

3. RESEARCH ACHIEVEMENTS

3.1: Consolidation and characterization of genetic diversity

Status of cotton germplasm

ICAR-CICR, Nagpur maintains one of the largest

cotton germplasm collections of the world with 11,648 accessions covering all the cultivated and wild species of *Gossypium* including perennials, landraces and interspecific derivatives (Table 3.1.1).

Table 3.1.1: Status of cotton germplasm at ICAR-CICR, Nagpur

Species	Number of accessions
<i>G. hirsutum</i>	8505
<i>G. barbadense</i>	312
<i>G. arboreum</i>	1936
<i>G. herbaceum</i>	565
Wild Species	24
Interspecific derivatives	40
Perennials and land races	254
Races and derivatives of species	12
Total Collection	11648

Exploration and collection of cotton germplasm

Exploratory survey was carried out to collect unique germplasm from Rayagada, Nuapada and Kalahandi districts of Odisha. Three morphological variants of *Gossypium barbadense*

were collected of which one belonged to *G. barbadense* var. *brasiliensis* possessing a unique character of fused seeds (Kidney cotton). One sample of *G. barbadense* was collected from Mandya district of Karnataka (Table 3.1.2).

Table 3.1.2: List of perennials of cotton collected from exploratory surveys

Sl. No	Districts	State	No. of Accessions	Species	Perennials/Annuals/Landraces
1.	Nuapada	Odisha	2	<i>G. barbadense</i>	Perennials
			1	<i>G. barbadense</i> var <i>brasiliensis</i>	Perennial
2.	Mandya	Karnataka	1	<i>G. barbadense</i>	Perennial
Total			4		



Perennial cotton *G. barbadense* from Nuapada, Odisha

Conservation of germplasm in long term storage : Seeds of one thousand six hundred two (1602) accessions of *G. hirsutum* and three registered genetic stocks of *desi* cotton (Brown coloured linted) were submitted for long term storage at ICAR – NBPGR, New Delhi.

Maintenance of germplasm under Medium Term Cold Storage : Two units of medium term cold storage modules were maintained at ICAR-CICR, Nagpur for conservation of cotton seeds at 4^o to 5^o C temperature and 32% to 35% relative humidity.

Maintenance of *Gossypium barbadense* germplasm : Three hundred and twenty *Gossypium barbadense* germplasm lines were maintained at ICAR-CICR,

Regional Station Coimbatore during 2017-18 crop season.

Enrichment of Cotton Gene Bank : Fifty eight (58) exotic accessions consisting 56 *G. hirsutum* accessions and 2 *G. barbadense* accessions were procured from USA through ICAR-NBPGR, New Delhi to enrich Cotton Gene Bank maintained at ICAR-CICR, Nagpur. A set of 36 exotic accessions including 34 Coker variants and 2 accessions of CLCuD resistant accessions (EC881780 and EC881781) were grown in field, characterized and evaluated for economic and fibre quality traits. Superior accessions were identified for ginning outturn, staple length and fibre bundle strength for further utilization in breeding program (Table 3.1.3)

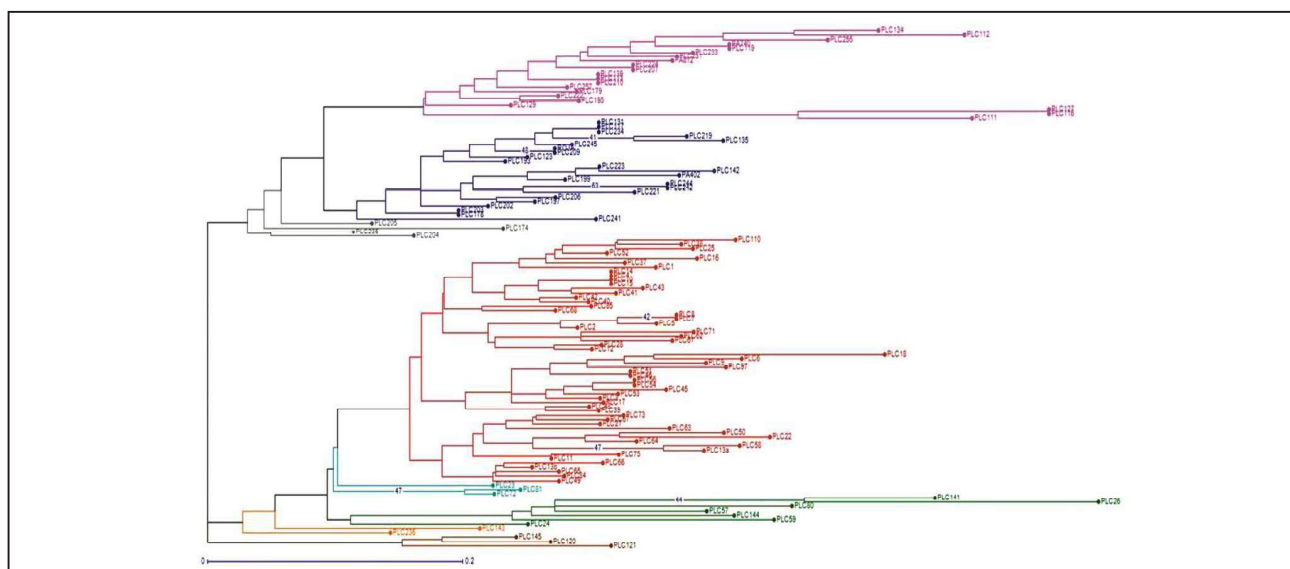
Table 3.1.3: Fibre quality characters of exotic Coker and CLCuD resistant accessions

S. No.	Accession Number	UHML (mm)	MIC (µg / inch)	Tenacity 3.2 mm (g/tex)
1	EC882398, EC882405, EC882408, EC882424, EC882425	32.0-37.0	3.0-4.4	29.3-35.0
2	EC882399, EC882403, EC882404, EC882407, EC882409, EC882413, EC882414, EC882415, EC882417, EC882418, EC882419, EC882420, EC882421, EC882422, EC882426, EC882427, EC882428, EC882429, EC882430, EC882431, EC796545, EC807817, EC881780	28.4-30.9	3.2-4.0	26.0-34.5
3	EC882400, EC882401, EC882402, EC882406, EC882410, EC882411, EC882412, EC882416, EC882423, EC881781	23.3-27.8	2.8-4.4	25.4-33.2

Morphological and molecular characterization of perennials of *desi* cotton

Morphological including DUS characterization was completed for 58 accessions of *desi* cotton and perennials. Molecular characterization was done for 114

accessions of perennials and landraces of *desi* cotton along with 5 popular *desi* cotton varieties using identified SSR markers. Out of 60 SSR primers screened, thirteen markers showed polymorphism (21.6%). Based on the study, 119 accessions were grouped into different groups using DARwin statistical package.



Dendrogram depicting genetic relationships among accessions of the perennials and landraces of *desi* cotton (Bootstrap values >50% are shown).

Germplasm evaluation of *G. herbaceum*

Identified 25 early maturing *G. herbaceum* germplasm accessions were evaluated for yield performance. Data on plant height, days to fifty percent flowering, days to harvest, days to 70 per cent harvest, number of bolls and seed cotton yield were recorded. Seed cotton yield ranged from 1210 kg/ha (IC 3712208) to 1560 kg/ha (IC - 371166). Baluchistan - 1 and IC - 371437 were found to be early in maturity as compared to standard checks

Evaluation of *G. arboreum* germplasm for fibre quality

A set of 1000 *G. arboreum* accessions were evaluated for fibre quality traits at ICAR-CICR, Regional Station, Coimbatore during 2017-18. Based on superiority for specific traits, germplasm accessions were identified as detailed hereunder:

Staple length : Eleven accessions namely 360-SP1 (29.3mm), Shamali (28.2mm), Desi-103 (28.1mm), 5974 (28mm), 6582 (28mm), 30859 (27.9mm), AK 606-SP1 (27.9mm), AC 3695 (27.9mm), Sarguja-NL-WF (27.8mm), 30843 (27.8mm) and 30839 (27.4mm) were identified for staple length.

Uniformity ratio : Seven accessions namely Gao 16 CB-9 (60), Obtusifolium-B-Indica (58), 360 (57), GDH 149 (Sel.) (57), Arboreum (Kanpur A) (56), Chinese broad

lobe (56) and PBN 48 (56) were identified for uniformity ratio.

Elongation: Twenty five accessions showing higher elongation of more than 6.2% were identified.

Strength: Fifty three genotypes possessing high fibre strength above 27.6 g/tex have been identified.

Strength to Length (SL) ratio: Set of twenty accessions showing SL ratio more than unity namely AC 3522 B, AC 3451, Arboreum (Kanpur A), AC 3284, AKA 14, AH 71, H 575, AC 3234, AC 3289, Desi-1, PS-135, SC 97, 79/BH-97, H 52-473, CC-1-1-3, AC 3368, 7763, 8410-2, PBN 6977 X AKH4, 30819-SP1 were identified. These accessions with long and strong fibre will be useful to textile industries.

Evaluation of *G. barbadense* germplasm

Eleven hairy germplasm accessions were evaluated for superior plant types in terms of yield, quality and earliness. The top performing five accessions viz., ICB-85, ICB-124, ICB-264, ICB-284 and HAG-02 have been identified as moderately resistant to sucking pests over the check Suvin which is highly susceptible. These identified lines may be useful for development of sucking pest tolerant/resistant lines in future.

