

3. RESEARCH ACHIEVEMENTS

3.1: Cotton Genetic Resources

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

Nagpur

Exploration for perennials

Exploration was done in Arunachal Pradesh, Madhya Pradesh, Maharashtra and Karnataka (Table 3.1.1) and 59 variants of cotton including 42 perennials and 17 landraces belonging to *G. arboreum* and *G. barbadense* were collected. These were established in the pot culture and conserved in the Gene bank.

Table 3.1.1: Details of Landraces of *desi* cotton and perennials collected

S. No.	Districts	State	No. of Accessions	Species	Perennial/Landrace
1.	West Siang, East Siang, Upper Siang and Dibang valley	Arunachal Pradesh	17	<i>G. arboreum</i>	Landraces
			14	<i>G. barbadense</i>	Perennials
2.	Indore and Khandwa	Madhya Pradesh	1	<i>G. arboreum</i>	Perennial
			2	<i>G. barbadense</i>	Perennials
3.	Wardha, Hinganghat, Chandrapur, Nandurbar and Dhule	Maharashtra	1	<i>G. arboreum</i>	Perennial
			8	<i>G. barbadense</i>	Perennials
4.	Raichur, Bijapur, Bagalkot, Gadag, Dharwad, Haveri, Uttarakannada, Davangere, Chitradurga, Bellary	Karnataka	10	<i>G. arboreum</i>	Perennials
			6	<i>G. barbadense</i>	Perennials
Total			59		



Landraces of *desi* cotton *Gossypium arboreum* from East Siang district, Arunachal Pradesh



Perennial cotton *Gossypium barbadense* from Uttarakannada district, Karnataka



Perennial *desi* cotton *Gossypium arboreum* from Bellari district, Karnataka

Conservation and Exploration of Wild Species

In the wild species garden, 26 wild species, 15 races of cultivated species and more than 45 synthetic polyploids are maintained. One new species (EC 669583) which was established in a pot during 2013-14 was found to be genetically distinct from other wild species as evident from the genotyping data (Fig. 3.1.1).

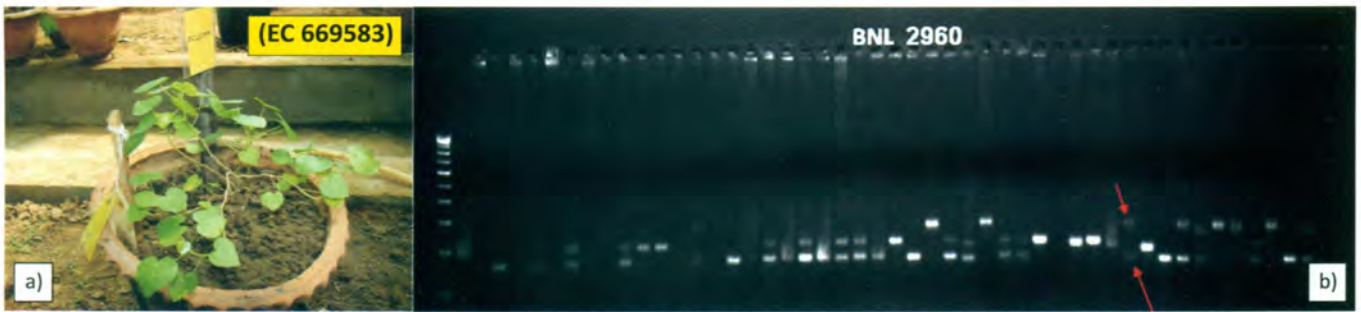


Fig. 3.1.1: a Exotic species EC 669583 established in pot & b - SSR marker BNL 2960 indicating the distinctness as compared to other species conserved in the wild species garden

DUS and Molecular characterization of perennials and wild species

One hundred and forty-two perennials and landraces of cotton were characterized for DUS traits.

96 lines, wild species, races of cultivated species, synthetic polyploids and introgressed derivatives were characterized by SSR markers, 52 of which were confirmed to be polymorphic.

F₂ plants of the mapping population were transplanted in the species garden from pots for their phenotyping:

S.No.	Derivative	Number of F ₂ plants transplanted
a.	<i>G. herbaceum</i> x <i>G. longicalyx</i>	525
b.	<i>G. arboreum</i> race indicum x <i>G. davidsonii</i>	170
c.	<i>G. arboreum</i> x <i>G. thurberi</i>	42
d.	<i>G. arboreum</i> (AKA 8401) x <i>G. davidsonii</i>	31

Genetic stocks registered

Three genetic stocks were registered with NBPGR, New Delhi.

S. No.	Name of the stock	Registration Number	Uniqueness
1	CNA 5	INGR14005	An inter-racial (<i>Indicum</i> and <i>Burmanicum</i>), pigmented and long linted <i>G. arboreum</i> (26.7 mm), tolerant to jassids, bollworm, bacterial blight and grey mildew
2	CNH CB-211	INGR 14058	Cluster boll bearing habit
3	CNH CB -212	INGR 14059	Compact and cluster boll bearing habit

Enrichment of Cotton Gene bank and maintenance of germplasm

One hundred and seventeen (117) exotic accessions (*G. hirsutum* : 112 from USA and *Gossypium barbadense* : 5 from Israel) were procured through ICAR NBPGR, New Delhi for enrichment of the cotton gene pool.

Evaluation of germplasm at Nagpur

Gossypium hirsutum

A set of 252 exotic accessions were evaluated for

economic and fibre quality traits. Sixteen long linted accessions with staple length (31.0 - 32.6 mm) and 28 accessions for high fibre strength (25.0 - 26.8 g/tex) were identified and documented.

Long fibre (>31 mm)

EC 697618, EC 700181, EC 700133, EC 700118, EC 676026, EC 700160, EC 700200, EC 700136, EC 700154, EC 700177, EC 700202, EC 700193, EC 700198, EC 697611, EC 700195, EC 700349.

High fibre strength

(>26 g/tex) EC 700133, EC 700181

(25-26 g/tex)

EC 692135, EC 692136, EC 700143, EC 700144, EC 700491, EC 700154, EC 700195, EC 700169, EC 700198, EC 700156, EC 700202, EC 700217, EC 700513, EC 700164, EC 700200, EC 700225, EC 700159, EC 700187, EC 700224, EC 700158, EC 700160, EC 700177, EC 700193, EC 70017, EC 700125, EC 700128.

Gossypium arboreum

Eight hundred and thirty six (836) accessions were evaluated for fibre quality traits. Twenty long linted accessions having staple length ranging from 26 to 28.3 mm and 14 accessions having high fibre strength (22.8 – 26.0 g/tex) were identified and documented.

Long fibre (mm)

CINA-341 (28.3), IC 440697 (28.1), IC 440484 (27.7), IC 439836 (27.3), CINA 333 (27.2), CINA 333- A-BLL (27.2), IC 564390 (27.1), AKH 4 (26.8), IC 440398 (26.8), PA 235 (26.6), IC 438922 (26.5), IC 440366 (26.5), CINA 349 (26.2), IC 439842 (26.2), IC 439829 (26.1), IC 440814 (26.1), IC 440828 (26.1), IC 440695 (26.0), IC 440473 (26.0), IC 440308 (26.0).

High fibre strength (g/tex)

IC 440695 (26.0), IC 440788 (26.0), IC 440583 (25.4), Comilla-1 (23.9), IC 412567 (23.8), IC 440567 (23.8), IC 440582 (23.7), IC 412342 (23.5), IC 412564 (23.4), IC 440371 (23.2), IC 412310 (22.8), IC 440076 (22.8), IC 440087 (22.8), IC 440554 (22.8).

Gossypium herbaceum

Five hundred and fifty (550) germplasm accessions were evaluated for seed cotton yield, boll weight, ginning outturn and fibre quality traits.

High seed cotton yield (g/plant)

IC 371351 (200.0), IC 371512 (198.3), IC 371353 (197.0), IC 371352 (195.3), IC 371603 (195.0), IC 371511 (192.0), IC 371394 (192.0), IC 371132 (190.0), IC 371395 (191.7).

High boll weight (g)

IC 371413 (2.6), IC 371407 (2.5), IC 371414 (2.5) and IC 371416 (2.5).

High ginning outturn (%)

IC 371425 (39.6), IC 371457 (39.0), IC 371392 (38.9), IC 371419 (38.9) and IC 371376 (38.8).

Fibre length (mm)

IC 371464 (30.6), IC 371406 (27.9), IC 371540 (27.5), Russian-5 (27.1), IC 371348 (26.9), IC 371341 (26.8), IC 371578 (26.8) and IC 371592 (26.8).

High fibre strength (g/tex)

IC 371515 (22.4), IC 371577 (22.0), IC 371578 (21.9) and IC 371364 (21.8).

Evaluation of promising cultures in AICCIP

Two promising cultures CNH-25 (*G. hirsutum*) and CNA 449 (*G. arboreum*) were identified and sponsored in AICCIP and cultures CNA 2019 and CNA 2043 (*G. arboreum*) were evaluated in station trial at CICR, Nagpur.

Conservation of Germplasm in Long Term Storage (LTS) and Medium Term Storage (MTS)

Seeds of 618 accessions (*G. arboreum* -268 and *G. hirsutum*-350) were kept for conservation in LTS at ICAR NBPGR and MTS at ICAR CICR, Nagpur.

Rejuvenation and seed multiplication of germplasm

Four thousand eight hundred and fifty (4850) germplasm accessions (*Hirsutum*-4000, *Arboreum*-300 and *Herbaceum*-550) were grown for rejuvenation and seed multiplication

Distribution of germplasm

Eight thousand three hundred and forty three (8343) germplasm accessions (*G. hirsutum*-8311, *G. barbadense*-1, *G. arboreum*-23 and *G. herbaceum*-8) were distributed to Breeders/Scientists of State Agricultural Universities for research purpose.

Sirsa

Three thousand eight hundred and eight (3808) accessions of *Gossypium hirsutum* were evaluated and the data recorded for different growth and economic characters (Table 3.1.2). Twelve compact germplasm lines were evaluated at 67.5 x 10 cm spacing alongwith checks F 1861, LH 2076 at 67.5 x 30 cm and Bt hybrid Bio Seed 6588 at 67.5 x 60 cm. The number of bolls per plant, boll weight and seed cotton yield/plant were significantly lower in compact lines than the checks. The closer spacing contributed significant increase in per acre yield in

accessions no. 5640 (36.7 q/ha), 5038 (36.5 q/ha), 6109 (36.2 q/ha), and SA 1521 (32.70 q/ha), than the

check varieties as well as Bt hybrid Bioseed 6588 (31.4 q/ha).

