4.1: Cotton Genetic Resources

Nagpur

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

Thirty one exotic accessions of G. *hirsutum* were procured from Uzbekistan and Hazera, Berurim and Israel through NBPGR, New Delhi.

Sourcel Country	Species	No. of Accessions	Characters
Uzbekistan	G. *hirsutum*	3	Early and highly susceptible to sucking pest, high boll weight
Israel	*G. hirsutum*	28	Early, high GOT and long capsule

A close wild relative of *Gossypium viz., Thespesia lampas* L. was established in existing wild species garden. Twenty wild species, 15 races of cultivated species and 32 synthetic polyploids were maintained in the species garden. Five new interspecific hybrids were added to the existing collection. These included Jawahar Tapi x G. *longicalyx* (F1), AKA7x G. *armourianum* (02-1), AK 8401 x G. *davidsonii* (D3-d), AK8401 x G. *trilobum* (08) and G. *davidsonii* (D3-d) x G. *arboreum* race *indicum* (A 1).

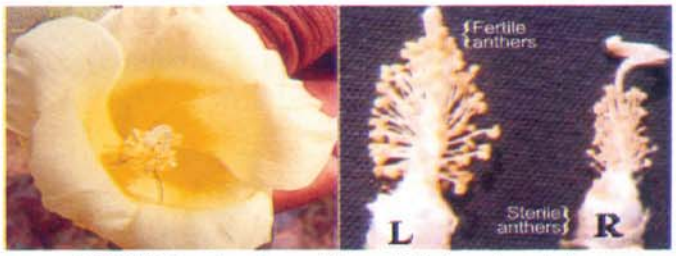
Seeds of one thousand five hundred seventeen G. *hirsutum* including 289 exotics and 350 accessions of G. *arboreum* were sent to NBPGR, New Delhi for long term cold storage, while another set of G. *hirsutum* and G. *arboreum* germ plasm was kept in Medium Term Cold Storage at CICR, Nagpur.

Twelve germ plasm lines *(Gossypium hirsutum-* race- *Latifolium-* 8 and *Gossypium arboreum* race *cernuum-* 3, *Gossypium arboreum* race- *Bengalense* -1 of unique and novel traits were added to the Gene Bank of CICR, Nagpur.

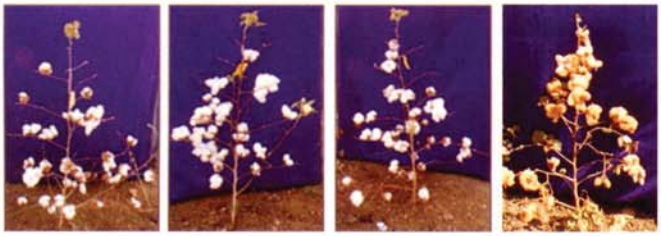
Thespesia lampas Linn. wild relative of *Gossypium*

Three genetic stocks of G. *arboreum* race *cernuum* immune to Grey Mildew *(Ramularia areola Atk)* disease *viz.,* 30814 (INGR No. 09117), 30826 (INGR No. 09118) and 30856 (INGR No. 09119) were evaluated and registered with NBPGR, New Delhi.

Eight genetic stocks were approved by Institute Germplasm Registration Committee for registration of the unique morphological traits at NBPGR, New Delhi. These included YPLL-9 (Yellow pigmented leaf lobed) G. *hirsutum,* SLL-3 (Single leaf lobed) G. *hirsutum* race *latifolium,* CINA-333, high seed cotton yielding G. *arboreum* culture, ABGMS (CSHN)-male sterile culture with curved stigma, NISC 40, 43 and 44 Jassid tolerant compact plant type introgressed genotypes suitable for organic cultivation and MSH 53 a dark brown-linted, introgressed derivative.



ABGMS line developed through induced mutation



NISC 40 NISC 43 NISC 44 MSH 53
Jassid tolerant compact introgressed *derivatives* of *G.hirsutum*

Two hundred *seventeen* accessions of *G.hirsutum* and 39 accessions of G. *arboreum* were distributed to various Cotton Research Stations/Centers of SAUs and *Government* Institutions for research purpose.

Sixty *seven* newly collected germ plasm lines of Asiatic cotton *(Gossypium arboreum-30,* G. *herbaceum-35,* G. *arboreum* perennials-2) collected from the coastal regions of Andhra Pradesh and were evaluated for yield and yield contributing characters for second *consecutive* year Table 1.

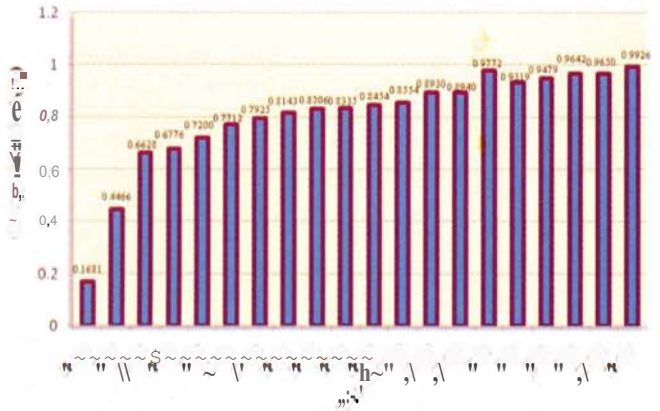
Table 1 : Evaluation of new Asiatic cottons

Species	Seed cotton yield/plant (g)	Sollwt. (g)	GOT (%)	MHL (mm)
G. *arboreum*	15.39-103.0	2.1-3.4	28.6-37.3	17.0-26.3
G. *herbaceum*	10.23-29.15	0.9-2.8	23.5-30.4	17.0-24.5

Assessment of Gossypol Content

20 G. *arboreum* germplasm lines were processed. Gossypol was extracted from the seed samples and colorimetric observations were recorded (Fig. 1). Per cent gossypol content was calculated with the help of a standard curve prepared with pure gossypol.

Fig. 1 : Seed Gossypol content(%) in *G.arboreum* germplasm lines



Germplasm lines

Molecular Characterization of cotton germplasm (Core Collection) using DNA markers

DNA fingerprinting of tetraploid cotton *(G. hirsutum)* using RAPD and ISSR markers

Twenty-four working germplasm of G, *hirsutum* (Boll weight group-4, Boll weight exotic cultivars group-2, GOT group-7, GOT exotic cultivars group-2, Mean hallow length group-4 and okra leaf group-5) were subjected to diversity analysis using RAPD and ISSR markers. Analyzed germplasm were found to form two major clusters A and B. Specificity of markers was evaluated by using 39 primers including 20 RAPD primers (OPA series) and 19 ISSR primers. Average number of bands produced per loci by RAPD and ISSR were 10-12 and 6-8 respectively. DNA fingerprinting of 24 working collections with one RAPD primer (OPA 11) and one ISSR marker (ISO 2) is presented in Figs. 2 & 3.

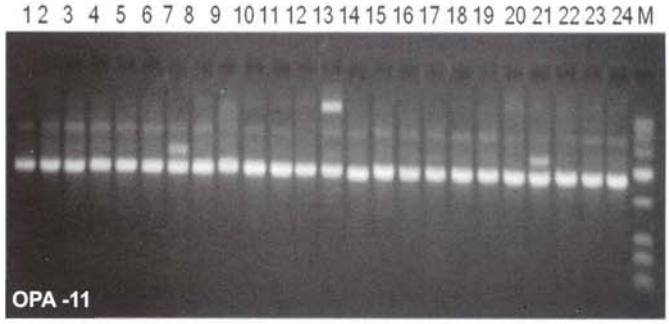


Fig. 2: RAPD profile of 24 cotton germplasm obtained with primer OPA-11, Lane 1 24 corresponds to cultivars taken in analysis. Lane 25, M = 3 kb ladder

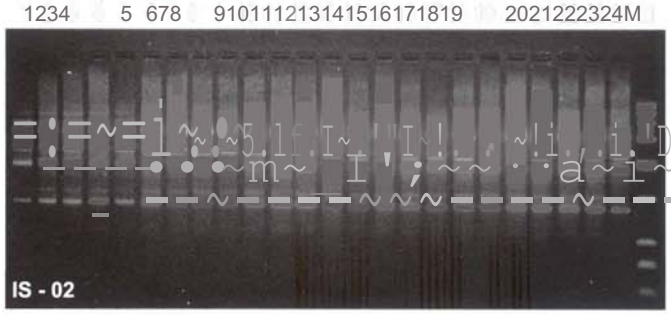


Fig. 3: ISSR profile of 24 cotton germplasm obtained with primer IS-02, Lane 1 24 corresponds to cultivars taken in analysis. Lane 25, M = 3 kb ladder.

Robustness of clustering pattern of 24 working germ plasm using RAPD markers was tested using 1000 resampling with 'Freetree software'. The UPGMA clustering pattern of 24 working germplasm using RAPD Markers showed STONEVILLE 213 from France to be the most distinct accession with bootstrap support of 100%. Rest of accessions could be grouped in two broad clusters, A and B. Cluster A consisted of 20 germ plasm with two sub clusters A 1 (19 accessions) and A2 (1 accession) with similarity coefficient 0.865. Cluster B consisted of three germplasm i. e BM Cot 167, Miscot 7913-83, RS 513, STONEVILLE 213. GP 187 and Mysore MDH 89 (both from USA) of Cluster A formed one cluster showing highest similarity of96% (Fig.4).

The UPGMA clustering pattern of 24 working germplasm using ISSR marker showed AC 241 to be the most distinct germ plasm with bootstrap support of 100%. Rest of germ plasm could be grouped in two broad clusters - A and B. Like RAPD marker, cluster A consisted of total of 22 germ plasm with two sub clusters A1 (20 accessions) and A 2 (two accessions) with similarity coefficient 0.848. Cluster B consisted of only single genotype MDH 38 sharing a similarity coefficient of 0.9004 with bootstrap support of 90%. Miscot 7913-83 and RS 513 of Cluster A formed one cluster which showed highest similarity of 97% though they are from different geographical region Sriganganagar (India) and USA, respectively (Fig. 5).

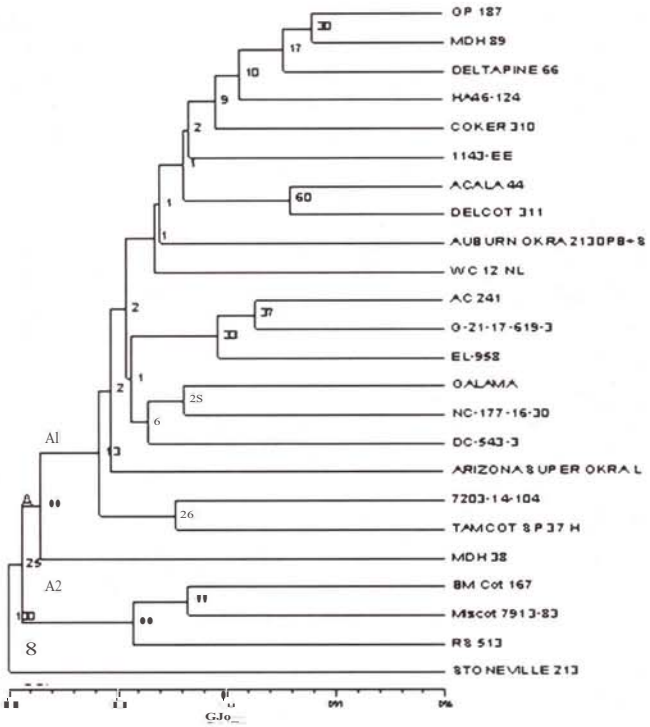


Fig. 4: UPGMAcluster analysis based dendrogram constructed from the RAPD profiles depicting genetic relationships among twenty-four working germ plasm of G. *hirsutum,* per cent bootstrap values depicted inside the figure

