09-111, CNH 2-1 and CNH 2-2 were identified for higher seed cotton yield and fibre strength. Lines CNH 09-9 recorded seed cotton yield of 2631 kg/ha followed by CNH 09-5 (2556 kg/ha) and CNH 09-7 (2025 kg/ha) with bundle strength of 23.1 g/tex, 23.4 g/tex and 25.7 g/tex, respectively.

From the segregating population F<sub>2</sub> and F<sub>3</sub>, 110 single plant selections were made based on earliness, boll weight, fibre strength and sympodial plant type. From inter-specific derivatives, 11 F<sub>5</sub> progenies were selected based on tolerance to jassids and yield potential from crosses NISC 261 × P 56-4, NISC 261 × AKH 081, NISC 261 × H 1252, NISC 289-4 × LRK 516, NISC 289-4 × EC 277959 and NISC 291 × EC 277959. Twelve cultures tolerant to jassids tested for seed cotton yield and fibre properties.

### Bollworm

Sixty-one promising genotypes from different agro-ecological zones were converted into Bt background through backcrossing. They are under different backcross generation (BC4F4 to BC2). Sixteen hundred individual plants were tested for presence of Bt using Elisa plates of which 428 plants were found positive.

### Testing of *G. hirsutum* cultures in AICCIP

Seven high yielding *G. hirsutum* cultures were sponsored for multi-location evaluation in All India Coordinated Cotton Improvement Project during 2013-14. Of these, four were tested in National trials and the remaining three were in advanced stage of testing in zonal trials.



## Compact plants suitable for HDPS

In two separate station trials, 14 compact plant genotypes each were evaluated along with Anjali, Supriya and Suraj as check varieties with a spacing of 45 x15 cm. Analysis of data on seed cotton yield indicated significant differences among the genotypes and the highest yield was recorded in PI 36-2-4-1 in Trial-I with 3539 kg/ha and in Surabhi x MM02-16-5-2-4 Bk in Trial-II

Several high yielding compact plant types with super okra leaf having good fibre quality amenable to closer planting were identified.



**Surabhi X MM 02-19-1-10-1-3** 2.5 % Span length = 30.4 mm Bundle Strength = 24.2 g/tex  
**MCU-13 X VNWH-1-7-2-3** 2.5 % Span length = 30.6 mm Bundle Strength = 24.5 g/tex



**Surabhi X M5 Z2-4-2-6** 2.5 % Span length = 30.7 mm Bundle Strength = 24.2 g/tex  
**Surabhi X M5Z2-13-3-4** 2.5 % Span length = 30.4 mm Bundle Strength = 23.8 g/tex

### Good quality super okra compact genotypes

### Evaluation of long staple cultures

Thirteen superior long staple *G. hirsutum* cultures were evaluated in replicated trial along with Suraj and Surabhi as check varieties. The highest yield was recorded in MM 03 - 39-2-4-3 with 1652 kg/ha and the culture also recorded the best fibre quality parameters of 33.4 mm 2.5% span length and 24.4 g/tex of tenacity at 3.2 mm gauge.

### Evaluation of medium staple *G. hirsutum* cultures

Fifteen medium staple cultures were evaluated along with Sumangala and LRA 5166 as check varieties. Data on seed cotton yield indicated significant differences among the genotypes and the highest yield was recorded in MM02-11-7 (2464 kg/ha). The culture MM03-12-1-2-4 recorded tenacity of 24.3 g/tex with 30.3 mm length.

### Evaluation of *G. barbadense* cultures

- Nine high yielding, early maturing genotypes (CCB-30, CCB-33, CCB-36, CCB-40, CCB-52, CCB-62, CCB-63, CCB-64 and CCB-74) were identified from two advanced yield trials.

- Twenty six single plants were selected from 76 progenies based on plant type, single plant yield, earliness and ginning outturn. Of these, 11 single plants were identified as superior with respect to yield and earliness.
- Five intermated population of Suvin were raised along with the existing Suvin as control and

evaluated for yield, GP and earliness.

- Third cycle of 478 random mating population were developed from nine diversified *G. barbadense*, L. genotypes. From the segregating population, some superior single plants were selected with typical boll shape combining high boll weight.



**CCH 526612 x VNW-1**

Boll Shape: Conical with typical beak  
Seed Cotton Yield = 115 g/plant  
Boll Weight = 5.5 g



**Surabhi x M522**

Boll Shape: Cylindrical  
Seed Cotton Yield = 95 g/plant  
Boll Weight = 4.8 g



**Surabhi x M522**

Boll Shape: Big Conical  
Seed Cotton Yield = 95 g/plant  
Boll Weight = 6.8 g

### Fibre strength improvement

High strength genotypes evaluated earlier in All India Coordinated Cotton Improvement Project were consolidated from various centres and two separate trials, with 20 genotypes each, were conducted to evaluate their yield potential and fibre quality parameters. In the first trial, the genotype CCH 10-2 recorded the highest seed cotton yield (1482 kg/ha). Quality wise, the genotype CCH 4474 was the best (33.4 mm length and 25.0 g/tex strength). Comparative performance of these cultures over the past two years *vis-à-vis* AICCP trial indicated consistent superiority of cultures *viz.*, CCH 4474, CCH LS 2, CCH 7122 and CCH 820 for fibre quality especially the bundle strength (Table 3.3.2).

In the second trial, the genotype SCS 1061 was the best in terms of seed cotton yield with 1672 kg/ha. The genotype also recorded the highest 2.5 % span length of 33.9 mm. The highest bundle strength of 23.7 g/tex was recorded for the genotype SH 2-4 in this trial.

### Sirsa

#### Tetraploid Cotton

#### Evaluation of CLCuV resistant cultures

##### Trial 1

In the first trial, 13 *G. hirsutum* cultures were evaluated against the check varieties RS 2013 and LH 2076 and susceptible check HS-6. The highest seed cotton yield was recorded in the culture CSH 2811 (1704 kg/ha) followed by CSH 2838 (1556 kg/ha) as against the check variety LH 2076 (1617 kg/ha). Maximum ginning out turn of 34 % was recorded in the variety CSH 2844 as compared to local check varieties 32.7 % in RS 2013 and

**Table 3.3.2 : Comparative performance of high strength genotypes for yield and quality (mean of 2012 – 13 and 2013 – 14)**

Genotype	Seed cotton yield (kg/ha)	2.5% Span length (mm)	Tenacity 3.2 mm (g/tex)
CCH 10-2	1110	29.9	21.7
CCH 4474	1008	33.5	25.5
CCH LS2	864	32.5	24.0
CCH 1831	858	29.2	22.7
Suraj (C)	807	32.4	21.9
CCH 7122	727	33.0	25.1
CCH 03-23	709	30.6	21.3
CCH 820	653	32.4	24.9
Surabhi (C)	425	33.1	23.8

33.8 % in LH 2076. *G. hirsutum* culture CSH 2908 recorded the minimum CLCuV incidence of 29 PDI and seed cotton yield of 1864 kg /ha as compared to 1617 kg/ha for LH 2076 with 37.5 PDI.

##### Trial 2

In the second trial, 11 cultures of *G. hirsutum* were evaluated against the conventional check variety RS 2013, LH 2076 and susceptible check HS-6. The highest seed cotton yield was recorded in the hybrid CSH 2931 (2494 kg/ha) followed by CSH 2932 (2247 kg/ha) as against the conventional check variety RS 2013 (1321 kg/ha). Maximum ginning out turn of 35.2 % was recorded by CSH 2924 as compared to local check varieties 32.7 % in RS 2013 and 34.0 % in LH 2076. The culture CSH 2947 had the highest 2.5 % span length (27.0 mm), and bundle strength of 21.9 g/tex having the



CLCuV incidence of 33.0 PDI as compare to 57.4 PDI recorded by HS-6 a susceptible check variety.

