



An *intra-hirsutum* hybrid CSHH 238 developed by the CICR Regional Station, Sirsa

CISA310, a *G.arboreum* variety, notified for commercial cultivation in the northern cotton zone.



Micro-tube drip system.

Profitable multi-tier cropping system for southern cotton zone.



## 4. Research Achievements

### 4.1: Cotton Genetic Resources

#### Nagpur

##### New germplasm collection

Three hundred and sixty eight germplasm lines were obtained from USA (345), China (4), Pakistan (10) and Iran (9).

##### Enrichment of Gene Bank

Three hundred and seventy one exotic germplasm accessions, belonging to *G. hirsutum* (368), *G. herbaceum* (1) and *G. arboreum* (2) were added to the gene bank of the Institute.

##### Conservation of germplasm

Base collection: Four thousand and five hundred and twenty accessions of *G. hirsutum* and 500 accessions of

*G. arboreum* were grown for seed multiplication.

Working collection: Four hundred accessions of *G. hirsutum*, 119 accessions of *G. arboreum* and 43 accessions of *G. herbaceum* were maintained.

Conservation: Six hundred and thirty six accessions of *G. hirsutum* were sent to NBPGR, New Delhi for long term storage. The same set is also being maintained at the Institute under medium term cold storage.

##### Evaluation of germplasm

One hundred accessions in each of *G. hirsutum* and *G. arboreum* were evaluated at Sirsa (North Zone) and Coimbatore (South Zone) under irrigated conditions and at Nagpur (Central Zone) under Rainfed conditions. Superior lines were identified for further exploitation (Table 1 and 2).

**Table 1: Superior *G. hirsutum* germplasm lines at Nagpur**

Sr. No.	Name	Seed cotton yield per plant (g)	Name	Boll wt, (g)	Name	Ginning (%)
1	LRA 5166	102.00	A02 N 46	5.2	A02 BN 32	42.7
2	A02 N 99	73.22	A02 N 71	5.1	A02N105	42.6
3	DH34	66.83	A02 N 98	5.0	A02 N 26	42.5
4	A02 N 106	62.95	A03 N 135	4.9	A02 N 46	42.2
5	BRR SP 1	62.18	DH6	4.9	A03N111	42.0
6	A02 N93	59.40	A02 N 100	4.9	A03 N 131	42.0
7	A03 N 119	58.75	A03 N 120	4.8	A03 N 132	42.0
8	DH 18	57.27	P 37	4.8	A03 N 136	42.0
9	CSH 3060	55.62	CSH 3213	4.8	A03 N 137	42.0
10	CSH 3213	55.50	A02 N 26	4.8	A03 N 144	42.0
	LRA 5166 Check	102.00	LRA5166 Check	3.6	LRA 5166 Check	36.5
	Range	(31.8 to 102.0)	Range	(1.8-5.2)	Range	(31.5-42.7)

**Table 2: Superior *G. arboreum* germplasm lines at Nagpur**

Sr. No.	Name	Seed cotton yield per plant (g)	Name	Boll wt. (g)	Name	Ginning (%)	Name	MHL (mm)
1	AC 3343	133	AC 3349	3.2	AC 3683	41.1	AC 3651	26.7
2	AC 3204	95	AC 3080	3.1	AC 3625	41.2	AC 3405	26.0
3	AC 3584	94	AC 3671	3.0	AC 3725	41.0	AC 3541	26.0
4	AC 3277	88	AC 3179	3.0	AC 3510	40.9	AKA_8401	26.0
5	AC 3188	83	AC 3277	3.0	AC 3276	40.5	AC 3045	25.7
6	AC 3096	82	AC 3720	3.0	AC 3187	40.4	AC 3169	25.3
7	AC 3443	82	AC 3188	2.9	AC 3474	39.0	AC 3710	24.7
8	AC 3747	81	AC 3098	2.9	AC 3062	39.0	AC 3257	24.7
9	AC 3237	81	AC 3584	2.9	AC 3045	38.9	AC 3021	24.7
10	AC 3326	81	AC 3018	2.8	AC 3014	28.8	AC 3237	24.0
	AKA 8401 (Check)	73	AC 8401	12.6	AC 8401	31.8	AC 8401	25.3

Two *G. arboreum* and one *G. herbaceum* accessions obtained from Andaman,

Nicobar and Neil Islands were evaluated (Table 3).

**Table 3: Evaluation of new accessions**

ICNo.	Species	Seed cotton yield per plant (g)	Boll wt. (g)	Ginning (%)	2.5 % Staple length (mm)	UR %	Micronaire	Fibre strength (g/tex)
IC 522212	<i>G. arboreum</i>	49.31	2.1	38.1	24.3	50	51	19.7
IC 522241	<i>G. arboreum</i>	71.25	2.7	35.2	20.7	49	4.8	21.2
IC 539849	<i>G. herbaceum</i>	40.17	1.3	34.6	17.9	48	5.7	19.0

Distribution of germ plasm lines: Seeds of 119 accessions of *G. hirsutum*, 213 of *G. arboreum* and 27 of *G. herbaceum* were distributed to various centres.

Gossypol Content in Germplasm lines: Gossypol estimation was performed in 100 *G. hirsutum* and 50 *G. arboreum* samples. Gossypol content in *G. hirsutum* lines ranged from 0.2 to 2.8 % and 0.6 to 2.3 % in *G. arboreum* lines.

Oil content in germplasm lines: Cotton seed oil was estimated in 49 lines. Oil content varied from 16.5 to 23.8 per cent in *G. hirsutum* and 17.2 to 24.7 per cent in *G. arboreum*.

Evaluation of germplasm through marker technique

Sixteen *G. arboreum* accessions were used for diversity analysis using PCR based DNA marker. Diversity analysis using both RAPD and SSR marker systems

indicated separate clusters. Specificity of genome characteristics was evaluated by using all 44 primers which includes 20 RAPD primers of OPA series and 24 SSR primers of JESPR-307 series. In both RAPD and SSR markers the cultivars of different origin formed a separate cluster and showed similarity coefficient between 0.55 to 0.87 in RAPD and 0.69 to 0.96 in SSR respectively. Thus from these observation it is estimated that SSR marker showed 8-15% more similarity than RAPD within these diploid cotton cultivars of different origin. The rare allele obtained in the study may be utilized for tagging and mapping of either simple or complex traits. They may possess useful genes controlling economically important traits. It was found that RAPD and SSR are effective in delineating the phylogenetic relationships among *G. arboreum* species. The result of cluster analysis indicated not only the separation of two major groups but also the separation of two major groups into sub groups.

### Physiological evaluation of cotton germplasm under rainfed condition

Hundred germplasm lines in each of *G. hirsutum* and *G. arboreum* were evaluated. Growth and physiological parameters were recorded at peak boll development stage in all the lines while in some contrasting lines process parameters (photosynthesis, transpiration rate and stomatal conductance) were recorded. Considerable variability was recorded for most of the parameters including yield in both the species. Yield realisation was relatively more in the *G. arboreum* lines as compared to *G. hirsutum* lines. *G. arboreum* lines in general were found to have slightly higher harvest index and lower leaf area as compared to *G. hirsutum* lines.

### Evaluation of germplasm lines for resistance to insect pests

Forty *G. hirsutum* germplasm with good yield of 50 g/plant, comprehensive pest tolerance and elite fiber properties (fiber length > 25 mm, bundle strength > 19 g/tex) were evaluated for two years (2004-2006) under unprotected conditions and the most promising were carried into the third year (2006-07). The peak jassid incidence and square damage was considered for comparison across years. 16 lines tested in 2006-07 demonstrated a jassid incidence of less than 2 nymphs per leaf and a Grade of I and II. 14 lines demonstrated insignificant square damage in 2006-07. Artificial screening over two years (2005-07) showed that IC 357857, IC 358338 and IC 357496 were the most promising in terms of their jassid tolerance and growth regulating effects on *H. armigera* larvae in addition to being good yielders. Selections were made from AR-27, ND-63, G-21-719, Piedmont Cleveland and JR-52 (elite fiber quality lines with comprehensive pest tolerance) for cultivation under low input rainfed systems with minimum need for plant protection interventions. The lines yielded a minimum seed cotton yield of 8 q/ha under on-farm conditions.

Out of 457 exotic lines evaluated in 2005-06, 25 lines were grouped as bollworm and jassid tolerant. Of these 11 lines (Ee 277959, EC 196390, EC 137600NLL, EC 232177, EC 129473B, EC 1266, EC 314228, EC 345786, EC 200766, EC 169749, EC 2) demonstrated elite fibre properties and were evaluated under unprotected condition during 2006-07. All the lines were found to be jassid tolerant of which one line (EC 2) possessed high bundle strength of 23.6 g/tex.