Table 3.1.4: Performance of hairy germplasm lines over the years

S. No.	Germplasm lines	Seed Cotton Yield (kg/ha)			Mean	GOT (%)	Fibre qualities			Sucking pest incidence
		2015-16	2016-17	2017-18			2.5% SL (mm)	Strength (g/tex)	Mic (µ/inch)	
1	ICB-124	736	712	601	683	31	35.2	31	3.1	MR*
2	ICB-284	801	786	546	711	30	34.7	33	3.1	MR*
3	ICB-85	688	634	504	609	30	33.6	31	3.0	MR*
4	ICB-264	702	621	542	622	29	34.2	30	2.9	MR*
5	HAG-02	623	569	497	563	31	33.5	32	3.3	MR*
	Suvin	619	587	364	523	30	36.7	30	3.0	S**

*MR-Moderately resistant, **S-Susceptible

Development of Mini-Core group

Geographically and genetically diverse 788 accessions of core germplasm assembly were grown for seed multiplication & DUS characterization. Data on morphological traits were recorded and lint samples are being analyzed for fibre quality traits. After, evaluation of fibre quality traits, the dissimilarity index and matrix will be prepared for the development of Mini Core group.

Distribution of cotton germplasm

A 'Germplasm Field Day' was organized at boll bursting stage so as to facilitate selection of suitable germplasm

lines by the cotton breeders across all three cotton growing zones (North, Central and South zone). Nine hundred forty three (943) elite germplasm lines of *G. hirsutum* including exotic accessions were distributed to breeders/scientists of different State Agricultural Universities to be used by them in their breeding programme.

Wild species of *Gossypium*.

Conservation

Twenty four wild species, 15 races of cultivated species and more than 45 synthetic polyploids were conserved

in the ICAR-CICR Wild Species Garden & green house. Herbarium of the available species, races of cultivated species and six registered unique genetic stocks of introgressed derivatives namely, MSH-91 SP, MSH 53 (Vaidehi-95), MSH 345 (Cleistogamous genotype), NISC 40, NISC 43, NISC 44 and CNA-5 were prepared.



**Herbarium of
*G. trilobum***

Utilization of wild species

Wild species were used in crossing programme and fresh crosses were attempted using *G. anomalum*, *G. trilobum* and *G. barbosanum* with cultivated species for introgression of economically important traits from wild species. Sterile F₁ crosses between *G. capitis virides*, *G. bickii* and *G. stocksii* were established in pots.



Interspecific crosses involving wild species of cotton:
a) AK 8401 x *G. bickii*; b) Suraj x *G. stocksii* and c) Phule dhanwantary x *G. capitis virides*

Plants of F₄ population of *Jawahar Tapti* x *G. longicalyx*, *G. arboreum* x *G. thurberi*, *G. arboreum* race indicum x *G. davidsonii* and *G. arboreum* Cv. AK 8401 x *G. davidsonii* were evaluated in the field. Single plant based selections were made and were advanced to next generation. The plants of *G. herbaceum* x *G. anomalum* cross were advanced from F₄ to F₅ generation.

Evaluation of introgressed derivatives:

A total of 461 introgressed derivatives were evaluated at Nagpur for fibre & economic traits. Among the 313 *G. hirsutum* based introgressed derivatives; fifteen (15) high yielding derivatives were identified. Among 60 *G. arboreum* based introgressed derivatives, ten high

yielding derivatives were identified. In *G. hirsutum* based introgressed derivatives, CNH 17297 ranked first with seed cotton yield of 2832 kg/ha followed by CNH 17293 with 2348 kg/ha and CNH 17295 with 2036 kg/ha. In *G. arboreum* based introgressed derivatives; CNA 17350 ranked first with seed cotton yield of 1472 kg/ha followed by CNA 17369 and CNA 17342 with seed cotton yield of 1334 kg/ha and 1288 kg/ha respectively.

CNH 16300 (*G. hirsutum* based introgressed derivative) evaluated in the Institute trial ranked third with seed cotton yield of 1986 Kg/ha as compared to the checks Suraj (1317 kg/ha), NH 615 (1350 kg/ha) and AKH 8828 (1662 kg/ha).

CNA 16383 (*G. arboreum* based introgressed derivative) ranked first with seed cotton yield of 2062 kg/ha as compared to the checks AKA 8401 (686 kg/ha) and PA 255 (706 kg/ha). The other parameters of CNA 16383 are also promising with fibre length of 27.9 mm and bundle strength of 27.6 g/tex.

Naturally coloured cotton:

A total of 128 coloured cotton genotypes of natural dark brown, light brown linted and other derivatives were evaluated for yield and tolerance to sucking pests during 2017-18. Single plant selections were made for high yield per plant.

Development of consensus genetic linkage map of *Gossypium*

A set of 227 RILs mapping population obtained from a cross of *G. arboreum* (KWAN-3) x *G. herbaceum* (Jayadhar) was grown as single row progenies. Two plants from each progeny (RIL) were selfed for maintenance and seed cotton of remaining plants was bulk harvested. Seeds of RILs shall be shared with the participating centers for phenotyping at three locations. Genomic DNA from each RIL progeny was extracted using cTAB method that will be shared with NBRI, Lucknow for SNP genotyping.

Molecular diversity analysis and DNA fingerprinting in cotton

Morphological characters based on DUS traits were documented for 50 public sector released varieties of tetraploid cotton (*G. hirsutum* and *G. barbadense*) as well as 27 *G. arboreum* released varieties and a fingerprint chart based on morphological traits was developed. Fifty additional SSR markers were screened among tetraploid varieties out of which 18 were found to be

polymorphic. Thus, over the years, screening of 400 SSR markers have been completed for tetraploid cotton and 86 polymorphic markers were obtained which can be used to complement DUS traits. A selected set of 20 SSR markers with high PIC could identify each variety under study including all CICR released varieties and a DNA fingerprint was developed. Screening of 27 diploid cotton (*G. arboreum*) released varieties using additional 100 markers revealed 16 to be informative. A total of 48 useful markers have been identified for *G. arboreum* varieties for further utilization. A selected set of 8 markers with high PIC could identify each *G. arboreum* variety under study at probability of identical match 2.68×10^{-6} .

3.2 Breeding for premium fibre quality and high yield as per global needs

Improvement of *G. arboreum* cotton

Evaluation of GMS based *G. arboreum* hybrids

Eight GMS based *G. arboreum* hybrids were evaluated for seed cotton yield with two check hybrids AAH 1 and CICR 2 at Sirsa. Three GMS based hybrids CISA 212 (3274.6kg/ha) and CISA 512 (2920.6 kg/ha) could record significantly higher seed cotton yield than the highest yielder check hybrid CICR 2 (2544.0 kg/ha) (Table 3.2.1).

Table 3.2.1: Performance of GMS based *G. arboreum* hybrids in replicated trial at Sirsa

Entry Name	SCY (kg/ha)	Lint Yield (kg/ha)	GOT %	Boll No. per plant	Boll Wt (gm)	UHML (mm)	UI	Strength (g/tex)	Mic (μ/inch)
CISAA 1312	2622.2	935.6	35.7	45.5	2.0	25.6	81	26.1	5.9
CISAA 1302	2264.3	815.8	36.0	46.6	2.1	25.6	81	26.2	5.8
CISAA 2012	2144.6	743.7	34.7	46.9	2.0	24.6	81	24.5	5.7
CISAA 2002	2323.9	834.2	35.9	39.3	2.1	25.0	81	24.7	5.6
CISAA 212	3274.6	1194.4	36.5	62.0	2.2	24.8	81	25.1	6.2
CISAA 202	2472.1	872.13	35.3	48.1	2.0	25.8	81	26.7	5.8
CISAA 512	2920.6	1127.9	38.6	47.5	2.1	23.4	80	24	6.2
CISAA 502	2541.5	948.9	37.3	42.0	2.0	23.6	80	24.2	6.2
AAH 1	2333.4	850.9	36.5	47.1	2.1	18.8	76	21.5	6.8
CICR 2	2544.0	1025.7	40.3	51.5	2.1	20.1	77	21.9	6.6
CD	248.56								
CV (%)	5.87								

Maintenance of GMS lines

Three GMS lines (DS5, CISA 2, GAK 413A) and 18 newly identified GMS lines [CISG-1, CISG-2, CISG-4, CISG-8, CISG-9, CISG-10, CISG-11, CISG-13, CISG-14, CISG-15, CISG-16, CISG-17, CISG-18 (narrow leaf), CISG-18 (broad leaf), CISG-19, CISG-21, CISG-22 (narrow leaf) and CISG-22 (broad leaf)] were maintained through sibmating at Sirsa. Pigmented GMS line CISG 20 is having red flower colour with petal spot. The plant is robust and the line is thermo-insensitive and no pollen shedders were reported.

Evaluation of Spinnable *G. arboreum* cultures

Fourteen spinnable *G. arboreum* cultures were evaluated under RBD along with two checks CISA 310 (3219.7 kg/ha) and PA255 (889.6 kg/ha) at Sirsa. None of the

genotypes could give significantly higher yield than the checks. However genotype CISA 33-9 showed numerically superior yield of 3380.2 kg/ha as compared to 3219.7kg/ha in check but had fiber length of 19.8mm. However genotypes CISA-6-295, CISA-6-350, CISA33-8, CISA-41-1 and CISA-6-209 having yield more than 30 q/ha and have promise for spinning. Four genotypes CISA-6-295, CISA-33-6, CISA 33-8 and CISA 6-256 were having UHML (mm) >25.0mm and strength ~25.0 g/tex.

Evaluation of High Yielding *G. arboreum* genotypes

Fourteen high yielding *G. arboreum* genotypes were evaluated in RBD design with two check varieties CISA 614 and Phule Dhanvantri at Sirsa. Two genotypes CISA-6-2 (3483.5kg/ha) and CISA 405 (3240.3kg/ha) gave significantly higher seed cotton yield (table 3.2.2) than high yielding local checks CISA 614 (2725.4 kg/ha).