Table 3.1.2 : Range for various characters in germplasm lines

Character	Minimum	Maximum
Flowering (DAS)	37	99
Plant height (cm)	40	155
Monopodia	1	7
Sympodia	4	13
Boll number/plant	4	38
Plant width (cm)	10	80
Boll weight (g)	2	3.6
Yield/plant (g)	10	100.2

Coimbatore

Germplasm lines of *G. hirsutum* (2719) were field characterized for various morphological parameters. So far, data were recorded on plant type, stem hairiness, stem colour, leaf shape, leaf lobing, leaf colour, leaf surface, leaf glands, leaf nectary, flower bracts, petal colour, pollen colour, boll bearing habit, boll shape and surface, glands on bolls and loculi / boll.

G. hirsutum

Evaluation of zero branching germplasm accessions

Six single plant selections were made from germplasm lines evaluated during 2013-14 viz., IC 291590, IC360026, IC356618, IC359978, IC358085 and IC 359992 exhibiting zero branching habit. The single plant progenies were sown in the field and evaluated in 2014.



IC 359992 plants showing zero branching

G. barbadense

Twelve compact accessions of *barbadense* were identified and evaluated with Suvin as control. Among them, ICB-194 recorded the highest yield of 130 g/plant followed by ICB-39 (125 g/plant). ICB-194 exhibits high ginning outturn (32%) and boll weight (3.6 g). Another set comprising seven dwarf germplasm accessions were evaluated for yield potential and economic characters. ICB-124 recorded the highest seed cotton yield (154 g/plant) and boll weight (3.6 g) followed by ICB-174 (125 g seed cotton yield and boll weight 3.5 g) and both were early maturing (169 to 170 days) than Suvin (183 days). EC-16 was also early in maturity (165 days). Twelve hairy accessions were identified from base collection of *G. barbadense* germplasm and evaluated for yield and sucking pest tolerance.



Among the 15 hairy accessions, ICB-264 exhibits highest seed cotton yield of (137 g per plant) and ginning outturn (30.1%) followed by ICB-284 (132 g/plant) and ginning outturn (27%). Both these accessions are tolerant to sucking pests and mature

in 170 and 163 days respectively, as against 184 days by Suvin.

Field evaluation was done with 31 advance genotypes from segregating population and Suvin multiple cross progenies. Eight high yielding, extra long staple genotypes yielded more than 1000 kg/ha while the control Suvin recorded an yield of 711 kg/ha. These genotypes also recorded more than 37 mm 2.5% span length and their strength ranged from 27-28 g/tex with > 3.5 micronaire and GOT that ranged from 30-31% as against 28% in Suvin.

3.2: Hybrid Cotton

Nagpur

Heterosis breeding

G. arboreum

Thirty three *desi* hybrids (10 GMS and 23 conventional) were tested. Among the GMS hybrids GAK-423 x G-135-49 recorded 2944 kg/ha seed cotton yield followed by CICR Hy 2 x 30838 (2515 kg/ha) while PA 255 x 30805 (3139 kg/ha) followed by AKA 8401 x G 135-49 (2528 kg/ha) and AKA-8401 x 30805 (2469 kg/ha) among conventional hybrids were good performers. Boll weight of (> 4.5 g) was recorded in all the F₁ crosses wherever 30838 was a parent.

Development of heterotic pool

G. hirsutum

Heterotic population was developed for superior medium staple (25-27 mm) category from parents selected on the basis of geographic diversity. Of the 37 lines developed, 21 lines were used in crossing programme for developing 132 F₁s. In order to find out percentage of heterosis for seed cotton yield and quality traits, these lines were crossed in two sets in diallel fashion. In the first set, 12 lines were crossed in half diallel to produce 66 F₁s. In second set, 9 lines were used along with three quality parents Suraj, NH 615 and G-21-19-619 for producing 66 F₁s.

Averaged over two seasons, 6 lines *viz.* CNH 20-4-3 (27.6 mm, 24.3 g/tex), CNH 16-3-1 (28.6 mm, 22.9 g/tex), CNH 16-3-5 (30.7 mm, 24.4 g/tex), CNH 10-

6-1 (28.7 mm, 25.0 g/tex), CNH 12-4-2 (30.3 mm, 24.5 g/tex) and CNH 18-8-3 (29.4 mm, 24.3 g/tex) were identified for better fibre properties. Line CNH 15-2-1, expressed its superiority as best general combiner for seed cotton yield with positive effects for all the yield contributing traits except sympodial length. Lines CNH 16-3-5 and CNH 12-4-2 exhibited best general combining ability for seed cotton yield and positive GCA (General Combining Ability) effects for all the traits studied. Lines CNH 7-3-3 and CNH 12-2-3 had desirable effects for reduced height while Line CNH 13-1-3 recorded significant GCA effects for number of bolls per plant.

GCA variances were significant for seed cotton yield and most of the yield components indicated importance of additive effects. The estimate of component of variance due to GCA was higher in magnitude than SCA indicating predominance of additive type of gene action for the inheritance of these traits. Both GCA and SCA variances were significant for seed cotton yield and boll number indicating importance of additive and non-additive gene actions in controlling these traits. Promising crosses CNH20-2-4 x CNH 20-4-3, CNH 20-2-4 x CNH 16-3-1, CNH 17-2-2 x CNH 14-1-1, CNH 20-4-3 x CNH 14-6-4 and CNH 12-12-4 x CNH 18-8-3 were identified for significant mid-parent heterosis.

Long staple lines CNH 20-4-3, CNH 16-3-1, CNH 16-3-5, CNH 10-6-1, CNH 12-4-2 and CNH 18-8-3 were identified, based on two years data. These lines can be used as a source for developing improved varieties and hybrids.

Sirsa

Evaluation of GMS based *G. arboreum* hybrids :

Six GMS based *G. arboreum* hybrids were evaluated for seed cotton yield with two check hybrids AAH 1 and CICR 2. CISAA-32 (2768 kg/ha) recorded significantly higher seed cotton yield than the check hybrid CICR-2 (2396 kg/ha) followed by CISAA-27 (2632 kg/ha). The GMS based hybrid CISAA-27 was sponsored in AICCIP Br25a/b national trial.

Evaluation of Line x Tester : Fifteen F₁ were produced using 3 lines and 5 testers and evaluated



with check CICR 2. Only one hybrid combination DS 5 x LD 909 (2833 kg/ha) gave higher seed cotton yield than check hybrid CICR 2 (2179 kg/ha). Based on GCA effects, parents DS-5, CISA-2, LD-909 and CISA 6-165 were best combiners and could be used for developing hybrids. Based on SCA estimates, crosses DS 5 x LD 909, CISA 2 x RG 540, CISA 2 x RG 526, CISA 20 x CISA 6-165, CISA 20 x DLSA 17 were adjudged as the best for seed cotton yield involving specific parents. Maximum heterosis was observed for lint (107%) followed by seed cotton yield (96%).

Maintenance of GMS lines : Three GMS lines DS5, CISA 2, GAK 413A4 and 21 newly identified GMS lines were maintained through sibmating. For genetic study of GMS line, CISA-20 having red flower was crossed with DS-5 and CISA-2 GMS lines to develop population and ascertain the gene for male sterility.

3.3 : Genetic Improvement

Sirsa

G. arboreum

Evaluation of spinnable *G. arboreum* cultures :

Fourteen cultures were tested in RBD along with two checks CISA-310 and CISA-614. The cultures CISA-6-295, CISA-10, CISA-33-6, CISA-33-7, CISA-33-7, CISA-33-8, CISA-54-1 and CISA-44-1 were found promising for spinning having 2.5% span length (>23.0 mm), micronaire between 5-6 and strength >19.0 g/tex. None of the genotypes record significantly higher seed cotton yield than the check varieties. However, the genotypes CISA 10 and CISA-44-1 were coarse with staple length 24.6 and 24.8 mm, respectively. Coarse genotypes with potential for high yield were CISA 33-6, CISA-6-165, CISA-6-123 and CISA-6.

Evaluation of high yielding *G. arboreum* genotypes :

Sixteen high yielding genotypes were evaluated in RBD along with two check varieties CISA-310 and CISA-614. Four genotypes CISA-6-2 (2493 kg/ha), CISA-405 (2540 kg/ha), CISA-8 (2491 kg/ha), CISA-7 (2555 kg/ha) significantly out yielded the check variety CISA-310 (2123 kg/ha). The other two genotypes CISA-33-3 (2391 kg/ha)

and CISA-6-187 (2218 kg/ha) showed promising seed cotton yield as they gave numerically higher seed cotton yield than check variety CISA 310 (2123 kg/ha) and CISA 614 (2059 kg/ha). Further, three genotypes CISA 9, CISA -6-209 and CISA-33-1 although not higher yielders, have good spinning quality as they recorded 2.5% span length >24 mm, micronaire 5.4-5.7 and strength >18 g/tex. The genotype CISA-6-2 was sponsored in AICCIP Br22a/b national trial.

Evaluation of Single Plant Progenies: Twenty five single plant progenies were evaluated in 3 replications with check variety CISA 310. SPS 127 (2798 kg/ha) progeny gave highest seed cotton yield followed by SPS 10 (2416 kg/ha). Other promising plant progenies were SPS 37 (2115 kg/ha), SPS 107 (2160 kg/ha) and SPS 103 (2103 kg/ha), respectively.

Nagpur

G. arboreum

One early maturing *G. arboreum* culture viz. CNA-2003 was identified amongst 29 cultures. Two cultures viz. CNA-2006 and CNA-2009 sponsored earlier in Br 22b were evaluated in the AICCIP trial this year. CNA-2003 and CNA-2023 were sponsored for the current season in Br 22 a/b. Six *G. arboreum* race *cernuum* collections from Mizoram were evaluated for plant height (cm), sympodial length (mm), boll number etc. A couple of very dwarf compact types were identified which could be explored for developing compact *G. arboreum* cultures with plant height (cm) values of less than 50 cm, sympodial length of <2 cm with open bolls ranging from 2 to 10 per cent indicating promise for evolving compact *G. arboreum*.