**Bacterial blight resistant germplasm :** Out of 127 germplasm lines of *G. hirsutum*, two lines viz. CN 425 45 Rand El 395 A were observed to be resistant to bacterial blight against the virulent race 18 of *Xam* under pot culture test of artificial inoculation.

Six hundred twenty five lines of *G. hirsutum* were evaluated for bacterial blight reaction under field conditions. Out of these, 27 lines were observed to be free from the incidence and 39 lines exhibited resistant reaction. Remaining 54 lines were moderately resistant, 198 lines moderately susceptible and 307 lines susceptible.

Out of one hundred lines of *G. hirsutum* of Br 01 trial evaluated under natural incidence of field conditions, two lines A 02 N 99 and A 03 N 119 were free from the incidence of bacterial blight whereas 8 lines viz. A 02 N 76, A02N83, A02N 100, A03 N 123, A03 N 135, A03 N 142, DH 34 and P 23 were resistant. Remaining 17 lines were moderately resistant, 31 lines moderately susceptible and 41 lines susceptible.

### Coimbatore

Two hundred germplasm accessions each of *G. hirsutum* and *G. arboreum* were evaluated for morphological and agronomic traits. Wide variability was noticed for all the characters studied. About 261 working collection of *G. hirsutum* were grown under field condition for seed multiplication and future storage.

Morphological characterization of 320 germplasm accessions of *G. barbadense* was done. Variability with regards to plant type, flowering and fruiting behaviour, and bursting types were identified. Majority of the *G. barbadense*, L accessions had three locule bolls, but 22 accessions showed four locule types. Seventeen *G. barbadense*, L accessions were identified with high micronaire value which ranges from 4.5 - 5.5.

### Sirsa

Seven hundred lines of *G. hirsutum* and 300 lines of *G. arboreum* were evaluated for various morphological and agronomical parameters including CLCuV incidence and bollworm infestation. The range for different parameters was worked out and superior genotypes of both the species for each parameter were identified. The 200 lines supplied from NBPGR New Delhi under safety multiplication programme were also evaluated (Table 4).

**Table 4: Working collection of *G. hirsutum* and *G. arboreum***

Character	<i>G. hirsutum</i>	<i>G. arboreum</i>
Seed cotton yield (g)	>150 = 11	>140 =5
Boll weight (gm)	>3.8 =11	>2.8 =10
Boll number	>50 =17	>50 =17
Ginning Outturn (%)	> 38 = 15	>38 =22
Seed Index (gm)	> 10 =22	>6 =40
Lint Index (gm)	> 5.8 =8	>3.5 =10
Number of Monopods	> 5 =28	>5 =30
Number of Sympods	>16 =23	>16 =12
Plant height (cm)	>150 =20	> 160 =16

#### Screening against important diseases

Two thousand germplasm lines were screened against Cotton leaf curl virus disease under artificial inoculation. Seven lines viz., BP-52-16 MBLYYH, JBWR-21, CNH 2773, AKH 9620, B59-1679-2, Super Okra Virescent and 59 CCD were found to be resistant. Out of 225 breeding lines screened against CLCuV disease, forty three lines showed resistance. Out of 99 *G. hirsutum* entries screened against root rot disease in the wilt sick plots, three lines were free from the disease. Similarly, six lines out of sixty-six *G. arboreum* genotypes were found to be root rot resistant.

#### Registration of germ plasm

Four genetic stocks with distinguishing characters have been registered under NBPGR, New Delhi.

(a) CPF-1 was identified as a spontaneous mutant with pink anther filament from the population of AKH-0308 (a strain derived from the combination of *Gossypium* species (*G. hirsutum* x *G. barbadense*) x *G. hirsutum*) x Derivative (*G. hirsutum* x *G. anornatum* x *G. sturtianum*). The mutant identified bred true to type and lacks petal spots at initial stage of flowering and with very light petal spots visible only at full blooming stage on the inner sides of the petals. The mutant has been registered with NBPGR (No. INGR07036).

(b) CSPF-1 is a spontaneous mutant with pink petals isolated from the  $F_3$  population of T-7 x LSC-5 cross. The mutant identified bred true to type. The mutant has been registered with NBPGR (No. INGR07035).

(c) BN-Okra and BN-Red: Two bollworm tolerant,

morphologically diverse strains BN-Okra and BN-Red have been registered as INGR 07050 and INGR 07049 in NBPGR. BN- Okra is a genetic stock with lanceolate (super okra) leaf shape. It was developed through back cross method ( $BC_3$  generation) using ROIL-3 as non-recurrent parent and BN as recurrent parent. BN- Red is a true breeding line with red pigmented plant body and semi naked seeds. The genetic stock has been developed through back crossing with Mc Namara Wine sap as non-recurrent parent and BN as recurrent parent. These morphologically diverse genetic stocks are tolerant to bollworms and possess higher amount of gossypol, flavanol and phenol contents in their plant parts and also had comparable agronomic properties and fibre characteristics.

#### 4.2: Hybrid Cotton

##### Nagpur

Eighty seven GMS and 10 CMS based hybrids were evaluated in three trials. In the first trial, hybrids NGMSH 57, NGMSH 35, NGMSH 27 and NGMSH 51 recorded more than 20 per cent yield increase over Hybrid 8. However, they were at par in yield over hybrid NHH 44. In the second trial, hybrid NGMSH 66 was the best with an yield increase of 17 per cent over NHH 44. In the third trial, hybrid NCMSH 192 was on par in yield with both Hybrid 8 and NHH 44.

Studies carried out through direct and reciprocal crosses indicated that the genes governing Gregg GMS and Abadhita GMS were the same.

## Coimbatore

### Development of high yielding conventional *intra-hirsutum* hybrids

Fourteen hybrids were evaluated in a replicated trial. Hybrid LS 2 x LS 22 with a mean seed cotton yield of 3067 kg/ha was better than the long staple hybrid check Bunny (2543 kg/ha); Quality-wise also with a mean fibre length of 31.6 mm and fibre strength of 24.6 g/tex, it was

better than the control. Among the medium staple hybrids, LK 1 x TK 22 with a mean seed cotton yield of 3,385 kg/ha was the best hybrid, with a mean ginning out turn of 36.7 percent.

Forty nine medium staple hybrids were evaluated for their yield and fibre quality. Four hybrids recorded significantly higher yield and had better fibre quality than the check hybrid Surya (Table 5).

**Table 5: Performance of medium staple hybrids in the initial evaluation trial**

Hybrid	Seed cotton yield (kg/ha)	Ginning (%)	2.5% Span length (mm)	Micronaire	Strength (g/tex)
LK1xTK2	3,519	33.0	31.0	4.1	24.4
LK 1xTK6	3,426	34.0	31.4	4.1	25.7
LK 1x TK49	3,299	34.0	30.6	3.8	24.7
LK22x TK6	3,191	33.0	31.1	4.6	24.6
Surya (C)	2,676		30.1	3.9	24.0
CD 5%	353				

Development and utilization of cytoplasmic and genetic male sterility for hybrid seed production and fertility restoration in cotton.

In a confirmatory station trial, 25 GMS based *intra-hirsutum* hybrids were evaluated along with Bunny and NHH 44 as the check hybrids. Analysis of data on seed cotton yield indicated significant differences between the hybrids. Some hybrids with better quality have been identified. New crosses were attempted to develop hybrids combining better fibre quality and yield using long staple high strength cultures as male parents.

Three different station trials were conducted to evaluate the performance of different CMS based *intra-hirsutum* hybrids. In the first trial, nine *intra-hirsutum* test hybrids were evaluated along with Bunny and NHH 44 as the check hybrids. The test hybrid RKR 4145 x AK 2 (with 3470 kg/ha) recorded the highest yield. In another trial, 19 *intra-hirsutum* test hybrids were evaluated along with Bunny and NHH 44 as the check hybrids. In this trial, the test hybrid 22- 29 HS x AK 1 was superior to the best check hybrid NHH 44.

Fifteen CMS based interspecific hybrids developed using two *G. barbadense* restorer lines viz., Suvin Restorer (SR) and Pima Restorer (PR) were evaluated in

a station trial along with Sruthi, DCH 32 and TCHB 213 as check hybrids. The highest seed cotton yield was recorded in the test hybrid 22-29 HS x PR with 1250 kg/ha as compared to 1145 kg/ha recorded in DCH 32, the best check hybrid.