Table 3.2.2: Performance of *G. arboreum* genotypes at Sirsa

Entry Name	SCY (kg/ha)	Lint Yield (kg/ha)	GOT %	Boll wt (gm)	UHML (mm)	UI	Strength	Mic
CISA-6-165	2679.30	936.48	35.0	2.2	19.0	76.0	21.4	6.7
CISA 6-2	3483.50	1342.36	38.5	2.3	19.4	76.0	21.6	6.8
CISA-6-123	1765.70	610.12	34.6	2.0	18.7	76.0	21.4	6.8
CISA-6-187	1854.80	647.40	34.9	2.0	19.0	76.0	21.4	6.8
CISA-6-214	1712.00	660.50	38.6	2.2	24.8	81.0	23.8	5.8
CISA 10	2247.50	794.95	35.4	2.1	27.8	82.0	26.7	5.9
CISA 6	2810.80	1100.64	39.2	2.1	20.0	77.0	21.8	6.8
CISA 405	3240.30	1235.78	38.1	2.2	19.9	77.0	21.8	6.7
CISA 8	2618.50	994.01	38.0	2.1	20.3	77.0	22.1	6.8
CISA 7	2636.60	1031.53	39.1	2.2	20.2	77.0	22.3	6.7
CISA 9	1743.30	629.25	36.1	2.0	22.9	80.0	24.0	6.3
CISA 294	1868.20	747.43	40.0	2.0	22.7	79.0	23.6	6.6
CISA 33-4	2589.40	982.37	37.9	2.1	25.1	81.0	26.0	6.1
CISA 33-5	2909.70	1115.94	38.4	2.0	20.0	77.0	22.0	6.6
Phule Dhanvantri	2623.00	940.21	35.8	2.0	21.5	78.0	22.6	6.4
CISA 614	2725.40	1013.26	37.2	2.1	19.4	76.0	21.6	6.8
CD	309.35							
CV (%)	7.52							

Thirteen *G. arboreum* selections were evaluated in two replications, of them SPS 16-3 (787 kg/ha) and SPS 1-1-1 (740 kg/ha) which found superior to check AKA 8401 (648 kg/ha).

Improvement of *G. herbaceum* cotton

Inter specific hybridization (*G. herbaceum* × *G. arboreum*): In a line × tester mating design involving seven *herbaceum* lines (GVHV-655, IC-371437, Jayadhar, IC-371158, Baluchistan - 1, IC-371136 and IC-371360) and three *arboreum* testers (PA-740, PA-785 and PA-812), twenty one F₁ crosses were made at Nagpur. Twenty one F₁ crosses, 3 standard checks (DDhC -11, Jayadhar and GN.Cot - 25) and 10 parents were analysed for general and specific combining ability. Parental lines, IC-371437 and PA-785 found to be the good general combiner for number of bolls and seed cotton yield. The inter specific crosses between *G. herbaceum* × *G. arboreum* exhibited higher level of heterosis than intra-*herbaceum* crosses.

Evaluation of F₁ intra specific *G. herbaceum* crosses: Twenty eight F₁ crosses were derived using line × tester mating design consisting seven lines (IC-371437, IC-

371360, GVHV-655, IC-371136, IC-371177, IC-371362 and IC-371527) and four testers (Baluchistan - 1, Jayadhar, IC-371158 and IC-371336). Relative heterosis and heterobeltiosis was studied in 21 F₁'s and standard heterosis was estimated using three standard checks (DDhC - 11, Jayadhar and GN.Cot - 25). Intra-specific *herbaceum* crosses IC-371437 × Baluchistan - 1 and IC-371437 × Jayadhar showed better performance for boll number and seed cotton yield over the mid parent.

Improvement of *G. hirsutum* cotton

Population Improvement

GMS based simple recurrent selection at Nagpur:

In the year 2016-17, all sterile plants were tagged and harvested separately from each of the trait specific GMS based recurrent populations. In the *G. arboreum* GMS based recurrent population, 1768 sterile plants were evaluated as plant to row progenies while, in *G. hirsutum* 2129 GMS sterile plants were evaluated. Observations were recorded on 3 fertile plants in each progeny for plant height, monopodia, sympodia, boll number, boll weight and GOT. Composite lint samples of each progeny were analyzed for fibre quality traits. Based on

the trait values and superiority for specific economic trait, about 5-7% superior plant progenies will be identified for boll weight, seed cotton yield, GOT, fibre strength and fibre length for making trait specific groups. Seed obtained from sterile plants of the identified trait specific superior progenies shall be bulked and grown as composite population during 2018-19.

Evaluation of single plant selection: About 1850 superior single plant selections from random mating populations and reselected plants from the segregating progenies were evaluated in plant to row progeny plots at Nagpur. The progenies were monitored for segregation, if any and also evaluated for uniformity, economic and fibre quality traits. Based on the performance, uniformity and fibre quality of plant

progenies 16 progenies of *G. arboreum* and 32 of *G. hirsutum* were identified for evaluation in replicated trial. From the remaining progenies about 2055 superior single plants were reselected based on economic yield and manual evaluation for fibre quality traits.

Evaluation of advance cultures:

130 cultures of *G. arboreum* and 85 cultures of *G. hirsutum* were evaluated in 8 replicated trials (4 rows plots in 2 replications) during the crop season 2017-18 at Nagpur. In all, 5 trials of *G. arboreum* and 3 of *G. hirsutum* were conducted following spacing of 60x45 cm and 60x 60cm, respectively. The seed cotton yield among the *G. arboreum* cultures ranged from 722 to 1886 kg/ha while, in *G. hirsutum* it ranged from 907 to 2562 kg/ha. The range of trait values for boll weight, GOT and fibre quality traits is given in the table 3.2.3.

Table 3.2.3: Range of variability for economic and fibre quality traits

Particulars	Range in <i>G. arboreum</i> selections	Range in <i>G. hirsutum</i> selections
Ginning percent	29.6 - 44.1	29.8 - 37.5
Boll weight (g)	1.52 - 2.60	2.8 -4.5
Fibre length (mm)	24.7 -29.3	24.0 - 32.4
Uniformity Index (%)	79 - 85	80.3 - 84.1
Micronaire value	4.4 - 5.9	3.7 - 5.0
Fibre strength (g/tex)	24.0 - 31.8	26.6 - 33.8

Based on performance for seed cotton yield and fibre quality traits, 79 cultures of *G. arboreum* and 55 of *G. hirsutum* were retained for second year replicated trial. From the evaluated selections, four *G. hirsutum* and six *G. arboreum* cultures entered in AICRP National trials.

The cultures entered in AICRP trials were grown on large plots for seed multiplication which includes CNA 1028 of *G. arboreum*, CNH 11-11, CNH 1123, CNH 1125, and CNH 1126 of *G. hirsutum*.



Plants with high boll number and big boll size; compact plant type selection from random mating population of *G. hirsutum*

GMS based random mating population

At flowering, the individual plant in the population was monitored for sterility/fertility at anthesis repeatedly at an interval of a week and tagged all the 400 sterile plants at Sirsa. All the out-crossed bolls from the sterile plants in the population were bulk harvested and ginned to constitute the next cycle of GMS based random mating population. After the 6th cycle of random mating 80 fertile plants having high yield potential and tolerance against CLCuV were selected for evaluation in progeny row trial.

Genetic enhancement

Cultures *viz.*, CNH-2073 (1320 kg/ha, boll wt 4.6) followed by CNH 2053 (1250 kg/ha, boll wt 4.1 g) and CNH 2046 (1050 kg/ha) were found superior to check Suraj (1050 kg/ha, boll wt (4.4 g). CNH 2073 (fibre length 28.8 mm and fibre strength 31.8 g/tex) was identified as a superior fibre quality line having semi compact plant architecture. CNH 2039, a synchronized bearing and bursting, uniformity in boll size within the plants was identified which had a boll weight of 4.4 g and 926 kg yield/ha. Amongst jassid tolerant cultures with grade 1, culture CNH 2053 was superior to check Suraj. In advanced generation selections SPS 13-2-1, SPS 14-1 and SPS16-3, zero monopodial closed sympodial plants (extreme compact types) with single plant yield ranging from 43 g to 116 g and a boll weight ranging from 2.5 g to 3.8 g were recorded.

Breeding for earliness and fibre quality

Forty-five (45) advanced selections based on early maturity of 145-150 days and better fibre properties were evaluated for seed cotton yield. Promising individual plants (200) having earliness and compact plant architecture were selected from the F₂ segregating populations of three-way and double crosses. 220 F₃ single plant selections were advanced to F₄ for evaluation in progeny rows and 35 F₄ progenies were selected for fibre quality, earliness, plant type and seed cotton yield.

In order to obtain recombinant and fertile progenies of interspecific crosses between *G. hirsutum* and *G. barbadense*, intermating between progenies of three-way and multiple crosses was attempted. Promising interspecific F₃ single plant progenies were evaluated. Based on specific trait performance and presence of

combination of introgressed characteristics of *G. hirsutum* and *G. barbadense* such as sub-okra leaf shape, cleistogamous flowers, stay green plants, high fibre quality and yield potential, promising single plants were identified. The selected BC₁F₃ progenies of a cross, (Suraj × Suvin) × Suraj had better fibre properties than Suraj. About 75 single plants were identified in F₃ with fibre length of 29.0 mm to 34.7 mm and fibre strength of 30.0 g/tex to 34.4 g/tex.

Four entries each for seed cotton yield (CNH 09-70, CNH 09-76, CNH 09-73 and CNH 14-5) and fibre length (CNH 09-52, CNH 09-54, CNH 6 and CNH 10-6-1) were identified based on replicated station trials. Two entries CNH 09-73 and CNH 09-11 were identified based on seed cotton yield in institute common trial for sponsoring to AICCIP Br 02 (b) trial 2018-19. In addition, two entries CNH 09-45 and CNH 09-11 were sponsored for compact genotypes trial Br 06 (b). Entries CNH 09-70 and CNH 09-9 were promoted to preliminary varietal trial, Br 03(b) and coordinated varietal trial, Br 06(b), respectively in AICCIP trial 2018-19. Entries, CNH 09-4 and CNH 09-62 were retained for second year in coordinated varietal trial of compact genotypes Br 06(b).