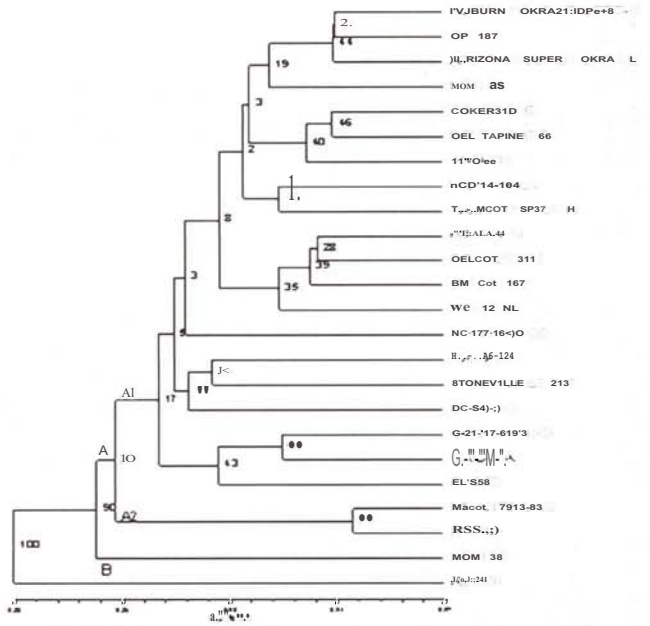


Fig. 5: UPGMAcluster analysis based dendrogram constructed from the ISSR profiles depicting genetic relationships among twenty-four working germplasm of G. *hirsutum,* per cent bootstrap values depicted inside the figure

Molecular Characterization of cotton germplasm using DNA Markers

One hundred working germplasm of G. *hirsutum* (Buri 0394, C 2686-5, Coker 417-68, EL 500, SA 117, UPA(57)-17, XAB-5X TANGUIS, (9-3X1311 C02)-1-3, 21-1-1-4-5, 65-2(5)2-3, SOBHAGYA, REBA PVT 9 (C-V), PEE DEE 0111(A), TAM COT SP37, TAMCOT SP215, TXMAROON 2-78, IRMA-323, BMCOT 95 BLL, BMCOT123(33 MLL), BMCOT 128 (182 MLL YY), DCB 348 CY, B4 EMPIRE, SAJAR 314, LAFRECOBRACT, Acala 8-1 X TamcotSP-21-1, DUNN 56 C-B, PEE DEE 4548 (A), RS 513, BMCOT 148, ACALA44, STONEVILLE 20, WC 12 NL, A 185, K 3822 (SORT 18819), MZ 561-3, PRS 72, A 72-62, AR 27, EWLS X TIDE WATER.ST, G21-17-619-3, COKER 310, NC 177-16-30, B-58-1290, M-4, MACHA, MEADE 9030 0, S 344, S 4727, X 82,5\44,21,561, ALPPO 40, Tashkent 3, ECV EARLY, SIMA-1, DC1116, DC 118, DC1120, DC1121, DC1122, CNH-36, CNH-151, CNH-152, CNH-154, CNH-1013, CNH-1020, NHBBR-38, CSH-911, ARIZONA SUPER OKRA LEAF (GREEN), AURBURN OKRA213-0PB-SPB1978, GP 187, MDH 90, TXORHU-1-78, TXORS-80, TXORSCE BO-1-79, 79-4303 P1, B56-181, B61-2038, BAR 12 18, COKER 413, GRS 60/15, KEKCHI (RED), SAENZE PENA TOBA, KW-61-276, UPA(62)31, 101-102 B, 150-3-1-1,6288, REBAB-50, BJR-JK-97-16-4, KH-113, JK-258, JK-259, JK-260, JK 344, M-1, M-7, M-15, M-18) were further subjected to molecular characterization by using STMS marker .

Twenty-eight STMS primers produced a total of 139 bands with an average of 4.96 bands per primer. Outof 139 bands 121 were found to be polymorphic, showing 86.12 per cent polymorphism. Average number of polymorphic bands per primer was 4.32. The number of DNA amplified fragment per primer ranged from 3 (JESPR 208) to 7 (MUCS 164 and BNL 2986). The average size of the fragments varied between 100-900bp in 100 germplasm when characterized using STMS marker M-04 (Fig. 6).

STMS Cluster analysis

Clustering of 100 working germ plasm lines of cotton with STMS marker M-04 is depicted in Fig. 7. Germplasm showing MZ-561-3 emerged to be the most distinct germplasm with bootstrap support of 100%. Rest of the germplasm could be grouped in two broad clusters-Cluster I and Cluster II with similarity coefficient of 0.652. Cluster I consisted of 93 germplasm whereas Cluster II consisted of six germplasm. Cluster I was further sub-divided into two sub clusters -Ia and Ib with similarity coefficient of 0.659. Subcluster Ia consisted of 86 germplasm of G. *hirsutum* whereas subcluster Ib consisted of seven germplasm. Cluster II was divided into two subclusters -Iia and Iib, with similarity coefficient of 0.662. Subcluster IIa consisted four germplasm i.e., KEKCHI, SENZE-PENA-TOBA, MACHA and S-4727 out of these KEKCHI and SENZE-PENA-TOBA from Bacterial blight resistant group showed highest similarity to each other. They were also known to share similar morphological characteristics i. e seed cotton yield (g), Ginning outturn (%), Mean Halo length (mm) and boll weight (g) etc. subcluster Iib consisted of only two germplasm i. e COKER-413 and M-1. Principal Coordinate analysis (PCA) based on genetic similarity matrices were used to visualize the genetic relationships between G. *hirsutum*. .

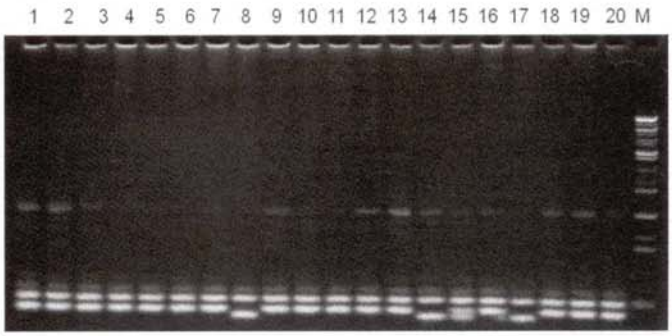


Fig.6:STMS profiling of 20 cotton working germplasm with primerM-04, M-100bp ladder

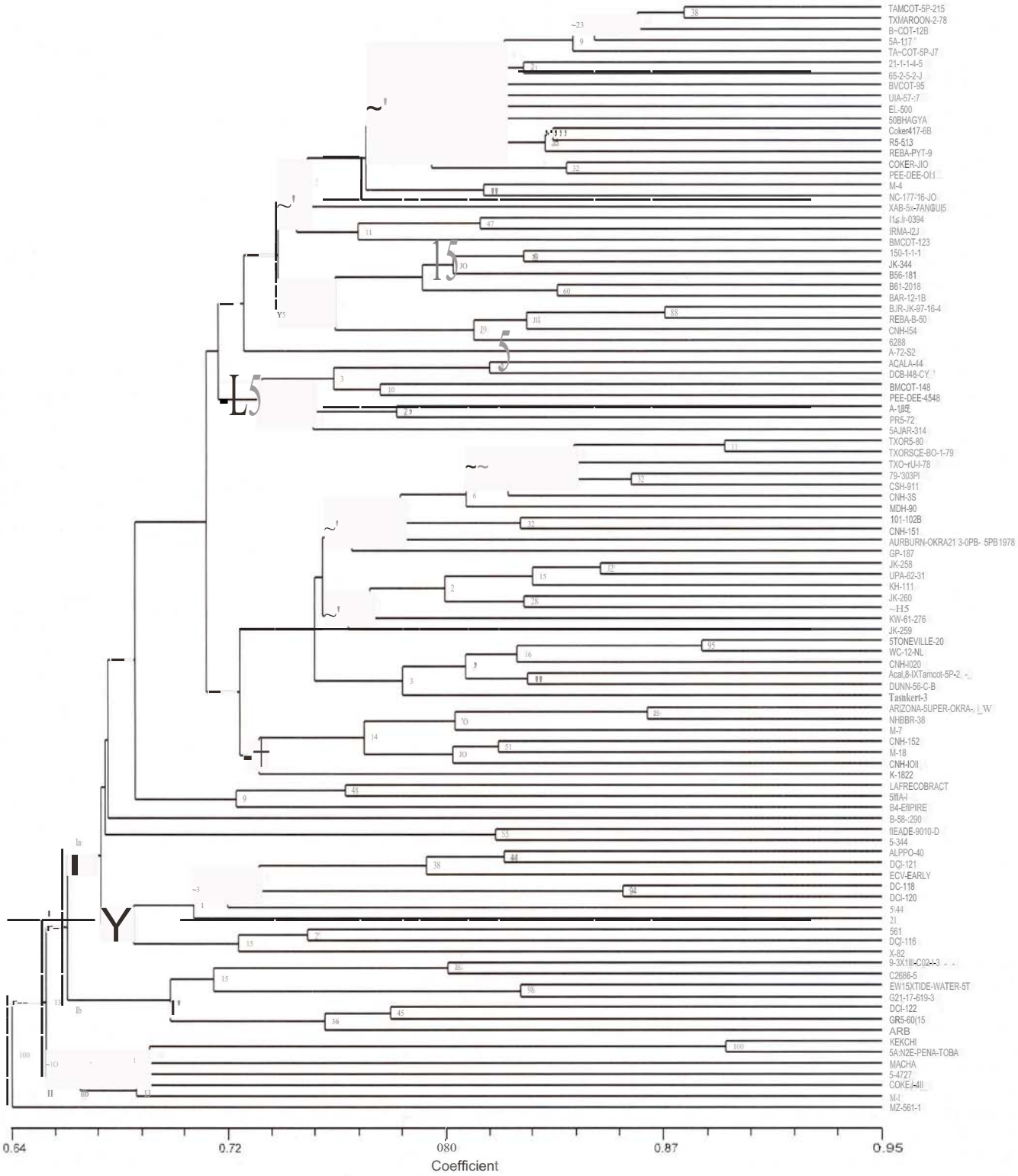


Fig.? UPGMA cluster analysisbased dendrogram constructed from the STMS profiles depicting genetic relationships among 100 working germplasm ofG. *hirsutum,* percent bootstrap values depicted inside the figure

A set of 96 core accessions were screened using the informative polymorphic markers. Combination of markers i.e.14 SSR, 17 SRAP and 15 RAPD markers (a total of 46) were employed to access genetic diversity and develop a marker profile. More informative markers are required to be used for reliably understanding the genetic diversity among the core accessions_

Coimbatore

Twenty two exotic G_*barbadense* accessions were raised in two row plots and characterized under field conditions. Seed cotton yield and fibre quality parameters of these new accessions are furnished below (Table 2).

Table 2: Performance of select exotic G. *barbadense* germ plasm accessions

Accession No.	Seed cotton Yield /Plot (g)	Ginning %	Lint Index (g)	Seed Index (g)	2.5%SI (mm)	Micronaire	Strength (g/tex)
EC 617835	485	34.5	5.7	10.8	35.6	3.7	**30.2**
EC 617837	614	22.2	2.4	8.4	26.4	4.5	24.8
EC 617838	532	34.9	7.6	**14.2**	34.5		
EC617852	275	35.5	5.5	10.0	35.3	4.5	29.1
EC 617862	500	35.4	7.0	12.8	**37.9**		
EC 617864	**1380**	**41.3**	7.4	10.5	30.1	**5.7**	22.7
Mean	601	34.4	5.8	10.9	33.0	4.4	26.4
Maximum	**1380**	**41.3**	**7.6**	**14.2**	**37.9**	**5.7**	**30.2**
Minimum	122	22.2	2.4	8.4	26.4	3.7	22.7

Note: Bold figures indicate the maximum value recorded among 22 accessions

Maintenance and evaluation of G. *barbadense* L. germplasm

One hundred sixty three G. *barbadense* germplasm lines were evaluated during 2009-2010 and good variability was noticed. There are 5 germplasm lines (ICB-67, ICB-86, ICB-218, ICB-244, ICB-273) expressing the short branching types. Thirteen G. *barbadense* L accessions (ICB-16, ICB-21, ICB-23, ICB-34, ICB-36, ICB-41, ICB-50, ICB-57, ICB-58, ICB-70, ICB-78, ICB-178, ICB-235) were early flowering types. Five accessions (ICB-41, ICB-212, ICB- 237, ICB-258, ICB- 261) had flowers without petal spot.. In boll bearing characters also, variability was observed. All short branching types bearing cluster bolls consisted of 2-6 bolls per cluster.

Seven single plant selection of G. *barbadense* were evaluated in a single row trial to assess their yield performance and fibre properties. Three best superior lines were identified (ICB-167, ICB-274, ICB-129) which possessed better yield potential and high span length than the check Suvin.