Out of 198 introgressed lines received from CICR, Nagpur and evaluated at RRS Abohar, a hot spot area for leaf curl virus, only two lines Rai 48 and MSH 126 showed tolerance.

### GMS based random mating population

At flowering, individual plants in the population was monitored for sterility/fertility at anthesis repeatedly at an interval of a week all 234 sterile plants watertagged. All the out-crossed bolls from the sterile plants in the population were bulk harvested and ginned to constitute the 4<sup>th</sup> cycle of GMS based random mating population.

### Fibre quality improvement

13 crosses were attempted between 5 long linted Coimbatore types with high fibre strength and 3 Sirsa cultures. F<sub>5</sub> progenies of the two crosses from these 13 crosses viz; CSH-3119-10-28-56 (3594.3 kg/ha) and MMO.3 (39-2-5)-3114-10-64 (3180.0 kg/ha) were early in maturity and gave significantly higher yield than the check H-1226 (2350.8 kg/ha). Fibre quality data indicated that CSH-3114 x MM-03-27-5-1-5 gave fibre strength more than 25 g/tex and fibre length > 30 mm. Six cultures with good fibre quality viz.; CSH-3129, 3114, 3047, 3312, 3313 and 3314 were contributed for testing under the marker assisted programme of Technology Mission on Cotton.

### Ginning outturn improvement

One hundred and forty eight F<sub>1</sub> crosses attempted between agronomically superior varieties of north zone and high GOT germplasm lines (37 to 41%) were evaluated in unreplicated trial. Sixteen F<sub>1</sub>'s of these gave GOT% > 37% and 9 crosses among these had > 75 g seed cotton yield / plant. Three F<sub>2</sub> populations of crosses RS-875 x SA-524, F-1861 x SA-668 and CSH-3129 x EC-358002 were evaluated for GOT % of individual plant in each of the cross. There were 11, 48 and 18 plants with more than 35 % GOT and 3, 4 and 1 plant(s) with > 40 % GOT in F<sub>2</sub> population of the above crosses. Thirteen F<sub>5</sub> progenies of the cross SA-977 (HG) x SA-112 (LG) were evaluated in replicated trial. Three progenies viz.; P-70, P-86 and P-164 were at par in seed cotton yield compared to check H-1226 (1876.5 kg/ha) and recorded more than 40 % GOT.

### Diploid Cotton

Under evaluation of advance culture trial, none of the genotypes could out yield the local check CISA 614 (2550 kg/ha). However, genotype CISA-6-123 (2253.1 kg/ha) gave higher yield over another local check LD 327 (2167.3 kg/ha) with 2.5% span length 19.8 mm,

micronaire 7.2 and strength 16.2 g/tex. The genotype CISA 6 had good fibre properties (2.5 % span length 25.8 mm, micronaire 5.6 and strength 18.7 g/tex). Under evaluation of varietal culture trial genotype CISA 6 (2777 kg/ha) gave numerically higher yield than CISA 310 (2600 kg/ha) and 6 genotypes gave higher seed cotton yield than the check LD 733 (1616 kg/ha). One hundred and thirty four single plant selections were made based on yield and fibre qualities from F<sub>2</sub> population.

## 3.4 : Genetic Diversity through Introgression

### Nagpur

#### Development of *Gossypium hirsutum* cultures with big bolls

One promising culture with big boll ranging from 5.9 to - 7.9 g was identified from a cross of Ganganagar Ageti x Acala B2. From the F<sub>5</sub> generation, 51 single plant progenies were raised. Twenty-three superior lines were identified for boll weight (3.70 to 5.03 g). The seed cotton yield ranged from 736 to 2717 kg/ha and GOT from 27.3 to 42.3 % in these progenies.

#### Conventional crossing based random mating population

The random mating population was constituted through conventional crossing and maintained by bulk harvesting.

#### Random mating population developed through exploitation of GMS

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

#### Evaluation of single plant selection

Twelve hundred single plant selections from random mating population were evaluated in plant to progeny row plots. One hundred and twenty plants were selected based on manual testing for fibre quality traits. About 750 superior single plants were reselected from the segregating single plant progenies. Forty six single plant progenies were uniform and exhibited better performance for seed cotton yield.



## Development of heterotic pool for superior medium staple cotton

Heterotic population was developed for superior medium staple (25-27 mm) from parents selected on the basis of geographic diversity. Of the 37 lines developed, 21 were used in crossing programme for developing 120 F<sub>2</sub>s. Some promising lines viz. CNH 16-3-5 (31.1 mm, 23.4 g/tex), CNH 10-6-1 (27.6 mm, 23.5 g/tex), CNH 12-12-4 (29.7 mm, 23.3 g/tex), CNH 12-4-2 (30.4 mm, 24.2 g/tex), CNH 16-3-1 (28.5 mm, 23.6 g/tex) and CNH 17-2-2 (27.5 mm, 23.6 g/tex) were identified for their utilization in crossing for developing heterotic F<sub>2</sub>s.

From heterotic population 18 cultures were developed for higher seed cotton yield with better fibre properties. Promising 8 cultures viz. CNH 7008-1, CNH 7012-13, CNH 7012-11, CNH 7022-4, CNH 7023-5, CNH 7020-1, CNH 7017-3 and CNH 7018-9 were identified.

## Coimbatore

### Development of variety

CCH 2623 recorded a mean seed cotton yield of 1725 kg/ha in Central Zone with 30.5 per cent yield increase over the zonal check variety of LRA 5166. It also recorded high yield of 1798 kg/ha as against 1589 kg/ha in the zonal check variety of Surabhi with 17.0 % yield increase in south zone. The culture is characterized by 27.2 mm 2.5% span length, 4.5 10-6 g/inch micronaire and 21.3 g/tex bundle strength.



### Evaluation of advance cultures

Twenty four entries of *G. hirsutum* and seven of *G. arboreum* were evaluated in Institute trials of which eleven were identified for sponsoring in AICCIP trials during 2014-15. The cultures identified were CNH 61, CNH 7022-4, CINH 1, CINH 2, CINH 5 and CNH 118 of *G. hirsutum* and CNA 2009, CNA 449, CNA 1022 and CNA 2006 of *G. arboreum*.

### AICCIP trials

Sixteen promising entries were sponsored in various AICCIP trials during 2013-14 (Table 3.4.1). Of these 6 entries were sponsored for Initial Evaluation Trial and 6 entries for High Density Planting System (HDPS) while 4 entries were promoted to various zonal trials.

**Table 3.4.1: The list of entries sponsored for AICCIP trials 2013-14 of CICR, Nagpur**

AICCIP Trial	IET/Zone	Name of the entry
<b><i>G. hirsutum</i></b>		
Br 02 (a)	IET	CNH 1116, CNH 19
Br 02 (b)	IET	CNH 7008, C NH 2001, CSH 1115, CSH 95
<b><i>G. arboreum</i></b>		
Br 22 (a/b)	IET	CNA 375, CNA 1013, CCA 1021, CCA 3
<b>HDPS Trial</b>		
Br 06 (a)	IET	CNH 121, CNH 3
Br 06(b)	IET	CNH123, CNH 1111, CCH 7012, CCH 1102
Br 06(a) & (b)	CZ & SZ	CNH 281, CNH 1109

## 3.5: State Multi-location Varietal Trial (SMVT)

### Nagpur

A State Multi-location Varietal Trial (SMVT) consisting of 19 genotypes each of *G. arboreum* and *G. hirsutum* was conducted.

The *G. arboreum* trial was vitiated due to heavy and continuous rains throughout the crop season. The range for seed cotton yield was 357-633 kg/ha. The maximum seed cotton yield of 633 kg/ha was obtained from CNA 2009 followed by JLA 0916 (632 kg/ha) and JLA 0794 (602 kg/ha).