Early maturing breeding lines identified

Two early maturing breeding lines namely, CNH 09-7 and CNH 09-9 with seed cotton yield of 1165 and 1192 kg/ha respectively were identified. The percentage of seed cotton in the first picking of the total seed cotton yield was 86% in CNH 09-7 and 82% in CNH 09-9. Both the entries recorded Bartlett's earliness index of 0.9 indicating early maturity of the identified genotypes.

Development of high yielding varieties with improved fibre quality

The high yielding good quality variety Central Cotton CCH 12-2 (Suchitra) has been recommended by Central Variety Identification Committee for release in Central Zone States of Gujarat, Maharashtra and Madhya Pradesh under irrigated conditions. The variety recorded a mean seed cotton yield of 1767 kg/ha as against 1644 kg/ha of the Zonal check. The variety has an yield potential of 2598 kg/ha. It has an upper half mean length of 28.0 mm, micronaire of 4.2 and tenacity of 29.0 g/tex (in HVI mode). The variety is tolerant to grey mildew and moderately tolerant to jassids. The proposal for notification has been submitted to Central Sub Committee for Release and Notification.



Central Cotton CCH 12-2 (Suchitra)

Central Cotton CCH 12-3 has been recommended by Central Variety Identification Committee for release in Central Zone States of Gujarat, Maharashtra, Orissa and Madhya Pradesh under rainfed conditions. It recorded a mean seed cotton yield of 1060 kg/ha as against 1053 kg/ha of the Zonal check variety. The variety recorded an upper half mean length of 27.0 mm, micronaire of 4.3 and tenacity of 28.7 g/tex (HVI mode).

Proposal for identification of high yielding good quality Central Cotton CCH 14-1 has been submitted to Central Variety Identification Committee for release in irrigated

condition in South Zone States. The variety possesses an yield potential of 3675 kg/ha. The variety combined excellent fibre quality viz., upper half mean length of 32.0 mm, micronaire of 3.7 and tenacity of 32.7 g/tex (in HVI mode) and 2.5% span length of 32.8 mm, micronaire of 3.6 and tenacity of 24.1 g/tex (in ICC Mode) matching the ICAR-CIRCOT norm for 50s count yarn. It is resistant to bacterial leaf blight, Grey Mildew and Tobacco Streak Virus and Immune to Root Rot. The variety is tolerant to jassids, white fly, thrips, aphids and stem weevil.



Central Cotton CCH14-1

Development of cotton genotypes with least short fiber content

In a station trial, 13 long staple cultures were evaluated along with Surabhi and Suraj as check varieties. Quality wise, YLS 21-4 was the best combining UHML of 34.1 mm and tenacity of 33.8 g/tex as compared to the best check variety Suraj (Table 3.2.4).

Table 3.2.4: Performance of select long staple cultures

Entries	Fibre length (mm)	Micronaire	Tenacity (g/tex)
YLS 19-2	33.0	2.9	30.3
YLS 21-2	34.0	3.4	31.0
YLS 21-3	34.1	3.4	33.4
YLS 21-4	34.1	3.9	33.8
YLS 25-1	32.7	3.7	32.9
Suraj	32.7	4.2	31.3
Surabhi	32.8	3.5	30.9

Evaluation of advance cultures of *G. hirsutum*: In this trial 20 *G. hirsutum* cultures were evaluated at Sirsa against the check varieties RS 2013, LH 2076 and F 1861 in RBD with three replications. The highest seed cotton yield was recorded in the advance culture CSH 1601 (2360.3 kg/ha) followed by CSH 2902 (2088 kg/ha) as

against the check variety LH 2076 (1694.5 kg/ha). Maximum ginning out turn of 38.2 per cent was recorded in the variety CSH 2924 as compare to local check varieties RS 2013 (37.1%) and LH 2076 (36.7%). The culture CSH 2924 also recorded the highest lint index of 3.9 and GOT of 38.2% (Table 3.2.5).

Table 3.2.5: Performance of promising advance cultures of *G. hirsutum*

Variety	Plant height (cm)	Number of monopods	Number of sympods	Number of bolls / plant	Boll weight(g)	Ginning outturn(%)	Seed Cotton Yield (kg/ha)	CLCuV (PDI)
CSH 2837	102.8	3.4	6.0	15.9	2.2	33.5	1967	27.1
CSH 2902	95.0	2.7	10.1	19.9	2.2	32.4	2088	24.1
CSH 2924	95.2	2.1	7.6	16.1	2.5	38.2	1725	22.4
CSH 1601	104.8	1.2	9.0	18.8	2.2	30.9	2360	26.4
RS 2013	111.2	3.4	7.6	16.8	2.5	37.1	1150	25.2
LH 2076	98.2	2.5	7.1	16.4	2.5	36.7	1695	21.9
F 1861	86.7	2.7	5.4	11.4	2.3	33.3	999	22.7
CD @ 5%	16.10	0.62	1.73	3.63	20.33	0.52	292.30	
CV %	10.23	14.25	14.53	13.81	13.57	0.93	13.54	

In another trial, 24 *G. hirsutum* cultures were evaluated against the check varieties RS 2013, LH 2076 and F1861 in RBD with three replications. The highest seed cotton yield was recorded in the advance culture CSH 1626 (1513 kg/ha) followed by CSH 1622 (1392 kg/ha) as against the check variety LH 2076 (1242 kg/ha). Maximum ginning out turn of 36.4 per cent was recorded in the variety CSH 1620 as compare to local check varieties RS 2013 (32.8%) and LH 2076(33.5%).

Breeding for high GOT and high yield

In large plot size (97.2 sq. meter area), 15 promising high GOT selections in F_5 generation were evaluated at Sirsa. Three F_5 selections P-10 (RS-875 × SA-524), P-4 (RS-875 × SA-524) and P-68 (SA-977 × SA-112) gave lint yield of 1127.2, 1016.1 and 1062.8 kg/ha and GOT of 38.8, 38.1 and 41.8% respectively against the 3 checks CSH-3129 (882.4 kg/ha, 33.4%), F-1861 (708 kg/ha, 34.6%) and Bt 773 (751.8 kg/ha, 34.2%).

Selection of single plant progenies from segregating populations:

Sixteen crosses were attempted among CLCuV tolerant germplasm lines of *G. hirsutum* in a Line × Tester fashion at Sirsa. Out of 91 single plants progenies 13 progenies having high yield potential and tolerance against CLCuV were selected in F_6 generation. The progeny CSH 1714 recorded the highest yield of 3145 kg/ha followed by CSH 1717 (3052 kg/ha) and CSH 1715 (2820 kg/ha) as compared to check variety F 1861 (1424 kg/ha). The culture CSH 1715 recorded the highest ginning out turn of 37.4 % followed by CSH 1718 (36.8 %). The mean fiber length and bundle strength ranged from 25.0 to 28.2mm and 24.5 to 29.3g/tex respectively.

Evaluation of compact lines

Fifteen compact accessions from *G. hirsutum* germplasm were evaluated at 67.5 × 10 cm. against Bt and Non-Bt promising checks in 3 replications at Sirsa. Accessions for seed cotton yield were EC140818 (36.7 q/ha), EC745226 (34.7 q/ha), EC 700495 (33.7 q/ha) against check F1861 (29.7 q/ha) and F 2383 (28.6 q/ha). The yield potential of these accessions was lower than Bt check hybrid Raghav (37.3 q/ha) but the differences were non-significant. Similarly, among 15 accession of *G. arboreum*, accessions AC-3562 (30.2 q/ha), 412090 (30.4 q/ha) were superior in yield over check HD432 (24.2 q/ha), CICR 1 (24.7 q/ha) and CICR 3 (25.2 q/ha).

Improvement of ELS cotton

Out of 15 single plant progenies isolated from BC₂F₄ generation, 5 promising selections have been identified for further evaluation. These promising selections possessed high yield potential and high ginning percentage and also exhibited high quality parameters which can rightly fit in to the ELS category. From the backcrossed F₂ segregating population, 167 single plants superior in terms of seed cotton yield, number of bolls and sucking pest tolerance, have been identified for further evaluation.

Mass multiplication of seeds of advance cultures for the Station as well as AICRP trials was done and a total of

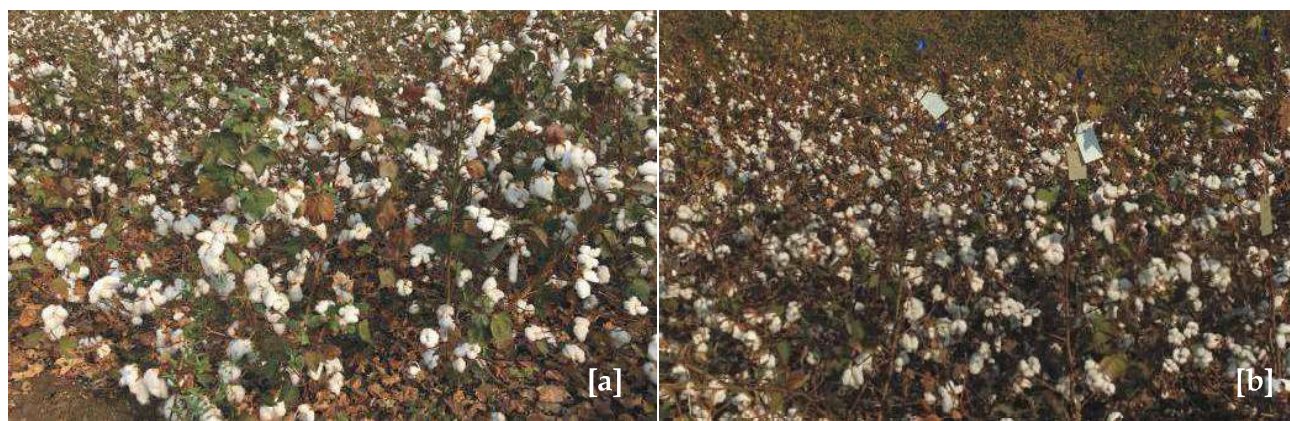
eight advance cultures (CCB-26, CCB-28, CCB-29, CCB-51, CCB-51-2, CCB-64, CCB-129, CCB-143B) were entered in three breeding trials (Br.12a, Br13a and Br.14a) during AICRP-2018-19.

