3. RESEARCH ACHIEVEMENTS

3.1: Cotton Genetic Resources

Nagpur

Exploration for perennials

Exploration and collection surveys were conducted in Wardha, Bhandara, Gondia and Yavatmal districts of Maharashtra, Kanyakumari district of Tamil Nadu and Nalgonda district of Andhra Pradesh. Fourteen perennials (Table 3.1.1) including nine of *Gossypium barbadense* and five of *Gossypium arboreum* were collected. They were established in pots and field conditions for further characterization and evaluation. The seeds were kept in the Gene Bank for conservation.

Table 3.1.1: List of perennials of cotton collected from different regions of India

S. No.	Districts	State	No. of accessions	Species	Annual/Perennial/ Landrace
1.	Wardha, Bhandara, Gondia and Yavatmal	Maharashtra	2	*G. arboreum*	Perennials
			9	*G. barbadense*	Perennials
2.	Kanyakumari (Western Ghats)	Tamil Nadu	1	*G. arboreum*	Perennial
3.	Nalgonda	Telangana	2	*G. arboreum*	Perennials

Thirty-six exotic accessions of *G. hirsutum* from USA were procured through ICAR – NBPGR, New Delhi for enrichment of the cotton gene pool.

Germplasm Evaluation

G. hirsutum

A set of 838 accessions (including 38 exotic accessions) were evaluated for economic and fibre quality traits. Nine long linted exotic accessions with staple length (30.2 mm – 31.8 mm) and 21 accessions for high fibre strength (26.3 g/tex – 28.4 g/tex) were identified and documented.

Long fibre (>30 mm)	EC 838248, EC 838253, EC 838266, EC 838263, EC 838249, EC 838268, EC 838255, EC 838256, EC 838252
High fibre strength (>26 g/tex) (HVI mode)	EC 838256, EC 838252, EC 838264, EC 838253, EC 838270, EC 838251, EC 838267, EC 838263, EC 838272, EC 838271, EC 838255, EC 838260, EC 838265,EC 838248, EC 838254, EC 807817, EC 838259, EC 838266, EC 838257, EC 796545, EC 838249

G. arboreum

One hundred and thirteen single plant selections of brown colour lint were evaluated for identification of lines with low micronaire (< 4.55) and fourteen lines were identified.

G. herbaceum

Work was initiated to identify germplasm lines of *G. herbaceum* with early maturity traits and identify genetically distinct genotypes suitable to climate change and enhanced productivity under local conditions. Seedlings of 582 germplasm were raised in paper tubes on two dates (27 June and 28 July 2015). Fifteen days old seedlings were transplanted in the field with 60 x 30 cm spacing in augmented design. Three standard checks were used (Jaydhar, DDhC -11 and G.Cot-25).

Early maturity traits *viz.* germination, plant height, days to first squaring, days to first flowering, days to first boll opening and percentage of open bolls at first picking were correlated with seed cotton yield per plant and direct path coefficient effect was estimated. Significant and negative correlation was found between yield and days for germination (-0.589), plant height (-0.420), days to first squaring (-0.463) and days to first flowering (-0.449). Path coefficient analysis revealed positive direct effect of number of bolls/plant (0.811), boll weight (0.335) and days to first flowering (0.314) on seed cotton yield.

Out of 582 germplasm, 31 genotypes showed early maturity traits (tested above). More than 350 genotypes were grouped in a range of 20-60 g seed cotton yield per plant. Six genotypes *viz.,* IC – 371437, IC- 371602, IC- 371587, IC – 371560, IC-371582 and IC-371575 recorded high yield ranging from 164 to 182 g/plant. Seasonal effect of two sowing dates and genotypes was highly significant in relation to yield.

Landraces of *desi cotton and perennials*

Evaluation for fibre quality traits

Fibre quality analysis was done for 119 landraces / perennials which included 80 *Gossypium arboreum*, 13 *G. herbaceum* and 26 *Gossypium barbadense* (Table 3.1.2).

Table 3.1.2: Range of fibre properties (ICC mode)

Name of the species	Staple length (mm)	Micronaire 10^{-6} g/in	Strength (g/tex)
G. arboreum	15.78-24.71	3.28-7.26	15.42-19.09
G. herbaceum	18.31-23.74	3.10-5.62	15.77-17.70
G. barbadense	25.09-34.21	2.94-5.88	18.29-25.67

Characterization of perennials and landraces

Morphological characterization including DUS characterization was completed for 19 germplasm accessions.

Diversity analysis

Diversity analysis was done by using MEGA 6 software, 24 unique *G. arboreum* accessions were identified and established in the "Perennial Species Garden" at Panjari farm, ICAR-CICR, Nagpur.

Wild Species

Twenty six wild species were conse-rved in wild species. One new species (EC669583), genetically distinct from other wild species flow-ered under field condi-tion.

Conservation in Long and Medium Term Storage

Seeds of 879 accessions (*G. hirsutum* – 294 and *G. herbaceum* – 585), 68 accessions of perennials and landraces (*G. arboreum*- 59 and *G. herbaceum*-9) were stored for Long Term conservation in the National Gene Bank facility for at ICAR-NBPGR, New Delhi as well as at medium term storage at ICAR – CICR, Nagpur.

Distribution of Germplasm

Seven thousand and fifty one (G. *hirsutum* – 4400, *G. arboreum* – 2063, *G. herbaceum* – 569 and Wild species – seeds and cuttings – 19), 14 perennial and landrace samples (*Gossypium herbaceum)* were distributed to breeders/scientists of CICR, state agricultural universities for utilization in cotton improvement programme.

Rejuvenation and Seed Multiplication

A set of five hundred accessions of *G. hirsutum* and 900 accessions of *G. arboreum* were grown for rejuvenation and seed multiplication.

Genetic Stock Registered

Five genetic stocks were registered with ICAR – NBPGR, New Delhi (Table 3.1.3).

Table 3.1.3: Features of unique genetic stocks registered

S. No.	Name of the stock	Species	Registration number	Uniqueness
1.	CNA 405	*G. arboreum*	INGR 15005	Narrow leaf lobed and brown linted
2.	CNA 407	*G. arboreum*	INGR 15024	Narrow leaf lobed, spotted petal blotch and light brown linted
3.	CNA 407 (SLP)	*G. arboreum*	INGR 15025	Spotless petals and brown linted
4.	CNA 1051	*G. arboreum*	INGR 15016	Distinct yellow top leaves virescent mutant
5.	CNH 1102	*G. hirsutum*	INGR 15015	High ginning outturn, resistant to bacterial leaf blight disease, long staple and high fibre strength

CNA 405

CNA 407

CNA 407 (SLP)

CNH 1102

Coimbatore

Germplasm evaluation

G. hirsutum

One thousand germplasm lines of *G. hirsutum* were characterized for various morphological parameters. Data were recorded on plant type, stem hairiness, stem colour, leaf shape, leaf lobing, leaf colour, leaf surface, gossypol glands on leaf, leaf nectary, flower bracts, petal colour, pollen colour, boll bearing habit, boll shape, boll surface, gossypol glands on bolls and loculi/boll.

Zero branching germplasm accessions

Six elite *G. hirsutum* germplasm lines were found to exhibit zero branching habit during 2013-14. Their single plant progenies were carried forward to fix the character.

G. arboreum

A set of 1059 accessions of *G. arboreum* were received from Gene bank of CICR, Nagpur. Forty one released genotypes were obtained from different centres of AICRP on Cotton; altogether 1100 genotypes were raised in a single row in augmented design. Traits namely number of monopodia, number of sympodia, days to fifty percent flowering, days to boll opening, leaf size, leaf shape, leaf texture, leaf colour, leaf lobing, stem colour, stem type, plant stature, gland and nectar glands, petal colour and lint colour were recorded. All these traits showed wide range of variation. Principal Component Analysis (PCA) based on qualitative traits revealed that traits *viz.,* boll shape, leaf shape and bract size distinguished all genotypes in higher order than the other traits. All the genotypes were grouped into six clusters.

G. barbadense

Three hundred and ten *G. barbadense* germplasm lines were maintained during 2015-16. Five new germplasm lines *viz.*, NGB-555, NGB-556, NGB-557, NGB-558 and NGB-559 were evaluated. NGB-556, a hairy line, is moderately resistant to sucking pest. NGB- 556 and NGB-559 were high yielding with better fibre qualities compared to control Suvin. NGB-557 is a short branching type, however, square drying was observed in this line. Variability for flower colour and boll characters was also observed. Ten compact germplasm lines *viz.*, NDGB-1, NDGB-2, ICB-7, ICB-39, ICB-71, ICB-145, ICB-161, ICB-174, ICB-199 and ICB-244 were evaluated for yield and yield attributes. All the cultures were cluster boll bearing types and the cluster varied from 2 to 6 per axil. Seed cotton yield of ICB-174 (1209 kg/ha) and ICB-244 (1007 kg/ha) were higher than the check variety Suvin (804 kg/ha). Both these accessions had high ginning outturn of 31% and 32% respectively. ICB-264 exhibited highest seed cotton yield of 137g per plant with 31% followed by ICB-284 (132 g) with 27% GOT. It is observed that both accessions were comparatively tolerant to sucking pest.

Eleven hairy germplasm accessions were evaluated for wax content. EC-18 had the maximum amount of wax (343 µg/cm2) followed by HAG-02 (305 µg/cm^2). Yield performance of ICB-124 and ICB-284 were superior to the other hairy germplasm lines. Among the fifteen accessions, ICB-264 had highest seed cotton yield of 137 g per plant and recorded GOT of 31% followed by ICB-284 (132 g) with GOT of 27%. Both the accessions were comparatively tolerant to sucking pest.