Table 3: Performance of the superior germ plasm lines

S. No	Accessions	Seed cotton yield /Plot (g)	Ginning (%)	2.5%SI (mm)	Micronaire	Strength (g/tex)
1	ICB-22	131	32	38.4	3.7	29.1
2	ICB-129	179	32	39.4	3.1	27.7
3	ICB-167	191	32	38.6	3.2	25.9
4	ICB-198	124	31	37.7	3.3	24.8
5	ICB-200	129	28	37.5	3.1	26.1
6	ICB-260	112	30	37.2	3.2	26.4
7	ICB-274	186	30	38.3	3.0	27.2
8	Suvin	97	32	36.7	3.5	28.4

Sirsa

Sixty new exotic germ plasm lines were evaluated and the superior accession for yield/ plant EC 599553 (109 g/plant), boll weight EC 599536 (3.7 g), boll number EC 599553 (33), GOT EC 599569 (37.9%), seed Index EC 599536(10.4 g) and lint Index EC 599536 (4.9 g) were observed. In addition, 288 accessions of G. *hirsutum* and 326 of G. *arboreum* available at the station were evaluated for yield parameters and reaction to diseases and pest and superior accessions were identified. The required germ plasm lines were supplied to breeders in the Institute as well as Universities of the zone.

Out of 58 *G.hirsutum* genotypes screened for salt tolerance, ten genotypes *viz;* CSH-612, KH140, RS2525, SCS451, L801 ,F2168,CNH11 04,RS2524,LH21 08,GISV218 were salt tolerant at Ec around 10. In another experiment at farmer's field out of 76 genotypes screened at 3.1 EC to 3.5 EC, twelve salt tolerant genotypes found were: BS-279, CCH-03-23, BN-TOM-277, CSH-3118,3119, F-846, RS-875,ANJALI, Pink Filament, BN-Okra, BN-TOM-277.0ut of 19 *G.arboreum* genotypes only 4 genotypes *viz;* CISA 6-214,5R, RG-18 and cernuum types were found tolerant to salt..

4.2: Hybrid Cotton

Nagpur

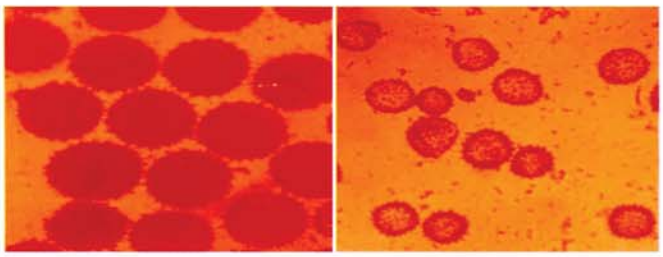
Maintenance of Male sterile lines

One hundred thirty seven CMS *(harknessii),* 15 CMS *(aridum),* 19 GMS and 57 restorer lines were maintained through crossing, sibmating and selfing. For improving the CMS and GMS lines, AK 32 CMS *(aridum),* Rajat CMS *(harknessii)* and G 67 (GMS) lines were treated with physical and chemical mutagen (gamma rays and ethyl methane sulphonate). Individual sterile plants were identified and maintained through crossing.

TGMSsystem

Sterility ofTGMS lines employed in the development of hybrids were ascertained by pollen staining. Three experimental hybrids of TGMS were evaluated. All the three experimental hybrids showed lower seed cotton yield than the released GMS hybrid of central zone. However the seed cotton yield of one experimental hybrid TGMS 9-1 X PA 255 (1789 kg/ha) was higher compared to the yields of CISA-2 (1647 kg/hal, the popular *desi* hybrid released for north zone and the seed cotton

yields of popular varieties of central zone *viz.* PA-402 (1597 kg/hal, PA-255 (1434 kg/ha) andAKA8401 (1472 kg/halo



Pollen sterility of TGMS lines
(b) ascertained with acetocarmine staining

EGMSSystem

Out of the eight EGMS lines evaluated for stability and seed cotton yield, EGMS-35 recorded the highest seed cotton yield of 2474 kg/ha followed by EGMS 08093-10R (1558 kg/ha) and EGMS 18 (1477 kg/halo Three EGMS lines namely EGMS 18, EGMS 35 and EGMS 36 were found stable showing sterility in the month of May, 2010 when the temperature was above 40 DC. Out of twenty experimental hybrids evaluated in replicated trial at Agricultural Research Station, Mudhol five hybrids were found promising for seed cotton yield.

Apomixis

Boll setting was observed in four lines namely 1060(B), AP1-4, AP 4-15 and AP 5-10 following methods of EMS and RSS. However, the percentage of boll setting was very low ranging from 1.30 %(AP 5-10) to 4.35% (1060 B). Cytological studies of these apomictic lines (IS 244-4-1, IS 244-4-2, IS 181-7-1) revealed the presence of aneuploidy. Cytologically pollen mother cell of these apomictic lines showed abnormalities in pollen development. . The triad formation was observed instead of normal tetrad at the end of meiosis. Apomictic lines, when crossed with the dominant marker Bikaneri Narma (Red), their F,s showed both normal green and pigmented plants indicating the presence of low percentage offacultative apomixis.

Coimbatore

Interspecific (G. *hirsutum* x G. *barbadense)* hybrids

In confirmatory trial, yield evaluation was done with forty-nine interspecific hybrids with the best check DCH-32. Hybrids CCHB-110 (2423 kg/hal, CCHB- 215 (2404 kg/hal, CCHB-260 (2400 kg/ha) and CCHB-123 (2391 kg/ha) performed well continuously during last three years over the check DCH-32 (1997 kg/halo The advanced hybrid CCH-110 is having better fibre properties than the other hybrids and DCH-32 (Table 4).

Pooled yield data over past three years revealed that hybrid CCHB-51 with mean yield of 2521 kg/ha was better than the check DCH-32 (1905 kg/ha) with better fibre properties. The hybrid CCHB-51 has been entered in the National trial through AICCIP.

Sirsa

Diploid Cotton

Maintenance and heterosis breeding

Eleven G. *arboreum* GMS lines were maintained through sibmating.

Table 4. Performance of identified interspecific hybrids

Hybrids	Seed cotton yield (kg/ha)	Ginning (%)	2.5% SI (mm)	Micro-naire	Strength (g/tex)
CCHB-110	2423	33	37.1	28.5	3.5
CCHB-215	2404	30	36.7	27.4	3.7
CCHB-260	2400	32	37.0	29.0	3.5
CCHB-123	2391	29	37.6	28.6	3.6
DCH-32 ©	1997	30	36.0	29.1	4.3
CD@5%	149	-	-	-	-
CV%	11.9	-	-	-	-

Performance of G. *arboreum* hybrid CISAA . 14

The GMS based hybrid CISAA 14 recorded seed cotton yield of 2773 kg/ha against local check 2617 kg/ha, ranked 4th and was retained in North Zone trial.. The hybrid was having 38.0% GOT, 2.5% span length 22.9 mm and bundle strength of 17.7 g/tex.

Performance of G. *arboreum* hybrid CISAA . 15 and CISAA . 16

The GMS based hybrids CISAA 15 and CISAA 16 gave higher seed cotton of 1595 kg/ha and 1373 kg/ha than the local check (1116 kg/ha) (Table 5). In respect of Ginning percent the hybrids were at par with the zonal and local checks. The hybrid CISAA 16 has been promoted to south zone trial for large scale testing.

Table 5: Mean Performance of G. *arboreum* hybrid CISAA . 15 & 16 (GMS based)

Entry/Character	Seed cotton yield (kg/ha)	Rank	Ginning (%)
CISAA 15	1595	3	32.3
CISAA 16	1373	6	32.6
LC	1116	14	32.7

Tetraploid Cotton

Maintenance of CMS lines

Eleven CMS lines were maintained through sib mating. Twenty two cotton restorer lines were maintained through selfing.

Performance of GMS based hybrids CSHG 1862

In the AICCIP North Zone trials, based on mean performance of last three years GMS based hybrid CSHG 1862 recorded 13 % increased seed cotton yield and 18 % for lint yield over the conventional check hybrid CSH H 198.

Performance of intra-hirsutum hybrids CSHH 3008

In the Br 05 (a) CHT AICCIP trial, intra-hirsutum hybrid CSHH 3008 recorded mean seed cotton yield of 1994 kg/ha with 33.3 percent ginning outturn and ranked at 5th position as compared to 1688 kg/ha of zonal check hybrid CSHH 198. The hybrid also recorded a higher 2.5 % span length of 27.9 mm, and bundle strength of21.8 g/tex (Table 7).

Table 7: Performance *intra-hirsutum* hybrid CSHH 3008

Sr. No.	Name of the hybrid	Seed cotton yield (Kg/ha)	Lint yield (kg/ha)	Boll weight (g)	GOT (%)	2.5 % span length (mm)	Tenacity (g/tex)	Mic. value
1.	CSHH 3008	1994 (5)	663	4.0	33.3	27.9	21.8	4.7
2	Local Checks	1856 (11)	640	3.9	34.7	27.7	23.9	4.7
3.	CSHH 198 (ZC)	1688 (19)	574	4.0	33.8	28.0	24.3	4.6
	CD	317.3	-	-	-	-	-	-
	CV(%)	11.5	-	-	-	-	-	-

4.3: Genetic Improvement

Nagpur

A.G.arboreum (diploid cotton)

In the Institute trial, CNA 1009 recorded the highest yield of 2128 kg/ha and was marginally superior to the check variety AKA 8401 (Table 7).

In the coordinated (AICCIP) trials, Culture CNA 1003 recorded the highest mean seed cotton yield of 1255 kg/ha and stood first in the South Zone trials (Table 8). Similarly, Culture CNA 1007 recorded the the second highest yield in the south zone trials (Table 9).

Table 7: Institute trial of *G.arboreum*

Sl. No.	Code	Seed cotton yield(kg/ha)	Lint yield (kg/ha)	2.5% span length (mm)	Strength (g/tex)	Micronaire
1	CNA-1008	2065	637	25.7	19.4	5.2
2	CNA-1009	2128	702	26.9	21.3	5.5
3	CNA-1010	2033	668	27.1	19.3	4.5
4	CISA-6-123	1657	543	-	-	-
5	CISA-105	1534	564	25.4	16.9	4.8
Check	AKA 8401	1937	608	25.2	19.3	4.2

Table 8: Performance of culture CNA 1003

Genotype	Dharwad	Nandyal	Mudhol	Kovilpatti	Mean (SZ)	Rank
CNA 1003	1247	2040	1069	666	1255	1
DLSa-17 (SZ)	854	1515	787	672	957	8
CD at 5%	500	134	86	226		
CV	13	23	7	20		

Table 9: Performance of culture CNA 1007

Genotype	Dharwad	Nandyal	Mudhol	Kovilpatti	Mean (SZ)	Rank
CNA 1007	967	2371	1627	617	1395	2
DLSa-17 (SZ)	843	2712	1109	765	1357	3
CD at 5%	206	517	178	280		
CV	12	15	15	13		

In G. *arboreum* station trial, among 10 entries tested, entry CNA 1009 recorded the highest seed cotton yield of 2128 kg/ha followed by CNA 1008 (2065 kg/ha) and CNA 1010 (2033 kg/ha). None of the test entry recorded significantly higher yield than the check variety AKA8401 (1937 kg/ha).

Random mating population through conventional crossing:

Gain in seed cotton yield was to the extent of 61.93% after the fifth cycle of random mating as compared to base population.

GMS based random mating population

The out-crossed bolls obtained from the second cycle of GMS-RM were bulk harvested, and this composite population was grown. At flowering, the individual plants in the population was checked for sterility / fertility at anthesis at an interval of a week and all sterile plants were tagged. Open pollination was allowed in the population. The out crossed bolls from all the sterile plants were bulk harvested. A fourth cycle of GMS based random mating has been completed.

Sirsa

Release and Notification of CISA-310

Gossypium arboreum variety CISA 310 was released for commercial cultivation in the North Zone states. The new variety recorded a mean seed cotton yield of 21.7 q/ha as against 19.7 q/ha of RG 8 (Zonal Check) in the variousAICCIP trials conducted during 2000-2004. The variety CISA 310 recorded a mean ginning outturn of 36.5 per cent which was higher than that of zonal check varieties. Variety CISA 310 has been notified vide Gazette of India NO.171 dated January, 2010.

Gossypium arboreum variety CISA 310

Notification of Variety CISA 614

Gossypium arboreum variety CISA 614 has recorded an overall mean seed cotton yield of 22.04 q/ha as against 18.34 q/ha of HD 123 (zonal check) and 19.90 q/ha of local checks in the

various AICCIP trials conducted during 2004-2007. The increase in seed cotton yield of the new variety CISA 614 *over* the common (Zonal check) check was of the order 20.17 per cent.. It was identified by Variety Identification Committee Meeting (AICCIP) held atANGRAU, Hyderabad 6-8April, 2009 and notified vide Gazette of India NO.608 dated April 1,2010.