3.3. Breeding for climate resilience and biotic stress tolerance

Bt varieties carrying *cry1Ac* (Mon 531) gene were approved for commercial cultivation under rainfed conditions of Maharashtra (Table 3.3.1). These varieties have recorded more than 15 percent increase in seed cotton yield over the BG II check hybrid. The fibre quality and seed cotton yield of these Bt varieties are presented in as Table 3.3.1.

Table 3.3.1 Yield and fibre quality parameters of ICAR-CICR Bt varieties approved for commercial cultivation

S. No.	Varieties	SCY (kg/ha)	FL (mm)	FS (g/tex)	Mic (g/in)
1.	ICAR-CICR Bt 14 (CPT2)	3066	28.1	25.4	4.8
2.	ICAR-CICR Bt 9 (SRI1)	3109	25.7	25.5	4.4
3.	ICAR-CICR GJHV 374 Bt	2577	28.2	26.8	4.4
4.	ICAR-CICR PKV 081 Bt	2743	28.5	27.9	3.9
5.	ICAR-CICR Rajat Bt	2660	26.8	26.1	4.5
6.	ICAR-CICR Suraj Bt	2407	29.1	26.0	4.3



Bt varieties developed by ICAR-CICR, Nagpur: a) ICAR-CICR Suraj Bt; b) ICAR-CICR PKV081 Bt

Sixty-one genotypes from different agro-ecological regions have been converted into Bt background at ICAR-CICR, Nagpur and were also tested for presence of Bt gene and maintained through selfing. About 144 random Bt positive plants were tested for homozygosity and 114 plants were identified as homozygous for *cry1Ac*.

Bt variety CICR-Bt6 was developed from Sirsa and was

approved for commercial cultivation in Haryana state. It recorded the seed potential of 3046 kg/ha under HDPS and was found to be significantly better than local check hybrid RCH 773 BGII in Haryana. This Bt variety also recorded UHML of 26.1 mm, Micronaire of 4.7 µg/in, bundle strength of 26.6g/tex and uniformity index 81.5% which is at par with the local check BG II hybrid. The variety is moderately resistant to CLCuV.



Field view of ICAR-CICR Bt6 variety (RS 2013 Bt)

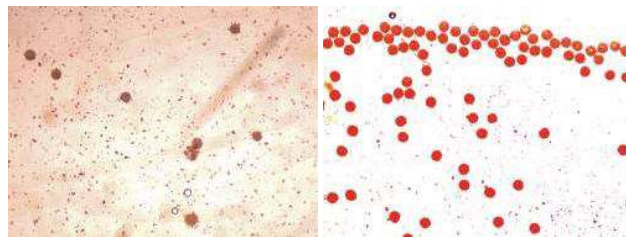
Introgression of genes for whitefly and CLCuD resistance in upland cotton

Following the standardized protocol for embryo rescue/immature embryo culture, three interspecific F_1 plants (*G. hirsutum* × *G. arboreum*) were established in the crossing block at Nagpur. An interspecific F_1 plants were examined for pollen fertility and found completely sterile. Few of the sterile flowers of the F_1 plants were crossed using fertile pollens from elite *G. hirsutum* parental lines. However, we could not succeed in getting fertilized backcrossed embryo/boll setting. Alternately, growing axial buds of the F_1 plants were subjected to 0.5% colchicine treatment using cotton swabs. Treatment of axial buds repeated 3-4 times on every alternate days.

At Sirsa, interspecific crossed bolls were harvested from a cross of *G. hirsutum* CSH 3129 × *G. arboreum* and also from crosses between *G. hirsutum* (GMS lines- 14, 17, 20, 22 & 32) × *G. arboreum*. Crosses have also been attempted using pollen from colchicines treated plants. The F_1 seeds obtained from the above crosses will be raised during 2018-19.



Interspecific cross between *G. hirsutum* and *G. arboreum*



Pollen sterility in interspecific F_1 (*G. hirsutum* × *G. arboreum*) hybrid and normal stained fertile pollen in control variety.

Breeding cotton for drought tolerance

One hundred and sixty six (166) cotton germplasm lines were evaluated in rainfed and irrigated regime in the field at Nagpur. Seventeen promising drought tolerant lines were identified based on physiological parameters viz., relative water content and mid-day leaf water potential. Lines DTS-405, DTS-413, Nagpur-9, N-2924, CNH-09-4 and 2853 were identified as promising for yield, drought tolerance and compact plant architecture.

Among the F_5 populations of twelve crosses, the cross 28I × Suraj recorded highest seed cotton yield of 1201 kg/ha with 52 percent increase over the check LRA 5166 which recorded seed cotton yield of 789 kg/ha. The cross combinations 28I × P3, 28I × HSD, NH 615 × Rex and LRA 5166 × N 170 recorded more than 15 percent increase over the check LRA 5166. The crosses, 28I × HSD, PKV 081 × CCH 510-4 and PKV081 × P3 showed good fibre quality.

F_3 generations of 33 single, three-way, double, six and eight parental crosses were evaluated. The genotypes showed significant difference between the treatments for seed cotton yield which ranged from 739.94-1651.16 kg/ha. Three-way cross [(28I × Moco) × 28I] showed best performance and recorded good fibre quality. Twenty nine crosses were at par to the check NH 615 which recorded seed cotton yield of 942.24 kg/ha. Multi-parental crosses, NH 615 × Suraj, PH 93 × Suraj, (28I × HSD) × (28I × HSD 11), (PKV 081 × Suraj) × (NH 615 × Rex) × (PH 93 × Rajat) and (CCH510-4 × Moco) × (NH 615 × Rex) × (PH 93 × Rajat) and (CCH 510-4 × Moco) × (NH 615 × Rex) × (PH 93 × Rajat) where some of the crosses which recorded fibre strength of 28.5-32.1 g/tex and fibre length of 28.4-32.1mm. Thirteen advance cultures along with check LRA 5166 were evaluated and the seed cotton yield ranged from 876-1539 kg/ha. with non-significant difference between the treatments. Ten cultures were at par to the check LRA 5166 which

recorded seed cotton yield of 956.73 kg/ha. Eight cultures showed more than 15 percent increase over the check variety. High GOT of 42.3 percent was recorded in case of NHP2 followed by DTS 108, DTS 155 and DTS 39. Culture DTS 44 performed well recording highest seed cotton yield and fibre quality.

Attempt was made to improve the fibre quality of identified drought tolerant cultures. Culture 28I has been selected for improving the fibre quality using Suraj, Suvin, P3 and HSD as donors. (28I × HSD) × 28I recorded high SCY of 1647 ka/ha and GOT of 39.6 percent. Promising single plants were selected from the cross (28I × Suvin) × 28I.

To develop compact plant type with early maturity, that can escape drought and good fibre quality, intermating was carried out between 15 inbred lines within cross which were stable for zero monopodia. Generation was advanced for development of MAGIC RILs for eight and ten parental cross. In eight parental crosses variation in selected F₂ plants (104), seed cotton yield ranged 17.4 to 116.2 g, boll wt from 1.7 g to 4.4 g and GOT from 27.3 to 44.9%.



Promising single plant selections

Introgression of indigenous transgenic events for effective bollworm management

In order to introgress the indigenous transgenic events *viz.*, Tg2E13 (*cry1Ac* gene) and CH12 event (*cry2A_{x1}* gene) into elite cotton genotypes, the BC₁F₁ population of three crosses *viz.*, Suraj × Tg2E13, NH615 × Tg2E13

Breeding for early maturity, compact plant type and jassid tolerance in cotton

Segregating populations *viz.*, F₂, F₃, F₅ and F₆ including 151 selected single plant derived progenies were evaluated for jassid tolerance, earliness, compact plant type, boll weight, yield and quality characters under normal spacing (60×60 cm) at Nagpur. Most promising plants were selected and advanced. Among the promising entries selected for earliness, jassid tolerance and compact plant type, 31 Bt (*cry1Ac* gene; Mon531 event) entries and 15 non-Bt entries were evaluated in replicated trial under high density (spacing 60×10 cm) along with standard checks to assess their potential. More than 1000 plants belonging to 72 promising plant to progenies were tested for *cry* toxin expression through ELISA and zygosity test through PCR. Of the 72 progenies, 13, 48 and 11 progenies were identified as homozygous, hemizygous and azygous for *cry1Ac* gene (Mon 531 event), respectively. The promising homozygous progenies having desirable characters like early maturity, jassid tolerance, compact plant architecture with good yield and fibre quality characters were identified for seed multiplication.



Evaluation of promising entries under normal (60x60cm) spacing and high density (60x10cm)

and CISH3178 × Tg2E13 were raised in seedling trays along with the recipient or recurrent parents under contained facility at Nagpur. ELISA was carried out at 30 days after sowing (DAS) to identify the event positive plants and event negative plants were discarded. Event positive plants were transplanted to larger pots and

high toxin expressing plants were identified among the BC₁F₁ population of each of the three crosses through ELISA at 60DAS. Backcrossing of event positive, high toxin expressing BC₁F₁ plants of Tg2E13 event and backcrossing of F₁ plants of CH12 event to their respective recipient cotton varieties was successfully

attempted in contained facility at Nagpur. Embryo culture protocol was standardized and successfully explored for accelerated generation advancement. Accordingly, excised embryos excised from old crossed boll were cultured and established for further backcrossing in contained facility.



Introgression of Tg2E13 (*cry1Ac* gene) and CH12 event (*cry2Ax1* gene) into elite cotton genotypes in contained facility. Backcross population of three crosses in seedling trays (a); Segregating F₁ (b) and backcross populations (c).



Backcrossing of event confirmed high toxin expressing plants recipient genotypes

Embryo culture in cotton: Culturing of embryo on culture media (a); Hardening of seedlings on soil rite (b); transplanted hardened seedlings in bigger pots (c).