Sirsa

Evaluation of *G. arboreum* and *G. hirsutum* germplasm

Eight hundred and twelve accessions of *G. hirsutum* and 815 accessions of *G. arboreum* were evaluated and superior accessions for various morphological and agronomic traits are summarized in Table 3.1.4.

Table 3.1.4: Accessions for superior morphological and agronomic traits

Character	*G. arboreum*		*G. hirsutum*	
	Range	Superior accessions	Range	Superior accessions
Earliness (days)	62- 97	G4-B-5 (62), Million Doller (62)	50-121	EC-700400 (50), EC-700280 (50)
Dwarf (cm)	84-187	355-E-6 (84), 78/1A(84)	39-115	EC-141677 (39); EC-142767 (39)
Monopodia	1-5	PBS 1549 (5), PBN 5664 (5)	0-4	EC-657087 (4), EC-657097 (4)
Sympodia	4-13	30816 (13), 30840-SP1(13)	2-9	EC-657097 (9), EC-158941 (9)
No. of bolls/plant	10-38	AKA62 (38), Desi-10 (38)	12-36	EC-158941 (34), EC-153524 (34)
Boll weight (g)	1.6-2.9	CC-2-1-11-15 (2.9), 1768 (2.9)	1.5-3.6	EC-692166 (3.6), EC-692161 (3.6)
Yield/plant (g)	12-94.4	1768 (94.4), 7444 (92.6)	18-104.7	EC-158941 (104.7), EC-700179 (102.0)
Ginning outturn (%)	28.8-36.6	AC-35P-1 (36.6), H503 (36.4)		

Fifteen compact *G. hirsutum* germplasm lines identified during the previous year were evaluated along with check (F 2383) at 67.5 x 10 cm. The number of bolls per plant, boll weight and yield per plant were significantly lower in compact lines than the checks. The closer spacing contributed significantly in increasing yield per unit area. Accessions SP-3897 (3400 kg/ha) and IC-358100 (3440 kg/ha) were superior than F 2383 (2850 kg/ha).

3.2: Hybrid Cotton

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. Three GMS based hybrids CISAA 15-38 (1841 kg/ha), CISAA 14-31 (1649 kg/ha) and CISAA 14-32 (1545 kg/ha) recorded significantly higher seed cotton yield than the highest yielder check hybrid CICR 2 (1366 kg/ha). CISAA 14-31 and CISAA 14-32 hybrids have been sponsored in National Br 25 (a/b) trial of AICRP on Cotton. Hybrid CISAA 14-31 is promoted to zonal trial Br 25(a) for further evaluation.

Maintenance of GMS lines

Three GMS lines DS5, CISA 2, GAK 413A and 16 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, CISG-19, CISG-21, CISG-22) were maintained through sib mating. A pigmented GMS line CISG 20 has red flower colour with petal spot. The plant is robust, thermo-insensitive and no pollen shedders are reported.

3.3 : Genetic Improvement

G. arboreum

Nagpur

Individual Cernuum plants of 5 accessions collected from Mizoram were evaluated. It showed compactness with plant height of 50 cm and sympodial length of 5 cm.

Sirsa

Evaluation of spinnable *G. arboreum* cultures

Fourteen (14) cultures were tested in RBD along with two checks CISA 310 and CISA 614. The seed cotton yield ranged from 1442 to 2395 kg/ha. None of the genotypes could give higher yield than checks. However, two genotypes CISA-10 and CISA-33-8 yielded more than 1700 kg/ha and have promise for spinning. Other genotypes CISA-6-350, CISA-6-295 and CISA-33-7 were having UHML (mm) >24.0 mm and strength >24.0 g/tex in HVI mode.

Evaluation of high yielding non-spinnable *G. arboreum* genotypes

Sixteen (16) genotypes were evaluated in RBD with two check varieties CISA 310 and CISA 614. The seed cotton yield ranged from 2218 to 2641 kg/ha. Three genotypes CISA-6-2 (2641 kg/ha), CISA-33-3 (2593 kg/ha) and CISA-6-123 (2583 kg/ha) gave higher seed cotton yield than local checks CISA 310 (2466 kg/ha) and CISA 614 (2570 kg/ha). The genotype CISA-33-3 is sponsored in AICCIP Br22a/b national trial.

G. barbadense

Coimbatore

Identification of promising extra long staple genotypes

Yield evaluation was done with 15 stabilized advanced generation (BC_3 F_9) genotypes during 2015-16. Four genotypes *viz.*, CCB-51 (1633 kg/ha), CCB-64 (1572 kg/ha), CCB-66 (1541kg/ha), and CCB-72 (1500 kg/ha) significantly out yielded the check variety Suvin (910 kg/ha) and had better ELS fibre quality. The promising genotype CCB-51 had maximum yield of 1312 kg/ha with high fibre length of 38.1 mm of span length, bundle strength of 28 g/tex with 3.3 μ/inch and ranked first in the list. In order to sponsor for AICRP trial for the year 2016-17, seed multiplication of 5 promising cultures (CCB-51, CCB-64, CCB-66, CCB-67 and CCB-72) was done (Table 3.1.5).

Table 3.1.5: Promising extra long staple genotypes for AICRP 2016-17

S. No	Genotypes	SCY (kg/ha)	% yield incr. over Check	GOT (%)	2.5% SL (mm)	Strength (g/tex) (ICC mode)	Mic (μ/inch)	Earliness Index
1	CCB-51	1312	41.1	34	38.1	28.2	3.3	0.74
2	CCB-64	1205	30.4	35	37.2	26.9	3.0	0.75
3	CCB-66	1242	34.4	32	36.1	27.9	3.0	0.73
4	CCB-72	1191	28.9	35	36.9	27.3	3.3	0.58
5	CCB-67	1175	27.2	35	37.1	28.2	3.2	0.74
	Suvin ©	924	-	31	36.5	27.0	3.0	0.71

In another advanced generation trial (BC_2 F_8), 16 stabilized ELS genotypes were evaluated. Pooled data of three years (2013-14 to 2015-16) showed that among the 16 genotypes, the yield performance of 6 ELS genotypes *viz*., CCB-143 (1281 kg/ha), CCB-93 (1272 kg/ha), CCB-142 (1207 kg/ha), CCB-141 (1119 kg/ha), CCB-140 (1118 kg/ha) and CCB-129 (1141 kg/ha) were significantly superior over the check Suvin (807 kg/ha). With respect to performance, fibre quality and earliness, CCB-143 was stable.

Evaluation of single plant selections from BC_2F_3

Twenty four single plant selections were made from 15 multiple cross combinations involving Suvin as one of the parent. Out of twenty four, six single plant progenies were found promising with high seed cotton yield. The single plant progeny from a cross

SN (SN x ICB 115) 2-7-1-1, SPS-3 produced 131 g seed cotton/plant respectively. A cleistogamous mutant in intra-*barbadense* cross (Suvin x ICB-125) x Pima S-4 was identified from the segregating population.

G. hirsutum

Nagpur

Improvement for earliness, compact type, jassid tolerance and fibre strength

Based on plant width (<60 cm) and number of monopodia (<1.5), N 170, Pratima, CSH 3178, Anjali, CNH 09-7, Arogya, DSC 99, CNH 1102 and AKH 8828 were identified as most promising for compact plant architecture. KC 3, G.Cot 20, CNH 2034, NISC 44, AKH 081, JK-4, CNH 409-9, CNH O7-34, ADB 532 and NH 545 were found promising for jassid tolerance as they had lowest jassid grade (=1) and lower nymph number (<1.8/3 leaves/plant). Based on percent bolls opened on 150 DAS (>95%), Pratima, CNH 409-9, CNHO 12, Sahana, IC 359478, Arogya, G.Cot 18, JK-4, CNH 2028 and F 1378 were identified as promising for earliness. Promising lines were also identified for boll weight, seed yield per plant and fibre quality attributes from 84 genotypes. Twenty-nine promising lines identified in the year 2014-15 were crossed in different combinations and hybrid seed was produced. Crosses attempted in 2014-15 were forwarded to F_2 generation. Evaluation of 154 selected F_3 families led to the identification of some of the promising segregants which matured within 130 DAS (>95% boll bursting), had jassid tolerance and compact plant architecture.

Compact plant architecture

Single crosses, backcrosses, three way crosses and double crosses made between high strength germplasm and high yielding varieties were evaluated for seed cotton yield and fibre properties. Nine hundred single plant selections were made in these segregating generations based on early maturity, tolerance to jassid and seed cotton yield per plant. These selected single plant selections were tested for fibre properties. Crosses (Suraj × CCH LS 2) × IC 359292, (Suraj × G-21-19-615) × (NH615 × CCH 4474), (Suraj × Suvin) × IC 292470, (Suraj × Suvin) × IC 356847 and (Suraj × CCH 4474) × IC 356847 recorded higher seed cotton yield with better fibre properties. Crosses (NH615 × CCH LS 2) × IC 359292, (NH615 × N 170) × (Suraj × CCH 7122), (NH615 × N 170) × (Suraj × CCH 4474), (NH615 × FS1) × IC 359292 were identified for tolerance to jassid coupled with higher yield and better fibre properties over check variety Suraj.