G. hirsutum

Breeding for compactness, earliness and jassid tolerance

Sixty-one genotypes possessing desirable characters like early maturity, jassid tolerance and compact plant architecture were evaluated in a replicated trial. Upon phenotyping, Sahana, G-Cot 16, CNHO-12, AKH-081, CNH-409-9, KC-3, IC-359691, CNHO7-12, EC-344092, DSC-99, Anjali, SGNR-24, AKH-8828, EC-344804 and CSH-3178

were found to be early maturing as they had more than 85% bolls opened by 150 days after sowing. On the basis of number of monopodia and width of plant canopy, Anjali, Arogya, Pratima, Supriya, Sumangala, CNHTi-1, CSH-3178, IC359478, PI-8-2-BK, Suraj, CNH09-7, CNHO-12, Sikandar Ageti, LRA 5166 and CNHO-7-34 were identified to have compact plant type. Based on jassid grade and nymphs / plant, CNH-2024, CNH-409-9, CNHO-7-34, DSC-99, CNH-2027, CNH-2033, NH-615, AKH-081, AKH-8828, CNH-2026, G-COT16, JK-4,

KC-3, CNH-2025 and CNH-2031 were identified as promising lines for jassid tolerance.

Coimbatore

A compact, densely hairy and pyramid shaped plant type was identified in back cross derived culture (Sumangala x ACT SP 16-1) ACT SP 16-1 C-5-1 which showed cleistogamous flower character and cluster boll bearing habit. It showed a high degree of tolerance to sucking pests and recorded yield of 52 g/plant.



Compact, densely hairy, pyramid shaped plants showing cleistogamous flower & cluster boll bearing

Evaluation and identification of F₂ segregants plants from three crosses

Individual F₂ plants of three different crosses *viz.* N-170 x IC 356750; N-170 x LRA-5166 (introgressed with *G. thurberi*) and N-170 x IC-358358 were raised and scored for plant architectural traits. Based on different numerical values the plants were classified into groups as dwarf and semi-dwarf compact height, short and closed sympodia, low and medium number of sympodia /plant, small, medium and high boll weight. One hundred and three plant progenies had boll weight >3.0 g.

Breeding for high strength with good fibre length and high yield

Sirsa

Thirteen crosses were attempted between 5 long

lented (Coimbatore) cultures with high fibre strength (Sirsa) cultures. Average of three years F₄ (2012-13), F₅ (2013-14) and F₆ (2014-15) progenies of the two crosses *viz.*; CSH-3119-10-28-56 (2744 kg/ha), fiber strength 22.9g/tex, fiber length 28.2 mm and MMO3 (39-2-5)-3114-10-64 (2498 kg/ha) were early in maturity and significantly higher yielding than the Check H-1226 (2071.57 kg/ha) and four were at par in seed cotton yield with the check H-1226. There was an improvement in yield in different crosses with advancement in generations. Further, in 2013-14, MMO3 (27-5-1-8)-3047-10-76 recorded 24.1 g/tex fiber strength and 28.7 mm fiber length.

From 13 F₄ populations of crosses of Coimbatore and Sirsa cultures, 72 early plants were selected in



2012-13 and their F_6 progenies were raised during 2014-15. High yielding individual plant progenies were CSH-3119-10-14-28-56 P3, CSH-3119-10-29-58 P2, CSH-3114-10-66 (27-5-1-5) P3, CSH 3047-10-78 22-1-2 P1, CSH 3047-10-78 22-1-2 P3, CSH 39-2-5 3047-10-80-P3.

Coimbatore

Eight superior long staple cultures were evaluated in a replicated trial along with Suraj and Sumangala as check varieties. Analysis of data on seed cotton yield indicated significant differences among the genotypes and the highest seed cotton yield was recorded in the test entry MM 03-12-1-2-3 (1881 kg/ha) as against 995 kg/ha and 837 kg/ha recorded in check varieties. Four test cultures recorded significantly higher yield than both the checks. Cultures MM 03-39-4-2-3 (Br-02a) and MM03-40-4-3-1 (Br-02b) were sponsored for multi-location testing in initial evaluation trial of AICRP on Cotton during 2014-15 in irrigated and rainfed trials, respectively.

Breeding for high GOT % and high yield

Evaluation of 13 F_6 (2013-14) and F_7 (2014-15) progenies of cross SA-977 (HG) x SA-112 (LG) indicated four promising progenies *viz.* P-69, P-86, P139 and P-164 for seed cotton yield and eight progenies *viz.* P-7, P-70, P-84, P-86, P-139, P1-64, P-174 and P-184 for lint yield. These lines also possessed GOT more than 40%. F_3 individual plant progenies were evaluated in which 6 out of 12 of cross (RS-875 x SA-524), 2 out of 85 of cross (CSH-3129 x EC-358002) and 12 out of 68 of cross (F-1861 x SA-668) recorded more or equal to 40% GOT. In two F_2 populations of crosses F-1378 x EC-359044 (20) and LH-2107 x EC-359044 (30), there were 8 and 4 plants with more than 40% GOT. 285 fresh crosses were attempted between 29 females and 13 high GOT F_6 lines of the cross SA- 977x SA-112. Five crosses recorded more than 40% GOT.

Breeding for big boll size

Twenty three single plant selections in F_6 generation of a cross Ganganagar ageti x Acala 44 (B2) were evaluated for big boll and other economic characters. The cross had shown continuous reduction in boll weight over the years

and during the crop season of 2014-15 it ranged from 3.0 to 5.1 g in different single plant selections (SPS). The selection SPS 131 recorded highest yield of 2112 kg/ha with GOT 37.3%, boll weight 4.5 g, fibre length 27.7 mm, uniformity ratio 53%, micronaire 4.6 $\mu\text{g}/\text{in}$, fibre strength 22.8 g/tex, elongation 5.9% and SFI 7.9%.

Two selections *viz.*; SPS 137-2 and SPS 140-1 which recorded good yield (17 q) also had good fibre quality with GOT >40 %, boll weight 5 g, 25-26 mm fibre length, uniformity ratio > 50%, micronaire 4.1 $\mu\text{g}/\text{in}$, fibre strength 21.0 g/tex, elongation 6.0% and SFI 8.3 and 11.1%, respectively were identified for multilocation trial.

Genetic Enhancement

Genetically enhanced lines of advanced generation and cultures were evaluated a spacing of 60 x 45 cm. Thirteen entries of the 55 were found to possess superior fibre quality parameters as compared to the check Suraj which recorded fibre length of 30.4 mm, fibre strength of 24.7 g/tex and micronaire 3.3 $\mu\text{g}/\text{in}$. Some of the lines with good fibre properties were SPS 9-6, SPS 9-7, SPS 9-19, SPS 9-27 and SPS 9-55.

G-21-19-619, a donor of high strength recorded high values for fibre length (32 mm), fibre strength (27.9), and a micronaire (3.3). It contributed to an improvement in fibre properties of the parent PKV-081 which has a fibre length of 27 mm, fibre strength of 20.4 g/tex and micronaire value of 3.2. Three entries SPS9-14, SPS9-15, SPS9-16 were identified as resistant to sucking pests with a grade score of 1 and boll weight of 3.4 g. *G. hirsutum* culture CNH-2015 was tested under HDPS trial and was sponsored for testing in AICCIP trial during 2015-16.

Population Improvement

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The GMS based random mating (RM) population of *G. arboreum* and *G. hirsutum* was grown on large plots. All sterile/fertile plants in both the population were tagged at flowering and allowed to open pollinate in the composite population. The out-crossed bolls from each of the sterile plant were harvested separately and will be raised as plant to

row progenies in the subsequent crop season. More than 110 single plants were selected from diploid composite GMS RM population and 230 from *G. hirsutum* GMS RM population based on seed cotton yield, boll number and of fibre quality *viz.* fibre length and strength.

Single plant selections (120 nos.) from random mating population and 750 re-selected plants from the segregating progenies were evaluated in plant to row progeny plots. A few of the progenies were uniform and exhibited good performance for seed cotton yield. Based on fibre quality and other attributes, 60 single plants of *G. arboreum* and 25 of *G. hirsutum* were identified and selected for evaluation in a replicated trial. From the segregating progenies, single plants were reselected for further evaluation as plant to row progenies.

Based on yield performance and fibre quality traits, 79 *G. arboreum* and 198 *G. hirsutum* cultures identified in 2013-14, were evaluated in replicated trial during the crop season 2014-15. Seed cotton yield ranged from 362 kg/ha to 1729 kg/ha in *G. arboreum* cultures while in *G. hirsutum* it ranged from 216 kg/ha to 1928 kg/ha. In all, four trials of *G. arboreum* and 9 of *G. hirsutum* were conducted in 2 replications of 4 row plots following spacing of 60 x 45 cm and 60 x 60 cm, respectively. Based on seed cotton yield, 26 cultures of *G. arboreum* and 68 of *G.*

hirsutum were retained for second year replicated trial. Five cultures were identified for promotion in AICCIP national trials. Seed multiplication of the cultures *viz.* CNA 1013, CNA 1016 and 1021 of *G. arboreum*, CNH 1110, CNH 1111, CNH 1118, CNH 1119, CNH 1120 and CNH 1121 of *G. hirsutum* were also done.

Sirsa

In GMS based random mating population, the individual plants in the population were monitored and tagged for sterility/fertility at anthesis repeatedly at weekly intervals. All the out-crossed bolls from the sterile plants were bulk harvested and ginned to constitute the next cycle of GMS based random mating population. From the fertile plants, 117 single plants showed tolerance to CLCuV, were selected for evaluation. Seed cotton yield of individual plants ranged from 15 to 190 g.

Breeding for Biotic Stress Tolerance

Nagpur

Jassids

Breeding programme was initiated to develop *G. hirsutum* varieties with improved yield, quality and tolerance to jassid. CNH 2-1 recorded seed cotton yield of 1725 kg/ha followed by CNH 07-16 (1594 kg/ha) and CNH 09-13 (1366 kg/ha) while quality check Suraj recorded seed cotton yield of 864 kg/ha. CNH 2-1 had recorded better fibre properties with bundle strength of 23.4 g/tex and staple length of 28.6 mm.

Eighty-five progenies comprising F_5 and F_6 generations were multiplied for testing in replicated trials. Fifty-one cultures were grown for seed multiplication and further evaluation for performance in replicated trials. From interspecific derivatives, 11 F_6 progenies were selected based on tolerance to jassid and yield potential.

F_1 crosses of high strength germplasm (IC 357608, IC 356751, IC 359292, GISV 267) and cultures (CCH 4474, CCH 7122, CCH LS 2) with Suraj and jassid tolerant variety NH 615 were evaluated for fibre properties.