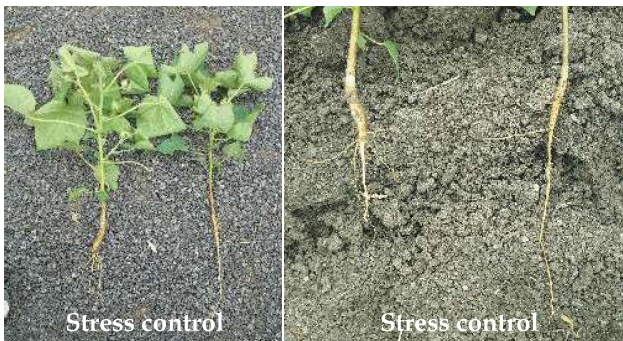
Water logging tolerance in cotton

Five accessions each of the identified water logging tolerant (IC 359979, IC 359245, IC 357235, and INGR 08092 & INGR 08093) and susceptible (IC 356708, IC 357607, IC 357558, IC 359242, IC 359915) genotypes were used in the crossing program to develop segregating populations for water logging tolerance at Nagpur and the crossed seed were harvested.



a) IC 359915 (Water logging susceptible);
b) INGR 08092 (Water logging tolerant)

Physiological studies were carried out on 80 cotton germplasm accessions which were exposed to continuous 20 days water logging in the field starting from 45 days after sowing. The phenotypic score was used based on leaf coloration in response to water logging like yellow and red leaves. Amongst the tested accessions, waterlogged tolerant lines *viz.*, IC 357235, IC 359979 and INGR 08092 had better root length and seed cotton yield (g/plant) compared to susceptible one (IC 357608 and IC 357607). The maximum root length of 40 cm was recorded in tolerant genotype IC 357235 under waterlogged condition. Difference in root growth was clearly observed under control and waterlogged plants



Root growth pattern in water logging stress imposed and control plants

Fifteen lines shortlisted from 210 lines based on replicated field trial conducted during the season 2016-17 were evaluated under pot culture experiment at Regional Station, Coimbatore. The lines were screened for water logging tolerance at 5 days after water logging under pot conditions. IC563997, IC357101, IC359925 and LRA 5166 (check) were identified as tolerant to water logging in terms of lenticel formation and absence of Fe deficiency.

3.4 Gene discovery, genomics and trait improvement

Gene expression analysis of *G. arboreum* LIM (*GaLIM*) gene family members

Protein containing LIM domains are documented for their role in regulation of gene expression at transcription level and cytoskeleton organization. Genome wide *in-silico* analysis identified 16 *GaLIM* members similar with animal cysteine rich proteins and 4 belong to plant specific LIM family. Gene expression analysis of plant specific *GaLIM* family members in response to pathogen *Fusarium oxysporum*, NaCl (200 mM), abscisic acid (100 μ M) and jasmonic acid

(100 μ M) treatments were quantified at different time intervals through q-Reverse transcription PCR (qRT-PCR). The plant specific *GaLIM* family members *GaDA1-1*, *GaDA1-2*, *GaDA1-3* and *GaDAR1* showed significant differential response to the above treatments. Significant up regulation of *GaDA1-3* and down regulation of rest of the three genes in response to abscisic acid treatment was observed.

Zinc finger proteins for cotton fibre initiation

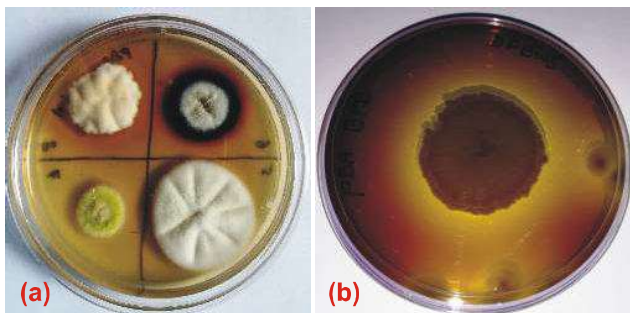
Quantification of gene expression of two putatively uncharacterized Zinc finger protein (ZFP) encoding genes *viz.*, *Ghzfp5* and *Ghzfp8* was carried out using MCU5 and its isogenic fibreless mutant, to aid the future research for the elucidation of their possible role(s) during cotton fiber initiation. Significant down regulation in the expression of *Ghzfp5* and *Ghzfp8* genes during 0 and 1 DPA (Days Post Anthesis) of MCU5 mutant compared to its wild type suggested the possible involvement of GHZFP5 and GHZFP8 transcription factors in the gene regulatory pathway of cotton fiber initiation.

Gossypol detoxification efficacy of bacterial clones expressing *CYP6AE14* Protein

Gossypol detoxification gene coding for CYP6AE14 protein was cloned in to expression vector *pET28c* and mobilised in to Rosetta-gami 2 (DE3) host strains. The overnight grown bacterium was inoculated (5%) on known amount of deoiled cotton seed cake and the gossypol content was estimated after 48 hrs of incubation by American Oil Chemists' Society (AOCS) method. Host cells with CYP6AE14 showed 73 and 56 per cent reduction in free and total gossypol content respectively over the control.

Isolation and characterization of microbes for gossypol detoxification

The gossypol detoxifying microorganisms were isolated from cotton rhizosphere soil and quantified for gossypol detoxification. Ten fungal and seven bacterial isolates were shortlisted and tested for gossypol detoxification. Total gossypol reduction efficacy of the microbes varied from 8.19 to 64.46 per cent over control, among them seven microbial isolates showed gossypol reduction more than 40 per cent. Earlier studies have reported increased expression of *laccase* protein in microbes growing on gossypol containing media; these isolates were further screened for *laccase* activity by guaicol assay and bromophenol blue assay. Screening resulted in identification of fifteen microbial isolates positive for *laccase* activity.

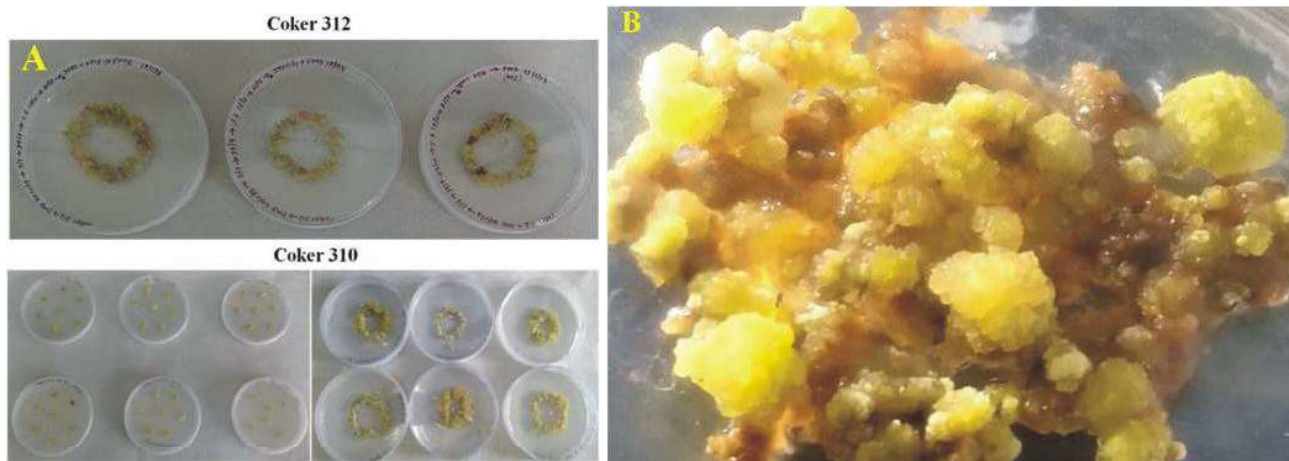


Screening of fungal isolates for laccase activity :

A. Brown discoloration in Guaicol assay B. Yellow discoloration in bromophenol blue assay observed due to laccase activity

An efficient regeneration system for transformation

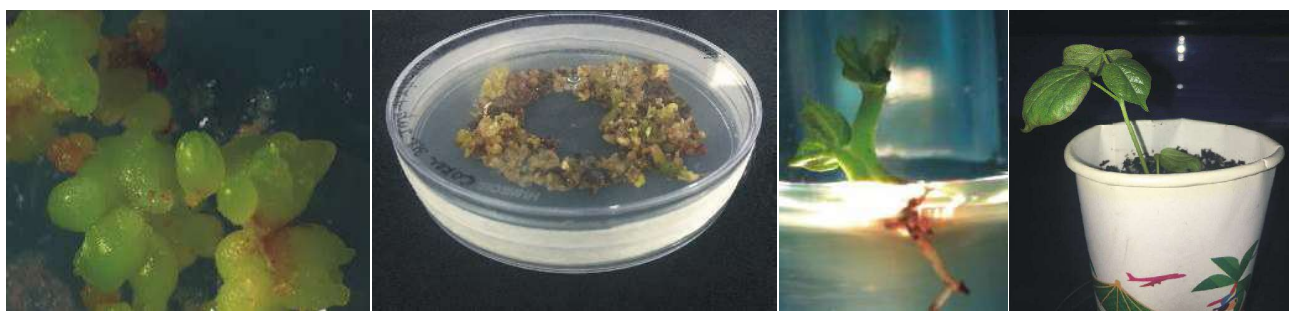
Induction of callus cultures for somatic embryogenesis : Callus cultures were induced on Murashige and Skoog (MS) medium supplemented with various combinations of auxins. Among them, media supplemented with 2, 4-D (0.1mg/L) and kinetin (0.5 mg/L) induced friable callus from *Gossypium hirsutum* Cv. Coker 310 and Coker 312. These callus cultures are being maintained through sub culturing for the generation of somatic embryos. The fresh calli were sub-cultured on MS medium with double the concentration of KNO_3 , without NH_4NO_3 , and growth hormone to obtain embryogenic callus.