In *G. hirsutum*, the seed cotton yield ranged from 1385 to 2351 kg/ha. Two cultures namely; PH 1060 (2351 kg/ha) and CNH 61 (2277 kg/ha) showed significantly high seed cotton yield over the others. The seed cotton yield of control variety NH 615 was 2177 kg/ha. Culture AKH-2006-2 showed promise for fibre quality traits for the second consecutive years with fibre length of 29.7 mm and bundle strength of 22.3 g/tex.

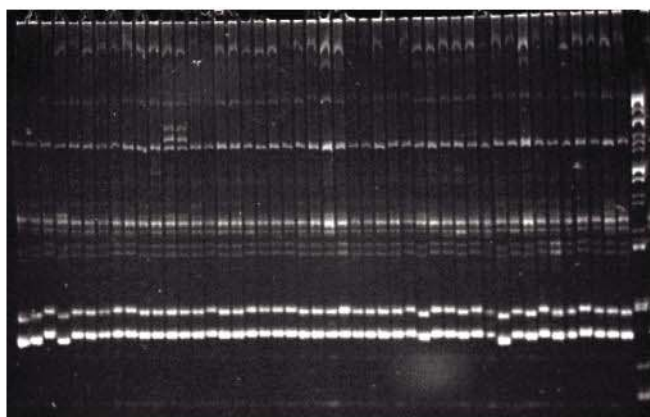
## 3.6 : Molecular Breeding

### Mapping QTLs for fibre quality traits

A cross between EL 958 and UPA 57-17 was effected and a large F<sub>2</sub> (EL 958 X UPA 57-17) population was grown. Few flowers of all the F<sub>2</sub> plants were selfed and carried forward to F<sub>3</sub> and subsequent generation following single seed decent method. At the end of F<sub>8</sub> 273 progenies of RILs were obtained; most of them were uniform for morphological traits. During 2013-14, RILs of *G. hirsutum* (248 No.) were grown in 2 row plots in two replications for fibre quality evaluation. Observations for sucking pests were also recorded.

So far, 3590 SSR markers were screened for parental polymorphism in *G. hirsutum* and 545 informative SSR markers were identified. Genomic DNA of 188 progenies has been subjected for genotyping using polymorphic SSRs (Fig. 3.6.1). Genotyping of *G. hirsutum* RILs using 59 SSR has already been completed. Genotyping of 172 RIL progenies with 2979 SNP markers was carried out by National Botanical Research Institute.

Parental polymorphism studies were also carried out in the diploid parental lines (*G. arboreum* cv. KWAN-3 x *G. herbaceum* cv. Jaydhar) used for development of RILs. A total of 1604 SSR markers were screened of which 171 SSR polymorphic markers were identified as polymorphic. So far 497 informative markers were identified after screening 3590 SSR markers.



**Fig 3.6.1: Genotyping of RILs with SSR marker CIR-203 in upland cotton**

#### **Maintenance of RILs in diploid and tetraploid cotton**

In *G. hirsutum*, 273 F<sub>9</sub> progenies were maintained by selfing. In *G. arboreum*, 193 progenies were selfed and maintained by single seed decent method. Both the populations are being put to use in genetic mapping of QTLs.

#### **Marker Assisted Breeding**

##### **CLCuV resistance**

From among 5000 germplasm lines screened, 30 lines were free from CLCuV under field conditions for two consecutive years. These lines were subjected for screening under epiphytotic conditions at Sirsa and also in the regular crop season at Hisar Faridkot and Abohar. None of the line was found to be resistant /immune. However, two lines with high degree of tolerance and minimum disease severity index were identified. Polymorphic survey of identified parental lines was carried out using 108 SSR markers and 54 polymorphic markers were identified.

Sufficient quantity of F<sub>2</sub> seeds was obtained at Sirsa from a F<sub>1</sub> crosses CP 15/2 x F 846 and LRA 5166 x HS-6.

Additional crosses have also been attempted among the parents selected based on minimum disease severity index and high economic yield.



**Susceptible *G. hirsutum* cotton plants affected with CLCuV**

##### **Bacterial blight resistance**

A set of 56 SSR primers were surveyed for polymorphism between bacterial blight susceptible and resistant lines of which 20 SSR markers were found to be polymorphic with Ganganagar Ageti and S295. Fifteen of the 20 polymorphic markers were used for genotyping the mapping population. Two hundred and thirty four germplasm lines and varieties (including AICCIP high strength lines) were screened for BLB resistance using CIR 246 marker. 27 germplasm lines including 3 high strength AICCIP lines have been identified as resistant for bacterial leaf blight using CIR 246 marker. Crossing has been made using variety Suraj as female parent and high strength lines CSH 3313, CSH 3047 and GTHH 032 as male parent. F<sub>1</sub> seeds have been harvested and sown at regional station, Coimbatore for back crossing with Suraj (BC1F1). Additional crosses have also been attempted using Suraj, Surabhi, Supriya and MCU 5-VT (long staple varieties) as recurrent parent with BLB resistant lines RKR4145 and TORSC78.

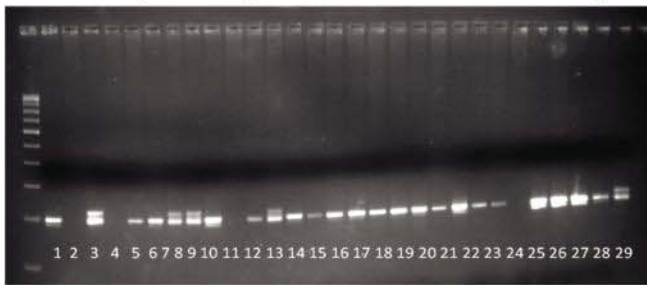
##### **Nematode resistance**

The identified parental lines namely Bikaneri Nerma (BN) for Root Knot Nematode (RKN) resistance; G. Cot 10 and American nectariless for Reniform Nematode (RN) resistance; and Suraj and Surabhi as susceptible parents for both RKN and RN were screened using 60 SSR markers of which 43 were polymorphic. Based on maximum divergence among the parental lines, crosses have been attempted and F<sub>1</sub>s were grown in pots for advancement of generation.

### DNA fingerprinting of public sector cotton genotypes

DNA fingerprinting of 28 *Desi* genotypes (3 of *G. herbaceum* and 25 of *G. arboreum*) was done using 15 SSR markers. Three (DPL 209, HAU 0058 and MCU 022) were found to be polymorphic (Fig. 3.6.2). DNA fingerprinting of 50 *G. hirsutum* genotypes was carried out using 80 SSR markers and 32 were found polymorphic (Fig. 3.6.3). Nine reproducible polymorphic markers were identified, which, in various combinations could be useful for differentiating varieties.