Three cultures were tested in various AICCIP trials during the year 2014-15. Culture CNH 7008 has



Compact plants with big bolls

been promoted from initial evaluation trial (IET) of 2013-14 to Br 03 (b) - Preliminary varietal trial (PVT). Culture CNH 7022-4 is being tested in IET of Br 02 (b) and CNH 2007-10 in high density planting system trial Br 06 (b). Early maturing five cultures (145-160 days) CNH 09-7, CNH 09-9 and CNH 2007-16 promoted to AICCIP trials Br 02(b) to be conducted during 2014-15.

CLCuD

Sirsa

Evaluation of advance CLCuD tolerant cultures :

Fifteen *G. hirsutum* cultures were evaluated against the check variety RS 2013, LH 2076 and susceptible check HS-6 in RBD with three replications. CSH 2932 recorded minimum 10.5 PDI whereas HS-6, RS-2013 and LH 2076 recorded 40.2, 55.4 and 36.0 PDI, respectively. The highest seed cotton yield was recorded in CSH 2811 (1913 kg/ha) followed by CSH 2836 (1872 kg/ha) as against the check variety LH 2076 (1615 kg/ha). Maximum ginning outturn of 36.0 per cent was recorded in the variety CSH 2924 as compared to local check varieties RS 2013 (34.9%) and LH 2076 (32.7%). The culture CSH 2837 recorded the highest 2.5 % span length of 26.6 mm whereas highest bundle strength of 21.6 g/tex was recorded in CSH 2837. Most of the *G. hirsutum* cultures recorded less than 21 PDI for CLCuV incidence.

Selection of CLCuD tolerant single plants from segregating populations :

To develop the segregation populations of *G. hirsutum*, 16 crosses were attempted among CLCuV tolerant germplasm lines in a Line x Tester fashion during 2012-13. Sixteen F₂ populations were grown to select the single plant progenies having high yield potential and tolerant CLCuD. In all, 91 single plants were evaluated in progeny row trials. Some of the single plants recorded more than 100 g yield per plant and were tolerant to CLCuD.

Selection of CLCuD tolerant single plants from progeny to row trials :

From the 3rd cycle of random mating 102 single plants were selected and grown in progeny to row trials with 4 rows each. Based on CLCuV tolerance 135 single plants were selected

for further evaluation for seed cotton yield and other characters of economic importance.

Evaluation of Introgressed lines against CLCuV resistance:

Out of the 164 introgressed lines received from CICR, Nagpur seven introgressed lines showed some tolerance to CLCuV (less than 30% PDI).

Abiotic stress

Nagpur

Drought

Seven single and four double crosses were evaluated in a replicated trial. The seed cotton yield ranged from 16.4 to 94.2 g/plant, boll weight 1.7 to 3.5 g, fibre length 24.5 to 31.0 mm, uniformity ratio 45.8 to 54.1%, fibre strength 17.8 to 22.8 g/tex, micronaire values of 2.8 to 4.6 and elongation 4.6 to 5.3%. Cross 28I x Moco recorded the highest seed cotton yield per plant (94.2 g) followed by 28I x HSD (88.7 g), PH 93 x Rajat (79.6 g), PKV 081 x Suraj (59.0 g) and NH 615 x Rex (58.1 g) respectively. 28I x HSD recorded fibre strength of 22.82 g/tex, fibre length of 27.15 mm and strength to length ratio of 0.84. The other single cross PKV 081 x Suraj involving high strength parental line recorded fibre strength of 22.5 g/tex and fibre length of 31.0 mm. In a cross LRA 5166 x N 170, where N 170 is a compact plant type. The F₁ in the second year of testing also showed compactness with bolls very close to the main stem. Among the double cross hybrids, cross (LRA 5166 x N 170) x (PKV 081 x Suraj) and (CCH 510-4 x Moco) x (NH 615 x Rex) recorded seed cotton yield of 50 g/plant.

In F₂ generation, the seed cotton yield ranged from 890 to 2378 kg/ha. The highest seed cotton yield was recorded in cross PKV 081 x CCH 510-4 (2378 kg/ha) with boll weight which ranged from 1.56 to 3.98 g.

In eight drought tolerant lines, fibre length ranged from 22.4 to 30.7 mm, uniformity ratio from 43 to 54%, micronaire from 2.8 to 4.1, fibre strength from 17.6 to 23.5 g/tex, elongation from 4.5 to 6.6% and short fibre content from 17.2 to 22.8%. Three top ranking cultures (DTS 155, DTS 62 and DTS 104) with more than 50 per cent yield increase over the check were identified.

Waterlogging

Nagpur

One hundred and fifty accessions selected during 2012-13 & 2013-14 were screened against waterlogging in pots to further select the most tolerant lines. Among the 36 tolerant lines selected, lenticels were prominent in some tolerant accessions. Based on lenticel formation, 23 accessions (IC 359979, SA 1287, IC 359245, IC 359206, IC 358177, IC 357930, IC 357477, IC 357235, IC 356701, AKH-07-R, IC 359943, IC 359941, IC 359881, IC 359868, IC 359854, IC 359590, IC 359306, IC 359838, IC 359726, 133, 139, 135 and 132) were classified as water logging tolerant lines.

Water logging



Waterlogging tolerant accession IC 359979 with lenticels



Waterlogging susceptible accession IC - 359380

G. barbadense Improvement

Coimbatore

Evaluation of Suvin Multiple Cross progenies

Field trials were conducted with 15 (BC₁F₆) genetically advanced Suvin multiple crossed

progenies at 60 x 45 cm spacing. Superior single plants with high yield and desirable fibre parameters were identified for further evaluation from the crosses (SN x ICB 75)-1-5-6, (SN x ICB 122) 3-5-6 and (SN x ICB 179)-3-8-9.

Evaluation of intermated population of Suvin

Five intermated population of Suvin were raised along with the existing Suvin as control for increased yield, ginning outturn and reducing the crop duration. The F₁'s of the crosses were raised during the season. The cross SS-2 recorded significantly higher yield (1314 kg/ha) than Suvin. Crosses SS-1(1147 kg/ha), SS-4 (1161) and SS-5 (1005 kg/ha) were on par with each other and had higher yield than Suvin (767 kg/ha). It was observed that except SS-4, all the four crosses were having high 2.5% span length ranging from 37.9-39.4 mm. For strength SS-1 recorded 31.2 g/tex followed by SS-3 (30.5 g/tex) and SS-4 (29.2). Maximum micronaire of 4.1 µg/inch was recorded in SS-2 followed by SS-3 (3.7 µg/inch) while in Suvin it was only 3.3 µg/inch. Cross SS-5 exhibited the highest elongation percentage of 6.7 which is close to control Suvin (6.6). The cross SS-2 was identified for high seed cotton yield with high length and other fibre properties.

Evaluation of Random Mating Populations

From the fourth cycle of random mating populations, 90 single plant selections were made and 28 lines were evaluated for performance during the crop season. RMS-33 (92.7 g) and RMS-49 (92.6 g) recorded high yield and were highly significant than Suvin (38.2 g) followed by the genotypes RMS-12 (87.6 g) and RMS-57 (82 g).

3.4: Genetic Diversity through Introgression

Evaluation of introgressed derivatives

Four hundred and seventy introgressed derivatives were evaluated at Nagpur for fibre and economic traits and those with unique traits were identified. The yield levels were invariably higher in introgressed *arboreum* cultures as compared to that in *hirsutum* cultures (Table 3.4.1 a & b).

Table 3.4.1a : Identified *G. hirsutum* based cultures

S. No	Cultures identified	Seed cotton yield (kg/ha)	25% SL (mm)	Bundle strength (g/tex)	Micronaire (µg/in)	UR %
1.	CNH- Intro14-1 (14005)	83	28.9	21.8	4.2	44
2.	CNH- Intro14-2 (14022)	49	29.2	19.6	2.7	45
3.	CNH- Intro14-3 (14023)	116	27.6	20.2	2.8	45
4.	CNH- Intro14-4 (14007)	524	25.9	19.8	4.2	47
5.	CNH- Intro14-4 (14032)	44	26.7	18.5	3.0	43

Table 3.4.1b: Identified *G. arboreum* based cultures

S. No	Cultures identified	Seed cotton yield (kg/ha)	25% SL (mm)	Bundle strength (g/tex)	Micronaire (µg/in)	UR %
1.	CNA-Intro14-1(14336)	908	26.6	19.8	5.5	50
2.	CNA-Intro14-2(14332)	914	22.5	20.1	4.2	51
3.	CNA-Intro14-3(14333)	1074	23.6	21.3	5.4	49
4.	CNA-Intro14-4(14334)	836	24.3	19.8	5.2	50
5.	CNA-Intro14-5(14338)	1540	25.5	22.5	4.0	51

***G. herbaceum* and *G. anomalum* cross**

The F₁ of *G. herbaceum* and *G. anomalum* cross showed only 43% pollen fertility. More than 50% of the bolls were deformed in shape and shed prematurely. However, high fibre strength upto 36.7 g/tex was recorded. This F₁ plant has been conserved as a perennial in the wild species garden

alongwith F₂ population of 58 plants and 40 selfed seeds were collected during 2014-15.

Out of the 11 fibre strength specific SSR primers which were screened during 2013-14, two of them (BNL 3644 and BNL 1317) were highly polymorphic for a set of 58 F₂ plants of *G. herbaceum* and *G. anomalum* (Fig. 3.4.1a-b).