To improve fibre strength, germplasm with high fibre strength were selected for developing a 10 × 10 diallel crosses without reciprocals. Two high yielding varieties NH 615 and Suraj were also used. General combining ability variance (GCA) was higher than specific combining ability (SCA) for upper half mean length, uniformity index, micronaire and fibre strength indicating the predominance of additive gene action in the inheritance of all these traits. CCH 7122 and Suraj exhibited significant GCA effects for all fibre properties traits except micronaire. However, CCH 7122 had significant negative GCA effects for seed cotton yield. G 21-19-619 and IC 356751 recorded highly significant positive GCA effects for seed cotton yield.

Sirsa

Breeding for high strength

Evaluation of F_7 progenies of the two crosses *viz*; CSH 3119-10-30-60 (2960 kg/ha seed cotton, 1067 kg/ha lint yield and fiber strength 27.4 g/tex in HVI mode) and MMO.3 (39-2-5)-3114-10-64 (2976 kg/ha seed cotton, 1020 kg/ha lint yield and fiber strength 26.7 g/tex) were early in maturity and were significantly higher yielding than the check H 1226 (2056 kg/ha seed cotton yield and 723 kg/ha lint yield and fiber strength 24.0 g/tex).

Breeding for high GOT and high yield

Based on the average performance of three years, 13 F_6 (2013-14), F_7 (2014-15) and F_8 (2015-16) progenies [cross SA-977 (HG) x SA-112 (LG) *viz.* P-7(2019 kg/ha seed cotton yield, 780 kg/ha lint yield and GOT 39%) and P-69 (2033 kg/ha seed cotton yield, 815 kg/ha lint yield and GOT 40.2%) were significantly higher than the check H 1226 (1839 kg/ha seed cotton yield, 625 kg/ha lint yield and GOT 34.4%) over three years. As many as seven progenies *viz.*; P-68, 69, 70, 84, 139, 164 and 184 possessed GOT of more than 40%.

Coimbatore

Identification of compact plants suitable for HDPS

Seven super okra leaf compact genotypes were evaluated with three check varieties, Suraj, Anjali and Supriya under spacing of 30 cm x 10 cm. The culture Surabhi x MM02-19-1-8-2 was the highest yielder (2071 kg/ha). The culture MCU 13 x VNWH 1-7-2 was the best for fibre quality (2.5% span length of 33.1 mm and bundle strength of 23.0 g/tex in ICC mode).

In another station trial with 13 normal leaf types, three cultures showed numerical superiority over the check variety Suraj and the highest seed cotton yield of 2315 kg/ha was recorded in the culture PI 36-3-5-2 as against 2053 kg/ha in Suraj. Quality wise PI 36-3-1-3 recorded 2.5% span length of 31.1 mm and bundle strength of 23.5 g/tex in ICC mode.

From the segregating population, brown colour linted progenies were identified from the cross MCU 13xVNWH-1-4-1-2, 44 single plant progenies were raised which showed four different shades of colour as cream, light brown, brown and dark brown visually. Progenies with good yield potential, big boll types, compact types, good length and strength were identified and selected for further evaluation.

From F_6 generation, 50 single plants were selected for further screening which showed maximum boll weight of 5.4 g. Similarly, 50 single plants were selected for further screening which showed the maximum lint index of 6.6 g. Apart from the above, 39 single plants were selected for further screening which showed the maximum GOT of 47.1%.

Genetic enhancement

Nagpur

In F_9-F_{10}, of the 55 cultures, a maximum of 1629 kg/ha seed cotton yield was recorded in SPS 9-32. Thirty six lines were tolerant to jassids (grade-1, score). BC_3F_7 generation of each cross LRK-516 x (LRK-516 x Deltapine-66) recorded GOT of 37%. Five entries in F_7 *viz.* SPS 8-19, SPS 8-22, SPS 8-59, SPS 8-1, SPS 8-17 recorded high fibre strength, a maximum of 30.4 g/tex and fibre length of 29.1 mm in HVI mode over the check, Suraj (fibre length-29.1 mm, fibre strength-24 g/tex). In F_8, a maximum of 2173 kg/ha seed cotton yield was recorded for SPS8-53 with a boll wt. of 3.9 g. Eight entries recorded >2000 kg/ha seed cotton yield in HDPS (60 x 10 cm) trials.

Among 149 F_3 families evaluated for compact and semi-compact types, eight entries recorded >2000 kg/ha seed cotton yield in HDPS (60 x 10 cm), with a maximum of 2800 kg/ha.

CLCuD resistance

Sirsa

Evaluation of advance cultures : In this trial, 15 *G. hirsutum* cultures were evaluated against the check variety RS 2013, LH 2076 and susceptible check HS 6 in RBD with three replications. The highest seed cotton yield was recorded in CSH 2931 (1818 kg/ha) followed by CSH 2837 (1574 kg/ha) as against the check variety LH 2076 (1495 kg/ha). Maximum GOT of 35.9% was recorded in the variety CSH 2924 as compared to local check varieties 32.4 % in RS 2013 and 32.6% in LH 2076. The culture CSH 2924 also recorded the highest 2.5 % span length of 25.7 mm and bundle strength of 26.1 g/tex.

Selection of single plants progenies : To develop the segregating populations of *G. hirsutum* cotton, 16 crosses were attempted among CLCuV tolerant germplasm lines in a Line x Tester fashion. Out of 91 single plant progenies, six progenies having high yield potential and tolerance against CLCuV were selected in F_4 generation. The culture CSH 1607 recorded the highest yield of 2019 kg/ha followed by CSH 1603 (1898 kg/ha) and CSH 1601 (1806 kg/ha) as compare to check variety LH 2076 (1495 kg/ha). The cultures also have desirable fibre quality traits and lowest CLCuV reaction.

Selection of single plants from progeny row trials : From the fourth cycle of random mating, 102 single plants were selected and raised in plant to progeny row trials having 4 rows each. Based on CLCuV tolerance 65 single plants were selected for further evaluation for seed cotton yield and other economic characters.

Drought tolerance

Nagpur

Three set of experiments were conducted under rainfed and irrigated condition. First set comprised of 13 advance cultures and showed significant difference for seed cotton yield under both rainfed and irrigated condition which ranged from 432-1797 kg/ha and 926-2098 kg/ha. DTS 100 ranked first and second in performance under rainfed and irrigated conditions respectively followed by DTS 62, DTS 44 and DTS 104. Culture DTS 67 had recorded fibre strength of 25.6 g/tex (ICC mode) in the previous year, recorded seed cotton yield of 716 kg/ha and DTE (Drought Tolerant Efficiency) of 45%.

Second set of experiment comprised of 48 single plant selections in which thirty-three selections were similar to check LRA 5166 under rainfed and 10 selections under irrigated conditions, respectively. Top 10 performers recorded more than 40% increase over the check LRA 5166 under rainfed condition. Some of these single plant selections also recorded high drought tolerance efficiency (>91%). Seed cotton yield ranged from 454 – 1846 kg/ha under rainfed and 926 – 2098 kg/ha under irrigated conditions. DTS 150 was the best performer under rainfed conditions and ranked sixth in performance under irrigated conditions with 73% increase over the check LRA 5166 and DTE of 87.4%. DTS 86, DTS 15, DTS 75, DTS 110 were some of the other good selections. DTS 126 and DTS 67 possessed good fibre quality.

Among the F_3 generations of twelve crosses, eight crosses recorded more than 10% increase of yield over the check. Cross PH 93 x Rajat recorded highest seed cotton yield. High DTE was recorded for two crosses *viz.* LRA 5166 x N 170 (98.7%) and 28 I x Suraj (94%). Individual F_2 plants evaluated for fibre quality recorded maximum variability for fibre length (22.6-30.2 mm), fibre strength (19.4-26.7 g/tex), micronaire (2.9-4.3 g/in) and uniformity index (43-55%) in cross 28 I x Suraj in ICC mode followed by cross PKV 081 x P3 and PKV 081 x Suraj, respectively.

Evaluation of single, double, six and eight parental crosses under irrigated conditions recorded high GOT in some of the double and six parental crosses (>38%), UHML in these crosses ranged from 26.1 to 30.5 mm and fibre strength from 24.3 to 27.6 g/tex in HVI mode.

Evaluation of 19 cultures for biochemical and physiological parameters under field and pot conditions was carried out. Based on effect of drought stress on leaf temperature, photosynthetic rate, transpiration rate, NR activity, prolein, protein content, reducing sugar, phenol, amino acid and chlorophyll content, four genotypes DTS 4, DTS 6, DTS 9 and NHP 3 were identified for drought tolerance.

Waterlogging tolerance

Nagpur

In a pot experiment, the identified 150 tolerant accessions were evaluated in three replications for 48 days of waterlogged condition along with a control and morphological adaptations like lenticels and adventitious roots were prominently observed in tolerant accessions. Differences in root growth pattern were also very conspicuous.

Lenticel and adventitious roots in tolerant accessions IC 357477 and IC 359380

Population Improvement

Nagpur

Conventional random mating population : The random mating population developed through conventional crossing was maintained by bulk harvesting one open boll from each plant in both *G. arboreum* and *G. hirsutum*.

GMS based random mating population : In the previous year, all sterile plants in GMS based RM population were tagged at flowering, allowed open pollination and each plant was harvested separately. Seeds of all sterile plant were divided in two lots. One seed lot was used for planting as plant to row progenies during 2015-16 while the other seed lot reserved for next season sowing. Plants in each progeny were tagged (sterile and fertile) at flowering and observations were recorded on 3-5 fertile plants in each progeny for important economic and quality traits.