The GMS based interspecific hybrid CCHB 727 which was tested in co-ordinated hybrid trial of South Zone in AICCIP during the past three years has been sponsored for agronomic trial during the year 2007-08. The CMS based interspecific hybrid CCCHB 05-1 has been promoted to CHT in Central Zone. Apart from these, one CMS based *intra-hirsutum* hybrid CCCHH 07-1 (CMS RKR 4145 X A) has been entered in preliminary hybrid trial of AICCIP during the year 2007-08.

### Development of interspecific (H x B) hybrids

Two high yielding interspecific hybrids (LS-25 x P-28 and LS-26 x P-28) have been identified from three years of field evaluation (Table 6). The hybrid LS-26 x P-28 has been sponsored for multi-location testing in All India Coordinated Cotton Improvement Project during the year 2007-08. This hybrid is characterized by high yield potential (2750 kg/ha), better 2.5 % staple length (35.8 mm), ginning outturn (30%) and micronaire (3.5).

**Table 6: Mean performance of promising interspecific hybrids for yield and quality (2004-07)**

Hybrids	Seed cotton yield (k~/ha)	2.5% SL(mm)	Ginning (%)	Micronaire
LS 26 x P 28	2750	35.8	30	3.5
LS 25 x P 28	2560	36.3	29	3.6
Sruthi (c)	1700	35.2	33	3.4
TCHB-213 (c)	1990	35.4	33	3.6

In a confirmatory yield trial, 56 interspecific hybrids (HxB) were evaluated along with Sruthi, TCHB-213 & DCH-32 as checks (Table7). ISC-4 was found

to be the best combining both high (2245 kg/ha) & superior fibre quality (2.5% Span length-33 mm, bundle strength-24 g/tex and micronaire 4.0).

**Table 7: Performance of promising interspecific hybrid over last two years**

Hybrids	Seed cotton yield (kg/ha)	Ginning (%)	2.5% S.L (mm)	Strength (g/tex)	Micronaire
ISC-4	2245	32	33	24	4.0
ISC-48	2190	33	34	24	4.0
ISC-56	2190	33	35	22	4.0
DCH-32 (c)	2190	33	36	26	3.7
Sruthi (c)	1207	33	34	25	3.6
TCHB-213(c)	1257	33	35	26	3.8
CD@5%	688				

In another yield trial, 31 interspecific hybrids were evaluated along with TCHB-213 and DCH-32 as checks. The hybrids LS-22 x P-30 (1914 kg/ha) and N x P-30 (1832 kg/ha) recorded higher yield than the best check hybrid DCH-32 (1772 kg/ha).

Sirs a

**Demonstration of promising hybrids:** Among the CMS hybrids, hybrid CSHC 195 gave the highest seed cotton yield of 2370 kg/ha. Among the OMS hybrids, CSHO 1633 gave the highest yield of 2556 kg/ha, which was better than the conventional check hybrids CSHH 198 and CSHH 238. The highest ginning outturn was recorded by CSHC-797 (39.0 %) followed by CSHO 1862(37.0%).

**Demonstration of Bt hybrids:** Among the 13 Bt hybrids evaluated in the demonstration plot, the hybrid MRC 6301 gave the highest yield of 3194 kg/ha followed by NCS-913 (3083 kg/ha) and RCH-317(3074 kg/ha). The highest ginning outturn was recorded by RCH-308 (39.0 %) followed by RCH-134 (38.5 %).

**Diversification and utilization of male sterility system in *G. hirsutum***

**Evaluation of GMS based hybrids:** In the first trial, 98 OMS based hybrids were evaluated against the conventional check hybrids CSHH 198 and CSHH 238. The highest seed cotton yield was recorded in OMS 15x 60 (223 g/plant), OMS 15x 27 (220 g/plant) and OMS 15x 64 (175 g/plant) as against 166 g/plant and 168 g/plant of conventional check hybrids CSHH 198 and CSHH 238, respectively. Maximum ginning out turn of 40.0 % was recorded in the cross combination OMS 22 x 74.

**Evaluation of CMS based hybrids:** Thirty six CMS based hybrids were evaluated along with local check hybrids CSHH 198 and CSHH 238. The highest seed cotton yield was recorded in CMS based hybrid LRA 5166 x E (CIR70) (138 g/plant) followed by in F505 x D (136 g/plant). The highest ginning out turn of 40.2 % was recorded by the cross F 505 x E followed by 39.5 % in SH2379 x C and Jhorar x E (39.2). The hybrid SH 2379 x

Ahas shown the highest 2.5 % span length on 1.3 mm in comparison to local check CSHH 198 (26.5 mm). The hybrid LRA5166 xE had bundle strength of 22.9 g/tex.

Effect of alien cytoplasm on yield and fibre properties: To study the effect of male alien (sterile) cytoplasm on seed cotton yield and fibre properties of hybrid cotton, five cytoplasmic male sterile line (A) and their maintainers (B) were crossed with six restorer lines and two types of hybrids i.e. A x R and B x R were

developed and evaluated. The results showed that, sterile cytoplasm has detrimental effect on yield in hybrids. The hybrids produced by using restorers with *G. harknessii* cytoplasm as the female parent showed about 1.06 to 38.21 % reduction in yield. However, no negative effect was observed on ginning out turn and other fibre quality traits. Mean performance of A x R and B x R cross combinations for seed cotton yield, boll weight, ginning out turn and fibre traits is given in Table 8.

**Table 8 : Mean performance AxR and BxR crosses for seed cotton yield and its component traits**

Sr. No.	Type of crosses	Seed cotton yield/plant (g)	Boll wt (g)	GOT (%)	2.5% span length (mm)	Micronaire	Tenacity (g/tex)
1	LRA 5166 Ax R lines	105	3.2	35.2	28.2	4.5	21.6
2	LRA5166 B x R lines	174	3.6	38.5	27.5	4.7	21.0
3	SH 2379 A x R lines	94	3.1	37.7	29.2	4.2	20.7
4	SH 2379 B x R lines	105	3.2	37.7	29.0	4.2	21.5
5	Jhorar Ax R lines	103	3.5	34.8	28.1	4.8	20.7
6	Jhorar B x R lines	155	3.6	37.2	28.2	4.6	20.6
7	H 777 Ax R lines	91	3.1	36.5	28.4	4.3	20.8
8	H 777 B x R lines	123	3.3	37.0	27.8	4.4	21.1
9	F 505 A x R lines	113	3.3	37.5	27.5	4.7	20.4
10	F 505 B x R lines	155	3.6	37.2	28.2	4.6	20.6
	CV%	13	11	5			
	CD (5%)	9	0	0.5			

Effect of male sterility on seed cotton yield and its component traits

To study the effect of male sterility on yield and fibre quality traits, three type of hybrid combinations A x R, B x R and CMS lines x R lines (CIR23A, CIR26, CIR28A, CIR 32A, CIR 70 and CIR 720) were attempted. The

hybrids along with check hybrid CSHH 198 were evaluated. Seed cotton yield of conventional hybrids was found to be highest followed by CMS hybrids and CMS hybrids. However for ginning out turn and other fibre quality traits no significant differences were observed between three type of hybrids (Table 9).

**Table 9: Effect of sterile cytoplasm on seed cotton yield and fibre quality traits**

Sr. No	Type of crosses	Seed cotton yield /plant (g)	Boll wt. (g)	GOT (%)	2.5% Span length (mm)	Micronaire	Tenacity (g/tex)
1	K 34007 B x R lines	134	3.6	37.0	27.9	4.4	20.7
2	K 34007 A x R lines	117	3.3	36.3	27.1	4.4	20.2
3	K 34007GMS x R lines	101	3.0	36.8	26.9	4.4	21.5
	CSHH 198 (LC)	145	3.9	35.0	-	-	-
	CV%	22	2.1	15.6			
	CD (5%)	NS	2.8	NS			



**Identification and maintenance of new restorer lines**

The new restorer lines viz. CIR 8, CIR 12, CIR 15, CIR 23, CIR 26, CIR 32, CIR 38, CIR 47, CIR 70, CIR 72, CIR97P1, CIR97P3, CIR119P1, CIR119P3, CIR126P1, CIR526P1, CIR526P3, CIR 920 P1, CIR 926 P2, CIR 926 P3, CIR 1169 P1 and CIR 1169 P2 were identified and maintained through selfing.

**4.3: Genetic Improvement**

**Nagpur**

**Diploid Cotton Improvement**

Eight promising cultures from CICR, Sirsa and CICR, Nagpur were tested in a trial. The entries CAN 1003 (1987 kg/ha) and CAN 1005 (2126 kg/ha) recorded significantly higher seed cotton yield than the check variety AKA 8401 (1561 kg/ha). Cultures CAN 1001, CAN 1003 have been sponsored for Br 22 trials (Table 10).