Molecular characterization of introgressed derivatives

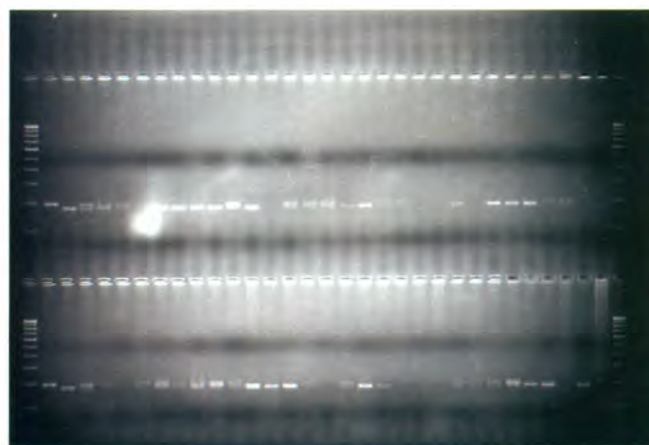
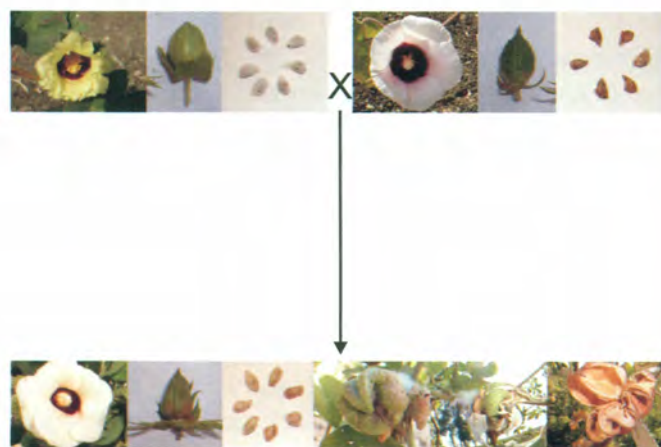


Fig. 3.4.1 a : Morphological observations in parents, F₁ and F₂ of *G. herbaceum* and *G. anomalum* cross ; b: Genotyping of F₂ mapping population with SSR marker BNL 1317

Naturally Coloured Cotton

Nagpur

Sixteen naturally coloured cotton introgressed genotypes (*G. hirsutum* and *G. arboreum*) were evaluated and used for mass scale seed production for evaluation in farmers field. Seeds of ten

MSH 53 planted in High Density Planting System



From the segregating population, brown colour linted progenies were identified in the cross MCU 13 x VNWH-1-4-1-2. In total, 44 single plants were selected which showed 4 different shades of colour

coloured cotton genotypes supplied by Cotton Research Station, Lam, Guntur were also multiplied.

Dark brown linted MSH-53 (Vaidehi-95), planted in high density at a spacing of 60 x 10 cm yielded 3055 kg/ha.

Yarn and cloth made from coloured cotton



as cream, light brown, brown and dark brown visually. Other characteristics of these progenies are furnished below.

Colour	Cream	Light Brown	Brown	Dark Brown
Number of selected single plants	8	19	3	14
Seed cotton yield (g/plant)	8.4-70.7	7.1-51.8	15.0-40.2	4.9-36.5
Lint Index (g)	4.7-6.6	3.0-5.6	3.1-5.0	1.8-9.1
Seed Index (g)	9.3-11.7	8.2-12.0	8.4-10.5	8.5-19.5
Ginning Outturn (%)	29.5-39.8	22.7-38.2	23.8-37.3	14.0-32.1



Compact colour cotton genotype (MCU 13 X VNWH-1-4-1-2)

From the F₅ generation, single plants were selected which had boll weight (6 to 7 g), lint index (6.5 to 8.5 g) and ginning outturn (39.2 to 47.7%).

3.5: State Multi-location Varietal Trial

Development of Variety

Coimbatore

The proposal for notification of medium staple central cotton CCH 2623 for south zone under irrigated conditions has been submitted for



consideration of Central Sub-Committee on Crop Standards, Notification and Release of Varieties.

Testing of *Hirsutum* cultures in AICRP on cotton

Thirteen high yielding *G. hirsutum* cultures were sponsored for multi-location evaluation in All

India Coordinated Research Projects during 2014-15 (Table 3.5.1). Of these, eight were tested in National trials and the remaining five were in advanced stage of testing in zonal trials (Table 3.5.2).

AICRP on Cotton trials

Table 3.5.1: List of entries sponsored for AICRP on cotton trials 2014-15 of CICR, Nagpur

Species	Culture Name	Trial No.
<i>G. hirsutum</i>	CNH 61, CNH 7022, CSH 1, CSH 2, CNH 5, CNH 1118	Br 02 (b)
<i>G. arboreum</i>	CNA 2009, CNA 449, CCA 1022, CCA2006	Br 22a/b
HDPS trials <i>G. hirsutum</i>	CNH 29I, CNH 2015, CNH 25, CNH 1120,	Br 06 (a)
HDPS trials <i>G. hirsutum</i>	CNH2007-10, CNH 1121 CNH 1119, CNH 29I	Br06 (b)

Table 3.5.2: Entries promoted/retained for 2014-15

Sl.No.	Culture Name	Zone	Trial No.
1	CNH 7008	Central Zone	Br 03 (b)
2	CSH 1110	South Zone	Br 03 (b)
3	CNA1016	Central Zone	Br 24 (b)
4	CNA1016	South Zone	Br 24 (b)
5	CNH 28I	South Zone	Br 06(b)
6	CNH 1111	South Zone	Br 06(b)

Multilocation Varietal Trial

A State Multilocation Varietal Trial (SMVT) consisting of 16 cultures of *G. arboreum* and 3 control varieties of *G. arboreum* and 16 cultures of *G. hirsutum* and 4 control varieties of *G. hirsutum* was conducted with normal recommended package of practices at CICR, Nagpur. The crop was affected due to drought following the sudden recession of monsoon after mid September 2014.

In *G. arboreum*, the range for seed cotton yield was 472 to 1284 kg/ha. Maximum seed cotton yield (1284 kg/ha) was obtained with JLA-794 followed by AKA 2004-29 (1200 kg/ha) and AKA-2009-6 (1184 kg/ha). The control variety PA-08 recorded seed cotton yield of 940 kg/ha. Compared to the control, only JLA 794 produced significantly higher yield.

In *G. hirsutum*, 16 cultures were compared with four control varieties. Seed cotton yield ranged

from 734 to 1198 kg/ha. Three cultures namely; AKH-09-5 (1198 kg/ha), CNH-25 (1073 kg/ha) and CNH 1110 (1070 kg/ha) recorded numerically higher yield than all control varieties. The seed cotton yield of control variety NH 615 was 923 kg/ha.

3.6: Molecular Breeding

A set of recombinant inbred lines (RILs) were developed from a cross between EL 958 and UPA 57-17 following single seed descent method from F₂ individual plants. At the end of F₈ generation, 273 progenies of RILs were obtained; most of them were uniform and are being used for genetic mapping.

During 2014-15, 857 SSR markers were screened for parental polymorphism and, 157 were found to be polymorphic/ informative. So far, 4417 SSR markers were screened for parental polymorphism in *G. hirsutum* and out of that, 702 informative SSR

markers were identified. Genotyping of 188 RILs using 162 SSR markers was completed.



Fig. 3.6.1: Genotyping of RILs with SSR marker Gh0132 in upland cotton

Genotyping of 172 RIL progenies with 2979 SNP markers using 50K SNP chip was done by NBRI under collaborative programme. The same sets of RILs were subjected to SNP genotyping using 70K SNP chip of Illumina through outsourcing. Results indicated that 3589 SNPs are polymorphic with the population.

DNA Fingerprinting

A set of 60 SSR markers were obtained which were polymorphic among 48 public sector released cotton varieties including those of *G. hirsutum* as well as *G. barbadense*. The polymorphic information content of selected 10 best markers ranged from 0.3 to 0.5. These markers were effective in distinguishing each of the 48 tetraploid cotton varieties, stability of which needs to be tested in the next generation selfed progenies. Among the 50 SSR markers studied in 30 cotton varieties (Fig.3.6.2) including those of *G. arboreum* as well as *G. barbadense*, 12 were polymorphic and capable of distinguishing all the 30 genotypes.

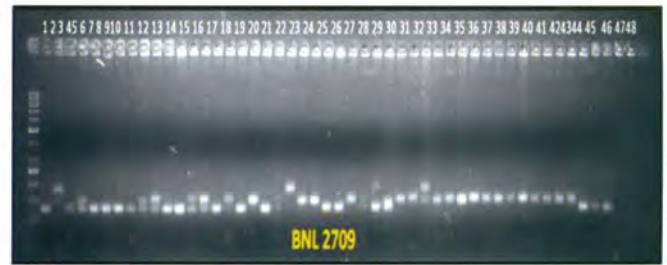
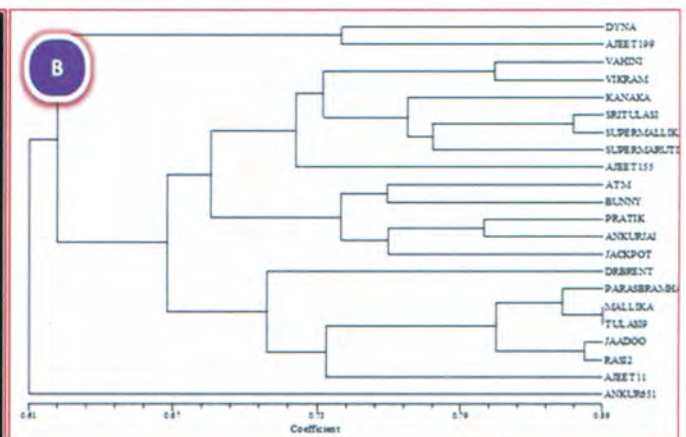
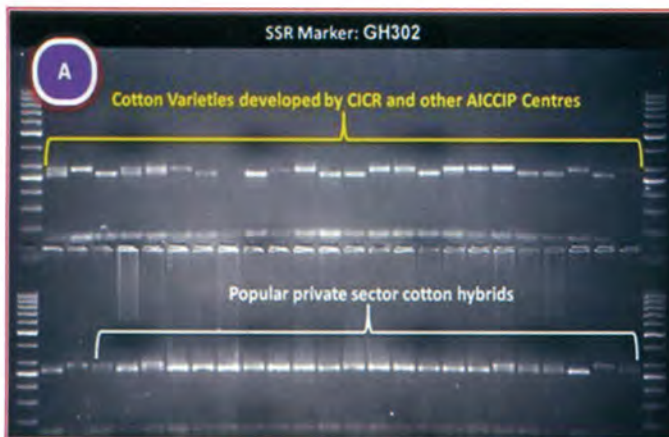


Fig.3.6.2: Fingerprinting of desi cotton varieties using SSR markers

Twelve varieties of CICR (Anjali, LRA 5166, MCU 5VT, Pratima, Sujata, Sumangala, Supriya, Surabhi, Suraj, Suvin, CNHO 12 and Arogya), 14 popular cotton varieties (AKH 8828, Khandwa 2, Khandwa 3, MCU 5, PKV Rajat, RS 2013, RS 810, Sahana, G. Ageti, Narsimha, DHY 286, KC 3, Kanchana and PKV 081) along with 22 (Dyna, Ajeet 199, Ankur 651, Vahini, Vikram, Kanaka, AJEET 155, SRI Tulasi, Super Maruti, Super Mallika, ATM, Pratik, Ankur Jai, Jackpot, Bunny, DR. Brent, Paras Bramha, Mallika, Jaadoo, Tulasi 9, Ajeet 11 and Rasi 2) private sector cotton hybrids were profiled for DNA polymorphism using identified 64 polymorphic markers. Utilizing the allelic profiles generated by these distinctly polymorphic markers (Fig. 1A), robust DNA fingerprint was developed which will assist in unambiguous identification of cultivar, its registration and protection under IPR regime. Higher genetic diversity was observed among cotton varieties (47%) as compared to private sector hybrids (32%). SSR markers effectively distinguished varieties (capturing both inter and intra species variation) and hybrids compared to DUS characters (Fig. 3.6.3 A -D).