Evaluation of single plant selection : Large number of single plant selections from random mating population and reselected plants from the segregating progenies (2676), were evaluated in plant to row progeny plots. Based on manual checking for fibre quality traits, 112 single plants were selected from the composite random mating population and about 1650 superior single plants were reselected from the segregating plant progenies. Twenty-two single plants of *G. hirsutum* and 48 of *G. arboreum* were identified for evaluation in replicated trial.

Evaluation of advance cultures: About 152 *G. arboreum* and 149 *G. hirsutum* selections identified in 2014-15 were evaluated in 16 replicated trials (4 rows plots in 2 replications) during 2015-16. In all, eight trials each of *G. arboreum* and *G. hirsutum* were conducted with a spacing of 60 x 45 cm and 60 x 60 cm, respectively. Seed cotton yield among the *G. arboreum* cultures ranged from 428 to 1466 kg/ha while in *G. hirsutum,* it ranged from 532 to 1600 kg/ha. Based on the significant seed cotton yield, 77 cultures of *G. arboreum* and 82 of *G. hirsutum* were retained for second year testing. Three cultures were identified for promotion in AICRP on cotton national trials.

Sirsa

GMS based random mating population: A GMS based composite random mating population of *G. hirsutum* was grown. At flowering, all plants in the population were monitored for sterility/fertility at anthesis. About 220 sterile plants were bulk harvested to constitute next cycle of random mating. Single plant selections (150 No.) were grown as plant to row progenies and further selection were made based on CLCuV tolerance and other yield components.

3.4 : Genetic Diversity through Introgression

Selection of introgressed derivatives

In *G hirsutum* based introgressed derivatives; genotypes with fibre length ranging from 25.7 to 28.9 mm and fibre strength ranging from 19.6 to 21.8 g/tex were selected. While in *G arboreum* based introgressed derivatives genotypes with fibre length ranging from 23.2 to 26.5 mm and fibre strength ranging from 20.0 to 23.2 g/tex were selected.

Naturally coloured cotton

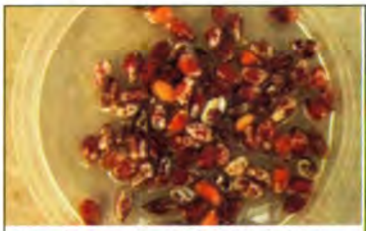
Sixteen naturally coloured cotton introgressed genotypes (*G. hirsutum* and *G. arboreum*) were evaluated during the season. Seed multiplication of MSH-53 (Vaidehi-95) was also taken up and 650 kg seed was produced.

An experiment was conducted to record the effect of black cloth on development of lint colour of MSH-53 (Vaidehi-95). It was found that the bolls developed dark brown colour inspite of covering them with black cloth bag which indicates that the colour of lint is genetically controlled.

Development of CLCuV resistant genotypes through introgression

Nagpur

Various breeding approaches were followed to develop populations/genotypes resistant to cotton leaf curl virus (CLCuV) like crossing *G. hirsutum* lines (Suraj, AKH081, NH615 & KC3) with *G. arboreum* lines (Phule Dhanwantri, Cernnum, Roja and PA 255); Colchicine treatment of *G. arboreum* and *G. hirsutum* genotypes; development of synthetic tetraploids of cultivated cotton by crossing *G. arboreum* and *G. herbaceum* with *G. raimondii*; crossing *G. hirsutum* lines (Arogya, DSC 99, KC 3, AKH 081, Anjali, CNH 1102, G.Cot. 16 and Sahana) with PAIG lines (PAIG 62, PAIG 326 and PAIG 346; crossing *G. hirsutum* lines (AKH 081, Anjali, CISH3178, LRA5166, NH615 and Suraj) with the registered genetic stock, IS-376/14/21 (INGR No. 04106; IC427815) reported to possess anatomical features of *desi* cotton in *G. hirsutum* background. The crossed bolls were harvested in all the approaches except *G. herbaceum* × *G. raimondii* crosses.


Seeds treated with Colchicine


G. arboreum


IS-376/14/21
(INGR No. 04106; IC427815)

Sirsa

Crosses involving *G. hirsutum* and *G. arboreum* were attempted and cross seed was obtained. The autotetraplodization of *G. arboreum* was attempted.

3.5 : Development of Variety and Multi-location testing

Proposal for release of varieties

Sirsa

The proposal for CSH 3075 was submitted for consideration of Central Varietal Release Committee on the basis of average performance of 11 AICCIP HDPS trials (67.5 x 10 cm) conducted in north zone from 2012-13 to 2014-15. CSH-3075 recorded seed cotton yield of 2467 kg/ha. The proposed variety recorded mean fibre length of 26.7 mm, micronaire of 4.13 and fibre strength of 21.6 g/tex in the spinning test (35.5%). The proposed variety was similar to the check varieties for majority of diseases and showed field tolerance to jassids.

Coimbatore

The proposal for identification of long staple high strength culture CCH 4474 for South Zone states under irrigated conditions was submitted for consideration of Central Variety Identification Committee. The culture was found to combine good fibre length of 30.6 mm and bundle strength of 24.0 g/tex and was capable of spinning upto 60s count yarn.

Testing of cultures in AICRP on Cotton

Nagpur

Two entries, CNH 147-1, CNH 126 were entered in Br 02 (a). Four entries, CNH 09-9, CNH 07-16, CNH 1122, CNH 1123 were entered in Br-02(b). In HDPS (Br-06 (a/b) trial, eight entries namely, CNH 140-1, CNH 95-04, CNH 131, CNH 09-7, CNH 09-5, CNH 1124, CNH 20 and CNH 1560 were tested. Five *G. arboreum*, entries namely CNA 2023, CNA 2019, CSA 2043, CSA 1028 and CCA 2033 were tested in Br 22 (a / b). In south zone trials, the entries promoted/retained were CNH 1111 (Br 06 (b)), CNH 25 (Br 06 (a)) and CNA 449 (Br 24 (b)).

Coimbatore

Fourteen high yielding *G. hirsutum* cultures were sponsored for multi location evaluation in AICRP during 2015-16. Of these, eight were tested in National trials and the remaining six were in advanced stage of testing in zonal trials.

The culture Surabhi x M5Z2 18-5 (CCH 15-1) was tested in initial evaluation trial of *G. hirsutum* varieties under irrigated condition. It recorded an upper half mean length of 27.1 mm in North Zone locations, 31.8 mm in Central Zone locations and 32.5 mm in South Zone locations. Further, the culture also exhibited the highest bundle strength of 30.7 g/tex, 32.8 g/tex and 36.1 g/tex in HVI mode in North, Central and South Zone locations, respectively. At Bhawanipatna, the highest strength of 40.9 g/tex was recorded. When the fibre sample was analyzed in Advanced Fibre Information system, it was found to register the least immature fibre content of 4.5 % and the maximum maturity ratio of 0.92 and notably, the short fibre content (<12.7 mm) by weight was only 3.9%. All these factors might have contributed for the high strength in the culture.

The culture MM 03-40-4-3-1 (CCH 15-4) was tested in initial evaluation trial of *G. hirsutum* varieties under rainfed condition in central and south zone locations. The culture recorded an upper half mean length of 31.2 mm in Central Zone locations and 30.9 mm in South Zone locations with bundle strength of 30.5 g/tex and 31.5 g/tex in HVI mode respectively.

The long staple culture MM 03-39-4-2-3 (CCH 14-1) tested in preliminary yield trial under irrigated conditions of both central and south zones exhibited superior fibre quality combining good length (31.6 mm) and strength (33.3 g/tex to 33.8 g/tex).

The compact super okra culture Surabhi x M5Z2 4-2 (CCH 15-5) was tested in AICRP trials under irrigated conditions, and the cultures Surabhi x MM 02-16-5-2-3 (CCH 15-7) and MCU 13 X VNWH 1-7-2 (CCH 15-8) were tested in rainfed situations. All these cultures exhibited excellent fibre quality in the initial evaluation trial and were promoted for evaluation in coordinated varietal trial in central and south zone locations.

In preliminary varietal trial of AICRP 2015-16 (Br 13a), an elite promising *barbadense* genotype CCB-29 ranked third and fourth in yield in Central and South zone, respectively. With regards to quality parameters it ranked fourth in both the zones. In Initial yield evaluation trial of AICRP 2015-16 (Br 12a), a promising ELS selection from an intermated population- CCB-11a had ranked fourth in yield and third in fibre quality parameters.

Sirsa

G. hirsutum cultures CSH 2920 & CSH 2932 in Br 02(a) National trial, CSH 3232 & CSH 3088 in Br 03(a) Zonal trial, GMS based *G. hirsutum* hybrid CSHG 2911 in Br 05(a) National trial, *G. hirsutum* hybrid CSHH 2012 in Br 05(a) Zonal trial, compact cultures CSHH 3158, CSH 5038 and CSH 6109 in Br 06(a) National trial, compact cultures CSH3113, CSH 3178 & CSH 73 in Br 06(a) Zonal trial, *G. arboreum* culture CISA-6-123 in Br 22(a/b) National trial, CISA-6-2 in Br 24(a) zonal trial and *G. arboreum* GMS based hybrids CISAA 14-31 & CISAA 14-32 in Br 25(a/b) National trial were sponsored/promoted during 2015. The *G. hirsutum* hybrid CSHH 2012 was recommended for agronomy based on overall performance in AICRP trials during 2015.