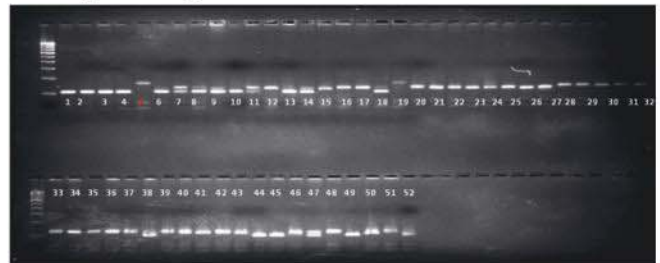
Further, genomic DNA was extracted separately from CICR released varieties viz. CHNO12, LRA5166, Pratima, Anjali, Suraj, Kanchana, Surabhi, Sumangala,



**Fig. 3.6.2: Molecular profile of released *desi* varieties by SSR markers (DPL 209)**

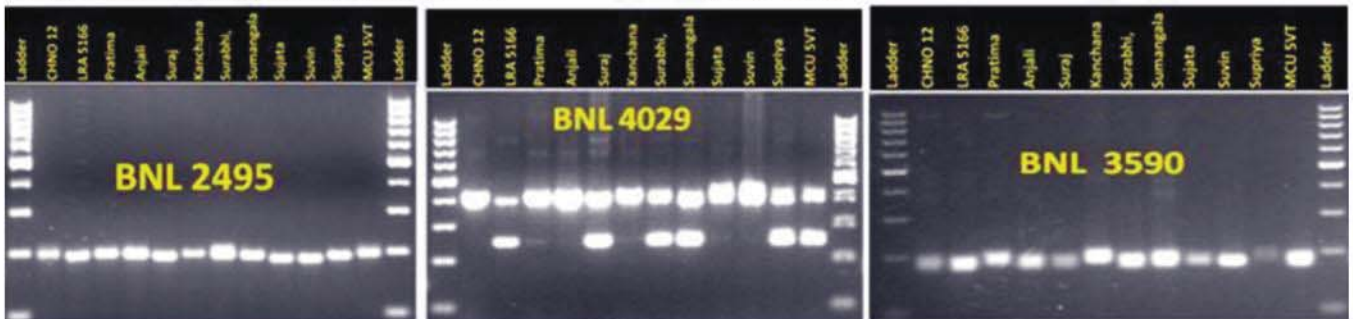
Raghavendra, JLA794, Digvijay, AKA8, AKA7, AKA8401, RG18, RG8, HD324, HD266, HD11051, HD123 G Cot 15, G Cot 19, G Cot 23, LD 210, LD 694, LD 327, LD 451, PA 183, G 27, GAK 423, C-29, Veena, Jawahar Tapti, Arvinda, Y1, Jayadhar, HD 107

Sujata, Suvin, Supriya and MCU5VT and genotyped using 330 genome-wide SSR markers reported to be polymorphic. Fifty-two distinctly polymorphic SSR markers were identified (Fig. 3.6.4) with 15.8% polymorphism. Molecular markers effectively and efficiently differentiated *G. hirsutum* varieties from *G. barbadense* varieties (Sujata and Suvin) and grouped them in separate clusters (Fig. 3.6.5). On an average, CICR varieties were found to be similar by 53%. Draft DNA fingerprint of varieties released from CICR was developed using the SSR data.

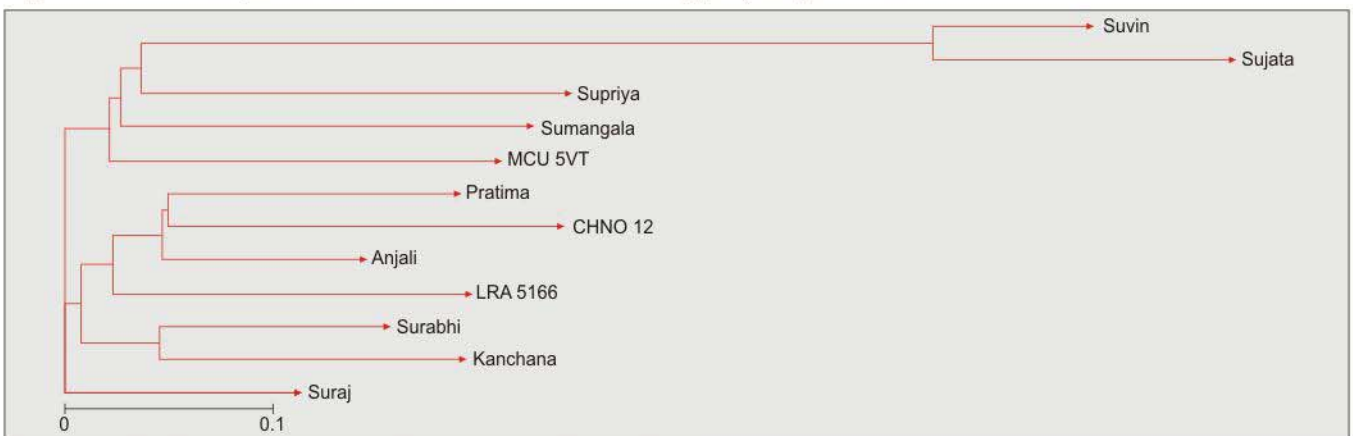


**Fig. 3.6.3: Molecular profile of public sector released varieties (*G. hirsutum*) by SSR markers (BNL 2709)**

1. Surabhi 2. Supriya 3. Sumangala 4. Suraj 5. MCU5VT 6. MCU5 7. MCU10, 8. Sahana, 9. LRA5166, 10. Anjali, 11. MCU12, 12. Arogya, 13. Sujatha, 14. Laxmi, 15. Pratima, 16. Abhadita, 17. BN1, 18. Narasimha, 19. CNHO12, 20. F1861, 21. F1054, 22. F1378, 23. F846, 24. Khandwa2, 25. Khandwa3, 26. Gcot18, 27. Gcot16, 28. Gcot20, 29. Gcot12, 30. Gcot10, 31. RS2013, 32. RS875, 33. RS810, 34. RST9, 35. HS6, 36. H1226, 37. H117, 38. AKH8828, 39. AKH081, 40. JCC1, 41. LH168, 42. NH545, 43. NH452, 44. NH615, 45. JK4, 46. Surat dwarf, 47. G-67, 48. KC-3, 49. DHY-286, 50. Deviraj, 51. G. ageti, 52. RMPBS155



**Fig. 3.6.4: Molecular profiles of CICR varieties as revealed by polymorphic SSR markers.**



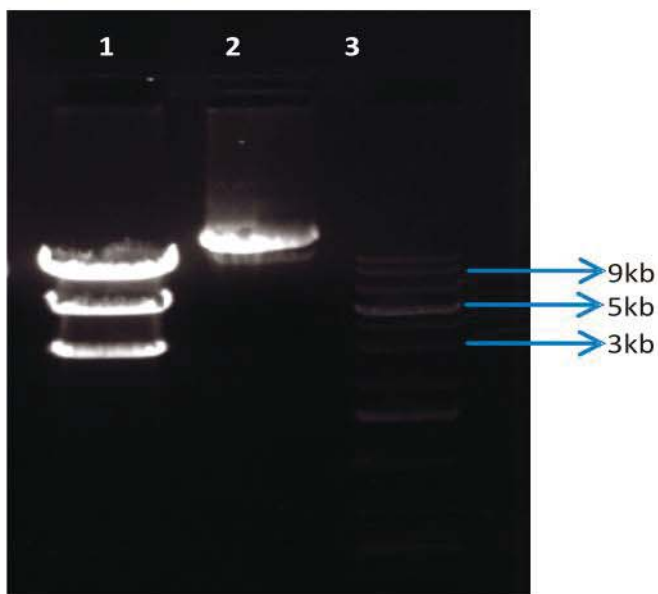
**Fig. 3.6.5: Dendrogram constructed based on genetic similarity**

### 3.7 Development of Transgenic Cotton

#### Development of multi-gene construct and Bt cotton varieties for sustainable pests management

*ChiA*, a gene for chitinase has been identified, codon-optimised and was synthesized. This gene along with cotton (*Gossypium hirsutum*) rubisco small subunit chloroplast transit peptide, fused in frame, has been cloned in plant expression vector with double 35S promoter, AMV enhancer and NOS terminator and also in *E. coli* expression vector.

To generate multigene construct (dual gene initially), chitinase gene cassette in plant expression vector with double 35S promoter, AMV enhancer and NOS terminator was sub cloned downstream of the CICR truncated *cry2Ab1Ac* gene cassette and confirmed through restriction analysis and transformed in to *Agrobacterium tumefaciens* (Fig. 3.7.1).