**Table 10: Promising *G. arboreum* cultures**

Name of entry	Seed cotton yield (kg/ha)	Ginning (%)	Fibre quality			
			2.5% Span Length (mm)	UR (%)	Micronaire	Fibre Strength (g/tex)
CAN 1005	2126					
CAN 1001	1860	40.6	24.3	52.0	4.6	19.8
CAN 1003	1987	39.2	24.2	49.0	5.1	20.8
AKA 8401 (Check)	1561	36.2	26.8	48.0	4.6	18.2

Two promising cultures viz. CINA 347 and CINA 348 with superior seed cotton yield (934 to 1329 kg/ha), Ginning Out turn % (37.8 to 38.2%), staple length (27.5 mm to 28.2 mm) and fibre strength (20.2 to 21 g/tex) were superior to the check variety AKA 8401. These two cultures were sponsored in AICCIP trial (Br 22) for Multilocation evaluation.

Two grey mildew resistant genotypes CINA 357 and CINA 358 recorded good fibre properties with a fibre length of 26.3 mm and 27.8 mm and fibre strength of 20.1 and 19.5 g/tex and Micronaire of 5.2 and 4.6, respectively.

Twenty eight cultures of *G. arboreum* received from eleven centres were evaluated. Culture KWA 228 recorded the highest yield of 883 kg/ha followed by MDL

2463 with 863 kg/ha. Long linted and high ginning cultures were also identified in the trial.

In the *G. arboreum* (*bengalense* x *cernuum* cross) x *G. anomalum* cross lines with five loculed bolls have been isolated.

**Tetraploid Cotton Improvement**

Development of heterotic pool for superior medium staple in tetraploid cotton

Sixty six F1s developed by adopting 12x12 diallel without reciprocals were raised for studying the combining ability and heterosis. The following crosses were identified on the basis of combination of seed cotton yield and halo length (mm) (Table 11).

**Table 11: Seed cotton yield and halo length (mm) of five F<sub>1</sub>s**

Cross	Seed cotton yield (kg/ha)	Halo length (mm)
GSH-2 x MCV-9	1592	28.1
MCV - 9 x IC 356590	1728	27.9
LRK 516 x LH 1948	2086	27.0
NH 545 x R 1252	1963	23.6
Sahana x DRY 286-1	2630	25.7

### Breeding of the upland cotton for improved yield, quality and resistance to biotic stress (bollworms and jassid)

One hundred and twenty germplasm cultures were sown in an augmented design. Crosses were made between identified donors (Code 504, 509, 514) for jassid resistance with seven cultivated varieties in line x tester mating design. Similarly, bollworm resistance germplasm [(N 6-63 (IC 356665), Cat 2535, Cat 2566 (IC 357243))] were crossed with cultivated varieties in Lx T mating design. The lines were AKH 080, DHY 286-1, Khandwa2, NH 545, Khandwa 3, G.Cot 18 and MCU9.

Table 12: Promising *G. hirsutum* cultures

Name of Entry	Seed cotton yield (kg/ha)	Ginning %	Fibre quality			
			2.5% Span Length (mm)	UR(%)	Micro uaire	Fibre Strength (g/tex)
CNH 1102	1476	42.0	30.4	50.7	4.2	23.2
CNH 1101	1104	37.6	31.2	51.0	3.8	23.6
LRK 516 (Check)	1027	35.6	26.2	51.0	4.2	20.7

Twenty one cultures received from various centres were evaluated. H 1250 recorded the highest yield 00634 kg/ha with a ginning out turn of 37.9%. H 1250 was also found to be jassid resistant. CNH 1102 was resistant to bacterial blight.

#### Abiotic Stress.

Studies were conducted with thirty advanced cultures to identify drought tolerant lines in tetraploid cotton. DTS 303 recorded the highest yield under rainfed conditions and ranked 3<sup>rd</sup> under irrigated conditions and was superior to the best check Anjali by over 16 per cent. It recorded the drought susceptibility index of 34.1 per cent. DTS 312 recorded aDSI of 5.1 %.

In another trial, SPS 28 UR (DSI of 33.9 %) and SPS 43 (DSI of 38.9%) were promising selections and out yielded the checks by 15 per cent.

#### Biotic Stress

Five lines viz., CAI 740, CAT 1557, CAT 2622, CAT 2633 and N 99 were found to be immune to jassids and have been deployed in the breeding programme.

#### Development of TGMS lines in *G. arboreum*

### Testing of early generation material

Early generation segregating material (F<sub>3</sub>-F<sub>4</sub>) was evaluated. About 389 single plant selections were evaluated in plant progeny rows. Few of the advanced selections were also tested in two replications (of two rows). The data on mean hallow length indicates fibre length of up to 35 mm and single plant yield up to 120 g/plant. The selections shall be further evaluated. Based on manual testing for fibre length and strength about 387 single plant selections were made.

Two cultures with improved fibre properties namely CNH 1101 and CNH 1102 have been sponsored under Br.02a and Br.02b trials of the AICCIP (Table 12).

Twenty male sterile lines were evaluated for their thermo sensitivity to pollen production. The critical sterility point was observed during March-April period and the critical fertility point was noticed during October-November period.

#### Population Improvement

In an effort to develop a random mating population in *G. hirsutum* and *G. arboreum*, the third cycle of the random mating population was completed in both the species. Utilising GMS lines, twenty F<sub>1</sub>s were advanced to develop a composite F<sub>2</sub> in *G. hirsutum*. Similarly, a first cycle of GMS based random mating population was also completed in *G. arboreum*.

#### Coimbatore

#### Breeding *G. hirsutum* variety with new plant types-Development of medium staple varieties

Culture CCH 510-4 was tested under the Coordinated varietal trial in both Central and South zones. Culture 510-4 out performed the zonal check in both the zones in seed cotton yield and was also on par with them in quality (Table 13 & 14).

**Table 13: Agronomic performance of culture CCH 510-4**

Culture	Mean seed cotton yield (k2/ha)		Ginnin2 (%)	
	Central zone	South zone	Central zone	South zone
CCH 510-4	1,299	2,195	36.5	37.5
Zonal check	1,082	1,807	34.4	34.4
Local check	1,489	2,336	33.6	35.9

**Table 14: Quality performance of culture CCH 510-4**

Culture	2.5% Span length (mm)	Micronaire	Strength (2/tex)
CCH 510-4 (CZ)	27.7	3.7	20.3
CCH 510-4 (SZ)	30.3	4.6	22.8
LRA5166(ZC/CZ)	23.6	4.3	20.0
Surabhi (ZC/SZ)	30.6	4.2	21.5

Development of high yielding and high spinning extra long staple cotton  
Ten high strength cultures were evaluated in a replicated trial,

Eight out of ten cultures tested possessed high fibre strength ranging from 24.5 to 26.3 g/tex. These cultures were also characterized by high ginning outturn of 38 to 41 % (Table 15).

**Table 15: Performance of ELS high ginning cultures**

Culture	Seed cotton yield (kg/ha)	Ginning (%)	2.5% Span length (mm)	Micronaire	Strength (g/tex)
CCHE 4-3-13	2,248	39	32.0	4.3	25.5
CCH E 5-2-5	2,208	39	31.1	4.0	22.3
Surabhi	2,189	34	32.6	4.0	24.0
CCH E 4-2-4	2,180	39	31.8	4.2	24.9
CCH E 5-1-9	2,077	40	30.6	4.1	25.9
CCH E 4-2-1	2,010	39	31.9	4.2	24.5
CCH E 5-2-7	1,957	39	31.3	3.9	26.3
CCH E 5-5-10	1,892	41	31.6	3.9	25.3
CCH E 4-3-7	1,884	38	31.6	4.3	25.6
CCH E 5-1-5	1,768	38	31.7	3.8	26.1
CD 5%	519				

### Cotton Seed Oil Improvement

Among the single plant selections that were made from the segregating populations, Anjali x (A x F 1861) - 1- 1- 1-3, Anjalix(AxF 1861)-1-3-3-3, Anjali x (Ax F 1861) -4-1-1-1, CBR 3 x F 1861 - 2 -2-1-1 were seen very promising with regard to single plant yield and also

possessing seed oil content of 24-26%. Materials from specific crosses viz., Sumangala x F776, M5KD933 x F 776, CBR3 x F 776, Anjali x F 776, Surabhi x F 776, Supriya x F 776, Sumangala x FI861 generated from the previous year trials exhibited good amount of variability for Nitrate Reductase activity and other biochemical constituents like reducing sugars, soluble protein and

specific enzymatic activities. Lines with good yielding ability and supported with effective biochemical and physiological attributes shall be further utilized in breeding programme.