3.6: State Multi-location Varietal Trial

Nagpur

A State Multi-location Varietal Trial (SMVT) of *G. arboreum* consisting of 19 genotypes and 4 control varieties and of *G. hirsutum* consisting of 13 genotypes+4 control varieties with three replications following recommended package of practices was conducted.

In *G. arboreum*, the range for seed cotton yield was 528 to 1114 kg/ha. The maximum seed cotton yield of 1114 kg/ha was obtained with PA-812 followed by JLA-0910 (1108 kg/ha) and CNA 1028 (9970 kg/ha). The control variety JLA 505 recorded highest seed cotton yield of 967 kg/ha which is quite higher than other control and genotypes under evaluation. PA 812 found to be promising with respect to fibre quality traits particularly for fibre length (31.9 mm) and uniformity index (83 %) combined with seed cotton yield. AKA-2004-29 and AKA-2010-10 recorded highest strength of 25.3 g/tex in HVI mode.

In *G. hirsutum*, seed cotton yield ranged from 895 to 1665 kg/ha. PH 1060 recorded significantly high seed cotton yield (1666 kg/ha) followed by culture Phule 688 (1594 kg/ha). Seed cotton yield of control variety NH 545 was 1512 kg/ha. AKH 2006-2 exhibited highest fibre length (31 mm) and strength (26.5 g/tex).

3.7: Molecular Breeding

Nagpur

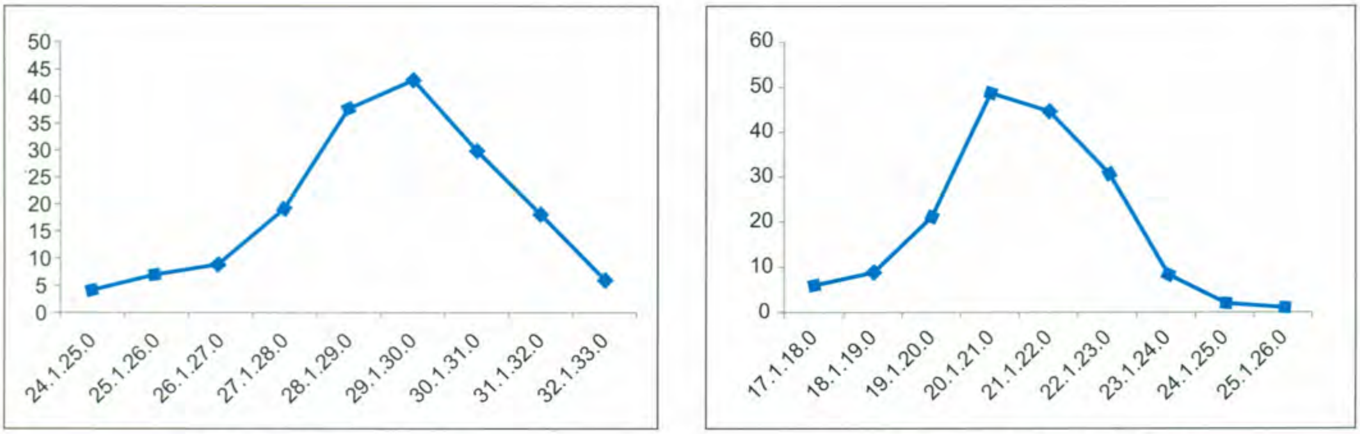
A set of recombinant inbred lines (RILs) developed from a cross between EL 958 and UPA 57-17 following single seed decent method was used for genetic mapping. About 240 progenies were used for phenotyping of fibre quality traits while 188 progenies for SSR marker genotyping.

Survey for informative SSR markers : During 2014-16, 4417 SSR markers were screened for parental polymorphism. Of the 4417 markers, 702 were found to be polymorphic/ informative which accounts for 15.9% polymorphism with the parental lines.

Genotyping of *G. hirsutum* RILs : Of the 240 progenies, the progenies showing more similarity for morphological and quality characters (based on early evaluation) were excluded, and the progenies showing distinct variation for morphological or quality traits were retained for genotyping with informative SSRs.

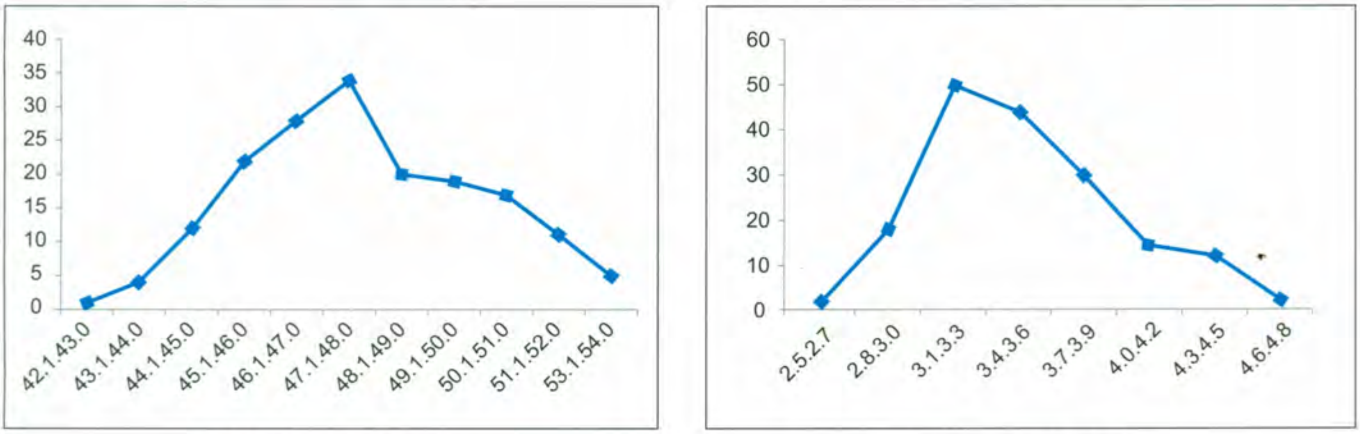
Genomic DNA of 188 progenies was used for genotyping using informative polymorphic SSRs. About 520 informative SSRs were used for genotyping a set of 188 RILs. Polyacrylamide gel electrophoresis (PAGE) with 8 to 10% concentration was used for fragment separation. About 50% of the markers appeared to be good and the polymorphic fragments were scorable. SSR markers showing polymorphism in parental screening using touchdown PCR turned out to be monomorphic while genotyping RILs and in cases where fragment size difference was very narrow, resolution of fragments was not proper making scoring of the genotypic marker data difficult in many instances and hence ambiguous marker scoring was avoided. The markers /segregating loci showing abnormal segregation pattern or drift in segregation were excluded from the final SSR marker data for linkage analysis. Finally, 227 anchor marker loci were used for making an integrated SSR-SNP genetic linkage map of AD genome *G. hirsutum* cotton. The purpose of the anchor loci was to identify linkage groups to specific chromosome and to make comparison with the already published linkage maps of AD genome.

Phenotyping for fibre quality traits : The harvested seed cotton of each RIL progeny was ginned and the bulk lint of each RIL progeny was prepared separately for fibre quality evaluation at GTC, CIRCOT, Nagpur. All the fibre quality evaluation was carried out using HVI Uster using ICC mode for both the years 2013-14 and 2014-15. The frequency distribution for pooled data is shown in Fig. 3.7.1



(a) Fibre Length (mm)

(b) Fibre Strength (g/tex)



(c) Uniformity Ratio

(d) Micronaire Value

Fig. 3.7.1: Frequency distribution for fibre quality parameters

The frequency distribution for fibre quality traits, namely fibre length, uniformity ratio, micronaire and fibre bundle strength, clearly indicates normal distribution pattern. It is to be noted that the data was not transformed for plotting the graphs. Normal distribution for all traits clearly indicates quantitative nature of fibre quality traits and merit of the population for linkage and QTL mapping particularly for the above fibre quality traits.

SNP genotyping : Genotyping of 172 RIL progenies with 2730 SNP markers was done by NBRI and data analyzed. The preliminary analysis showed 29 linkage groups with 1.31 cM distance between the consecutive markers.

QTL mapping for fiber traits : Phenotypic data for individual year as well as pooled over the years were used for QTL mapping. Genotyping data for 2730 SNPs (mapped on 29 linkage groups) were used for QTL mapping. QTLs were mapped using composite interval mapping (CIM) approach with the help of software QTL Cartographer. Threshold LOD was calculated for individual experiment. Peaks that qualified LOD threshold were considered as true QTL.

A total of 10 QTLs were identified for staple length, tenacity and micronaire. For staple length, two QTLs were identified each on LG 18 (year 2013/14) and LG 4 (pooled data) with LOD score ranging from 3.72–5.15 and phenotypic variance (R^2) from 8.7 – 10.6%. Similarly, for tenacity, total six QTLs were identified, two on LG 3 and one each on LG 10, LG 4, LG 5 and LG 8, respectively. Out of six QTLs, three were identified in the year 2013-14, two were identified in 2014-15 and one was identified in pooled data. LOD scores for these six QTLs ranged from 3.8 to 7.5 and phenotypic variance (R^2) from 8.88 to 16.65 %. For micronaire, two QTLs were identified, each in year 2014/15 and pooled data, on LG 18 and LG 4, respectively with LOD score for these two QTLs ranged from 4.1 to 5.8 and phenotypic variance (R^2) from 9.8 to 14.3%.

Maintenance of RILs in diploid and tetraploid cotton: In *G. hirsutum*, 240 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 put to use in genetic mapping.