- EcoRI and HindIII digest of CICR truncated *cry2Ab1Ac* + Chitinase gene cassette
- Uncut CICR truncated *cry2Ab1Ac* + Chitinase gene Plasmid
- 1 Kb ladder

Fig. 3.7.1: Restriction confirmation of CICR truncated *cry2Ab1Ac* and chitinase gene cassette in plant expression vector

#### Bollworm resistant transgenic cotton

Transgenic plants carrying Bt *Cry1Ac* for insect resistance in *G. hirsutum* variety Suraj are under different stages of molecular confirmation. The presence of “transgene” with gene specific primers was confirmed and 1.8kb full length genes were amplified (Fig. 3.7.2). Presently, 4-lines of independent transgenic plants of Suraj variety were established for event selection trial

along with previous lines of ILK-Bt77 (Anjali, LRA-5166 and Suraj varieties). Concurrently, 3-lines carrying Bt *Cry1F* in Suraj variety was positive with gene specific primers were also selected for event selection trial. The new fusion genes of CICR-*Cry2Ab1Ac* were subjected to transformation and regeneration through somatic embryogenesis in Coker 310. Putative transformants were obtained via callus based somatic embryogenesis in MS media with B5 vitamins + 0.1 mg/l 2, 4-D and 1.0 mg/l 2ip + 4.0 g/l phytigel+30 g/l Maltose+ 0.1 g/l PVP. (Fig. 3.7.3).

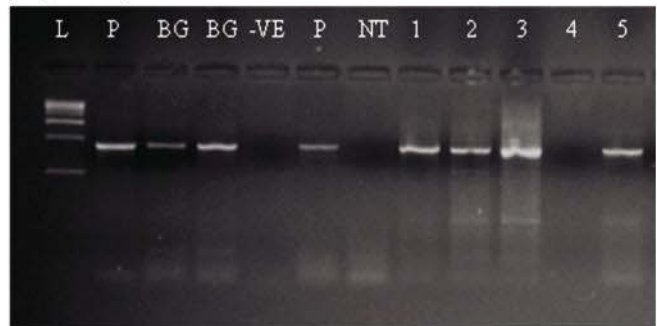


Fig. 3.7.2: L- 1kb ladder; P-Plasmid; BG-Bollguard; BGII-Bollguard-II; -VE- Negative Control; NT- No template; Sample – 1-6 Suraj-*Cry1Ac* samples.



Fig. 3.7.3: Transformation of fusion gene *CICR-truncated Cry2Ab1Ac* with Coker 310/Suraj callus and regeneration through somatic embryogenesis.

#### Transformation of cry-fusion gene by direct shoot organogenesis/multiple shoot induction

Transformation of newly developed cry-fusion gene was carried out by *Agrobacterium* using shoot tip meristem cells. One hundred and eleven explants of *G. hirsutum* cv Suraj and 146 explants of *G. arboreum* on AKA 7 were selected on Kanamycin (50 mg/L) containing medium. The Kanamycin resistant explants were regenerated by direct shoot organogenesis or multiple shoot induction. The shoots that were sufficiently elongated were rooted on MS medium supplemented with 0.1 mg/L NAA and putative transformed plants were established.



**Selection of shoot explants on Kan (50 mg/L) medium**



**Multiple shoot induction from infected explants**



**Rooting of putative transformed shoot**

### ***In-planta* transformation**

The germ line cells from younger *in-vitro* raised seedlings were manipulated for transformation by *Agrobacterium*. In all, 359 embryo axis of Suraj were agro- inoculated and 11 T<sub>0</sub> plants were established. In another method of transformation, 328 seedling meristem were treated with *Agrobacterium* by removing one cotyledon and 105 T<sub>0</sub> plants were established. In AKA 7 however 20 plants were established from the treatment of 260 seedling meristem.



**Removal of one cotyledon**



**Boll set with transformed Pollen**



**T<sub>0</sub> Progeny**

### **Pollen and pollen tube transformation**

Freshly opened flowers were collected from field and their pollen were collected in the laboratory. The pollen was transformed by *Agrobacterium* containing cry-fusion gene. The transformed pollen were carried to field and the emasculated flowers were pollinated. In all, 444 flowers were pollinated and 30 bolls were harvested.

In pollen tube transformation, 120 flowers were injected

with *Agrobacterium* suspension and 30 bolls were harvested.

### **Leaf curl virus resistance**

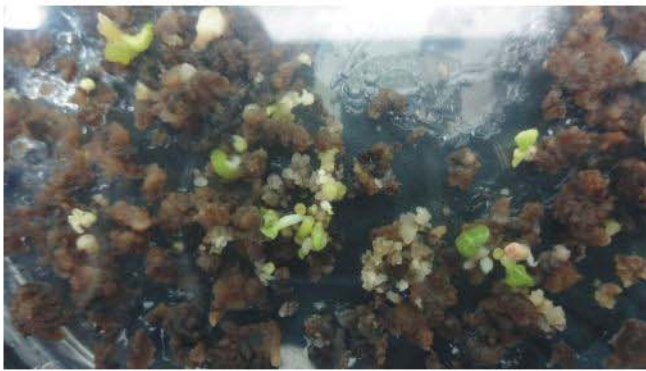
*G. hirsutum* varieties viz., H 777, F 846 and HS 6 carrying ACP (Anti-sense coat protein) gene was confirmed by PCR with gene specific primers (700 bp) and were selected for event selection trial.

### **Fungal resistance**

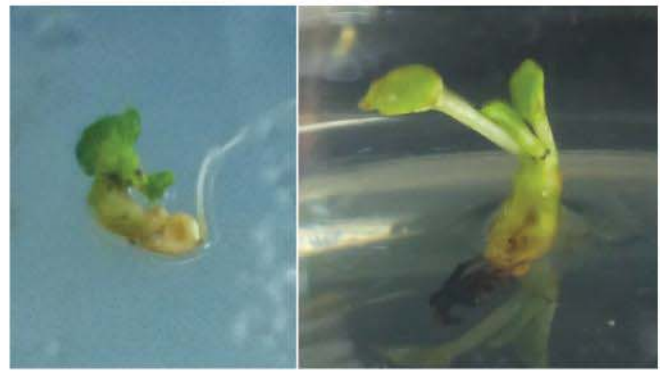
The PCR positive plants expressing chitinase gene in *G. arboreum* cv PA 255 were selected. The selfed seeds from these plants representing three events were collected for event selection trial.

### **Genomics of cotton boll and fibre development**

*Agrobacterium* – mediated transformation was carried out with embryogenic calli using 11 genes provided by consortia partner viz., Expansin, E6, RD22 like protein, Aquaporin, Galactinol-3-synthase, Arabinogalactan-3, Fatty acid chain elongase, Proline rich protein-5, Cytochrome p450 like protein, Osmotin like protein and R2R3 Myb transcription factor. Among them putative transformants were generated with Aquaporin, Expansin, Arabinogalactan 3, Galactinol- 3- synthase and RD22 like protein genes and the plants are under establishment.