### Sirsa

#### Tetraploid Cotton

Development of heterotic pools for medium and superior medium staple cottons

Six non inbred lines and six inbred lines were used for the development of heterotic pool. The non inbred lines include the locally adapted varieties/ germplasm lines (LRA5166, F 505, SH 2379, K34007 and Jhorar) and six inbred lines were used as testers. In order to group the germplasm lines in to two heterotic pools seed cotton

yield and component traits data were utilised.

#### Diploid Cotton

Release and Notification of variety, CISA310

The variety CISA 310 is an early maturing variety. It recorded 29.0% higher yield over the zonal check RG 8. It possesses a high ginning out turn of 41.7 per cent. Variety CISA 310 notified for general cultivation under irrigated conditions in the North Zone.

#### Evaluation of advanced cultures:

Fifteen advanced cultures were evaluated. Culture CISA-6-350 gave significantly higher seed cotton yield (2538 kg/ha), with a 2.5% span length of 27.5 mm and a fibre strength of 22.0 g/tex. The highest Ginning Out turn (43.2) was recorded by CISA-6-212 (Table 16).

**Table 16: Mean performance of top four advance cultures**

Sr. No.	Entry	Seed cotton yield (k2/ha)	Boll wt.	Ginning (%)	2.5% Span length (mm)	Micronaire	Bundle Strength (g/tex)
1	CISA-6-165	1966	1.9	37.7	23.8	5.5	20.6
2	CISA-6-187	1980	2.1	35.0	24.4	4.1	21.0
3	CISA-6-214	1891	2.0	38.7	23.7	5.3	20.5
4	CISA-6-350	2537	3.1	37.8	27.5	5.5	22.0
5	RG-8	2074	2.1	37.8	19.8	6.9	19.7

CV=7.20

CD kg/ha at 5%=240

Performance of CISA 614 in Zonal trial  
The culture CISA 614 was tested in North Zone centres under AICCIP. It was on par with the zonal check and out

yielded the local check. Quality wise, it was a shade better than the check varieties in fibre properties (Table 17).

**Table 17: Performance of culture CISA 614**

Cultivar	Seed cotton yield (kg/ha)	Boll wt. (g)	Ginning (%)	2.5% Span length (mm)	Micronaire	Strength (g/tex)
CISA 614	2024	2.3	36.9	19.7	6.7	15.5
ZC (HD 123)	1967	2.5	38.7	17.3	7.0	13.9
LC	1787	2.6	40.7	18.7	7.0	14.9

#### 4.4: Genetic Diversity through Introgression

##### Nagpur

Maintenance and utilization of wild species.

Twenty four wild species, 20 perennials, six races of *G. arboreum* and seven races of *G. hirsutum*, one race of *G. barbadense*, one race of *G. herbaceum* and 32 interspecific hybrids are maintained in the species garden. Twenty four crosses were effected on the cultivated species, using 11 wild species and 6 races as pollinators. The per cent boll set was observed to be more when Band D genome species was used as the pollinator, rather than E, F or G genome.

Further selections were carried out in 258 cytologically stable and morphologically uniform lines developed from the cross ((*G. hirsutum* x *G. raimondici*) x *G. hirsutum*) for resistance to sucking pests, bollworms and various diseases. Similarly, selections were carried out in the multi species hybrid derivatives of ((*G. hirsutum* x *G. raimondici*) 2 x (*G. barbadense* x *G. thurberi* )2). Plants with zero monopodia and short sympodia (MSH 99) have been identified.

*G. anomalum* and *G. stocksii* were successfully crossed and the resulting F<sub>1</sub> showed partial sterility. Cytological investigations showed a chromosomal configuration of 6.34 I, 8.88 II, 0.46 III and 0.14 V.

From the segregating population of introgressed breeding material involving *G. hirsutum* and wild diploid species several single plants with better fibre length (up to 32 mm) and better yield potential have been selected. Similarly, in the *intra-hirsutum* crosses also medium to long staple genotypes with superior yield have been obtained.

##### Coimbatore

In a common trial 18 stable introgressed lines obtained from various cooperating centres were evaluated for yield and other characters. The check variety Sumangala recorded the highest yield. When nine introgressed *arboreum* cultures were tested along with DLSa 17 as the check variety, none of the test cultures were found better than the check variety.

Several superior single plants were selected in segregating lines obtained from different cooperating centres based on morphological characters. Some of the progenies combined both yield and fibre quality as compared to check varieties viz., Anjali and Surabhi.

When near stable introgressed lines were evaluated in a

station trial, highest yield was recorded in the medium staple check variety Sumangala. However, several progenies with good fibre quality had better yield than the long staple check variety Surabhi.

In an Institute common trial, the high quality stable derivative MM 03-23 have been tested in Nagpur, Coimbatore and Sirsa along with 27 other entries including the check varieties LRA 5166 and Sumangala. The introgressed culture (1401 kg/ha) was superior to Sumangala (1383 kg/ha). But quality wise, the culture was far superior to the check variety recording 30.4 mm of 2.5% span length and 24.5 g/tex of fibre strength. At CICR, Nagpur also the culture showed superiority in terms of yield over the check varieties viz., LRA 5166 and Anjali (1316 kg/ha as against 810 and 1206 kg/ha, respectively).

#### 4.5: Development of Transgenics

##### Development of Transgenic cotton

Attempts are underway to transfer Bt gene into promising cultures and popular cultivated varieties through back cross procedure. TwentyBC<sub>2</sub>F<sub>1</sub>, sevenBC<sub>1</sub>F<sub>1</sub>, F<sub>1</sub>, and eleven F<sub>2</sub> populations were advanced to the respective F<sub>2</sub> generations.

Four Bt hybrids were evaluated along with their non Bt counterparts in the RCGM Trial, NHH 44 Bt recorded the highest yield in the trial and was superior to the non Bt counterpart by over 26 per cent. In another trial, four Bt varieties were tested along with their non Bt counterparts. BN Bt and Anjali Bt recorded higher yield than their non Bt counterparts.

In the Institute trial, 58 Bt hybrids were tested along with NHH 44 Bt and Ankur 651 Bt as checks. Bt Hybrid 4 was the best hybrid with 28.9 per cent increase in seed cotton yield over NHH 44 Bt.

Pollen outflow and out crossing studies conducted in Bt cotton hybrids indicated a pollen outflow of 1 m in North, West and East directions and 6 m in the South direction. The out crossing was approximately 1 per cent.

Cultivars Anjali Bt and LRA 5166 Bt (*G. hirsutum*) and RG 8 Bt (*G. arboreum*) were evaluated in all the three zones. The expression of Cry protein was assessed by ELISA test at different stages of plant growth viz., 60, 90, 130 and 160 days. Bt protein expression was found highest, (5-7 ug) in early stage and later it was found reduced to 1.12 to 1.85 ug.

Through Agrobacterium mediated gene transfer procedures the Bt gene transformation was attempted in

the *G. arboreum* varieties RG 8, PA255 and PA402. The available seeds were grown in boll to row progenies. The plants tested positive in "Dipstick" method before

flowering were further subjected to ELISA test on 90th and 125th days. The number of plants showing positive reaction are furnished in Table 18.

**Table 18: Transgenics in *G. arboreum***

Genotype	Generation	Gene Construct	No. of plants tested +ve		
			Pre-flowering	at 90 days	at 125 days
RG8	T3	<i>cry</i> 1Ac	119	66	60
PA255	T2	<i>cry</i> 1Ac	35	28	17
PA402	T2	<i>cry</i> 1Aa 3	17	15	14

The plants were also quantitatively assessed for the expression of Bt protein in the leaf sample (Table 19).

**Table 19: Quantitative assessment of transgenics**

Gene construct	Genotype	Plants tested	Expression of Bt Protein. (ug/mg)	
			90 days	125 days
<i>cry</i> 1Ac	RG8	119	0.019 to 3.1	0.012 to 3.55
<i>cry</i> 1Ac	PA255	35	0.13 to 4.93	0.06 to 4.50
<i>cry</i> 1Aa 3	PA402	17	1.07 to 3.48	1.07 to 3.90

Plants with high expression of Bt protein were selected for further evaluation.

#### Indigenously synthesised genes

Transformation of shoot tips were carried out by infecting the explants of 7 *G. arboreum* genotypes with

Agrobacterium containing Bt genes. In all 518 shoot tip explants were co cultivated with *cry*1Aa3 gene and 736 with *cry* 1F gene. Of these, 65 explants containing *cry* 1 Aa3 gene and 140 explants containing *cry* 1F gene were isolated from kanamycin medium (Table 20).