A. Callus cultures induced from coker genotypes of *Gossypium hirsutum*; B. Friable callus

Somatic embryogenesis of cotton : A medium recipe for healthy root and shoot growth in germinated somatic embryos has been standardized. Among different concentrations of indole-3-butyric acid IBA treatments,

woody planting medium with 1mg/L IBA has showed significant improvement in root growth of germinated somatic embryos in Coker 310 genotypes followed by 0.5mg/L of IBA.



A. Somatic embryos initiation B. Germination and maturation C. Shooting and rooting D. Hardening

Standardization of In planta transformation method for transient gene expression in cotton

A protocol for genotype independent sonication and vacuum infiltration assisted *Agrobacterium*

tumefaciens mediated in planta method for gene delivery into cotton was standardized. Mature excised embryos with intact apex, when subjected to a combined treatment of sonication (for 3 min) followed by vacuum

infiltration (10 min at 500 mmHg) of *Agrobacterium* suspension (OD at 600nm; 0.6-0.8), resulted in successful, deeper and uniform penetration of bacterial cells into explant. The infected explants when further co-cultivated on 1.5 to 2ml co-cultivation medium at lower

temperature (23°C) in dark resulted in desired gene delivery and transient gene expression in to the plant cells. The present standardized method can be used as a tool to study transient gene expression in cotton tissues for functional gene characterization.

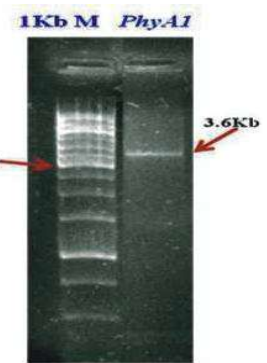


Steps in in-planta gene delivery in cotton A) excised mature embryo with apex B) co-cultivation on filter paper with minimum co-cultivation medium C) Transient GUS gene expression assay D) Plantlet regeneration

Isolation of *PhyA1* gene from cotton fiber for CRISPR/Cas9 mediated mutagenesis

PCR variation was employed for the amplification of 3.6 Kb full length *PhyA1* gene from cotton genome. It will be utilized for sequence confirmation through cloning and sequencing before proceeding with CRISPR/Cas9 mediated mutagenesis.

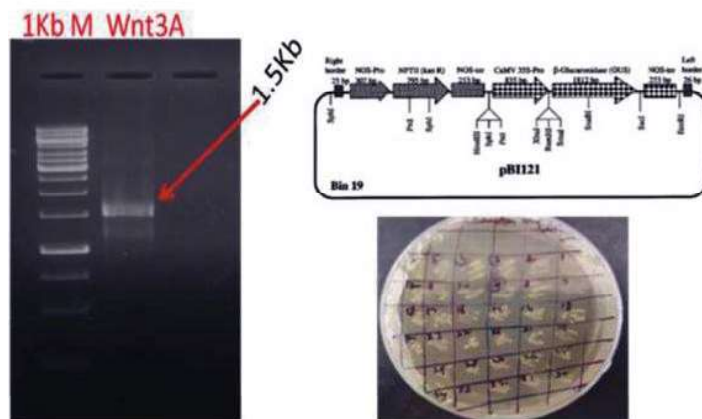
product was cloned into plant binary vector *pBI121* as per the principle of directional cloning and was confirmed through colony PCR, plasmid PCR and restriction release. This construct will be utilized for the functional validation through plant genetic transformation studies.



PCR amplification of 3.6 Kb *PhyA1* gene

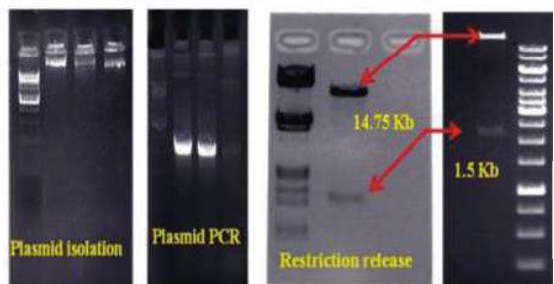
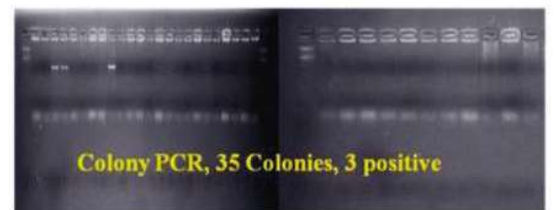
Isolation and molecular cloning of *Wnt*-like putative candidate gene expressed during cotton somatic embryogenesis

Full length *Wnt*-like gene was amplified from cDNA of *Gossypium hirsutum* fiber. The purified PCR gene



PCR Amplification of *Wnt*-like gene

Colonies after transformation and plating



Amplification, directional cloning and confirmation of cotton *Wnt*-like gene

Unapproved Herbicide tolerant GM cotton testing at ICAR-CICR, Nagpur

Commercial cultivation of herbicide tolerant (HT) Bt-cotton seeds shocked everyone, since these transgenic cotton seeds were not permitted for sale or commercial cultivation in India by Genetic Engineering and Appraisal Committee (GEAC). Earlier ICAR-CICR, Nagpur tested 9 Bt hybrid samples (Kharif-2017; Feb 2017) submitted by Shri. Amol Pusadkar, Nagpur and 6 Bt hybrid were reported to be positive for HT event (RRF MON88913). During Kharif 2017-18, scientists of ICAR-CICR, Nagpur conducted field surveys and collected 379 leaf samples from different cotton fields suspected to be growing HT cotton from Wardha, Chandrapur, Yavatmal and Nagpur Districts of Vidarbha, Maharashtra. Out of 379 leaf samples tested 171 samples were positive for roundup ready Flex (RRF) harbouring CP4-EPSPS gene confirming illegal cultivation of HT cotton. Different print and electronic media have also reported about illegal cultivation of HT cotton in Maharashtra, Gujarat, Karnataka, Telangana and Andhra Pradesh. The state government seed inspectors have also sent 531 seed samples for testing presence of HT gene (*cp4-epsps*) in the seed samples. ICAR-CICR, Nagpur tested all the samples by qualitative detection method and it was found that 8 samples were positive for the presence of CP4-EPSPS protein. The gene and event was confirmed by PCR amplification using RRF-event (MON88913) specific primers.

3.5 Seed production and quality improvement

Storability of cotton seed

Different packaging materials were evaluated for better storability at quarterly intervals. Genotypic differences were observed for Suraj (*G. hirsutum*) and Phule Dhanwantari (*G. arboreum*) when stored for 12 months. Irrespective of storage containers, packaging materials and storage conditions, Suraj exhibited relatively higher seed germination (69%) as compared to Phule Dhanwantry (58%) at the end of 12 months from the initial seed germination of 85% and 81%, respectively. Vacuum packing followed by orange paper envelop packing showed better storage results and maintained higher seed germination as compared to brown paper packet, polylined aluminium packet and polythene heat sealed packets. Storage conditions had significant influence on seed germination. Seed stored under cold storage conditions maintained higher seed germination till 12 months as compared to ambient storage

conditions. Seed stored under vacuum packing conditions exhibited higher seed germination as compared to non-vacuum packing containers. Seeds stored in airtight acrylic box with inbuilt hygrometer along with zeolite beads maintained higher viability and germination after six months of storage both in ambient as well as refrigerated conditions. Among the different seed treatments with organic materials after six months of storage, seed treated with Tulsi leaf powder had recorded highest seed germination (87%) followed by Moringa leaf powder (80%).

Effect of pollination systems on boll and seed setting

With augmented pollinators, in GMS crop of DS 5, the boll setting of 14.25% and seed setting of 56.0% was obtained as against 10.64% and 47.3% without augmented pollinator. Similar results were observed in GMS 16 line emphasizing the contribution of pollinators in boll and seed setting in sterile population. In its fertile plants of GMS lines, the boll and seed setting were 63.6% and 93.6% in DS 5 and 64.8% and 78.8% in GMS 16, respectively. Delayed picking of bolls after opening was observed to affect the boll and lint characters in cotton. If picking of opened bolls is delayed by 25 days after its opening, the reduction of boll weight up to 0.2 gm, seed index up to 0.4 gm, lint index up to 1.2gm and micronaire up to 0.4 was observed in *G. hirsutum* cultivars. While, in *G. arboreum* genotypes, reduction of boll weight up to 0.5 gm, seed index up to 0.2gm, fibre length up to 0.5 mm and increase in micronaire up to 0.1 was observed. The other traits remain unaffected.

Enhancement of quality of primed seeds through pulsed magnetic seed treatments

A laboratory experiment was conducted to assess the effectiveness of seed priming with chemicals and botanicals followed by treatment with pulsed electromagnetic field using acid delinted seeds of cotton variety Suraj. The priming of seeds with KH_2PO_4 @ 450 ppm followed by pulsed electromagnetic treatment has recorded 93% germination which was higher by 18% over untreated seeds (75%), however, priming with KH_2PO_4 @ 450 ppm alone (87%) enhanced germination by 12%. Seed priming with CaCl_2 @ 2%, KCl @ 1.0%, MnSO_4 @ 0.1%, Prosopis leaf extract @1.5% with pulsed electromagnetic treatment has also significantly increased the germination over control. The seedling length, vigour and dry matter of seedling have shown significant increment corresponding to the above treatments. For assessing the effectiveness of these seed treatments towards seed and seed cotton yield, treated

seeds of Suraj variety were sown in field in two replications with RBD. The plant height was recorded at 90 DAS, 120 DAS and 150 DAS. At 90 DAS, among the treatments, seeds primed with CaCl₂ @ 2% (92cm), Prosopis leaf extract @ 1.5% (89cm), KH₂PO₄ @ 450 ppm (89cm) recorded significantly higher growth when compared to control. The boll number, dry matter of plant, boll weight, seed index and ginning % were also positively influenced by seed priming with chemicals

and botanicals followed by pulsed electromagnetic field treatment showed to enhance of seed germination, seedling vigour and plant establishment.