Marker Assisted Breeding

Sirsa

Cotton Leaf Curl Disease (CLCuD)

Screening of new germplasm under field conditions : A total of 940 lines were screened against CLCuD under field conditions along with susceptible check variety HS 6 sown at every 10th line. Three lines which remained free from CLCuD were confirmed under pot conditions through artificial inoculation. Two lines *viz.* PIL 8 and PIL 43 were screened against CLCuD in polyhouse under artificial inoculation using viruliferous whiteflies showed least PDI of 14.5 & 11.4, respectively, as against 61.8 in the susceptible check HS 6.

Bacterial Leaf Blight (BLB)

Nagpur

Screening of segregating populations : Three each of F_2 and BC_1 populations were grown during 2015-16, the population size ranged 150- 200 plants for F_2 and 100-130 for BC_1. The F_2 populations include- LRA 5166 x S-295, Surabhi x S-295 and Surabhi x 102 B and their corresponding BC_1 populations. Individual F_2 and BC_1 plants were artificially inoculated with *Xam* bacterial culture twice. The inoculated plants were covered with transparent polythene bags spread on inner side with water for maintaining humidity inside the polythene bags. After 15-20 days, observations for bacterial blight incidence were recorded on individual plants. Variety Suraj (*G. hirsutum*) and three BC_1 populations were grown in the field. All three BC_1 populations were artificially screened after one month of sowing with *Xam* culture (race 18) sprayed on pin pricked plants in the field. The inoculated plants were covered with polythene bags. After 7 and 15 days of inoculation, water soaked lesions and BLB symptoms were recorded and BC_1 plants were categorized as resistant and susceptible based on symptoms.

Molecular screening : DNA was isolated from BC_1 plants and screened using SSR marker CIR 246. BC_1 plants were categorized as resistant (156 bp+146 bp) and susceptible (166 bp +156 bp) and compared with 101-102B which is universal resistant for BLB having 146 bp. On the basis of phenotypic and polymorphic marker screening, individual plants were selected for crossing. Backcrossing of positive BC_1 plants was done with Suraj to obtain BC_2 seeds during peak flowering. BC_2 crossed seeds were harvested for further advancement. Similarly, at CICR, Regional Station, Coimbatore, BC_2F_1 of the Suraj x CSH 3313 were raised and marker positive plants for BLB were backcrossed with Suraj.

Nematode resistance

The segregating populations namely F_2 and BC_1 developed using nematode resistant and susceptible lines were grown in the field. Genomic DNA of individual F_2 and BC_1 plants was extracted and subjected to screening using SSR markers BNL 3279 and NAU 2152. The marker positive plants in BC_1 were backcrossed with recurrent parent Suraj to obtain BC_2 seeds while F_2 marker positive plants (10 plants each) were tagged and selfed to get F_3 seeds.

Phenotyping of F_2 plants was carried out for reniform and rootknot nematode population. Marker positive plants were resistant to nematode and nematode reproduction rate in these plants was close to the resistant parent G. Cot 10. Further, genotyping and phenotyping of the additional population is being carried out.

At Coimbatore, crosses were attempted between G Cot-10 and American Nectariless (resistant) with susceptible cultivar (Suraj). F_2 population supplied from CICR, Nagpur was grown in pots and genomic DNA of 305 F_2 plants was extracted and the population continued to be genotyped for validation of marker with reniform nematode resistance.

DNA Fingerprinting of public sector released genotypes of cotton

Sixty additional SSR markers were screened for 48 public sector released tetraploid cotton varieties (*G. hirsutum* and *G. barbadense*) and 150 markers for 24 *G. arboreum* varieties during the year 2015-16. For tetraploid varieties, 12 robust markers were obtained, in addition to the 10 already identified during previous year. Thus a total of 22 useful markers found highly informative which are unambiguous, repeatable and able to distinguish each tetraploid cultivar. These markers when subjected to genetic diversity analysis (Darwin software) showed maximum dissimilarity for *G. barbadense* cultivars, Sujatha and Suvin from *G. hirsutum* varieties. Three markers found specific for *G. barbadense* varieties, Suvin and Sujatha; were further confirmed in 20 germpasm lines of *G. barbadense* (Fig. 3.7.2).

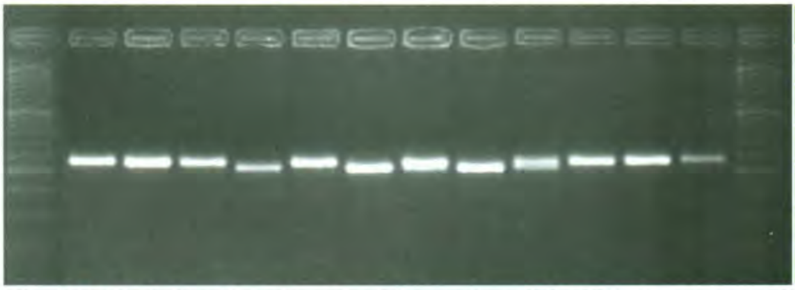
Fig. 3.7.2: DNA fp *barbadense*

Among *G. hirsutum* varieties, DHY 286, CNHO 12, Anjali and PKV Rajat were placed unique and showed maximum dissimilarity from other varieties.

For *G. arboreum*, only 20 markers were polymorphic of the 150 screened showing less genetic diversity (13%) among the varieties. Sixteen of 20 informative markers for *G. arboreum* were found robust and repeatable with PIC values ranging from 0.5-0.7. These markers were effective in distinguishing each *arboreum* variety (Fig. 3.7.3).

Fig. 3.7.3: DNA fp *arboreum*

Above varieties of both tetraploid and diploid cotton were also morphologically characterized for DUS traits. These are being maintained in perennial form in a Varietal Garden. Five CICR hybrids from RS Sirsa, were also characterized morphologically as well as at molecular level. The parents of five hybrids (4 *G. hirsutum* and 1 *G. arboreum*) and two *G. arboreum* varieties were screened using 117 SSR markers with high PIC values (as observed in initial studies). Distinctly polymorphic markers (ranged between 10 and 20) were identified among male and female parent of each of the five hybrids (Fig. 3.7.4). These would be further confirmed in respective hybrids so that a robust DNA fingerprint could be developed for these hybrids.



Lane #	Genotype	Lane #	Genotype
1	CICR2-M	7	CSHH243-M
2	CICR2-F	8	CSHH243-F
3	CSHH198-M	9	CSHH1862-M
4	CSHH198-F	10	CSHH1862-F
5	CSHH238-M	11	CICR1
6	CSHH238-F	12	CICR3

Fig. 3.7.4: Distinctly polymorphic markers identified among male and female parent of each of the hybrids

3.8 : Development of Transgenic Cotton

Development of Bt cotton varieties through marker assisted pyramiding of indigenous events

G. hirsutum cv. Coker 310 plants carrying Cry1 Ac (Tg2E-13 event) were received from Delhi University. The seedlings were raised into plants in a contained glass house facility (Fig. 3.8.1a). Six elite cotton genotypes *viz.,* AKH 081, Anjali, CISH 3178, LRA 5166, NH 615 and Suraj were also established to use them as recipients in marker assisted transgene introgression breeding. Presence of Cry1Ac gene in the event was confirmed using gene specific PCR and uniqueness of the event claimed was also confirmed through PCR using specific primers designed based on gene cassette/vector sequence (Fig. 3.8.1 b). Spatial and temporal variation in Cry toxin expression was carried out. Expression was consistent upto at 90 DAS. Leaf bioassays indicated larval mortality to range between 90-100%, 5 days after release of one day old larvae.

Crossing between donor and recipients were attempted and hybrid seed of each cross was produced in the contained glasshouse facility.

Parental polymorphism survey identified many polymorphic markers for marker assisted background selection (Fig. 3.8.1 c).

Fig. 3.8.1 (a)

Transgenic Coker310 line carrying Cry1Ac gene (Tg2E-13 event)

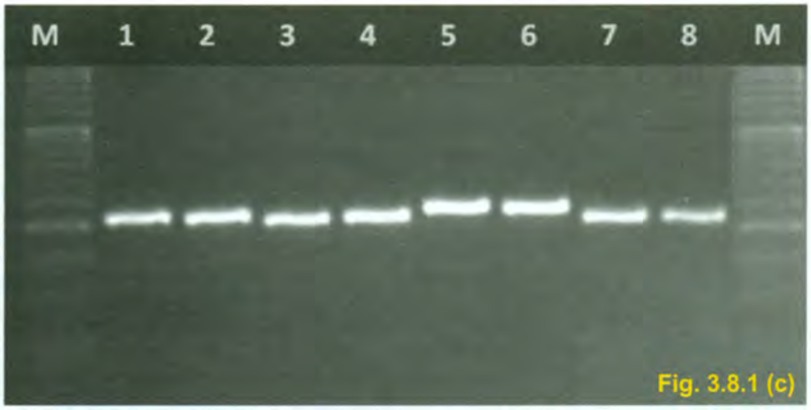


Fig. 3.8.1 (c)

Identification of polymorphic markers between donor and recipient genotype for marker assisted background selection [L: Ladder, 1:Coker310 with Cry1Ac (Tg2E-13 event), 2: AKH081, 3:Anjali, 4:CISH3178, 5: LRA5166, 6: NH615, 7: Suraj, 8: Coker310 (non-transgenic)]