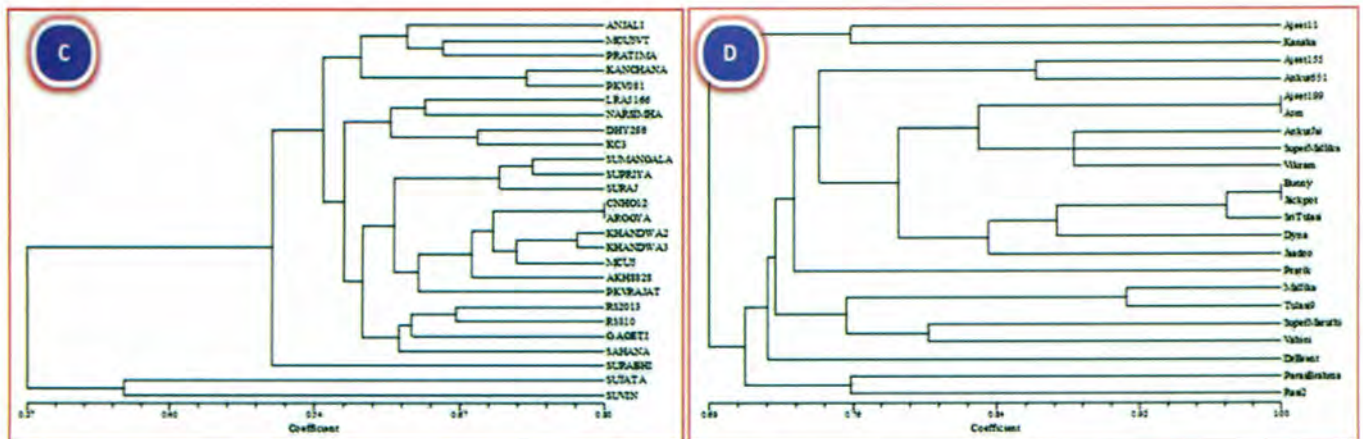


Fig. 3.6.3A - D : Molecular profiling (A) and SSR marker based cluster analysis of private Bt hybrids (B) and CICR varieties (C); DUS trait based cluster analysis of private Bt hybrids (D).

Replicated trial of RILs: During 2014-15, 248 RILs of *G. hirsutum* were grown in 2 row plots in two replications for fibre quality evaluation following incomplete block design. Rajat was the common control variety after every 30 progenies. Observations for boll number, seed cotton yield and GOT was recorded in each RIL progenies.

Phenotyping of RILs : The harvested seed cotton of each RIL progeny is being ginned. The bulk lint of RIL progenies were subjected to fibre quality evaluation using HVI Uster at GTC, CIRCOT, Nagpur. Average seed cotton yield of RIL progenies was 667 kg/ha and is in the range of 186 to 1475 kg/ha.

Maintenance of RILs in diploid and tetraploid cotton: In *G. hirsutum*, 273 progenies were grown in 5 dibble rows for maintenance. Selfed bolls were obtained from each progeny for further maintenance. In *G. arboreum*, a set of 193 progenies were selfed and maintained by single seed descent method. Both the populations were used in genetic mapping.

Marker Assisted Breeding

Cotton Leaf Curl Disease

Sirsa

Screening of new germplasm under field conditions : A total of 3808 lines provided from CICR, Nagpur were sown in the field along with susceptible check variety HS 6 sown at every 10th line at CICR RS Sirsa. The 2128 germinated lines

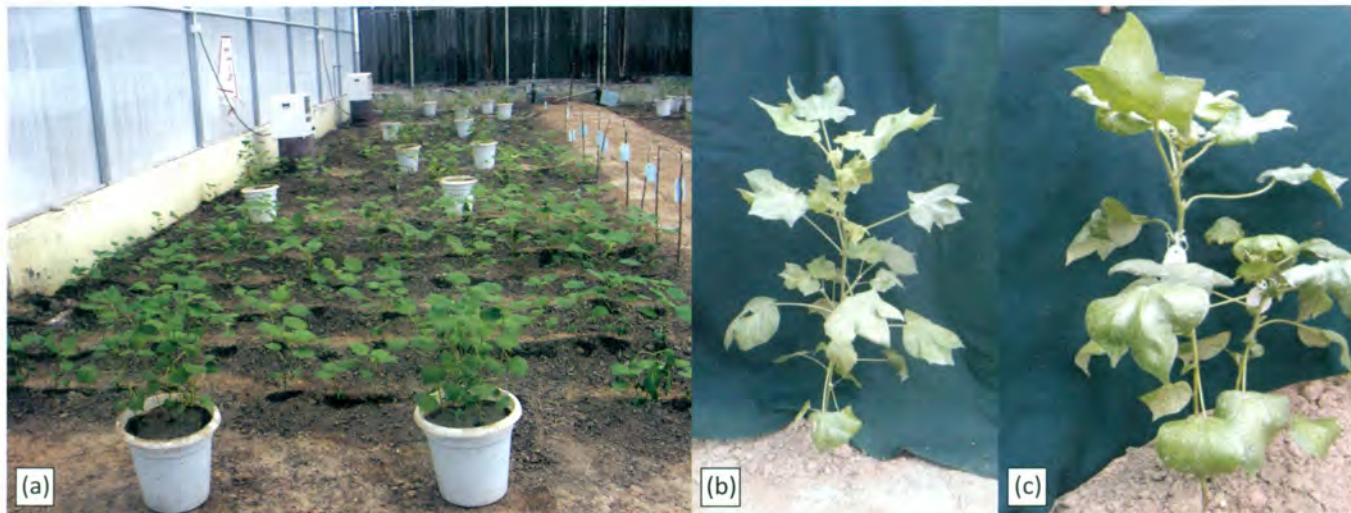
were screened against cotton leaf curl virus disease under field conditions. All the lines showed disease reaction and the PDI ranged between 12.5 to 100. Twenty one lines showed PDI less than 30. The lines with PDI in parenthesis are as follows : BMK-189 (12.50), AKG2/49 (16.67), LD-132 (16.67), N-2944 (16.67), NH-452 (16.67), NH-603 (16.67), NHBBR-2 (16.67), N-148N (18.18), GSH-8 (22.22), LFH-10 (22.22), NHBBR-31 (22.22), IC-356556 (25.00), IC-358353 (25.00), CPH-5728 (25.00), ORS-22 (25.00), NHBBR-5 (26.67), IC-356546 (27.78), IC-356893 (27.78), NAC-1 (27.78), EC-344253 (29.17) and IC-356745 (30.00).

Screening of selected germplasm in nursery : Sixty-one germplasm lines identified as tolerant in 2013-14 were sown in disease screening nursery. Recommended package of practices were followed except plant protection measures against sucking pests. Observations on the incidence and severity of cotton leaf curl virus disease were recorded. All the entries showed symptoms of CLCuD. However based on their disease rating, 6 lines (RS2733: 29.1, F 2276: 28.7, SCS 1061:19.4, RS 2711:27.3, MR 68:28.3 and GISV-251:25.8) showed tolerant reaction.

Confirmation of reaction of parental lines in screening nursery & poly house : Eight lines namely MR 786, Biyani-251, GCH-3, 893, LRA 5166, F 2164, CP 15/2 and RS 2013 and two susceptible varieties HS 6 and F 846 were tested in screening nursery and through artificial inoculation in poly-house for confirmation of their reaction against CLCuD. The PDI of these lines ranged

between 12.5- 25.6 in poly house whereas it varied from 16.4 – 46.0 under screening nursery. The two susceptible varieties HS-6 and F 846 showed PDI of

56.7 and 46.2 in poly-house respectively while in screening nursery, it was 54.1 and 49.1, respectively.



Screening for CLCuD. a) Early crop stage under poly-house conditions, b) CLCV free plant, c) Diseased plant under poly-house

Development of F₂ Mapping Population for CLCuD : Six F₁ crosses were advanced to F₂ generation for developing mapping population for CLCuD. The parents (except RS 2013 and F 2164) and F₁ crosses *viz.* Biyani 251 x F-846, F 2164 x HS-6, RS 2013 x HS-6 and F2164 x F-846 had PDI less than 30 indicating CLCuD tolerance.

Fourteen new crosses were attempted for developing mapping population. Seven tolerant parental lines RS-2013, 893, Bhiyani-251, F-2164, GCH-3, MR 786 and LRA 5166 were crossed with two susceptible parents HS-6 and F 846.

Phenotyping of F₂ mapping population: The data on CLCuD reaction was observed on individual plants and the PDI was calculated taking all the plants into consideration. The maximum grade IV was observed only on single plant in the whole population. Majority of plants were in grade of I indicating less disease spread. In parent HS 6, there was 100% disease but the PDI was 28.29 indicating that most of the plants were in Grade I and Grade II.

Coimbatore

The parental lines of CLCuD resistant varieties *viz.*, MR 786, GCH 3, F 2164, Bhiyani 251, and GCH 3

were sown in the field and crosses will be affected among these parental lines in diallel mating design. The CLCuD susceptible BG II hybrid RCH 134 BG II and resistant hybrids *viz.*, entries 27, 41 and 46 have been sown in the field for effecting crosses.

Bacterial Leaf Blight (BLB)

Nagpur

An elite variety Suraj was used as female parent and 17 F₁ crosses were attempted in 2013-14. F₁ combinations were grown in the field and screened for BLB. One month old plants were sprayed with *Xam* culture (pin pricked plant) and disease incidence was recorded after 7 and 15 days. Based on disease symptoms/reaction F₁ plants were categorized as resistant or susceptible. Molecular screening was also done using an SSR marker CIR 246 for disease resistance. The F₁ plants carrying CIR 246 desired amplicon were selected for crossing with variety Suraj, as a recurrent parent (Table 3.6.1).

On the basis of polymorphic primer screening, individual plants showing resistance to BLB and CIR 246 (146 bp + 156 bp) marker were selected from three F₁ crosses for obtaining BC₁F₁ (Fig. 3.6.4).