Regeneration of *Aquaporin* putative transformants



Plant under establishment with *Aquaporin* putative transformants

### Molecular characterization and validation of fiber strength genes

Gene expression analyses were carried out with mapping population obtained from crossing of divergent lines with contrasting fiber strength (High fiber strength 25 g/tex, medium 22 g/tex and low 17 g/tex). The selected candidate genes such as *SusA1*, *GhcesA1*, *GhcesA2*, *GhcesA7*, *Ghcobl4*, *GhMT1* and *GhFLA3*

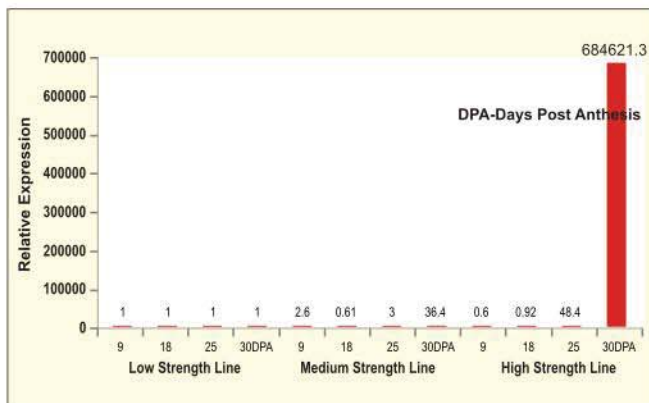


Fig. 3.7.4: Analysis of *GhCesA1* gene expression at different stages of fiber development in high, medium and low strength lines of RIL mapping population

were subjected to comparative transcriptomic analyses during mid elongation (18 DPA) to maturation phase (30 DPA) at different intervals viz, 9, 18, 25 and 30 DPA. The relative gene expression of *SusA1*, *GhcesA1*, *GhFLA3*, *GhcesA2*, and *GhcesA7* were highly expressed during secondary wall formation stage in high fiber strength lines (Fig. 3.7.4 & 3.7.5). Full length of selected candidate genes such as *SusA1*, *GhcesA1*, *GhcesA2*, and *GhcesA7* were cloned and subjected to sequencing (Fig. 3.7.6 & Fig. 3.7.7).

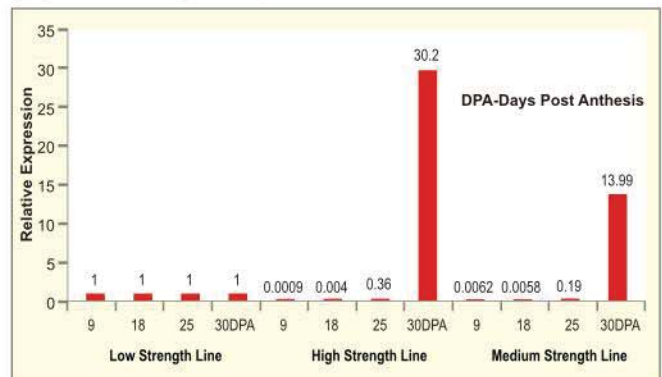


Fig. 3.7.5: Analysis of *GhCesA2* gene expression at different stages of fiber development in high, medium and low strength lines of RIL mapping population

### Cloning of *SusA1* gene from *Gossypium hirsutum* var *Suraj*



1-3 Lane *SusA1* amplicon  
1kbl- Marker

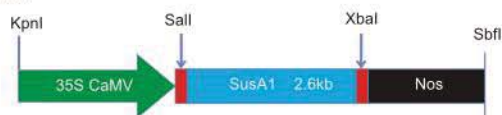


Fig. 3.7.6 : Cloning of *SusA1* gene from *Gossypium hirsutum* var *Suraj*

### Cloning of *CesA1* and *A2* gene from *Gossypium hirsutum*



Amplification of *CesA1* & 2 gene

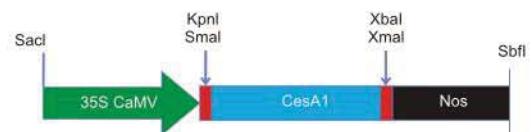


Fig. 3.7.7: Cloning of *CesA1* and *A2* gene from *Gossypium hirsutum*

### Identification of fibre strength associated candidate genes from the existing pool of genes available in public database

ESTs available for secondary cell wall synthesis stage of fibre were obtained from NCBI database, assembled into contigs and nucleotide Blastn analysis identified 63 contigs which were uncharacterized. Among them four contigs which showed differential expression during secondary wall synthesis stage (18 and 25 Days Post Anthesis (DPA) in *Gossypium hirsutum* variety Suraj were chosen for validation using mapping population.



qPCR analysis of selected genes using RIL mapping population identified a candidate gene (Contig 570), which showed 25.5 and 17.4 times higher expression at secondary wall synthesis stage at 25 and 30 DPA respectively in high strength line compared to low strength line of mapping population (Fig. 3.7.8). Protein sequence based homology search identified it as lim domain protein (actin binding family) of *Gossypium hirsutum* known to be associated with fibre strength and fineness.

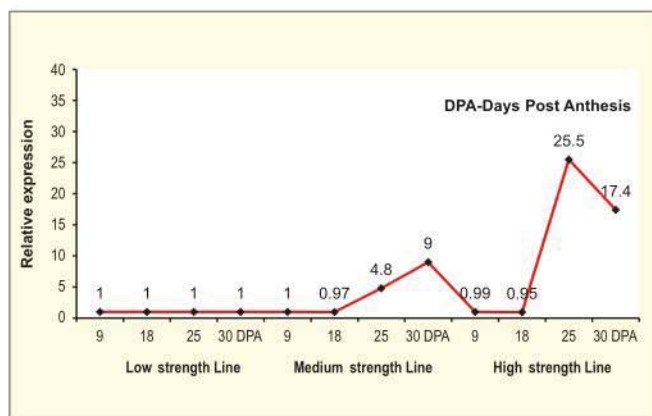
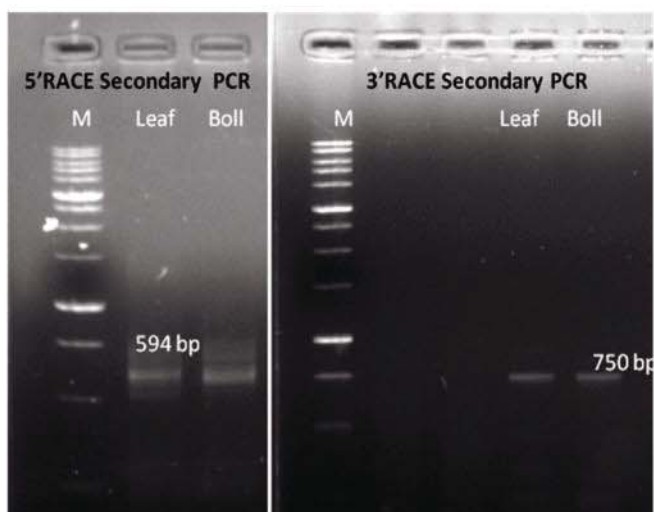


Fig. 3.7.8: Analysis of contig 570 expression at different stages of fiber development in high, medium and low strength lines of RIL mapping population

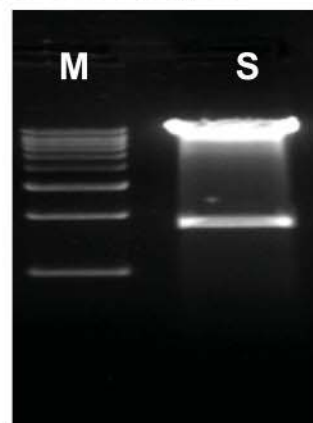
### RACE for isolation of full length nucleotide sequence of GhCOBL4

Full length nucleotide sequence of gene coding for *Gossypium hirsutum* cobra like protein (GhCOBL4) associated with fibre quality trait was isolated using 5' and 3' RACE PCR, cloned, sequenced and characterised for gene expression analysis.



### Seed specific reduction of gossypol biosynthesis

To develop RNAi construct for silencing gossypol biosynthesis in cotton seed, intron hairpin loop RNAi cassette for the target genes coding for  $\delta$  Cadinene synthase and  $\delta$  Cadinene hydroxylase under seed specific promoter in plasmid pBSK-int (3.1kb) was further successfully subcloned into plant expression vector, and transformed into *Agrobacterium tumefaciens*. *Agrobacterium* mediated transformation of *G. hirsutum* elite genotypes with dsRNAi construct for gene coding  $\delta$  Cadinene synthase driven by seed specific promoter is under progress.