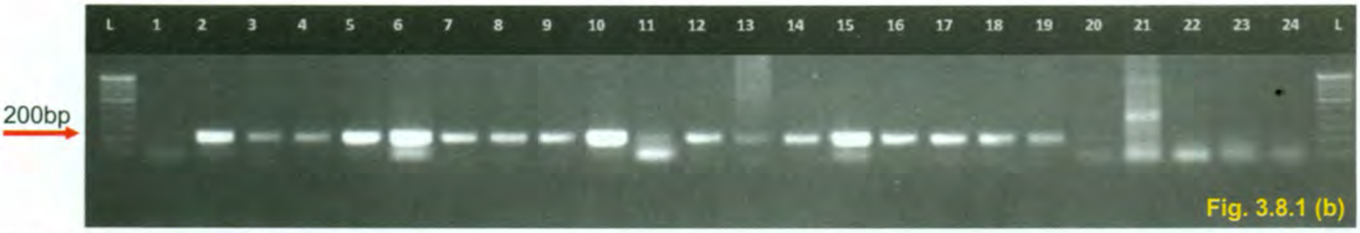


Fig. 3.8.1 (b)

PCR confirmation of Tg2E-13 events Octopine synthase terminator(ocsT) primers [L=Ladder, 1=No template control, 2-19=Tg2E-13 event samples, 20=Non transgenic sample, 21= JK Bt Sample, 22-24= Mon531 event samples]

In-planta transformation with *cry2Ab1Ac : Chitinase gene*

In the new *in-planta* transformation, two DAP flowers were selected and their corolla tube was removed exposing ovary without style. Transformation with Cry fusion gene was carried out by injecting suspension of Agrobacterium (500 µl Agrobacterium + 2 µl acetosyringone + 1 ml ¼ MS) with the help of syringe through the tip of ovary. Number of bolls set was observed and at maturity 111 open bolls were harvested.

Shoot tip meristem transformation with *cry2Ab1Ac : Chitinase gene*

For shoot tip transformation, during the year, 4020 seeds were surface sterilized and germinated under *in-vitro* conditions. Seven days germinated seedlings were used to isolate 2831 shoot tip meristem explants. These explants were inoculated for 30 min by direct shoot organogenesis and co-cultivated for four days, followed by regeneration of plants with Agrobacterium. In all, 75 plants were regenerated and established in polyhouse for screening.

Somatic embryogenesis of cotton

Hormonal regime for callus growth and proliferation of *in vitro* cultures for cotton genotypes was standardized. Based upon *in silico* studies, novel genes and transcription factor associated with cotton somatic embryogenesis were traced. Subsequent experimental studies have shown that those genes were differentially expressed in cotton callus and are being used to induce somatic embryogenesis followed by regeneration under *in vitro* culture conditions.

Development of protocol(s) for effective gene delivery into cotton

For *in vitro* transformation, complex mediated transgene delivery which was previously cloned virD2 gene from *Agrobacterium tumefactions*

LBA4404 is under expression optimization for designing *in vitro* transformation complex comprises of VirD2-SS T-DNA/ds T-DNA-recA protein for cotton transformation.

Development of transgenic cotton for cotton leaf curl virus resistance

CLCuV transgenic plants obtained by direct shoot organogenesis method were screened for the presence of the gene. Genomic DNA was isolated from the seedlings of genotypes HS 6, H 777 and F 846 with Sense coat protein (SCP), antisense coat protein (ACP), antisense replicase protein (ARep) grown in the polyhouse. The DNA template was used for PCR analysis with the gene specific primers.

F 846 with sense coat protein (SCP) 3 plants and antisense- replicase protein (ARep) 18 plants were positive. HS-6 with sense coat protein (SCP) 4 plants, antisense coat protein (ACP) 5 plants and antisense replicase protein (ARep) 1 plant were PCR positive. In H 777 with antisense coat protein (ACP), 12 plants and with antisense-replicase protein (ARep), 1 plant was PCR positive.

New events were generated in genotypes HS 6, H 777 and F 846 with Sense coat protein (SCP), antisense coat protein (ACP), and antisense replicase protein (ARep) through *Agrobacterium* - mediated transformation.

Validation of fiber strength genes using mutant variety

Lint-less mutant (MCU-5) and its counterpart wild type cotton was used to validate the role of identified candidate genes (*GhcesA1, GhcesA2, GhcesA7, Ghcobl4, and Ghfla3*) in fibre development. Relative gene expression of those genes were compared between wild and lintless mutant using leaf, square and ovules specific transcripts. The results showed that the selected candidate genes are radically expressed in wild type compared with mutant. Down regulation of gene expression in mutant fibre tissues at 10 and 20 dpa (days post anthesis) over wild type has established their role in fibre development. These results confirm our earlier selection of candidate genes based on Recombinant Inbred Lines (RILs) screening. Gene constructs for over expression and down regulation were synthesized with three potential genes such as *GhcesA1, GhcesA2,* and *Ghfla3* using plant transformation vector (pCAMBIA2300) driven by fibre specific promoter and the gene cassette was confirmed by restriction analysis. All the above genes are cloned under 990bp of *GhcesA1* promoter sequences which were chosen based on literature and bioinformatics analysis. To functionally characterize the promoter elements through deletion analysis, three gene cassettes in plant expression vector were generated by cloning 700bp, 990bp and 1.2 kb of promoter sequence in upstream of GUS reporter gene. Genetic transformation of cotton was initiated with all the above constructs for which *G. hirsutum* cultivars Suraj and Coker 310 hypocotyls calli were generated Embryogenic calli was subjected to genetic transformation with *GhcesA2* gene.

3.9 Seed Production and Technology

Seed Production

Nagpur

Around 2235 kg seeds of various categories were produced. Among these, 485 kg cotton seed (stock seed, hybrid seed, *G. arboreum* race cernuum seed etc.) was produced for further multiplication. For Gram cv. Vijay, 17.50 q of certified seed was produced for MSSC, Nagpur (Table 3.9.1).

Table 3.9.1: Seed Production

Crop	Stage	Production (kg)
Cotton -47 varieties (*G.arboreum*-31 & *G.hirsutum*-16)	TFL	274
Gram-Vijay	CS	1750
Cotton hybrid seed production	TFL	12
CICR-2 seed production	TFL	87
Germplasm (for maintenance)		33
Cernnum (for maintenance)		79
Total		**2235**

Seed cotton (15.5 q) of Suraj was obtained from seed producer and 6.87 q seed was processed for further distribution. Seed cotton of Phule Dhanwantry (10.75 q) was procured from farmers/ NGOs at the Institute and processed.

Resource of around Rs 6 lakhs was generated through the sale of these seeds or its by-products.

Sirsa

During 2015-16, 30 kg seed of female and 20 kg of male parent of CICR 2, 60 kg seed of *desi* cotton variety CISA 614 and 60 kg of variety CISA 310 were produced.

Coimbatore

Breeder seed production of Suvin, LRA 5166, Suraj, Surabhi, Sumangala and MCU-5VT was undertaken. During the year 2015-16, a total of 128 kg of breeder seed was distributed to various seed producers.

Seed Quality Improvement

Nagpur

Exogenous application of plant growth hormones and other chemicals on seed yield and quality of cotton variety Suraj was conducted during the season. The experiment included two foliar sprays at 65 and 85 days after sowing for all the treatments. Results indicated that the total seed cotton yield was significantly higher in spermine (0.1 mM) followed by putrescine (4.0 mM) (22.15 q/ha). The cotton seed yield was highest in spermine (0.1 mM) (14.0 q/ha) followed by putrescine (4.0 mM) (13.92 q/ha) and spermidine (0.1 mM) (13.74 q/ha) as compared to unsprayed control (12.31 q/ha).

Seed quality parameters indicated that the highest seed germination was in spermine (0.1 mM) followed by salicylic acid (1.0 mM). Vigour in terms of seedling length was highest in 5-sulphosalicylic acid (0.5 mM) followed by putrescine (2.0 mM) and spermine (0.5 mM). Lint quality parameters were non significant with respect to exogenous application of plant growth hormones.

Sirsa

In studies to improve the seed and boll setting efficiency in cotton, the effect of growth hormones, pollinator attractants and weather parameters were observed on boll setting and seed setting percentage. Highest boll setting of 85.5% were observed with hand pollination. Among other treatments significantly higher boll setting with CICR consortium (83.0%), NPK 2% (82.3%) were observed over control (80.0%). The seed setting efficiency (93.3%) and 3375 kg/ha yield was also higher in hand pollination.

Among the pollen attractants experiment the boll setting was 84.4% with hand pollination and 82.7% with mollases (10%) as against 80.4% in control. The seed setting was also significantly higher in hand pollinations system (93.1%) than all the attractant treatments.

The contribution of pollinators towards percent boll and seed setting in sterile parents GMS-DS-5 and GMS-16-A was estimated. In sterile plants, the boll and seed setting was 18% and 64% in GMSDS-5 and 17% and 59% in GMS-16-A than in fertile-80% and 84% in DS-5 and 85% and 91% in GMS-16-A, respectively.