Gossypium arboreum variety CISA 614

Evaluation of promising varieties

In the station trial CISA 6 (1677 kg/ha) was superior to the common check PA 255 (1235 kg/halo The genotype CISA 6 has a 2.5% span length 25.1 mm ,micronaire of 5.5 and strength of 18.6g/tex.

Tetraploid Cotton Improvement

Nagpur

Drought tolerance

Using Drought Tolerant Efficiency (DTE) based on the performance under rainfed and irrigated condition, the hybrid combinations were evaluated. Pusa 56-4 x 30 I recorded highest DTE of 76.08 per cent., 301 x Pusa 56-4 recorded 67.07 % and Pusa 56-4 x 29 I 66.81 %. Hence prediction based on high DTE will indicate a good measure of tolerance for drought and there is a likelihood of arriving at good drought tolerant genotype. Evaluation of advance generation of sixty nine SPS grown in two separate trials under rainfed condition revealed significant differences between the genotypes for seed cotton yield which ranged from 505.32 to 1534.31 kg/ha as inferred from statistical analysis. DTS 43-09 was the best selection recording 1534.31 kg/ha SCY followed by DTS 83-09, DTS 90-09, DTS 79-09 and DTS 86-09. DTS 83-09 and DTS 69-09 which were among the top ten performers for two years. DTS 76-09 and DTS 77-09 possessed good seed cotton yield ,fibre strength and strength to length ratio *above* 0.80. In another trial, 17 SPS were tested and DTS 31 (32) 13 wa~ identified as the best followed by DTS 31 (32) 07, DTS 1 (12) 11 and DTS 40 (38) 05.

Sixty three breeding lines (SPS) were tested for drought tolerance based on positive chlorophyll value, negative membrane stability value, range of reducing sugar 50-120 mg/g of FW, amino acid content 50-90 mg/g of FW and less of phenols (5.1 to 5.4 mg/g of FW). Twenty nine lines were found to *have* better adoption to stress in respect to yield compensation during stress. But when compared with check variety LRA5166 only six lines were superior. When the holistic approach *viz.,* physiological, biochemical and yield per se was analysed four lines DTS 155-09, DTS 108-09, DTS 100-09 and DTS 104-09 were found to be tolerant and suitable for high yield.

Jassid tolerance

Out of the 172 single plant selections made in both F_2 intercross and intra-cross population, based on earliness, tolerance to jassids and yield potential, 45 single plants were identified for further studies. Nine inter-specific derivatives (rai derivatives) were crossed with six cultivated genotypes of *G.hirsutum* resulting in 54 F,s. 25 F,s were selected based on earliness and tolerance to jassids and yield potential.. Crosses NISC 261 x AKH 081, NUSC 289 x EC 277959, NISC 306 x PKV Rajat and NISC 291 x H 1252 were found promising based on seed cotton yield. The crosses PKV Rajat x MDR 8, AK 32 x FQ 9, DHY 286 x CPT 426, JK 04 x MDR 8 and FQ 9 x MDR 8 were observed tolerant to jassids.

Oil Improvement

Fourteen advanced cultures were evaluated for seed cotton yield and oil content.. The culture CNHO 21 recorded the highest seed oil content (20.25 %) followed by culture CNHO 675 (19.95 %) and CNHO 5 (19.41 %). 80 single plant selections were made in F_3 progenies based on earliness, yield potential and fibre properties.

Gossypium hirsutum **variety, CNHO 12** has been identified for release in the central zone under irrigated conditions. The variety is characterized by its dwarf stature, early maturity (160-165 days), medium to high seed oil content of 21.8% with synchronous boll bursting, attributes suitable for central zone. The variety recorded higher strength/length ratio of 0.85 in comparison to zonal check LRK 5166 (0.80).

Gossypium hirsutum variety, CNHO 12

Genetic Enhancement

In advanced F. generation a row bulk populations with a pedigree Reba *pvt* 91yy x MCU 5 (1958 kg/ha) *gave* the highest yield as compared to check LRK 516 (720 kg/halo In advanced BC3F4 of Rajat x (Rajat x Rex) there was an increase of GOT % upto 40%. CIHS 18 has been entered in AICCIP trial (Br 02(b)) trial for 20 10-11.

Twenty nine G. *hirsutum* entries were tested in the institute trial.. Entry CISH 18 recorded the highest seed cotton yield of 1954 kg/ha followed by C 1068 (1953 kg/ha) and CNH 1106 (1812 kg/halo The seed cotton yield of the best check variety LRK 516 was 644 kg/ha.

Random mating population through conventional crossing

Gain in seed cotton yield was to the extent of 55.3% after the fifth cycle of random mating when compared to the base population.

GMS based random mating population

In the previous year, out-crossed bolls obtained from tagged sterile plants were bulk harvested and a composite population and planted. 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 sterile plants. 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. A third cycle of GMS based random mating has been completed.

Coimbatore

In the station trial of the eleven elite bulks evaluated, the highest seed cotton yield of 1393 kg/ha was recorded in MM 02-11-7 Bk, which was significantly higher than the best check variety Sumangala (1013 kg/ha). Among the eleven stable long staple cultures were evaluated, the highest seed cotton yield of 1243 kg/ha was recorded in the culture MM 03-27-5-1 as against 817 kg/ha recorded in Surabhi. Among the 18 stable introgression lines evaluated, the highest seed cotton yield was recorded in MM-04-29-515 with 2086 kg/ha as against 1300 kg/ha yield recorded in Sumangala, the best check variety.

Thirteen medium staple cultures were evaluated in a replicated trial with two check varieties for two years. A perusal of two years data indicated that CCH 815 and CCH 816 were superior to the check variety Suraj by 25 to 28 per cent.,

Medium Staple culture CCH 2623 was tested in the Preliminary Varietal Trial in both South and Central Zones. As in the previous year, CCH 2623, during the current year also, recorded the second highest yield of 1834 kg/ha in South Zone. By virtue of its higher ginning out turn of 38.1 per cent, it occupied the first rank in lint yield. In the Central Zone, culture CCH 2623 recorded the highest yield for the second year in succession. It recorded a mean seed cotton yield of 1932 kg/ha. A perusal of two years AICCIP data indicated that the culture CCH 2623 was superior to the respective zonal checks in both the zones by 31 to 52 per cent and was also superior to the local checks (Table 10).

Table 10: Combined analysis of culture CCH 2623 in AICCIP trials for seed cotton yield (kg/ha) and fiber quality

Culture	Central Zone			South Zone		
	2008-09	2009-10	Mean	2008-09	2009-10	Mean
CCH 2623	1995 (1)	1932 (1)	1964 (+52%)	1911 (2)	1834 (2)	1873 (+31%)
Zonal Check	1226 (17)	1360 (8)	1293	1537(31)	1333 (8)	1435
Local Check	1610 (16)	1745(6)	1678	1758 (10)	1289 (10)	1524
Culture	Central Zone			South Zone		
	2.5 % SL (mm)	Micronaire	Strength (g/tex)	2.5 % SL (mm)	Strength (g/tex)	Micronaire
CCH 2623	28.0	4.4	23.2	25.6	21.3	4.8
Zonal Check	32.7	4.5	23.9	32.0	24.5	4.3
Local Check	27.4	5.2	21.4	27.9	21.7	4.5

Development of early duration, compact genotype

Twelve compact genotypes were evaluated for the third year in succession. Culture HCT 8 recorded the highest yield of 3664 kg/ha followed by HCT 12 with 3355 kg/ha. HCT 7 recorded the highest ginning out turn of 39.7 per cent.. The best check Anjali recorded a mean seed cotton yield of 2692 kg/ha. A perusal of three years data also indicated the superiority of HCT 8 and HCT 12 by over 20 per cent as compared to the best check variety Anjali (Table 11).

Table 11: Combined analysis of promising compact genotypes

Sl.No	Entry	Seed cotton yield (kg/ha)			Mean Seed cotton yield(kg/ha)	% inc. over Anjali
		2007-08	2008-09	2009-10		
1	HCT-8	3751	2292	3664	3236	23
2	HCT-12	-	2066	3355	2711	21
3	HCT-6	4324	1852	2675	2950	12
4	HCT-9	3555	2027	3227	2936	12
5	Anjali©	3392	1788	2692	2624	0
6	MCU7©	3349	1524	2683	2519	-4
	CD@5%		414	570		

Development of long and extra long staple *G.hirsutum* cotton

Thirteen long and extra long staple cultures were evaluated in a replicated trial with Surabhi as check for two years. Culture CCH 818 with a mean seed cotton yield of 1957 kg/ha was superior to Surabhi by 30 percent, followed by CCH 820 with 1935 kg/ha (Table 12).

Table 12: Performance of promising long staple cultures in Station Trial

Culture	2008-09	2009-10	Mean seed cotton yield (kg/ha)	% inc. over Surabhi	2.5% SL (mm)	Micronaire	Strength *(g/tex)*
CCH 818	2329	1586	1957	30	30.2	4.0	25.6
CCH 820	1908	1962	1935	29	31.8	3.8	23.0
CCH 819	2008	1635	1822	21	30.5	3.9	21.7
CCH 822	1870	1699	1784	19	32.2	3.6	25.2
SURABHI©	1540	1460	1500	0	31.6	3.5	22.0
CD@5%	360	280					

Sirsa

G. *hirsutum* culture CSH-3158 and CSH 10 were superior to the check varieties in the Initial Evaluation Trials. (Table 13).

Table 13: Performance of G. *hirsutum* culture CSH 10 in Sr 02(a) PHT Trial

Sr. No.	Name of the hybrid	Seed cotton yield (kg/ha)	Lint yield (kg/ha)	GOT (%)	2.5 % span length (mm)	Mic. Value	Tenacity *(g/tex)*
1.	CSH-3158	2616 (6)	893	34.1	27.6	4.7	21.6
2.	CSH10	2585(8)	936	36.1	27.7	4.0	20.9
3.	Local Check	2432 (10)	869	35.0	25.3	4.2	20.0
4.	RS 2013 (ZC)	2307 (12)	767	33.5	27.4	4.0	20.4
	CD at 0.05	292.7	-	-	-	-	-
	CV(%)	10.4	-	-	-	-	-

Similarly, CSH-3129 ranked second(2572 kg/ha) against zonal check(2338 kg/ha) in the North Zone Preliminary varietal Trial (Table 14).

Table 14:Mean performance of CSH-3129

Sr. No.	Name of the hybrid	Seed cotton yield (kg/ha)	Lint yield (kg/ha)	GOT (%)	2.5 % span length (mm)	Mic. value	Tenacity *(g/tex)*
1.	CSH-3129	2572 (2)	914	35.6	30.4	4.5	22.6
2	Local Checks	2437 (7)	881	36.1	26.4	5.2	19.9
3	Zonal check RS 2013	2338 (9)	808	34.93	25.4	4.5	20.6
	CD at 0.05	392.1	-	-	-	-	-
	CV(%)	11.3	-	-	-	-	-

Evaluation of CLCuV resistant cultures

Coimbatore

Forty five CLCuV resistant cultures were evaluated for yield and other parametes. CSH 2838 (2745 kg/ha) and CSH 2827 (2429 kg/ha) as against the check variety RS 2013 (2070 kg/ha) in the first trial and CSH 2912 (2353 kg/ha) and CSH 2907 (1993 kg/ha) as against the conventional check variety RS 2013 (1765 kg/ha) in the second trial were found to be superior in yield.

4.4: Genetic Diversity through Introgression

Nagpur

Utilization of wild specIss, synthetic polyploid.

Established 126 F_2, four BC, plants and phenotyping was done for identification of genetic markers and characterization of fibre strength and drought tolerance traits in interspecific cross between G. *herbaceum* and G. *anomalum.Using* cultivated diploid species as female successful crosses were made with G. *anomalum* and G. *davidsonii* species for diversification of male sterility.

Crosses were made between cultivated and wild species (G. *hirsutum* x G. *aridum* and G. *hirsutum* x G. *armourianum).* The seeds of resultant F_1 hybrids were treated with 1 % colchicine for 24 hours. In seed treatment, 3 out of 7 seedlings showed successful polyploidisation.

4.5: State Multi Varietal Trial (SMVT)

A State Multilocation Varietal Trial (SMVT) consisting of 21 genotypes of G. *arboreum* and 12 of G. *hirsutum* was conducted at CICR, Nagpur. The promising G. *arboreum* genotypes were JLA 705, JLA 794 and CAN 1008 and the promising G. *hirsutum* varities wereAKH 9916 and NH 634.