**Table 20 : Transformation of shoot tip explants by Agrobacterium containing *cry* 1 F and *cry* 1 Aa3 genes**

Genotypes	Gene construct	No. of shoot tips inoculated with <i>Af(robacterium)</i>	No. of explants growing on Kanamycin medium
PA402	<i>cry</i> 1 Aa3	187	28
PA405	<i>cry</i> 1 Aa3	30	11
PA 183	<i>cry</i> 1 Aa3	10	03
AKA 5	<i>cry</i> 1 Aa3	97	10
AKA 7	<i>cry</i> 1 Aa3	194	13
PA255	<i>cry</i> 1 F	696	140
PA405	<i>cry</i> 1 F	40	-

Indigenously developed gene constructs were used for the transformation of *G. hirsutum* cultivars Anjali & LRA

5166 through *Agrobacterium* mediated transfer. The transformation frequency ranged from 0.15% to 0.45%. (Table 21)

Table 21: Bt *cry 1 genes* Transformation and transformation frequency in *G. hirsutum* Cultivars

Genotypes	Gene construct	No. of explants	No. of Putative transformants	Npt-II positive plants	Transformation frequency (%)
Anjali (LRK - 516)	<i>cry 1 Ac</i>	1418	12	4	0.28
	<i>cry 1 Aa3</i>	1840	34	7	0.38
	<i>cry 1 F</i>	1509	22	4	0.26
	<i>cry 1 Ac(E)</i>	1265	10	2	0.15
LRA-5166	<i>cry 1 Aa3</i>	1095	06	2	0.45

#### Development of Bt. transgenic cotton for Insect Resistance

*G. hirsutum* variety viz. LRA 5166 was co-cultivated with indigenously synthesized genes *cry 1 Aa 3*, *cry] Ia5* and *cry IF.*. In LRA 5166, total 867, 130 and 903 explants were used for transformation with gene construct *cry 1 Aa3*, *cry] Ia5*, *cry IF* respectively. Positive transformants were identified with each of the genes, through PCR for *npt 11*. The putative transgenic plants are under various stages of testing in glass house and under field testing through RCGM approvals.

#### Development of disease resistant transgenic cotton through RNA interference mediated targeting of cotton leaf curl virus

Plasmid vector for generating double stranded RNA (dsRNA) of CLCuV sequences targeted for disruption of

CLCuV was constructed by modifying the cloning vector pBluescript (Stratagene). Two vectors, pKSB-Gus1 and pKSB-Gus2 were constructed using ca. 800 bp and 900 bp fragments of Gus gene respectively as a stuffer fragment (Fig. 1a). The primers were synthesized to amplify fragments of the Gus gene, flanked with restriction sites EcoRI and BamBI.. Plasmid pBluescript was digested with EcoRI and BamBI and the stuffers were cloned in the polyclonal sites, resulting in pKSB-Gus1 and pKSB-Gus2 of 3.8 and 3.9 kb respectively. Five sets of primers were synthesized to amplify genes of DNA-A and DNA ~ of CLCuV (Fig.1b). The primers were so designed that the amplified sequences would be flanked with specific restriction sites and cloned on either side of the stuffer sequence. The strategy would enable cloning the same sequence but in opposite orientations on either sides of the stuffer DNA.

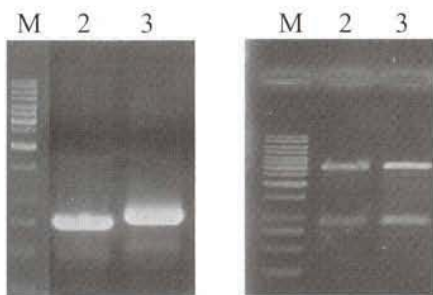


Fig.1a : PCR amplification (a) and cloning (b) of Gus fragment of 800 bp (2) and 900 bp sizes in plasmid pKSB to construct pKSB-Gus 1 and pKSB-Gus2 respectively

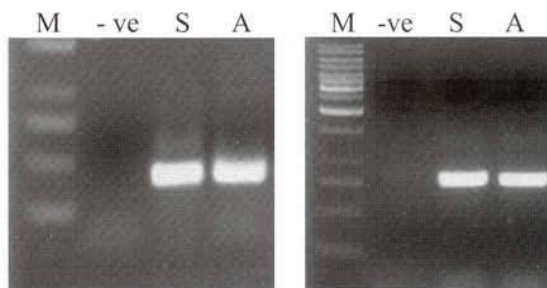


Fig.1b: PCR amplification of CLCuV ~DNA(a) and coat protein gene (b) for cloning in sense and antisense orientations in plasmid pKSB-Gus 1 and pKSB-Gus2

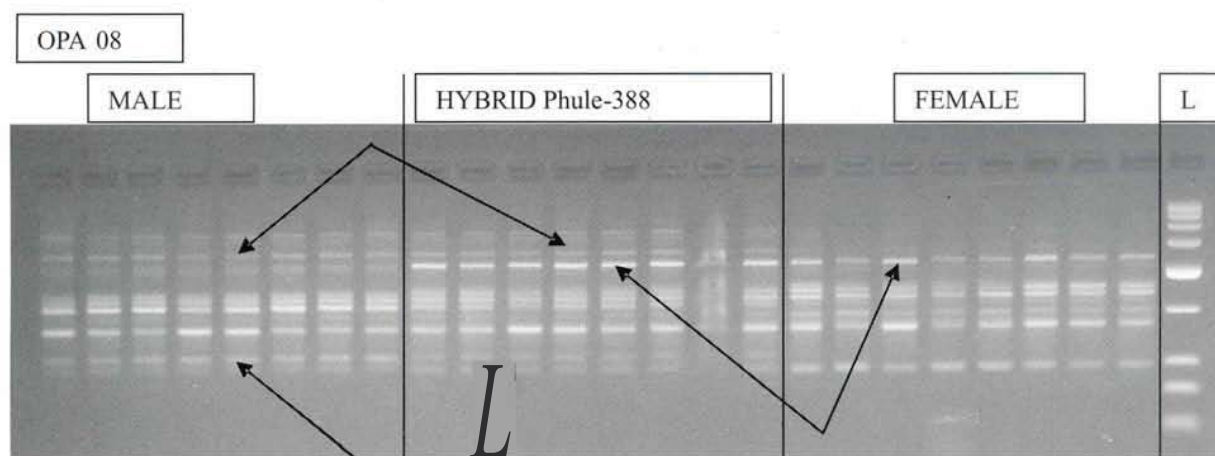
#### 4.6: Molecular Breeding

##### Nagpur

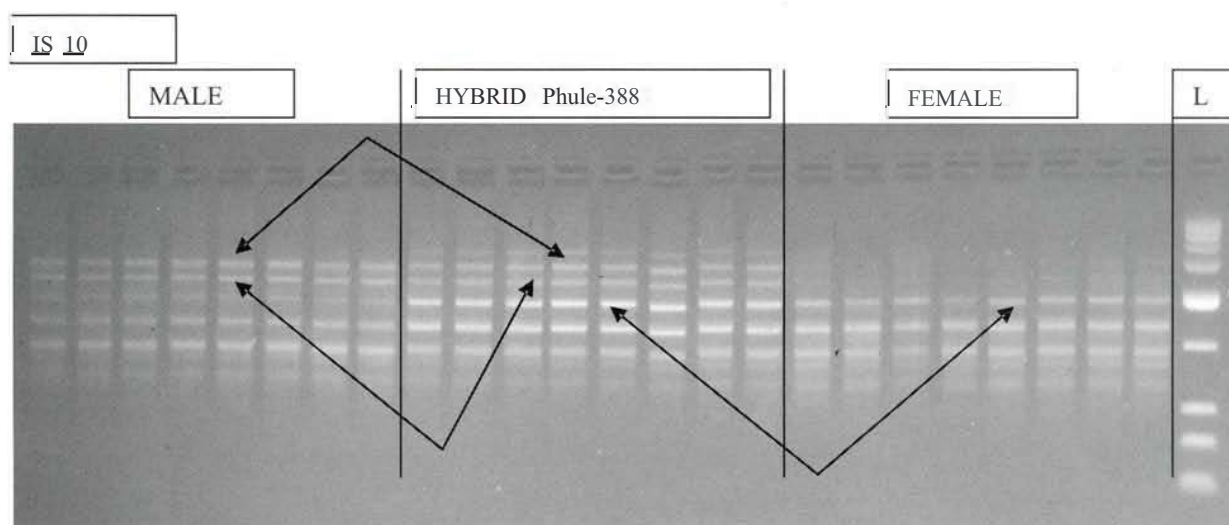
##### Studies on marker based purity evaluation of hybrids

RAPD primer, OPA 08 (Fig. 2) and OPA 11 ; ISSR primer, IS 10 (Fig. 3) and ISI2; SSR primer, S 28 and S

29 (Fig. 4) led to the conformation of hybridity of Phule-388 and hence can be used as a discriminating marker for testing hybridity and purity Phule-388. RAPD primer OPA 07 shows the male specific band and ISSR primer IS 15 shows the female specific band which led to the conformation of hybridity of DHH-II and hence can be used as a discriminating markers for testing hybridity of DHH-II.