Seed Production

Seed belonging to different stage of certification was produced as under at ICAR-CICR, Nagpur, Coimbatore and Sirsa during 2017-18 (Table 3.5.1).

Table: 3.5.1: Seed Production during 2017-18

Crop	Variety	Stage	Quantity produced (q)
Cotton	Suvin, Surabhi, LRA-5166, MCU 5VT, CICR-2 Female, CICR-2 male, CISA 614, CISA-310, CSH 3075, CSH 3129	BS	3.36
	Suraj, LRA-5166, LRK-516, PKV 081, NH-615, , NH 452, CNA 1003 (Roja), AKA 7	TFL	0.98
	Bt Varieties (3)	NS	1.73
	Stock Seed (48 varieties)	TFL	1.32
	Suraj*	TFL	4.38
Wheat	WH 1142	CS	265
Red gram	BSMR-736	CS	4.70
Gram	Vijay	CS	0.50
Red gram	BSMR-736	TFL	5.14
Grand Total (Cotton & other crops)			286.13

* Produced through Farmers Participatory Approach in mediation with Suyash NGO, Wardha; BS – Breeders Seed; TFL – Truthful Labeled Seeds; NS – Nucleus Seed; CS – Certified Seed

PVP legislation and DUS testing

Five separate trials were conducted to undertake DUS testing of 4 entries (first year trial), 4 entries (Second Year trial), 2 entries (Varieties under Common Knowledge trial), 2 entries (Essentially Derived Variety and Initial variety trial) and 12 entries (Reference trial). Twenty four *desi* cotton varieties were sown for maintenance and seed multiplication. The monitoring of DUS trial conducted at ICAR-CICR, Nagpur was held on 26.10.2017 under the Chairmanship of Dr. Phundan Singh, Former-Director, ICAR-CICR, Nagpur.

During the year 2017-18, the data base on extant cotton varieties has been updated. Seed multiplication, characterization and maintenance of 142 extant cotton varieties (118 of *G. hirsutum*, 15 of *G. arboreum*, 3 of *G. herbaceum* and 6 of *G. barbadense*) was undertaken.

At ICAR-CICR, Regional station, Coimbatore, field trials for DUS testing of new cotton genotypes, varieties

of common knowledge, and essentially derived variety was conducted. There were 5 new candidate varieties in the second year testing trial. In the first year trial, 2 new candidate varieties were grown along with 15 reference varieties. The trial was conducted as per test guidelines for tetraploid cotton. Morphological characters were recorded from seedling stage to harvested fiber.

Promising entries of ICAR-CICR under evaluation in different trials

State Multi Varietal Trial (SMVT) at CICR, Nagpur

A State Multi-location Varietal Trial (SMVT) of *G. arboreum* (21 entries + 3 control varieties) and *G. hirsutum* (16 entries + 5 control varieties) with three replications following recommended package of practices was conducted at CICR, Nagpur during 2017-18. At the terminal stage of the crop, 7-12% infestation of pink

bollworm was observed on *G. hirsutum* as well as *desi* cotton. In *G. arboreum*, seed cotton yield ranged from 1364 to 2176 kg/ha. The maximum seed cotton yield of 2176 kg/ha was obtained from JLA 1321 followed by CNA2016 (2147 Kg/ha). In *G. hirsutum*, the seed cotton

yield ranged from 847 to 1917 kg/ha. Rajat, a control variety recorded highest seed cotton yield of 1917 kg/ha while the genotypes under evaluation had seed cotton yield less than the control. The range of variation for fibre quality traits are presented in Table 3.5.2.

Table 3.5.2: Fibre quality parameters of the entries tested in SMVT at Nagpur

Particulars	Range in <i>G. arboreum</i>	Range in <i>G. hirsutum</i>
Ginning percent	31.4 – 38.1	27.4 – 38.0
Boll weight (g)	2.3 – 3.0	2.7 – 4.9
Fibre length (mm)	24.8 – 30.8	26.4 – 30.2
Uniformity Index (%)	81.7 – 83.5	81.7 – 83.4
Micronaire value	4.7 – 5.6	3.9 – 4.8
Fibre strength (g/tex)	25.3 – 30.2	27.2 – 30.8

Testing of ICAR-CICR entries under AICRP on Cotton

Seven AICRP trials viz., Br 02 (b), Br 22 (b), Br 22 (b) LL, Br 24 (b), Br 24 (b) LL, Br 03 (b) and Br 06 (b) and two

Institute common trials of *G. hirsutum* and *G. arboreum* were conducted at ICAR-CICR, Nagpur.

Trial name	Entries sponsored for testing under AICRP on Cotton in 2017-18
Br 02(a)	CSH 3419, CSH 1604
Br 02 (b)	CNH 1126, CNH 25-09, CNH 11-11, CNH 12-4-2, CNH 2050, CNH 09-70
Br 06(a)	CSH 3824, CSH 1613
Br 06 (b)	CNH 1127, CNH 1128, CNH 136, CNH 09-9, CNH 09-98, CNH 2048
Br 12 (a)	CCB 64, CCB 129, CCB 143, CCB 102
Br 15 (a)	CCHB 32, CCHB 14
Br 22 (a/b)	CNA 1033, CNA 1034, CNA 2016, CNA 2031, CISA 7, CISA 33-5
Br 22 (a/b) LL	CNA 1058, CNA 1067, CNA1037, CISA 33-7, CISA 33-8
Br 25 (a/b)	CISAA 17-1, CISAA 17-2

Zone	Trial name	Entries Promoted / Retained*
NORTH	Br 25 (a/b)	CISAA 17-1, CISAA 17 -2
	Br 06(a)	CSH 3129-2, CSH 5640
	Br 24(a)	CISA 33-3
	Br 24(a)	CISA 6-2*
CENTRAL	Br 03 (b)	CNH 11-11, CNH09-70
	Br 06 (b)	CNH09-9, CNH09-4*
	Br 13 (a) PVT <i>G. barbadense</i>	CCB 64, CCB 129, CCB 143-b
	Br-24 b CVT – <i>G. arboreum</i>	CNA 2031, CNA 1054, CNA1031*, CNA1032*
	Br-24 b CVT -LL- <i>G. arboreum</i>	CNA1037
	Br25 (b)	CISAA 17-2
	Br 03 (a)	CCH16-1
	Br 06 (a)	CSH 1613, CSH 3129-2*

Zone	Trial name	Entries Promoted / Retained*
SOUTH	Br 13 (a)	CCB 143 b, CCB 64, CCB 129, CCB29*, CCB51*
	Br-03 (b)	CNH09-70
	Br06 (b)	CNH 1128, CNH09-62
	Br-24 (b): CVT -G. <i>arboreum</i>	CNA 1054, CNA1031*
	Br-24 CVT -LL- G. <i>arboreum</i>	CNA1037
	Coloured Cotton Trial: G. <i>hirsutum</i>	16315 LB, 16301 DB, 16337 LB
	Coloured Cotton Trial: G. <i>arboreum</i>	CNA407 SLP, 16378 LB-1, CNA405, CNA407 and 16377 LB-A

Note: entries marked with asterisk (*) denotes retained entries

Entries Proposed for Agronomy Trial

Central zone: CCH15-1, G. *hirsutum*, Variety (Irrigated), CSA 1028, G. *arboreum* Variety, (Rainfed); **South zone:** CCH15-1 G. *hirsutum*, Variety (Irrigated)

In multilocation evaluation of pre-release Bt varieties with deregulated event Mon 531, six, three and two entries were promoted in North, Central and South zones, respectively (Table 3.2.7).

Table 3.5.3: Bt entries from ICAR-CICR under testing in AICCIP trials

Year (2017-18)	ZONE	Name of the entries
11 entries	North	CICR 242 BT, CICR 562 BT, CICR76 BT, CICR 98 BT, CICR 38BT, CICR 861 BT,
	Central (Rainfed & Irrigated)	CICR 81 BT, CICR16 BT, CICR 2017 BT
	South (Rainfed & Irrigated)	CICR 902 BT, CICR23 BT

3.6 : Enhancing resource use efficiency through climate smart agro-techniques

Nagpur

Allelopathy an alternative weed management strategy in cotton

Timely weeding is a major issue in the sticky black cotton soils on which cotton is grown. Furthermore, cost of weeding is substantially higher that leads to a reduction in profitability of cotton production. Therefore, devising alternate weed control strategies using allelochemical producing cover crops were considered as a possible solution. The cover crops were evaluated under rainfed conditions at Nagpur and winter irrigated conditions at Coimbatore.

Field studies were conducted to screen cover crops in the rainy season as well as the winter season to study their efficacy in controlling weeds and on cotton productivity. Among the 12 cover crops evaluated, sunnhemp, sorghum, sesame were found to not only smother the weeds effectively but also had high seed cotton yields (Fig. 3.6.1).

Using the GC-MS, cover crops were analyzed for the allelochemicals. The allelochemicals belonged to the category primarily of fatty acids and their derivatives, terpenes, sterol, aliphatic hydrocarbon and aldehydes. The major compounds identified in cover crops under study were, phytol and pentadecanoic acid, 1,4-methylene methyl ester in sorghum; 9,12-octadecadienoic acid (Z,Z) - methyl ester and neophytadiene in pearl millet; squalene & linolenic acid in sunnhemp; 9,12-octadecadienoic acid, 9,12-octadecatrienoic acid (Z,Z) & their methyl esters in sesame; quinic acid & decanal in marigold; γ -sitosterol & octatriconyl pentafluoropropionate in bitter cumin; 9,12,15-octadecatrienoic acid and stearic acid methyl ester in desmodium.

With regard to soil biochemical properties, the plots with the sunnhemp as a cover crop had better enzymatic activity (Acid phosphatase, Alkaline phosphatase and β -glucosidase) as compared to the other cover crops as well as mulch of the polythene and newspaper. Sorghum as a cover crop had the highest dehydrogenases activity.