4.6: Molecular Breeding

Nagpur

Diploid Cotton

For developing a framework linkage map in diploid cotton, interspecific F_2 mapping population involving G. *arboreum* (A2) Cv. KWAN-3 and G. *herbaceum* (A 1) Cv. Jaydhar is being used.

Phenotyping of F_2 mapping population and F_2 derived F_3 plant progenies for quality traits have been completed. The data on fibre quality traits and ginning outturn for F_2 population and F_2 derived F_3 progeneies showed significant variation for important fibre quality traits. The frequency distribution for ginning outturn, fibre length, maturity ratio and fibre fineness was normal, indicating the traits under consideration are polygenic controlled by QTLs. Extraction of genomic DNA of parental genotypes and individual F_2 plants in the population was done using standard CTAB method.

Polymorphism survey using 408 SSR, 238 pair combinations of SRAP, 283 combinations of TRAP markers was carried out during 2009-10 and identified 53 SSR, 9 SRAP and 40 TRAP polymorphic markers. So far, we have identified 101 SSR, 9 SRAP and 40 TRAP and 20 RAPD markers, a total of 170 (12.89%) markers (from a total of 1319 markers screened) were polymorphic. Survey for additional polymorphic markers is in progress. For genotyping, we are using 190 F_2 plants and genotyping with 60 informative SSR, 9 SRAP and 1 RAPD (a total of 70 markers) has been completed. Genotyping of F_2 mapping population with identified informative markers is in progress.

Transgressive segregants with combination of quality characters have been identified in F_2 and F_3 population. Efforts will be made to stabilize the progenies in advanced generation.

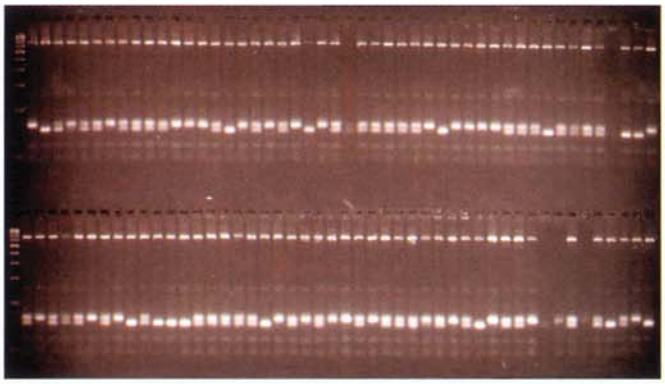


Fig. 8: Genotyping of F_2 (G *arboreum* x G. *herbaceum)* mapping population using NAU 758 SSR marker

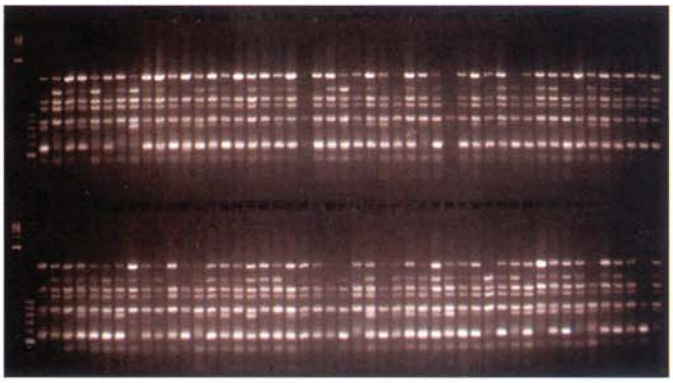


Fig. 9: Genotyping of F_2 (G *arboreum* x G. *herbaceum)* mapping population using OPE 12 RAPD marker

Transgressive segregants with combination of quality characters have been identified in F_2 and F_3 population. Efforts will be made to stabilize the progenies in advanced generation.

Upland Cotton *(Gossypium hirsutum* L.)

Development of framework linkage map in tetraploid cotton was carried out under the FAST TRACK Scheme of DST. An intraspecific F_2 mapping population developed involving G. *hirsutum* (AD1) cv. EL 958 and UPA 57-17 was used. Genomic DNA extraction from parental genotypes and individual F_2 plants extracted using CTAB method. Survey for the informative markers has been carried out using 408 SSR, 238 pair combinations of SRAP and 283 pair combinations of TRAP markers. From the total of 921 markers surveyed for parental polymorphism 94 (10.21 %). i.e. 50 SSR, 20 SRAP and 24 TRAP markers, were found to be polymorphic. Survey/ screening for more informative markers is in progress. Genotyping of F_2 mapping population (190 plants) are being used for extensive genotyp ng. So far we have employed about 50 polymorphic markers to the mapping population. Genotyping with more informative markers is in progress.

Development of permanent mapping populations (RILs) for fibre quality traits In diploid and upland cotton

Recombinant inbred lines (RILs), permanent mapping populations for fibre quality are being developed using the F_2 mapping populations developed for construction of a linkage map for diploids and tetraploids cotton. In diploids, 193 F_8 plant progenies WEre raised. One random plant in each progeny was selfed which shall be carried forward to F_6 as boll to row progenies in 2010-11. In G. *hirsutum* a set of 273 progenies were grown in boll to row fashion, single random plants from each progeny was selfed and further those shall be carried forwardtoF$_6$ generationin2010-11.

4.7: Development of Trans genies

Nagpur

Targeted Gene integration- Transgenic cotton

Bollworm resistant transgenic cotton in tetraploid and diploid cotton

The advanced T_4 generation single event G. *hirsutum* cultivar Anjali (LRK-516) carrying *cry1Ac* was raised under contained condition. Plants showing uniformly high concentrations of Cry protein expression were selfed, rouging out the segregating non-Bt plants. CRY protein expression to the extent of 2.70 ppm was recorded. In case of diploid cotton, 848 plants of *Gossypium arboreum* cv. RG 8 (T_6), PA 255 (T_4) and PA 402 (T_3) containing cryl *Ac* (Fig. 9) and *cry IAa3* genes, expression of CRY proteins was determined by ELISA.. Bt protein in boll to row progeny of 253 plants of RG 8 ranged from 2.54 to 4.6 ppm. Boll to row progeny of PA 255 culture 366 showed protein up to 3 ppm. In other promising cultures *viz.,* 355, 358 and 365, the protein concentration ranged from 2-3 ppm.

G.hirsutum cv. Anjali Bt (ILK Bt- 77) carrying Bt *cry1Ac* gene under green house

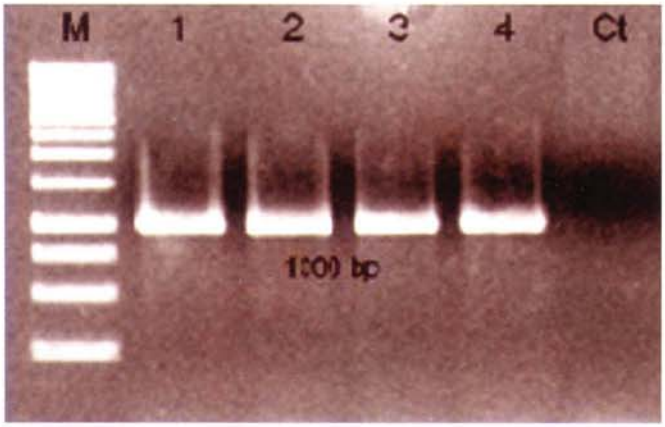


Fig.10: PCR detection of *cry1Ac* in diploid cotton. Lane *1- cry IAc (* PA 255, plant no. 43); 2- *crylAc* (RG 8 plant no, 306; 3- Bunny Bt, 4- positive control (Agrobacterium containing *crylAc* gene); Ct- negative control; M- 1kb ladder

The new transformation events generated during the previous year were characterized. Among 18 putative transformants, one plant (LRK-516) showed single copy gene *(cry1Ac)* integration. Embryonic explants of elite *Gossypium hirsutum* cultivarsAnjali and LRA-5166 were also subjected to transformation with *cry1 F*. Southern hybridization of 7 transformants (4 Anjali and 3 LRA5166 plants) showed single copy integration of *cry1F* gene in only oneAnjali cotton (Fig. 11).

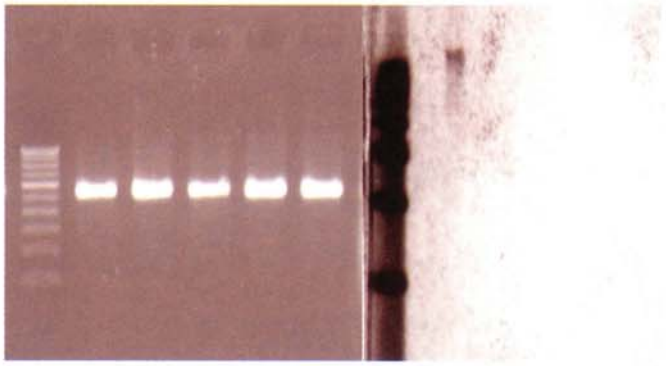


Fig. 11: a. PCR:. G. *hirsutum cv.* LRK-516 carrying Bt *cry1* F ; b. Southern Blot:. G, *hirsutum cv.* LRK-516 carrying single copy of *cry1 F* gene

G. *hirsutum* variety *viz.* LRA 5166 were taken for Co-cultivation with indigenously synthesized gene *crylAa3, crylla5* and *crylF*.. Total 600 explants with gene *cry1 Aa3,* 360 explants with *cry11a5* and 450 explants with *cry* 1*F* were used for transformation. With *crylF* construct, 50 putative transformants, in *cry1la5,* 25 putative transformants and in *cry I Aa3, 20* putative transformants were obtained.

Characterization of new transformed events

New transformation events were generated with G. *hirsutum* cultivars *viz.*, Anjali and LRA 5166 with *cry1Ac* gene. Total 2076 embryonic explants were subjected to Agrobacterium mediated transformation and 11 primary putative transformants were selected in the kanamycin medium. Five *cry1Ac* transformed plants (3-Anjali and 2 LRA) showed integration of gene when checked by PCR.. Southern hybridization of 5 plants showed single copy gene integration in two plants. Southern positive To plants were hardened and established in the soilrite. In case of diploid G. *arboreum,* 26 new events (To) containing *crylAa3* and *crylAc* gene were established in cultivars PA 255 and PA402.

Pollen tube pathway transformation

A new method of gene transfer was standardized to overcome limitations associated with recalcitrant, genotype dependent somatic embryogenesis and tissue culture protocols. A number of parameters were standardized. The characteristics of the *cry1 F* transformants developed through this novel approach, including transformation frequency, transgene expression, copy number integration, stability of gene integration, etc. were studied.The superiority of the new method will be re-established before filing a patent on the protocol..

Genetic Engineering for Abiotic Stress Tolerance in crops and identification of new genes for high water use efficiency

Elite cultivars *viz.,* LRA 5166 and LRK-516 were subjected to genetic transformation with PATRD 29A::AtCBF31 DREB1A+PoSMYB02::AtAVP1gene construct by *Agrobacterium* mediated transformation. Putative transformants selected in the antibiotic medium were tested for the presence of transgene by PCR confirmation. The transformation frequency of 0.88% in LRA5166 and 0.73 % in LRK 516 was recorded.

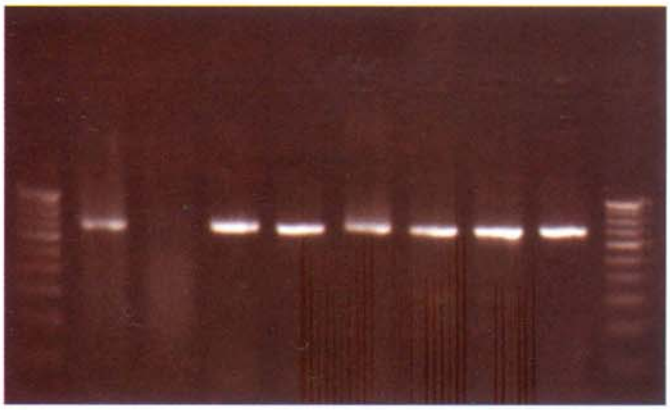


Figure 12:PCR detection of DREB1a positive plants

Development of disease resistant transgenic cotton

Cloning of Chitinase genes for fungal resistance

Chitinases are known to hydrolyse chitin polymers and are effective against fungi having chitin content in their cell walls. Chitinases belong to group of PR proteins that constitute the second line of plant defense. Using conserved and degenerate primers, a 1.3 kb full length novel class I chitinase gene was amplified and cloned from *Gossypium hirsutum* variety LRA5166. Analysis of sequence revealed the gene to be unique to upland cotton (GenBank # HM 125506). The gene-specific primers failed to amplify the sequence from G. *arboreum* lines including cultivar PA255, PA402 and RG-8 (Fig. 13).