Coimbatore

Effect of pulsed magnetic seed treatment on cotton seed quality enhancement

A laboratory experiment was conducted with differentially aged *viz*., 12, 24 and 36 months aged seeds of five cotton genotypes namely Sahana, Supriya, Surabhi, JLH-168 and JCC-1 which were treated with pulsed magnetic field strength of 500 nT, 750 nT and 1500 nT for five hours a day for 15 days continuously. Seeds subjected to 1500 nT (15 days), 750 nT+1500 nT (30 days), 500 nT+750 nT+1500 nT (45 days) were evaluated for seed viability, seedling vigour and biochemical status along with control seeds. The mean data on germination of 36 months stored Sahana seeds indicated a significant improvement of 22%, 22% and 29% respectively when seeds were exposed to 1500 nT, 750 nT+1500 nT and 500 nT+750 nT+1500 nT pulsed magnetic field strength, over untreated seeds. Whereas, in 24 months stored seeds the improvement was 17, 20 and 24%, respectively for the same treatments. In respect of 12 months stored seeds this was 14, 11 and 11 % respectively indicating less response to treatments as against the highly aged seeds. The seedling growth measured in terms of root length, shoot length and dry matter of seedling expressed a similar trend as that of seed germination. The seedling vigour improvement was recorded in all levels of seed treatment in 36, 24 and 12 months stored seeds, however, the rate of improvement was less in 36 months stored when compared to 24 and 12 months stored seeds. This indicated that seeds maintain vigour proportional to their period of storage. The same pattern of observation was recorded in Supriya, Surabhi, JLH-168 and JCC-1 genotypes and indicated non selective response of seeds to pulsed magnetic treatments due to genotypes.

Another experiment with seeds of ten genotypes (Sahana, Supriya, JCC 1, Abadhita, Surabhi, Pratima, JLH 168, NH 545, Narasimha, Laxmi) with initial vigour levels ranging from low to high was subject to pulsed magnetic field strength of 500 nT, 500 nT+750 nT, 500 nT+750 nT+1500 nT, 750 nT, 750 nT+1500 nT and 1500 nT. Significant improvement was observed in germination of seeds of Sahana, Supriya, JCC 1, Abadhita, Surabhi, Pratima, JLH 168 to the tune of 19,19,22,18,19,6 and 13% respectively over untreated seeds, when subjected to magnetic field strength of 500 nT for 15 days followed by at 750 nT for another 15 days (Fig. 3.9.1). The seedling growth and vigour index also expressed positive response to seed treatment with magnetic field strength of 500 nT for 15 days followed by at 750 nT for another 15 days.

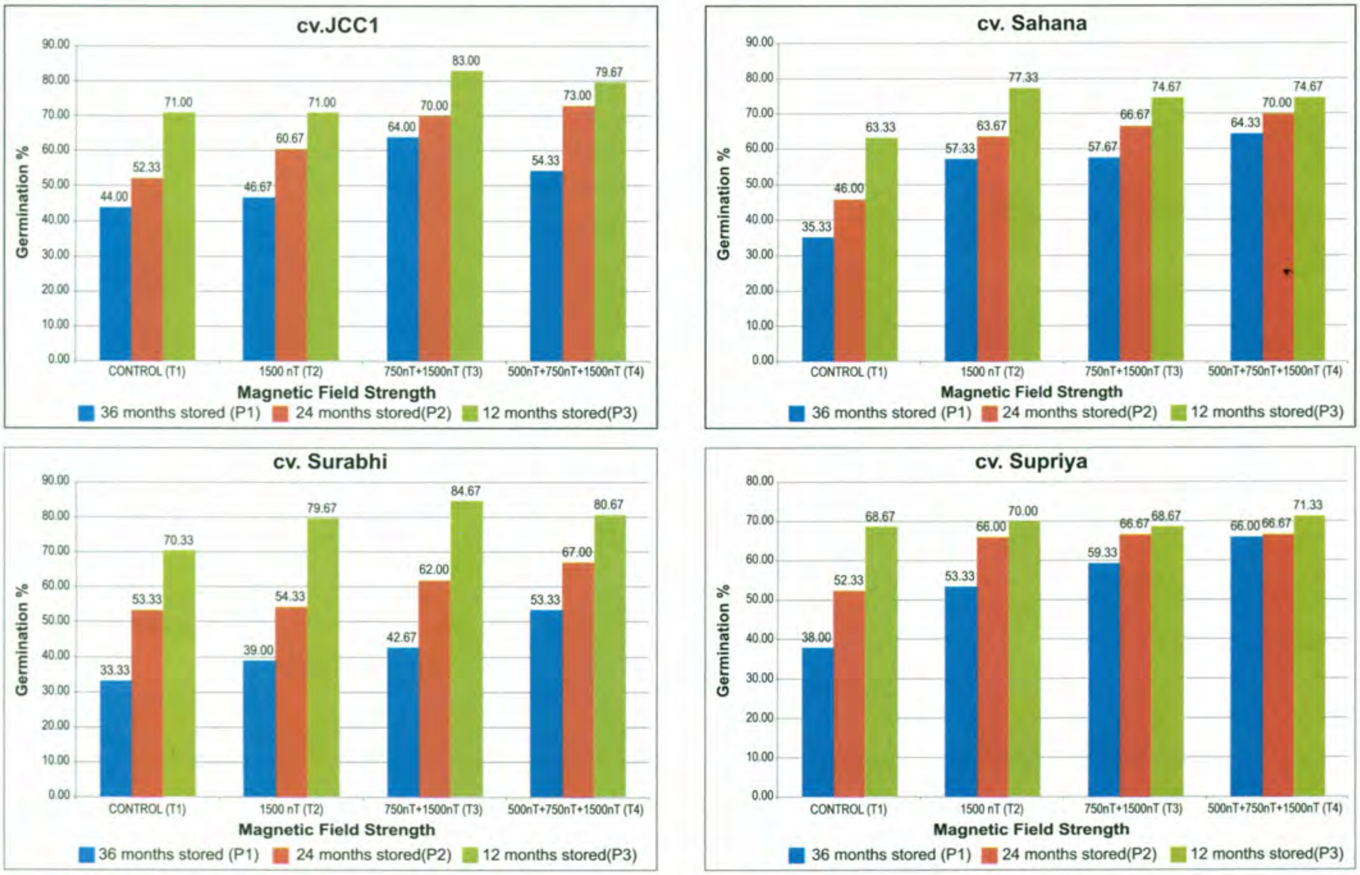


Fig. 3.9.1: Effect of pulsed magnetic seed treatment on germination of stored cotton seeds

Studies on the performance of pink pigmented facultative methylotrops (PPFM) seed treatment on germination enhancement under differential soil moisture

A pot culture experiment was conducted under ambient condition using delinted Suraj cotton seeds with an objective to find out the efficacy of seed priming with PPFM bacterial formulation and subsequent establishment of seedling under regulated soil moisture as per the formulation of TNAU. Delinted cotton seeds were primed with one percent PPFM culture formulation by taking equal volume of seed and solution. Parallely, untreated seeds were sown for all the soil moisture levels. The observation on seed germination, shoot and root length of seedling, vigour index and dry matter production of seedling was recorded. The mean data showed an improvement in seed germination of 71.5% as against 57.5 % in untreated seeds at 10% soil moisture, maintained throughout growth period. The arithmetic increase of seed germination of primed seeds was noticed up to 35% soil moisture. Sowing primed seeds in 15 to 25% soil moisture followed by watering once in two days also favoured seed germination. Though there was a marginal increase in germination over untreated seeds, when watering was done once in five days in different soil moisture levels, they were significantly low when compared to germination enhancement under continuous maintenance of soil moisture. Therefore, cotton seed priming with PPFM would be useful for taking up seed sowing even when soil moisture is sub optimal provided that the same soil moisture would be maintained till the end of germination period.

DUS testing

Nagpur

Five trials were taken up for DUS characterization which include seven Essentially Derived Varieties and their respective Initial varieties; 13 genotypes under Varieties of Common Knowledge (VCK), 23 new genotypes under first year of testing and 54 genotypes under second year of testing.

In addition to the regular phenotyping of genotypes as per DUS test guidelines, additional traits were studied for its suitability in variety characterization. These included leaf petiole length (4th leaf from top), peduncle length, marginal bract trichome density, seed fuzz percentage. Considerable variability was observed for above traits among the tested genotypes and they could be classified into high, medium and low groups. The method of leaf trichome stain was improvised and a new staining method using toluene blue was developed for a better determination of the density count.

Coimbatore

Three field trials for the establishment of Distinctness, Uniformity and Stability (DUS) of new cotton genotypes, varieties of common knowledge (VCK), farmers variety (FV) and essentially derived varieties (EDV) were conducted as per the test guidelines of tetraploid and diploid cotton. First year testing had 49 new candidate varieties of which 12 were VCK and one FV; 68 new candidate varieties of *G. hirsutum* for second year of testing trial and 19 each of EDV and Initial varieties were taken up in two environment *viz.*, unprotected and protected in third trial. Expression of characteristics of candidate varieties were compared with 60 reference varieties in the trial one and two for establishment of DUS.

One application for registration of extant cotton variety, under PPV&FR Act, 2001 was submitted through NBPGR and the database on varieties released through CVRC and available in public domain, varieties of common knowledge, farmer's varieties, etc., was updated.

In addition to above, seed multiplication and maintenance breeding of 70 cotton varieties of all four cotton species were taken up and the harvested seed cotton was preserved for future use as reference seeds.

3.10 : Nutrient Management

Nagpur

Gadget to detect N stress and correlation of leaf colour transmittance with soil / plant nutrient status

Sand culture experiments were conducted twice from December 2014 to May 2015 and from June 2015 to December 2015 with varying levels of nitrogen (N) to obtain a range of foliar leaf N deficiency symptoms. The first sand culture experiment was used to correlate nitrogen, chlorophyll and anthocyanin contents of third, fourth, fifth and eight position leaves of plants subjected to varying levels of N ranging from 0 to 100% to RGB values of leaves. Digital images were taken in transmittance mode. Good correlation was obtained between