Table 3.6.1: F₁ Combination with Suraj (*G. hirsutum*) as female parent

S.No.	F ₁ population	S.No.	F ₁ population
1	Suraj x CSH 3047	10	Suraj x RKR 4145
2	Suraj x GTHH 032	11	Suraj x IC356719
3	Suraj x CSH 3313	12	Suraj x IC 356901
4	Suraj x IC 356798	13	Suraj x IC359098
5	Suraj x IC 356816	14	Suraj x IC357695
6	Suraj x IC356945	15	Suraj x IC357008
7	Suraj x IC356976	16	Suraj x TXORS80
8	Suraj x IC357392	17	Suraj x EC152280
9	Suraj x IC 358481		

Positive F₁ plants were tagged after molecular screening using marker CIR 246 and backcrossed with Suraj.

Coimbatore

The BC₁F₁ progenies of the crosses Suraj x CSH 3313, Surabhi x SIMA, Surabhi x RKR, MCU 5 VT x SIMA, MCU 5VT X RKR were raised and back crosses were effected with the corresponding recurrent parents. The BC₂F₁ progenies *viz.*, Suraj x GTHH 032, Suraj x CSH 3047 and Suraj x CSH 3313 were sown in the field for effecting back crosses with recurrent parent during Summer 2015.

Nematode resistance

Nagpur

Boll to row progeny of F₁ cross each between nematode resistant parents (American Nectariless, G.Cot 10 and Bikaneri Narma) and elite parents (Suraj, Surabhi and NH 615) were raised. F₂ seeds (G.Cot 10, American Nectariless and Bikaneri Narma with Suraj as elite parent) were sent to Regional Station, Coimbatore for phenotyping with respect to reniform and root knot nematode resistance. Markers CIR 316, BNL 3661, BNL 3279, NAU 2152 on chromosome 11 and 14 were found to be polymorphic. Nematode reproduction would be taken as criterion to evaluate reaction of these F₂ populations to nematodes alongwith genotypic characterization.

Coimbatore

The F₁ hybrids of the cross combinations Suraj x

BN, Suraj x AN and Suraj x G Cot 10 were sown and back crosses were effected with the recurrent parent.

3.7 : Development of Transgenic Cotton

Cloning of VIRD2 protein in bacterial expression vector:

To generate *in-vitro* nano complex of VirD2-ssTDNA-VirE2- with cell penetrating peptide (Tat2) for transgene gene delivery into cotton, VirD2 from *Agrobacterium tumefaciens* LBA4404 was successfully cloned into TA cloning vector. It was further subcloned into a bacterial expression vector (pET28c) using EcoRI and XhoI restriction sites and confirmed through restriction digestion (Fig. 3.7.1 & 3.7.2).

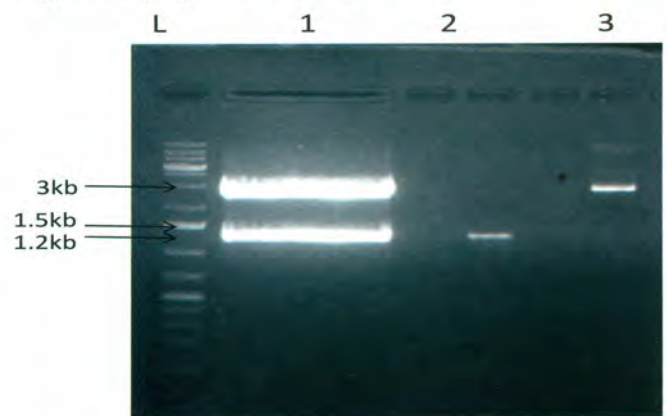


Fig. 3.7.1 : Restriction confirmation of virD2 Clone in TA cloning vector with EcoRI and XhoI

L: 1Kb Molecular ladder ; Lane 1: Restriction Confirmation of virD2 plasmid with EcoRI and XhoI.

Lane 2: virD2 gene product; Lane 3 : pGEMT easy vector with virD2 insert.

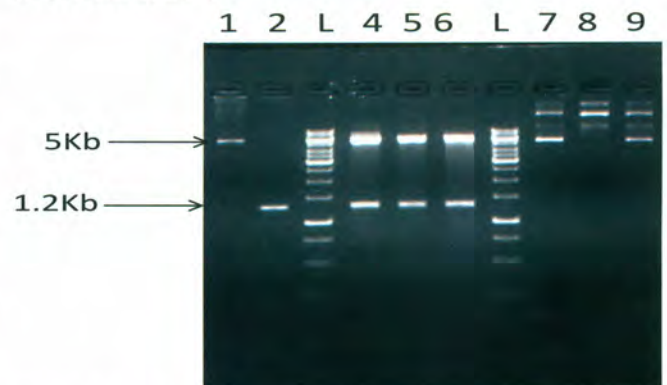


Fig. 3.7.2 : Restriction confirmation VirD2 Clone in pET28c expression vector with EcoRI and XhoI

L: 1Kb Molecular ladder; Lane 1: Linearized pET28c(+) plasmid, Lane 2: virD2 gene fragment,
Lane 4-6 : Restriction digested pET28c(+)VirD2 with EcoRI and XhoI
Lane 7-9: Uncut pET28c(+)virD2 plasmids

Transformation of gene construct - *CICRcry2Ab1Ac:chitinase*

In-planta transformation: Two methods of *in-planta* transformation were adopted to incorporate the gene into var. Suraj (*G. hirsutum*).

1. In the first method half of the portion of style was cut after pollination and *agrobacterium* suspension was injected in to the style of 422 flowers.
2. In the second method, a drop of *Agrobacterium* suspension was added over the stigma gently of 215 flowers. Flowers were covered with paper bag to avoid contamination. Flowers were covered with paper bag after injection of *agrobacterium* suspension.

Seed setting in *in-planta* transformation in var. Suraj (*G. hirsutum*)

Method	Flowers treated	Bolls harvested	Seeds Obtained
1	422	11	230
2	215	06	072

Shoot tip transformation of *CICRcry2Ab1Ac:chitinase* gene

In-vitro germinated seedlings were raised in the laboratory. Shoot tip explants containing meristem cells were isolated aseptically. The shoot tip were treated with *Agrobacterium* suspension for 0.5 h and subjected to co-cultivation and kanamycin selection.

In all 2274 seeds were inoculated, of which 1550 seeds germinated. Explants (1010) were treated with *agrobacterium* and 152 shoots were selected on kanamycin medium. In all, 36 plant-lets were elongated and grown under control condition.

Agrobacterium mediated transformation of *CICR-cry2Ab1Ac* gene construct and regeneration through Somatic embryogenesis

CICR Bt-multi gene construct (*CICR-cry2Ab1Ac*) was subjected to transformation and regeneration through somatic embryogenesis using Coker as well as Suraj cultivar. Profuse callus was induced initially with Coker 310 and Suraj varieties using MS media with 0.1 mg/l 2, 4-D and 0.5 mg/l kinetin and transformed with multi gene construct by *Agrobacterium* transformation. Putative transformants were induced without antibiotic MS media with B5 vitamins and Maltose 2%. Fortnightly the transformed calli were sub-cultured and somatic embryo formation was observed after 4-5 weeks. Regenerated plants were transferred to H1 medium (Stewart and Hsu) with 1% sucrose. Three year independent putative transformants were regenerated and are under root induction and establishment process.



Transgene delivery using cell penetrating peptide (CPP) through pollen tube pathway
Linearised RNAi gene cassette for gossypol biosynthesis gene, coding for delta *cadinene synthase* driven under Beta Globulin seed specific promoter(BGP) with Tat2 cell penetrating peptide was injected into 40 flowers through pollen tube pathway and six bolls that survived till maturity were harvested.

Validation of fibre strength genes

Gene expression analysis was done with selected genes (*GhcesA1*, *GhcesA2*, *GhcesA7*, *GhcesA8*, *Ghcobl4*, *Ghfla3* and *GhMT1*) through qPCR using RILs mapping population to establish correlation with high fibre strength. Among them *GhcesA1*, *GhcesA2*, *Ghfla3* and *Ghcobl4* showed strong association and higher gene expression during

secondary wall synthesis especially at 25 and 30 days post anthesis. Thus all these genes were cloned and characterized for validation through transgenic approach. The promoter region for *GhcesA1* was also cloned and characterized (2.8kb). The other genes of cellulose synthase such as *GhcesA7* and *GhcesA8* were cloned and sequenced. The nucleotide sequence was submitted to NCBI GenBank (*GhcesA7*: Accession No. KJ777132). Similarly sucrose synthase gene (*GhSusA1*) is one of only two enzymes which can decompose sucrose into hexoses by catalysing a reversible reaction but preferentially converting sucrose into fructose and UDP-glucose in-planta, was also cloned, sequenced and submitted to GenBank (*GhSusA1*: KJ777133).

The candidate genes such as *GhcesA1*, *GhcesA2*, *Ghfla3* and vectors were digested with *KpnI* and *EcoRI* restriction enzyme. The fragments were eluted from agarose gel and ligated into pCAMBIA vector with NOS terminator. The vector was transformed into DH5 alpha. The transformants were confirmed by screening with colony PCR, restriction digestion and PCR amplification.

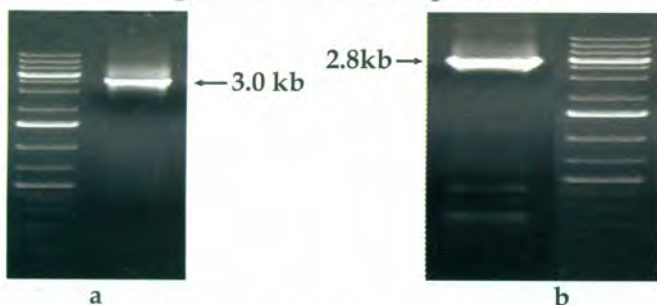


Fig.3.7.3 a. Amplicon of *GhcesA1* gene (3.0kb). b. Amplicon of *GhcesA1* promoter region (2.8kb)

Apart from cellulose synthase, genes encoding other protein genes such as *Fascillin-like-Arabinogalactan-3* (*Ghfla3*), *Ghfla-11* and *Ghfla-12* were studied. Among them *Ghfla-3* showed higher expression during secondary cell wall synthesis. Hence the *Ghfla-3* gene was cloned for validation. The other *fla* genes such as *Ghfla-11* and *Ghfla-12* were sequenced and nucleotide sequences were submitted to NCBI GenBank (*Ghfla11*: KM457622 and *Ghfla12*: KM457623). The clones were reconfirmed with restriction digestion analysis.