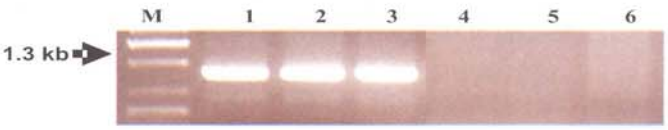


Fig. 13: Amplification of a unique class I chitinase gene from G. *hirsutum* (lanes 1-3) and absent in G. *arboreum* ~anes 4-6)

Diploid cottons G. *arboreum* (4-6) did not possess the same. The forward and reverse primers were engineered with EcoRI sites flanking the intitiation and termination codon respectively. Using them the 1.3 kb chitinase gene flanked with EcoRI sites were amplified and cloned in plasmid pGemT (3.0 kb). The

recombinant plasmid was subcloned in the binary vector pBInAR (Fig.14 a&b) and transformed in *Agrobacterium tumefaciens* strain EHA 105, by triparental mating.

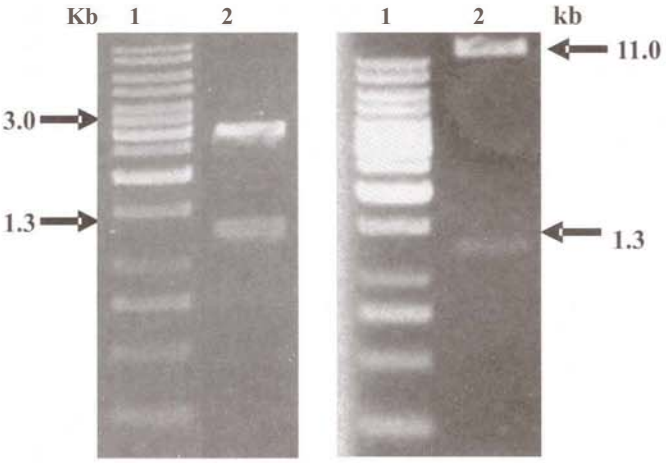


Fig.14: Chitinase gene cloned in pGEM-T (a) and sub cloned in pBIN-AR19 (b). The resultant plasm ids were checked by digestion with AatII and *PstI* in the former and EcoRI in the latter

Transformation and characterization of putative transformants

The grey mildew susceptible G. *arboreum* cv. PA255 was transformed with chitinase gene by direct shoot organogenesis. Putative transformants were selected on MS supplemented with 50 IJg/ ml Kanamycin. Integration of transgene was confirmed by PCR amplification using gene-specific primers (Fig 15). **The** genomic DNA isolated from the putative transformants (To) showed presence of 1.3 kb gene which was not present in the wild type PA255.

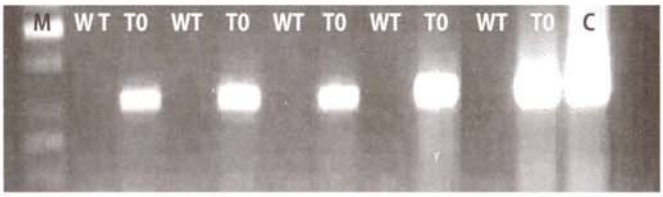


Fig.15: Putative chitinase transformants of G. *arboreum* cv. PA255 were tested for the presence of transgene by PCR.. WT, wild type cotton; C, positive control

Southern hybridization of genomic DNA extracted from two putative transformants, with DIG labeled chitinase gene as DNA probe showed insertion of the transgene in both of them (Fig. 16). The transgenic plants are being characterized forthe novel gene product..

Development of transgenic cotton for Cotton Leaf Curl Virus resistance

Antisense approach

Southern hybridisation of CLCuV transgenic G. *hirsutum* HS6 (T,) plants showed integration of the Sense coat protein, antisense coat protein and antisense Rep gene. Three transgenic events one each involving ACP and ARep in HS6 and SCP in H777 proved tolerant against CLCuV when tested in CICR Regional Station at Sirsa with viruliferous whitefly. Fresh transformation events were generated with each of the three gene constructs. The putative transformants were characterized for the presence of genes using gene specific primers. Further characterization of the T, and To plants is in progress.

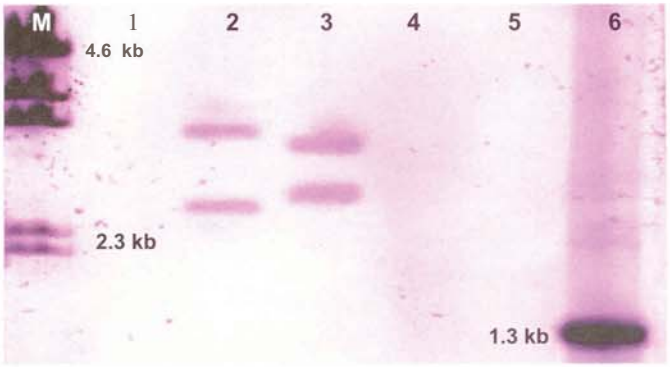


Fig. 16: Southern hybridization of two putative transformants of G. *arboreum* cv PA255 using chitinase gene as DNA probe. Lanes: 2-3, Transformants 1 and 2; lane 6, chitinase gene (positive control); M, AI HindIII

Two (HS 6 ACP and H 777 - SCP) of the three events (T,) showing resistance against CLCuVs

RNA interference Approach

Based upon the conserved sequences, five sets of primers specific to five different target regions of DNA A and ~ DNA components of CLCuV genes were designed. The restriction sites were so designed that each amplified sequence can be cloned in two orientations, on either side of a stuffer fragment in the inverted repeat generating plasm ids, pBSK-Int (3.1 kb).

Construction of inverted repeat constructs of CLCuV target sequences

The sense and antisense stands of DNA-A *viz.* AC2 (150 bp), CP (185 bp), MP (109 bp) and ~-DNAviz., ~C1 (212 bp) and ~V4 (177 bp) were cloned individually on either sides of the intron sequence in plasmid pBSK-int creating inverted repeat constructs pBSK-int-AC2-SA (3.3 kb), pBSK-int-CP-SA (3.4 kb), pBSK-int-MP-SA (3.2 kb), pBSK-int-~C1-SA (3.4 kb) and pBSK-int-~V4-SAof3.4 . kb (Fig.17 and 18 a-e).

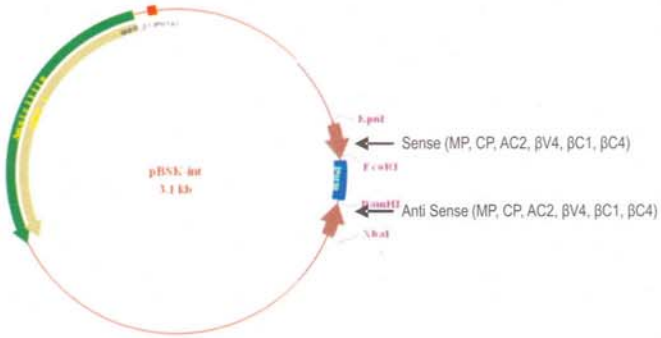


Fig.17: Inverted repeat plasmid pBSK-Int (3.1 kb) with sense and antisense strands of 6 viral target sequences

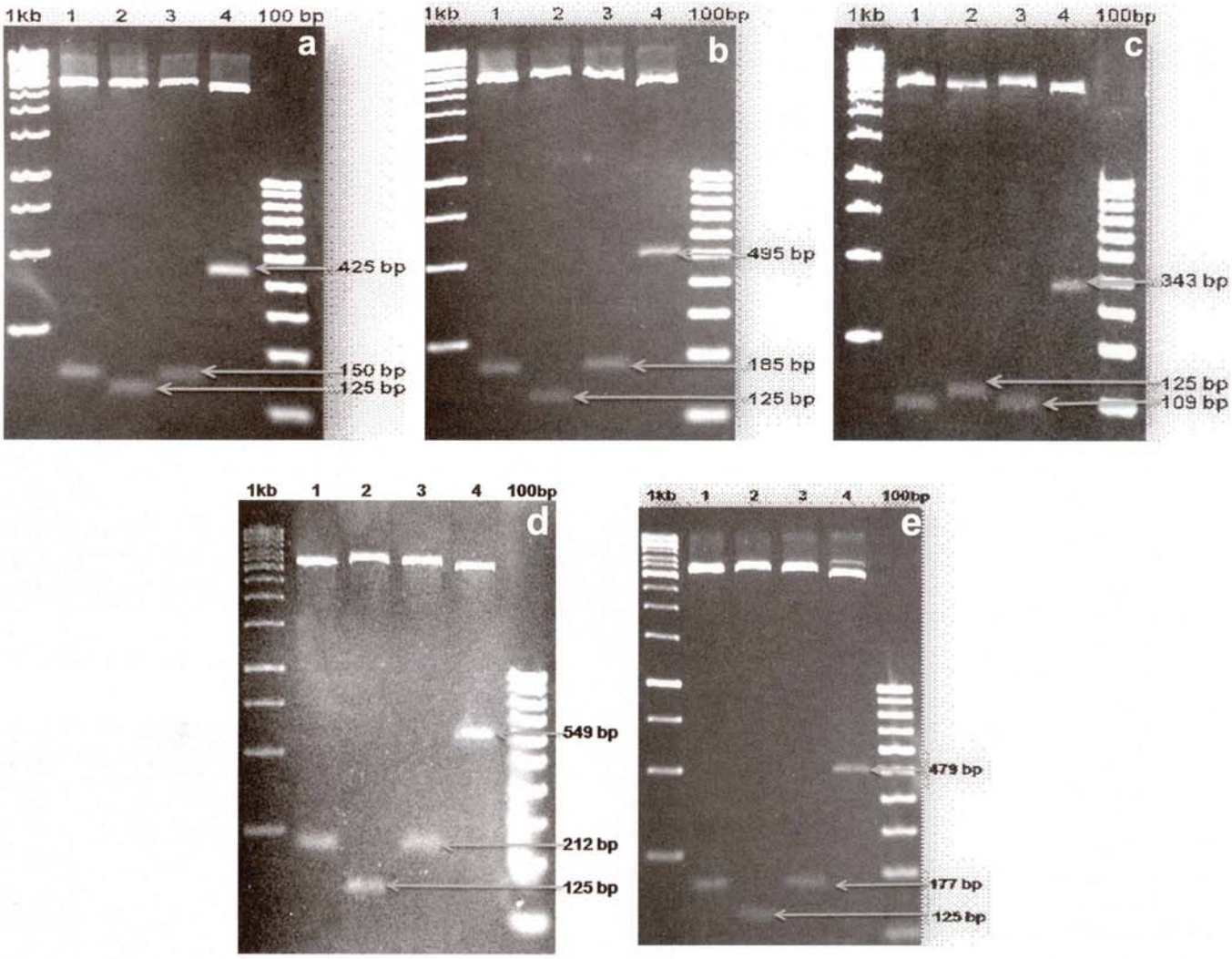


Fig.18 a-e: Inverted repeat construct of a. AC2 gene, b. CP gene, c. MP gene, d. ~C I gene e. ~V 4 gene in pBSK-int (3.1 kb), Lane I. *Kpni* + *EeaRI* = Sensestrand; 2. *EeaRI* + *BamBI* = Intron sequence; 3. *BamBI* + *Xbal* = Antisense strand; 4. *Kpni* + *Xbal* = Sense+ Int + Antisense fragment

The inverted repeat constructs of target viral sequences in pBSK-Int was sub-cloned into pBinAR and pGreen for transformation in *Agrobacterium tumefaciens* strain **EHA** 105 and subsequently in cotton. The presence of plasmid with inverted repeat construct for each target sequence of CLCuV in each binary vector was ascertained by PCR amplification using gene specific primers (Fig. 19 a & b) before initiating plant transformation.