M : 1.0 kb Ladder  
S : Restriction digestion with KpnI and SacI

Restriction confirmation of Intron hairpinloop RNAi cassette for  $\delta$  Cadinene synthase in plant expression vector

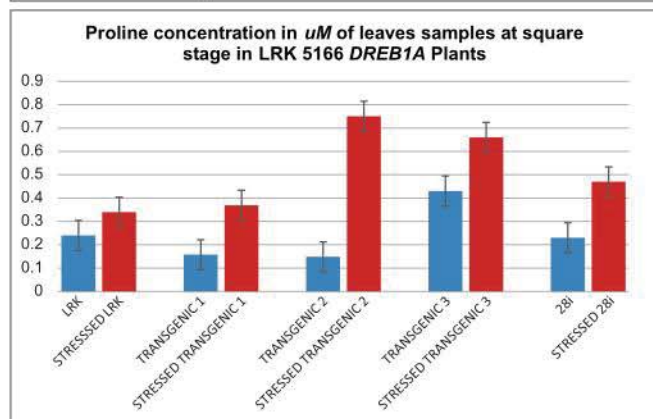
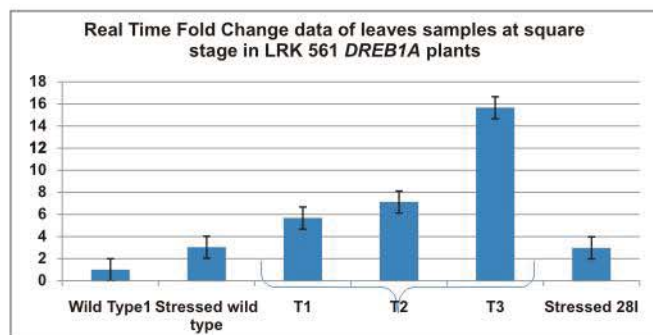
### Development of drought resistant transgenic cotton and identification of new genes for high water use efficiency

To evaluate the gene expression of candidate genes for drought tolerance in LRK 516, transgenic ( $T_4$ ) and non transgenic plants were grown in pots and drought stress was applied to plants at square formation (55–60 days)



and boll developmental stage for 15 days. Younger leaves from the main terminal branch and root samples were taken from stressed plant at above stages and were used for RNA isolation. Cotton glyceraldehyde-3-phosphate dehydrogenase (GAPDH) a house-keeping gene was used as an internal control. Two transgenic

#### Real Time Fold Change data of leaves samples at square stage in LRK 516 *DREB1A* Plants



**Fig. 3.7.9: Relative fold expression of *DREB1A* T<sub>3</sub> progeny and transgenic cotton plants at different growth stages. Expression of *DREB1A* in leaves at (a) squaring stage (left) and (b) roots (right). Plants were kept under water stress for 15 days at squaring stage.**

#### Developments of new transgenic events for cotton leaf curl virus resistance

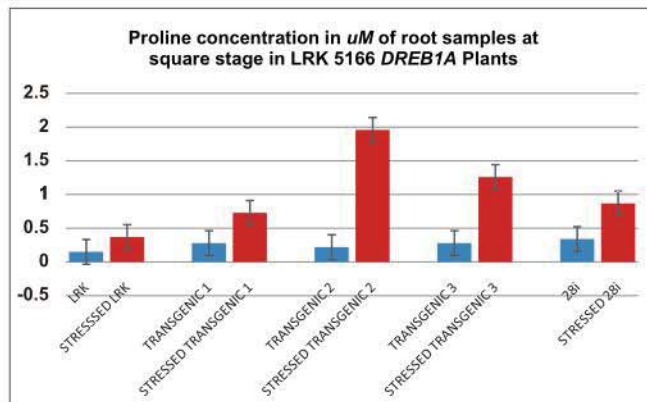
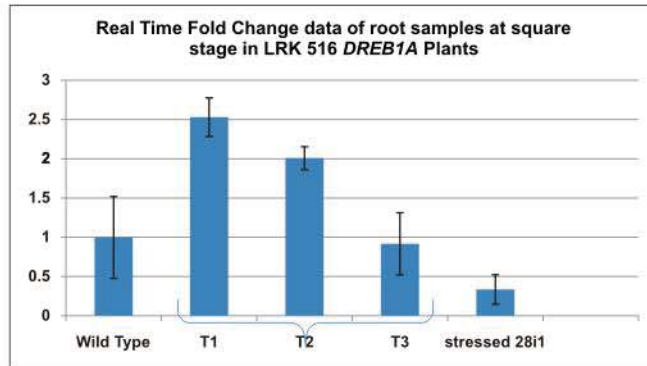
New events of transgenics for leaf curl virus resistance were developed using three genes, Sense coat protein (*SCP*), anti-sense coat (*ACP*) protein and antisense - replicase protein (*ARep*) in 3- genotypes viz., HS-6, H 777 and F 846. T<sub>2</sub> transgenic seedlings were raised in the polyhouse and screened for the presence of the specific gene. Seventy two plants were positive for *ACP* in H 777, 26 plants were positive for *ACP* in HS 6, 30 plants were positive for *ACP* in F 846.

#### Methods for screening of germplasm lines for glyphosate tolerance

Filter paper disc method for screening of glyphosate tolerance in cotton was standardized. Nine hundred and twenty one germplasm lines were screened with 2%

events were analysed for gene expression using qPCR and biochemical test for proline. The plants at square formation stage showed an average of 5 fold increase of transgene expression and 3-fold increase of proline content when compared to wild type and the drought tolerant variety 28.

#### Real Time Fold Change data of root samples at square stage in LRK 516 *DREB1A* Plants



glyphosate treatment. Few lines were shortlisted for molecular and biochemical characterization.

## 3.8 : Seed Production and Seed Quality Improvement

### Nagpur

#### Seed production

Under Mega Seed Project, Breeder seed of four varieties viz. Suraj, PKV 081, NH 615 and AKA 7 were supplied to 16 centres for the production of Foundation Seed and 135.96 q of Suraj and 17.34 q of NH 615 seeds (Foundation stage) were produced. Breeder seed of 1.14 q of Suraj was also produced.

TFL seeds of *G. arboreum* race *cernuum* (10 kg), Red Gram cv. BSMR-736 (2.0 q) and Certified seed of Gram cv. Vishal (7.0 q) were produced.

37 cotton varieties were sown for Stock Seed by Manual Driven Seed Drill at 60 x 15 cm spacing and about 73 kg seed was produced. Resources worth 13.94 lakhs was generated through the sale of seeds.

### Seed quality improvement

Plant vigour is better with good quality seeds. In order to enhance the seed quality, 42 different media were used for seedling nursery. Shoot, root length, stem girth and dry weight was superior with kelbrick with FYM in varying proportions as well as Kelbrick with FYM, cotton stalk compost, vermi-compost and soil mixed in equal proportion.

Higher seed cotton yield was obtained from seeds treated with H<sub>2</sub>O<sub>2</sub>, CICR Microbial Consortia individually or in combination, with and without Imidacloprid, in transplanted compared to that of direct sown seeds.

Vermi-compost (2019 kg/ha), cotton stalk compost (2080 kg/ha) and FYM (2015 kg/ha) gave best results among the 14 treatments when applied directly to the soil in the transplanted and direct sown seeds.

In root trainer experiment, 11 treatments were compared for their effect on seedling vigor. Treatment *Pseudomonas putida* and *P. fluorescens* were superior in terms of root, shoot and girth of the stem.

In an attempt to revive the old seed lots of PKV 081, seed treatment with GA3 @ 40-50 mg/lit gave 30-40% germination in 2008 seed lot compared to control which was non-viable.