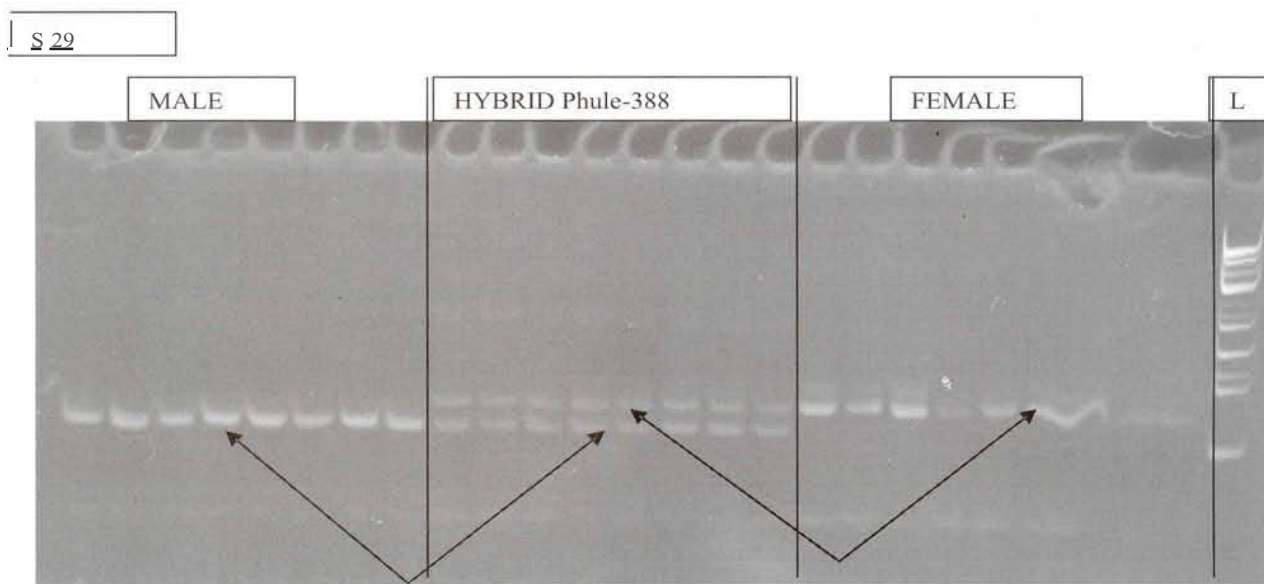


**Fig. 2: RAPD primer, OPA 08 led to the conformation of hybridity of Phule-388**



**Fig. 3: ISSR primer IS 10 led to the conformation of hybridity of Phule-388**





**Fig. 4: SSR primer, S 29 led to the conformation of hybridity of Phule-388**

#### Coimbatore

##### Genetic purity testing of hybrid seeds using electrophoresis technique in cotton

The Electrophoresis analysis for two cotton hybrids Surya and DHH 11, expressed polymorphic protein bands. The presence of protein bands, position and intensity of bands recorded for all the samples showed higher differences and can be utilized for varietal characterization and identification. The protein bands observed for surya (hybrid), M12 (Male parent) and HLS 329 (Female parent) are polymorphic in expression. There are nine in hybrid and eight in parents, the additional band expressed at  $R_m$  value 0,439 will delineate female parental seeds in the hybrid seed lot. The band expressed at  $R_m$  value 0,465 in hybrid and female parent confirm the parentage and also help to identify other cotton variety seed particularly the male seed in hybrid seed lot. Similarly the presence of protein band at  $R_m$  values 0.325 and 0,465 in CPD 423 (female) will aid for the identification of admixtures in hybrid DHH 11.

#### 4.7: Seed Production and Seed Quality Improvement

##### Nagpur

Seed vigour traits studied in six *G. hirsutum* varieties Viz., Surabhi, Anjali, F- 1861, K- 2 and NH 545 indicated that the seed germination and seed reserve quantity increased significantly for seeds with high seed index, whereas seed reserve utilization was unaffected by the seedling size of the seeds. Field studies revealed

significant increase in emergence, cotyledonary leaf area, seedling dry weight (15 DAS), sympodial number and seed cotton yield.

Testing for Distinctiveness, Uniformity and Stability (DUS) was undertaken in eighty genotypes using forty-one morphological traits as per National DUS Test Guidelines.

Seed yield and seed quality were assessed in seven released varieties in each of *G. arboreum* and *G. hirsutum* species. Seed yield and quality were assessed under low input management and under different soil depths. Preliminary studies showed that most of the varieties tested gave higher yields in medium type soil than in deep soils. In shallow soils, *G. arboreum* varieties gave higher yield as compared to the *G. hirsutum* varieties.

Studies were conducted to identify suitable areas for quality seed production. From the seeds sample received from seven centres in different zones and in terms of seed germination, seed index, moisture content and seed vigour, Banswara in Rajasthan and Surat in Gujarat appeared to be ideal centres for seed production. However, from the point of seed health, Banswara and Coimbatore (Tamil Nadu) appear to be the best as the seeds from these centres carried the least fungal load.

##### Coimbatore

##### Supplemental foliar nutrients on yield and quality of cotton seed

Application of DAP @ 2 %, Boron @ 0.6 kg/ha, Zinc @ 0.5% individually gave less improvement in seed quality.

When applied together (DAP@2 % + Boron@0.6 kg/ha + Zinc @ 0.5%) thrice during flowering and boll maturation phase of seed crop enhanced the seed quality significantly.

### Film coating of cotton seeds with Polymers

Polykote @ 3 ml kg<sup>-1</sup> + Thiram 75% WDP @ 2.5 g kg<sup>-1</sup> + Super red @ 5 ml kg<sup>-1</sup> + Cruiser 75% WP @ 5 g kg<sup>-1</sup> coating improved viability from 91% to 95%. The influence of polymer to maintain viability was found high in polykote than in polyloc over untreated seeds. At 26 months of storage seeds coated with Polykote @ 3 ml kg<sup>-1</sup> + Thiram 75% WDP @ 2.5 g kg<sup>-1</sup> + Super red @ 5 ml kg<sup>-1</sup> + Cruiser 75% WP @ 5 g kg<sup>-1</sup> have registered germination of 68% and 71% in cloth and poly bag, respectively, which was significantly higher than in untreated seeds (56% and 58%) stored in same containers.

Experiments conducted further to probe the sequence of coating / pelleting with nutrients and seed protectants indicated that coating of cotton seeds with Thiram @ 2g kg<sup>-1</sup> + Gypsum @ 60 g kg<sup>-1</sup> + Micronutrient @ 20 g kg<sup>-1</sup> + Imidacloprid @ 7g kg<sup>-1</sup> + DAP @ 20 g kg<sup>-1</sup> in five layers sequentially were found to significantly enhance the viability.

### Distinctiveness, Uniformity and Stability (DUS) testing of cotton genotypes

#### Developing National Test Guidelines

Draft National Test Guidelines for tetraploid and diploid cotton were developed to include subject, material required, conduct of tests, methods of observations, grouping of varieties, characters & symbols, table of characteristics, explanation on the table of characteristics and a technical questionnaire. In the table of 36 characteristics for tetraploid species, 21 essential traits were marked with an asterisk (\*), to be examined every season and included in the description of the varieties. The remaining characteristics are optional and helpful for characterization of a variety. Similarly for diploid species out of 31 characters 22 are essential which are to be observed each season to establish distinctiveness.

### Digitalization! Data base of extant notified varieties

Morphological characterization of 131 extant cotton genotypes was completed.

### Sirsa

#### Effect of seed size on field germination and yield

The effect of different seed size on field germination and

yield was evaluated in *G. hirsutum* (RS 2013) and *G. arboreum* (LD 327) varieties. The field emergence was better in seed lot of higher seed index. In RS 2013 the field germination percentage was 86%. The field emergence gradually declined with reduction in seed index. Similarly in LD 327 variety, the field germination was 84% when seed lot of above 5.4 gm seed index was used. In lower seed index lots, the reduction in field germination percentage was noticed. Similar trend for yield/ plant in each variety was observed.