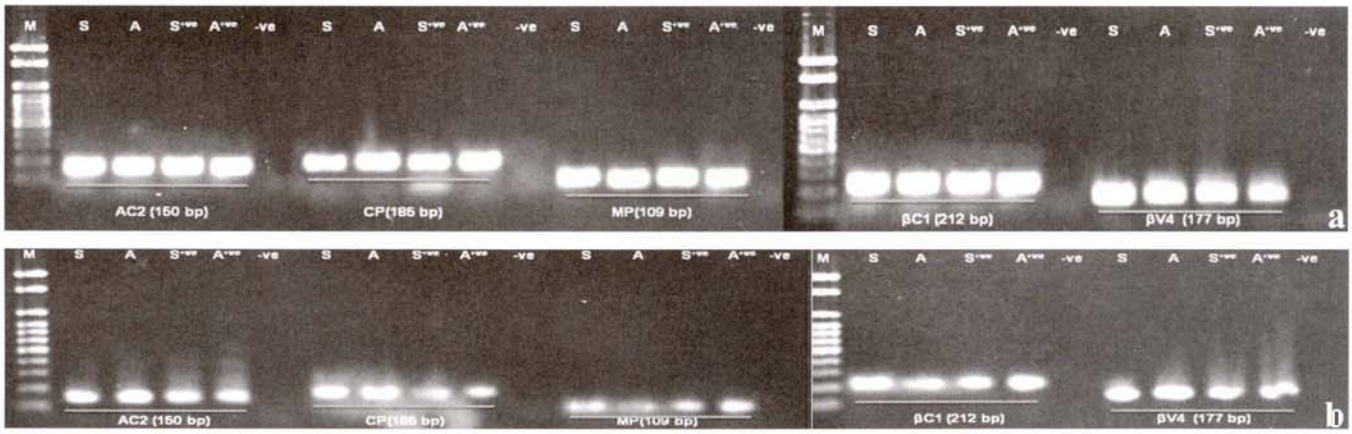


Fig.18 a&b: PCR amplification of sense and antisense strands in *A. tumefaciens* transformed with inverted repeat constructs in pBin-AR (a) and pGreen (b). M, 100 bp ladder; S, Sense; A, Antisense; S +ve and A +ve; positive control (amplified from CLCuV cloned DNA); -ve control

The inverted repeat constructs of target sequences in pBin-AR were transformed in CLCuV susceptible G. *hirsutum* cultivar HS 6. Shoot apex explants containing apical meristem cells were harvested aseptically and inoculated with log phase culture of *Agrobacterium tumefaciens* strain EHA 105 harboring inverted repeat constructs pBin-CP-S-int-A and pBin-~C4-S-int-A generated previously. Agro-infection, co-cultivation and regeneration was done as per procedure described by Nandeshwar et al (2009). Putative transformants of pBin-CP-S-int-A (12) and pBin-~C4-S-int-A (18) were selected on MS medium containing 50 mg/l Kanamycin.

Identification of molecular markers and tagging genes for Bacterial blight resistance

Five cotton lines were screened for resistance against race 18 of *Xanthomonas axonopodis* pv. *malvacearum* by syringe infiltration of bacterial cell. *Gossypium hirsutum* cotton Acala 44 and Ganganagar Agethi were highly susceptible against race 18 of X. *malvacearum,* while *G.hirsutum* cotton IM216, S295 and 101-102 B were completely resistant to race 18. Crosses were effected using two susceptible lines and three resistant lines *viz.,* IM216, S295 and 101-102B. Susceptible lines were employed as female parents while the resistant lines were used as male parents. The F_1s of each of the four crosses *viz.* Acala-44 x IM216; Ganganagar Agethi x IM216; Ganganagar Agethi x S295; and Ganganagar Agethi x 101-1 02B, were completely resistant against race 18 of X. *malvacearum.* The phenotyping **of F_2 mapping population of 122 plants of a cross between** Acala-44 x IM216 by inoculation with *Xanthomonas axonopodis* pv. *Malvacearum* showed inheritance of resistance and susceptibility at an expected 3:1 ratio indicating single gene dominance of resistance. Chi-Square analysis was performed for testing independent segregation for bacterial blight resistance and susceptible traits.

II. Genotyping of parental lines with SSR markers

The bacterial blight resistant and susceptible cotton showing contrasting phenotypes and their crosses were surveyed for genomic polymorphism using SSR and RAPD markers. Polymorphism among the parental lines was surveyed using 280 SSR primers and 60 RAPD primers. Preliminary screening revealed limited polymorphism with SSR primers used. Limited polymorphism ranging from 4.3 to 8.21 percent among the contrasting parents and specific crosses-Acala44xIM216, GAx1M216, GAxS295, GAx101-102B, were observed.The informative markers mostly showed co-dominance while few showed dominant pattern of inheritance (Fig. 20 a&b, Fig. 21 a & b).

Twelve out of 280 primers were polymorphic between resistant IM216 and susceptible Acala-44 with only 4.3 per cent of polymorphism (Fig. 16a & b). While 15 SSR markers were informative between IM216 (resistant) and Ganganagar Agethi (susceptible), providing only 5.34 percent polymorphism.

Isolation of seed specific promoter sequences for site-specific genetic engineering

Isolation of seed specific promoters for use in RNA interference mediated down-regulation of gossypol in cotton seed was done. Approximately 1100bp seed Alpha globulin promoter sequences from the four *Gossypium* spp *viz., Gossypium hirsutum,* G. *barbadense,* G. *arboreum and* G. *herbaceum* were PCR amplified using degenerate primers and cloned.

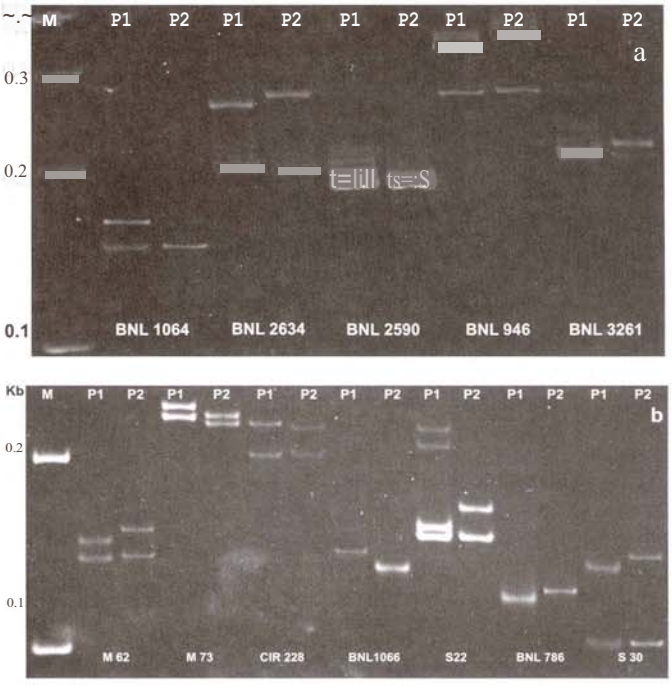


Fig. 20 a & b: Screening of SSR primers to survey polymorphism among bacterial blight resistant and susceptible parents; P1-Acala-44, P2-IM216, M-100 bp Marker

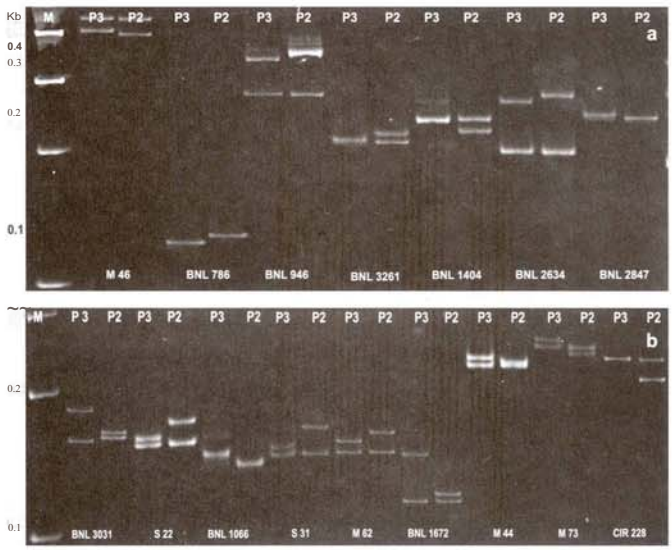


Fig. 21 a& b: Screening of SSR primers to survey polymorphism among bacterial blight resistant and susceptible cotton parents; P3- Ganganagar Agethi (GA), P2-IM216, M-1 00 bp Marker

BIOSAFETY OF Bt COTTON

Performance of *Osmanabadi* goats fed with Bt and Non Bt cotton leaves

Productive performance

Average milk yield of does (female goats) browsed on Bt cotton leaves was 1600 ml compared to group of that does fed upon non-Bt cotton leaves which yielded 1200 ml milk. The lowest volume of the milk yield of 1000 ml was recorded in the group of that browsed on non cotton crop. However, the percentage of milk fat (MF), solids-not-fat (SNF) and total solids (TS) in milk of does in all the three experimental groups did not differ significantly. No deleterious effect on the quality of milk and milk constituents was documented as a result offeeding on Bt cotton.

Nutritional parameters

The dry matter content of Bt and non-Bt cotton leaves was observed to be 20 per cent.. The crude protein percentage of leaves in Bt cotton was observed to be 26.19 per cent compared to 20.38 per cent protein in non-Bt cotton.

Physiological parameters

Feeding does on the Bt cotton did not have any significant effect on the physiology of the animal.. The rectal temperature, heart beat and respiration rate of the does browsed on Bt cotton, non-Bt cotton or non-cotton plant during the end of the year did not show any significant differences.

Haemato-biochemical parameters

Haemato-biochemical values of the does browsed on Bt and non-Bt cotton as well as non-cotton plants was characterised. Analysis of hematological values such as haemoglobin (Hb) percentage, packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC), MCH, MCV, MCHC, blood albumin, Serum glutamic pyruvate transferase (SGPT), alanine tri phosphate (ALP), calcium, phosphorous and TP etc. in the blood irrespective of their feed, whether Bt, non-Bt cotton or non-cotton plant revealed no significant effect..

Health status

The health of all the 12 experimental goats was observed to be good throughout the experimental period. No ailments/ gastrointestinal problems &/or any other diseases were observed in case of any of the goats. No adverse/ deleterious effect of feeding Bt cotton leaves on the health of goats was observed.

Survivability

As there was no mortality in any of the 3 experimental groups of goats during the entire length of experimentation, the survivability of experimental goats was 100 per cent..

Coimbatore

Development and promotion of Bt transgenic cotton for bollworm resistance

Using BN Bt as the donor parent, the popular cultivars released for South Zone like OS 28, Sahana, MCU 5 & Surabhi are being converted into Bt background through conventional back crossing. The back cross progenies have also been tested for their quantitative expression of Cry protein using ELISA (Table 15).

Table 15: No. of *cry 1Ac* positive plants identified through ELISA in BC_s populations

Genotypes	No. of plants Established	No. of *cry 1Ac* positive plant	Percentage of positive plants
OS28	53	18	34.0
Surabhi	73	27	37.0
MCU 5	58	17	29.3
Sahana	82	23	28.0

4.8: Seed Production and Seed Quality Improvement

Nagpur

Seed production and seed quality improvement

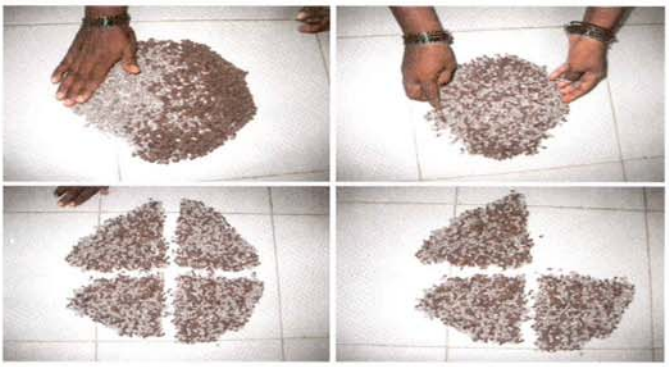
Enhancement of seed germination and vigor

The size graded seeds of six G. *hirsutum* varieties were evaluated to study the effect of seed index on field emergence and seed cotton yield. The seeds with highest index (ranging from 8.5 to 10.0g) gave highest emergence (96%) and seed cotton yield (780 kg/halo The seed cotton yield from the seeds with lower index (ranging from 7.0 to 8.0 g) followed next (662 kg/halo The seeds with lowest seed index (less than 7.0 g) gave the least seed cotton yield over varieties (530 kg/halo

Among the seed hardening treatments the controlled hydration of seeds over night was more effective for early and higher emergence under low soil moisture level (at 40%) compared to the control..