### Seed treatment for quality improvement

Two field trials (Trial 1: 31 seed treatments; Trial 2: 9 seed treatments) were taken up to compare their effect on seed germination, plant growth and seed cotton yield in popular cultivar, Suraj. Trial 1 consisted of 8 biologicals, 5 plant protectants, 2 growth hormones, 2 polymers, 9 reported chemical regulators and three natural products such as cow's milk, panchgavya and bio-fertilizer.

The fifth day count on seedling emergence was high with uniform seed germination for seed treatments with KNO<sub>3</sub> @ 0.5 %, Succinic acid @ 0.2 % and H<sub>2</sub>O<sub>2</sub> @ 80 mM (95 % - 100 %). The final seed emergence was above 80 % for all the treatments.

The treatment with plant protectants (thiram, thiomethoxam and imidacloprid) resulted in significantly higher seed cotton yield compared to all others. Among the microbials, seed treatment with *Pseudomonas putida* was found better. Among the other reported chemicals, succinic acid @ 0.2 % seed treatment resulted in highest seed cotton yield.

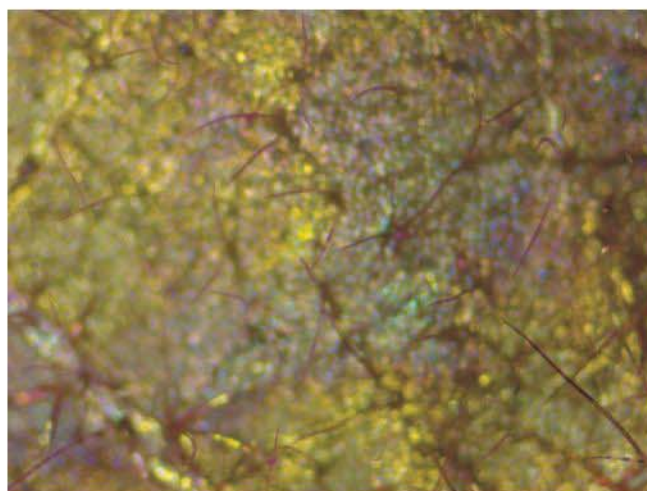
In trial II where seeds were treated with only plant protectants, the initial germination and seedling growth was uniform in seeds treated with imidacloprid and thiomethoxam. Seed treatment with thiomethoxam resulted in significantly high seed cotton yield (959

kg/ha) followed by Azoxystrobin (743 kg/ha) and untreated control (730 kg/ha).

### DUS testing

Two trials were conducted for DUS testing under "Implementation of PVP legislation, 2001" programme during the year. First year trial consisted of 40 candidate genotypes with 30 reference varieties and second year trial consisted of 15 genotypes with 7 reference varieties. Out of 37 traits to be observed, 27 characters were completed.

A method to quantify leaf hairiness and marginal bract hairiness was developed during the year. The selected leaf (fourth/fifth leaf observed at flowering stage) was observed in a unit area and stained to observe the hairs under microscope. Fifty genotypes were studied for their leaf hair density and considerable difference could be observed among the genotypes.



Leaf hairs stained for counting

The qualitative grouping provided for leaf hairiness in the National DUS test guidelines could be as follows:

Leaf Hair density	Variety
Sparse 8.5 ± SEm (0.55)	Kanchana, Sujata, Suvin
Medium 52 ±SEm (1.54)	Surabhi, MCU 5, Khandwa 2, Surat dwarf, MCU 5 VT, LRA 5166, Supriya, Anjali, Suraj, Sumanagala, F 1861, F 1054, MCU 10, Suman, Laxmi, MCU 12, PKV Rajat, Pratima, Sahana
High 119± SEm (3.073)	G Cot 12, Deviraj
Dense 190±SEm (1.75)	DHY 286

Other than leaf hair density, genotypes also showed variation in stellar branching (number of hairs originating

from one point). Suvin was unique with maximum of 8 trichomes originating from one point and F-1861 was unique with maximum of 6 branches arising from one point.

## Coimbatore

### Nucleus and breeder seed production

Nucleus seed production of Suvin, LRA 5166 and Suraj was undertaken and Breeder seed production was undertaken for Suraj, Surabhi, Supriya, Anjali and LRA 5166. In all, 129 kg of breeder seed was distributed to various seed producers.

### Seed production in agricultural crops and fisheries

Sunflower hybrid seeds (CO2) 114 kg have been produced and supplied to Department of Oilseeds, TNAU, Coimbatore.

### Implementation of PVP legislation 2001 and DUS testing of cotton under ICAR and SAU system

During 2013-14 new 82 candidate varieties of tetraploid cotton and two in diploid cotton were tested against 44 and three reference varieties, respectively. In addition to this 68 varieties of common knowledge were also tested for their Distinctiveness, Uniformity and Stability.

Registration certificate for four varieties was received. Seed multiplication and maintenance breeding was undertaken in 98 varieties of *hirsutum*, six of *barbadense* and 15 of *arboreum*.

## Sirsa

The effect of spray of various growth regulators and nutrient combinations on seed setting was not significant. The boll setting percentage was significantly higher in case of hand pollination (72%) against use of various pollinator attractants. Among the treatments it was higher in Mollases 10% (68.9 %) and Sugar 10% (68%).

During 2013-14, breeder seed of female parents (45 kg) and male parent (25 kg) of hybrid CICR 2 and variety CISA614 (65 kg) and CISA310 (55 kg) were produced at CICR regional station Sirsa. Three hundred and fifty quintals of certified seed of Barley (BH 393) was produced and supplied to the HSDC Sirsa.

## 3.9 : Nutrient Management

### Bio enriched compost potassium silicate

#### Nagpur

#### Evaluation of bio-enriched compost on growth and yield of cotton

A bio-enriched compost from cotton plant residues was evaluated for its performance on growth and yield of

cotton in a field experiment. Based on the two years field study, the present INM treatment (60:30:30 + 5T FYM) gave significantly higher seed cotton yield and boll weight as compared to the modified INM (M INM) treatment (60:30:30 + 5t Cotton compost). Use of cotton compost alone and the modified INM (MINM) treatments were similar to the recommended. The effect of microbial seed treatment on seed cotton yield and boll weight was not significant (Table 3.9.1).

**Table 3.9.1: Effects of cotton stalk compost with and without microbial seed treatment on cotton averaged over 2 years**

Treatments	Seed cotton yield (kg/ha)	Boll wt. (g)
<b>Main treatments</b>		
Without microbial seed treatment	2041	4.65
With microbial seed treatment	2291	4.77
<b>CD (0.05)</b>	<b>NS</b>	<b>NS</b>
<b>Sub treatments</b>		
Recommended NPK (90:45:45)	2389	4.76
Present INM (60:30:30 + 5t FYM)	2711	4.85
Modified INM (60:30:30 + 5t CC)	2252	4.65
Only FYM	1821	4.58
Only Cotton Compost	2118	4.80
Control (No nutrients)	1703	4.61
<b>CD (0.05)</b>	<b>362</b>	

### Evaluation of potassium silicate formulations on cotton production (yield and quality) and protection (pests and diseases)

Field experiments were conducted to evaluate the effects of potassium silicate formulation on cotton yield, quality, pest and disease in Suraj variety. The experiment was laid out in randomized block design with four main treatments [1. seed treatment with Agrisil (Potassium silicate solution), 2. seed treatment with potassium silicate powder, 3. Agrisil foliar Spray (Squaring), and soil application of potassium silicate powder] and 8 sub treatments [control, RDF, N & P only, 500, 1000, 2000, 4000 and 8000 ppm of potassium silicate formulations]. All the formulations were tested under natural and artificial conditions. In artificial study, Bacterial Leaf Blight (BLB) suspension @  $2 \times 10^{10}$  spores/ml was sprayed 80 DAS. Suraj (un-treated) seeds were sown at a spacing of 45 x 15 cm.

There was no significant difference in seed cotton yield between the treatments in Agrisil. Under artificial