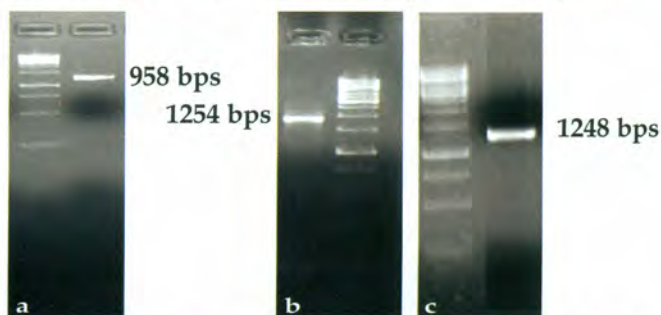


Fig. 3.7.4 a. *Ghfla3* amplicon (958bps) b. *Ghfla11* amplicon (1254bps) c. *Ghfla12* amplicon (1248bps)

3.8 Seed Production and Technology

Nucleus seed production of Anjali and Suraj was undertaken. Breeder seed production was undertaken in respect of Suraj, Anjali, Sumangala and MCU-5VT.

A total of 484.52 q seeds including 194.52 q of cotton seeds of different categories was produced (Table 3.8.1) and resource of around Rs 15-16 lakhs was generated through the sale of these seeds or its by-products.

Table 3.8.1 : Seed production achieved during 2014-15

Crop	Stage	Production (Q)
Cotton (8 varieties + parents of CICR -2)	Breeder Seed	4.65
Suraj	Foundation Seed	2.52
Suraj	Certified Seed	161.38
Suraj	Truthfully Labelled Seed	23.85
Barley cv. BH -393	Certified Seed	289.00
Cotton (48 varieties + germplasm lines)	Truthfully Labelled Seed	1.74
<i>G. arboreum</i> race <i>cernuum</i>	Truthfully Labelled Seed	0.378
Red Gram BSMR -736	Truthfully Labelled Seed	1.00
Total		484.518

Immortalization of cultivar germplasm through 'VARIETAL GARDEN'

Twenty-six *G. hirsutum* varieties (Anjali, Arogya, CNHO-12, Kanchana, LRA-5166, MCU-5, MCU 5VT, Pratima, Sumangala, Supriya, Surabhi, Suraj, PKV-081, AKH-8828, DHY-286, F-846, G. Ageti, HS-6, JK-4, KC-3, Khandwa 2, Khandwa 3,

Narasimha, RS-2013, PKV Rajat and Surat Dwarf), two *G. barbadense* varieties (Sujata and Suvin) and four *G. arboreum* varieties (H-1226, HD-123, HD-324 and ABC-5) were established at "Varietal Garden" to conserve them in a perennial form and also assure the continuous supply of genetically pure seeds of cotton varieties for future cotton research.



Varietal Garden established at ICAR-CICR, Nagpur

Nagpur

Planting methods and soil/seed treatments to enhance seed production

Seed cotton yield of 1 month old transplanted crop was superior than the 20 day old transplanted and direct sown crop. Boll number and sympodial number were higher in the transplanted crop than the direct sown crop.

Fourteen different soil treatments were given while sowing imidacloprid treated seeds of Jai Bt in the direct sown as well as in transplanted (1 month old seedling) crop. Treatments included, FYM, vermicompost, cotton stalk compost, potassium silicate (Agrisil), potassium silicate (powder), *Bacillus* sp, *Pseudomonas fluorescens*, *P. putida* sp, Tricocash, *Caedacea* sp., *Pantoea* sp., humus, *Trichoderma viride* along with control. There was no significant difference for seed cotton yield among the treatments. Single boll weight of the treatments varied from 2-3 g in direct sown crop whereas it was 2.5-3.5 g in transplanted crop. Among the

treatments given in direct sown crop, cotton stalk compost gave the highest seed cotton yield (1532 kg/ha) followed by potassium silicate powder (1445 kg/ha) and *Pseudomonas fluorescens* (1324 kg/ha). In the transplanted crop, soil treatment with *Pseudomonas fluorescens* (1592 kg/ha), gave highest yield followed by *Pseudomonas putida* (1504 kg/ha) and *Bacillus* (1472 kg/ha).

In a study on variety Suraj sown under high density with 60 cm x 10 cm spacing, seed treatment with Thiram @ 2.5 g/kg seed gave the highest seed cotton yield (2193 kg/ha.) followed by Dentop (containing Thiomethoxam) (2150 kg/ha) and imidacloprid combined with Thiram respectively (2013 kg/ha).

In another field trial, seeds were treated with microbial cultures *Pseudomonas fluorescens*, *Pseudomonas putida*, Microbial consortia, Tricho cash and untreated control. Among the treatments, *Pseudomonas fluorescens* gave significantly higher yield compared to other treatments.

Coimbatore

Assessment of seed vigour tests for relative storability and field performance

Assessment of seed vigour tests for relative storability and field performance was initiated to standardize the methods of estimating seed vigour in hybrid cotton 153. Seed lots with four vigour levels were evaluated initially and estimation of vigour was done at an alternate month of storage at recommended temperature (25°C) and at an elevated temperature of 35 ± 1°C. The mean data of seed germination, seedling root length, shoot length and dry matter of seedling were computed. The vigour index was computed and compared with seed germination taken at bimonthly intervals. The data on germination revealed that a significant improvement in germination was observed when tested at 35°C (79.9%) than at 25°C (72.3%) over four vigour levels and three intervals of test. Among the vigour levels while V1 (79.5%) and V2 (77.5%) at par with significance over V3 and V4 at 25°C. However, at 35°C V2 was significantly superior (87.7%) than the rest. The period of storage has significant influence on seed germination and a decline was observed with advancement of seed ageing. Across the temperature and interval of test V1 (80.2%) and V2 (82.6%) were on par but maintain its significant superiority over V3 (73.2%) and V4 (68.4%). The interaction of period of test with vigour levels and temperature of test and vigour levels were also significant. The mean data for root length, shoot length, dry matter of seedling and vigour index showed similar trend to that of germination.

Seed treatment for quality enhancement

An experiment conducted with RCH 2 BGII seeds to assess their responses for seed treatments such as priming with botanicals, nutrients, chemicals, growth regulators, bio inoculants and seed coating revealed that treatments of seed coating (15g/kg) with *Pseudomonas*, Turmeric (*Curcuma* sp), rhizome powder @ 20g/kg coating using polymer, polykote (5 ml polymer coating/kg), Molybdenum (sodium molybdate dehydrate) - Dry dressing (0.5 g/kg); seed soaking in Dasakavya (2.5%) KH₂PO₄ (450

ppm), CaCl₂ (2%), Panchakavya (10%), KCl (1%), *Prosopis* leaf extract (1.5%), neem leaf extract (1%), KNO₃ (0.5%), Zeatin (20 ppm), H₂O₂ (60 mM), CuSO₄ (0.2%), 6BAP (10 ppm); bio priming with *Pseudomonas* (2 g/kg) and pulsed magnetic treatment at 750nT 5h for 15 days significantly increased the germinability and seedling vigour.

Sirsa

Studies to improve the seed and boll setting efficiency in cotton

The effect of growth hormones, pollinator attractant and weather parameters were studied on boll and seed setting efficiency. The higher boll setting (93.7%) was observed with hand pollination. Boll setting in other treatments *viz.* 2%NPK (91.3%), CICR consortium (91.3%), Godrej double (90.7%), 2% DAP (90.2%) were observed significantly higher than control (87.2%). The seed setting efficiency (92.5%) was also significantly higher in hand pollination than other treatments which in turn was at par with respect to seed setting efficiency.

Among pollen attractants, the boll setting (93.0%) with molasses (10%), 92.0% with sugar solution (10%), 90.6% with rose extract (10%) were significantly higher than control (89.0%). The pollen attractants did not increase the seed setting efficiency.

The contribution of pollinators towards the boll setting % and seed setting % was studied using sterile as well as fertile plants of GMS-DS-5 and GMS-16-A with sufficient population of pollinator by keeping one box of honey bee colony in field. The data on boll and seed setting % was recorded at three interval of 15 day each. In sterile plants, the boll setting % was much lower i.e. 2.2 to 4.4% compared to 79.4- 88.3% in fertile plants of DS5 and 2.3-4.6% in sterile plants compared to 77.8-85.6% in fertile plants of GMS 16A. The seed setting percentage was also less in sterile plants i.e. 13.8-24.0 % in sterile plants and 79.5-81.4 % in fertile plants of DS 5 and 14.2-21.4 in sterile plants and 78.2-81.0% in fertile plants of GMS 16A.

The contribution of pollinator on boll and seed

setting efficiency was also estimated by planting the field at 500 m distance with assured availability of pollinator (one box of honey bee) and pollinator's attractant. Another field was purely under natural condition. The significant difference between these treatments were observed only in boll formation till 15th of September but the difference in seed setting efficiency was not significant. In another experiment, the contribution of pollinator was also estimated by keeping the pollinator in covered net in which significant difference was observed only in boll formation (76.1%) compared to open plots (74.5%).

To study the effect of prevailing environment on seed setting efficiency, minimum and maximum temperature, humidity, and rainfall was recorded during flowering and data was correlated with boll and seed setting percentage. Because of similar range of temperature, humidity and rainfall during first and second flowering stages, the boll formation percentage was 85 to 90% and seed setting was 74 to 89%.

DUS Testing

Five different trials were conducted under DUS characterization which included, 49 EDVs, 43 VCKs, 63 genotypes under first year testing and 30 under second year testing. The data on hypocotyls pigmentation, leaf and flower characters, boll and plant growth characters have been completed in three replications of all the above genotypes. Among the observation on EDVs, only thirteen were found to be EDVs with respect to their Initial Varieties and these were subjected to Bt testing by ELISA. Bt test results performed on seeds revealed clear segregation for Bt genes, *Cry 1Ac* and *Cry 2 Ab* in many of the BG II hybrids.

In addition to the regular phenotyping of genotypes as per DUS test guidelines, more traits were studied for suitability in variety characterization. These included variation among genotypes for leaf petiole length and peduncle length. The petiole length of fourth leaf from top on four plants was measured while peduncle length was determined from one boll each selected from upper, middle and lower canopies on three

individual plants and mean calculated. A clear genotypic variation could be observed for both leaf petiole length as well as peduncle length with some genotypes showing high and others showing low values. The consistencies in values will be corroborated next year.