Assessment of working seed sample size for Bt testing based on estimation Cry1ac protein

Experiment was conducted to determine the optimum sample size for seed-purity test of commercial Bt-seed lots. Seed lots were prepared artificially by mixing Bt cotton seeds with Non-Bt seeds in different combinations to obtain samples with 50, 60, 70, 80-99% Bt seeds. Seed samples (25 gm) were drawn from commercial Bt seed packet (450 gm) obtained from the market by halving method. The Cry1Ac protein expression was estimated through ELISA.. Chi test done on the observed data showed that the hypothesis of testing 10, 20 and 30 seeds as a representative sample to assess seed-purity of the entire seed lot did not hold good, indicating that the present sample size is probably inadequate .



Preparation of Bt seed samples for analysis using standard method

Studies on seed quality parameters of TFL seeds sold in the market

The genetic purity of seed samples collected from Central zone varied from a minimum of 3.33% to a maximum of 100%, compared to the samples from north and south zones where the purity ranged from 80 to 100%.

Expression of transgene in seeds of crosses developed from different parental combination with respect to Bt

The leaves from plants obtained from crosses using Bt as female or male parent with homozygous Bt were subjected to ELISA at 45 OAS. The t-test analysis (5%) of Cry-toxin indicated the level to be higher in plants obtained from the cross where Bt was used as female parent (5.54 ppm), compared to the plants obtained from the cross where Bt was used male parent (5.22 ppm). The crossed seeds were studied for Cry protein expression by ELISA in seed parts such as endosperm and

seed coat.. Cry toxin levels in the endosperm of seeds obtained from the cross where Bt was used as female parent was significantly higher (6.24 ppm) compared to the seeds obtained from the cross where Btwas used male parent (5.99 ppm).

Effect of trans gene on quantity and quality of seed reserves

The total storage protein profile performed on SDS-PAGE revealed no difference in the protein banding pattern of BN Bt as well as its non Bt counter part, BN-1 (Fig.22). Similarly the seed parameters, germination and vigor also showed the properties to be not different between Bt and Non Bt.

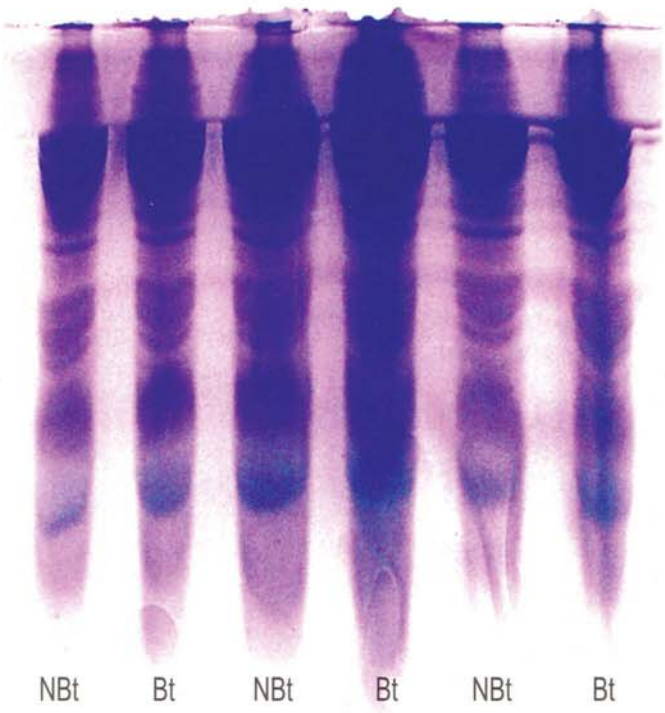


Fig.22: SDS PAGE profile of total seed protein in Bt and Non Bt seeds of BN-1

Development of efficient agro-techniques for enhancing productivity and seed quality in cotton

The application of salicylic acid @ 0.25% at 30 days after flowering gave higher seed cotton yield over control in first picking. However, application of lihocin @100 ppm at flower initiation gave higher seed cotton yield in second picking. The results indicated that salicylic acid application hastened the crop maturity compared to control and lihocin application. Seed index and seedling vigor was significantly superior in all treatments over control.

Testing and Documentation of extant varieties, hybrids and their Parents for Distinctness, Uniformity and Stability

Twenty five candidate varieties/hybrids from private sector along with twenty two reference varieties of G. *hirsutum* were characterized based on DUS test guidelines.

Coimbatore

Implementation of PVP legislation

Distinctiveness, Uniformity and Stability testing of tetraploid cotton genotypes were taken up in two trials comprising 5 and 23 candidate varieties, respectively. Similar trial was conducted for diploid cotton with one candidate and two reference varieties. The complete expression of desired morphological characteristics of seedling, leaf, flower, boll and fibre were measured. The characters were recorded from seedling to maturity adopting the procedure of approved national test guidelines for tetraploid cotton.

Registration of extant and new cotton varieties under PPV&FR Act, 2001 was initiated. In the first phase. Sixty one application forms comprising of new and extant cotton varieties were submitted to PPV&FRA through NBPGR.. Under the programme of maintenance of reference collection Fifty four G. *hirsutum* and ten G. *barbadense* genotypes were maintained. A database on varieties released by CVRC or State Varietal Release committee and in common knowledge/farmer's varieties, etc., was composed.

Film coating of cotton seeds with polymers

Seed deterioration can be prevented up to 18 months of storage and can retain the viability of 77% when pre cleaned seeds were coated with seed polymer polykote @ 3 ml/kg of seed diluted with 5 ml water combined with carbendazim (Bavistin) @ 2 g /kg and the seeds are stored in cloth bag under ambient condition. Viability can also be retained to 76% by coating the seeds with polykote @ 3 ml/kg of seed diluted with 5 ml water combined with carbendazim (Bavistin) @ 2 g /kg and Imidacloprid @ 7g /kg when seeds are stored in polythene bags.

Establishment of genetic purity of hybrid seeds through bio molecular profile

Profiling of salt soluble globulins seed protein through SDS PAGE electrophoresis of hybrid G.Cot.MDH 11and its parent's aid for genetic purity testing of cotton. The presence of additional bands in hybrid will help the seed analysts to identify, the % pure hybrid seed as well as admixtures such as parental seed and other variety seeds.

Sirsa

Technology to enhance the better crop establishment and yield in cotton

Studies on transplanting of raised seedlings indicated that significantly higher plant stand (up to 98 %) and yield (30.35 q/ha) than normal sown crop on the date of transplanting (85% and 25.1 q/ha) was recorded when the raised seedling in big container was transplanted at 25 days seedling stage.

The effect of different levels of seed index showed that the germination percentage (89.5% in CSHH 198 and 87.2% in CICR 2) was significantly higher in seed lot with superior seed index followed by medium seed index (83.3% and 81.6%) and declined in lower seed index lot (72.8% and 72.3%). The yield (32.3 q/ha in CSHH 198 and 32.8 q/ha in hybrid CICR 2) was significantly higher in the plot where seed lot with superior seed index was used than lower seed index seed lot (26.5 q/ha and 26.9 q/ha), respectively. Among the various pre sowing seed treatments, the plant stand was higher in seed lot treated with $KN0_3$ 100 mM + imidacloprid + vitavax (94.3 %) followed by treatment of DAP 1% + imidacloprid + vitavax (93.1 %) against the control water soaked seed (85 %). Significantly higher yield of 31.04 q/ha was recorded in treatment with $KN0_3$ 100 mM + imidacloprid + vitavax followed by DAP 1% + imidacloprid + vitavax (30.83 q/ha) and trichoderma (30.41 q/ha) than control (28.55 q/ha).

Standardization of seed coating with synthetic polymers and additives

Eight treatments with synthetic polymers and additives were applied on seed and stored in cloth bag as well as polythene under room conditions and observations on moisture content of seed, their germination per cent and vigour index were recorded at bi-monthly interval.. Up to 6 month of experiment period, higher germination in cloth bag (88.8%) and polythene bags (90%) was observed in seed lot in which fungicide and

insecticide was used along with polymers as treatment during storage.

Seed Production

Nagpur

Under the Mega Seed production programme, seeds of 13 elite cultivars of cotton along with wheat and pulses were produced. A sizeable resource of 6.72 lakhs approximately was earned through the sale proceeds.

Crop	Stage	Production (Q)
Cotton -13 varieties	TFL	32.89
Wheat cV.HD 2189	Foundation Seed	40.90
Red Gram-BSMR	TFL	73.80
Gram-Vijay	CS	29.80
Wheat-GW-496	CS	55.98

Coimbatore

Breeder Seed Production

Variety	Breeder seed production (kapas in kg)	Breeder seed distribution (seeds in kg)
LRA 5166	326	111
MCU 5 VT	-	69*
Surabhi	130	111
Suraj	192	54
Supriya	94	6
Suvin	235	33
Total	977	384

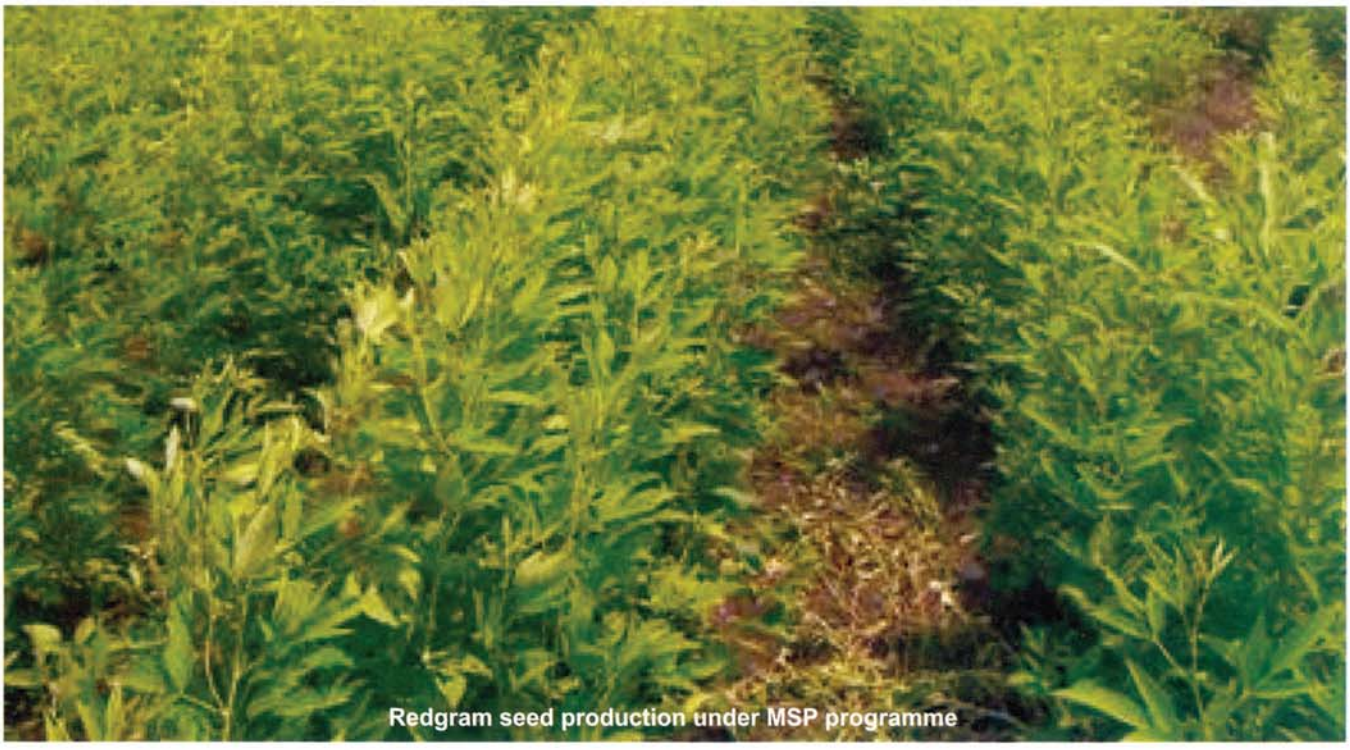
**From Carried over stock*

Sirsa

Maintenance of Nucleus and Breeder Seeds

No	Variety/Parents	Quantity (kg)
1	CSHH 198 (F)	45
2	CSHH 198 (M)	38
3	CSHH 243 (F)	25
4	CSHH 243 (M)	10
5	CSHH 238 (F)	35
6	CSHH 238 (M)	30
7	CICR 2 (F)	35
8	CICR 2 (M)	30
9	CISA310	85
10	CISA614	100

Seed production of wheat under MSP programme



Redgram seed production under MSP programme