#### Effect of different seedling vigour on field germination and yield

The effect of different seedling vigour on field emergence and yield was evaluated in *G. hirsutum* (RS 2013) and *G. arboreum* (LD 327) varieties. The higher field emergence was noticed in both the varieties i.e. 82% in RS 2013 and 88% in LD 327 when seed lot of higher seedling vigour was used. The yield was also more in seed lot of higher seedling vigour. The field germination per cent and yield per plant declined with reduction of seedling vigour of the seed lot.

#### Effect of pre sowing seed treatment on field germination and yield

Germination was highest in seed lots treated with KNO<sub>3</sub> (100 mM) with imidacloprid and vitavax (81%) followed by DAP (1%) with imidacloprid and vitavax (79%). The yield per plant, ginning out turn, seed index, boll weight and boll numbers were also higher in seed treated with these chemicals. In the seed lot treated with normal water, the field germination and value of yield parameter were lowest as compared to other treatments.

#### Effect of crop management practices on seed yield

The crop management practice of detopping at 60 DAS in *G. hirsutum* and *G. arboreum* species produced higher number of bolls and gave more yield. The yield in *G. hirsutum* variety was highest in plot detopped at 60 DAS (120 g/ plant) followed by 75 DAS (106 gm). In *G. arboreum* variety LD 327 the effect of topping was higher than the *G. hirsutum* variety. The yield /plant in topping at 60 DAS was 132 gm followed by topping at 75 DAS (115gm).

#### Standardization of seed pelleting/ coating with polymers

The seed coating with polyloc along with thiram and vitavax was superior with respect to germination percentage, vigour index and field emergence.

#### Economics of hybrid seed production

The net profit in hybrid seed production of conventional hybrid CSHH 198 was Rs. 74,813/ha, whereas it was Rs.1,15,063/ha in the case of male sterile based *Desi* cotton hybrid CISAA 2.

#### Maintenance Breeding

##### Nagpur

Maintenance breeding was undertaken in variety CNH 120 MB. Twenty five type plants were selected for further maintenance breeding.

##### Coimbatore

Cotton seeds of 85 varieties including parental lines and hybrids were collected and kept under reference collection

##### Sirs a

The selected five female, male progenies of each of hybrid Om Shankar, CSHH 198 and CISAA 2 were evaluated based on sca, gca and *per se* performance. Based on combining ability effects good combining lines were selected in the male and female parents of hybrids Om Shankar, CSHH 198 and CISAA 2. These will be used for production of nucleus seed.

#### Breeder Seed Production

Breeder seed production of the following varieties has been taken up would be commercially sold to the seed producers as per the Government of India allotment.

Name of variety	2006-07	
	Indent(q)	Production (q)
LRA5166	0.68	3.71
LRK516	0.75	1.84
Surabhi	1.05	3.50
Supriva	0.10	1.00
MCU5VT	0.02	2.50
CSHH 198		-
Female		50
Male		15
CSHH 238		-
Female		6.5
Male		10
CISA2		-
Female		30
Male		30
CISA310		50

#### 4.8: Integrated Nutrient Management

##### Nagpur

#### Long term effects of fertilizer and INM on productivity and fibre quality

Long term effect of fertilizer and integrated nutrient management on yield and quality of rainfed hybrid cotton in strip cropping system with pigeon pea indicated that with the combined application of major, secondary and micronutrients, significant increase in yield was observed over farmers' practice (FP) and FYM alone. Significant increase in seed cotton yield was observed in the treatment 2 t goat manure + 2 t FYM per ha with NPK: 60:30:30 (1387 kg /ha) over treatments RDF:NPK: 90:45:45 (1162 kg/ha), FP (819 kg/ha), organic manure (775 kg/ha) and control (403 kg/ha). Application of FYM, sulphur and zinc resulted in increasing yield of pigeonpea. Highest nutrient use efficiency in hybrid cotton at 110 DAS was observed in combined application of major nutrient + S + Zn + FYM. The organic carbon content increased from 0.39% to 0.57% at 0-20 cm soil depth, over 3 years in FYM treated plots. No significant improvement in fibre quality of NHH-44 hybrid was observed with the addition of secondary, micro-nutrient and organic manure.

#### Integrated nutrient management for high quality fibre and yield

Seed cotton yield was the highest with recommended INM practice (1660 kg/ha) and was significantly higher than the farmers' practice (1150 kg/ha) and application of recommended dose of fertilizers (1340 kg/ha). Application of recommended dose of N along with 2t FYM and 2t enriched compost yielded at par with the recommended INM. The results indicate that there is potential to save on fertilizer costs, especially P and K.

The average nutrient balance, as a consequence of all nutrient management practices, was in general negative for Nand K to the tune of 8.9 and 84.2 kg/ha, respectively, while it was positive for P, balance was positive (9.3 kg/ha). Fibre quality parameters were, in general, not affected by the nutrient management practices.

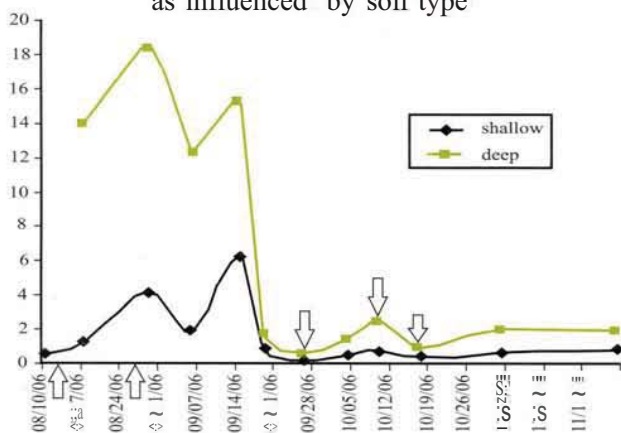
On-farm trials conducted indicated a significant impact of nutrient interventions. Application of recommended dose of fertilizer along with 20 kg ZnSo/ha yielded (1334 kg/ha) significantly more than the farmers practice (821 kg/ha). Effect on Ginning out turn and fibre quality parameters was also not significant.

### Effects of foliar-N application on crystal toxin expression in Bt Transgenic

Studies were conducted on Ankur 651 Bt on shallow (45 cm) and moderately deep soil (90 cm depth) to evaluate the response to foliar-N application. Averaged over soil types, application of N in 3 splits with or without foliar-N application resulted in significantly more yield (1410 to 1500 kg/ha) than application of N in 2 splits (1180 to 1220 kg/ha).

With regard to Cry toxin expression, application of N in 3 splits with foliar-N supplementation improved the toxin expression. In moderately deep soil, samples collected at 74 DAS in the plots with 3 splits had higher Cry toxin expression (6.84 Ilg/g) than in 2 splits (5.35 Ilg/g). Similar trends were noticed at later sampling dates; however, the levels were lower than the critical limit of (1.9 Ilg/g). Cry toxin expression was significantly higher in the moderately deep soil than the shallow soil (Fig. 5).

Fig. 5. cryIAc toxin expression as influenced by soil type



None of the fibre quality parameters were affected by the methods of N application. However, fibre length was significantly lesser in cotton grown on shallow soil (26.9 mm) than that on moderately deep soil (28.1 mm).

### Coimbatore:

#### Individual effect of bio-inoculants on cotton

Application of 75 % recommended N and P with Azospirillum + PSB + PPFM as seed and soil treatment along with two foliar sprays of PPFM at flowering to boll development recorded the highest (2106 kg/ha) seed cotton yield and it was on par with all other bio-inoculated treatments with 75 % recommended N and P or 100 % recommended N and P without bio-inoculants.

Application of 75 % recommended N and P without bio-inoculants recorded significant yield reduction to the extent of 438 kg/ha as compared to 75 % recommended N and P + Azospirillum + PSB + PPFM.

#### Field study to standardize the phyto-sphere application of PPFM

Application of 75 % recommended N and P (K 100 %) with Azophosmet as soil and seed treatment combined with two foliar application of PPFM at 60 and 90 DAS or at 45 and 60 DAS recorded numerically higher number of productive bolls/plant which was on par with all other treatments. The boll weight was not influenced by the treatments. Application of 75 % RDF with Azophosmet as seed and soil treatment was on par with 100 % RDF without bio-inoculants.

#### On farm testing

On farm testing of bio-inoculants on farmers field during 2006-07 using RCH 2 Bt as test cultivar indicated that application of bio-inoculants + 75 % recommended N and P recorded the highest (2612 kg/ha) seed cotton yield which was 15.8 % higher yield than 75 % recommended N and P alone without bio-inoculants.

### 4.9: Integrated Water Management

#### Nagpur

#### Yield maximization of hybrid cotton under drip irrigation

Results on fertigation through drip in hybrid NHH 44 grown on shallow soil indicated that significantly higher seed cotton yield (1900 kg/ha) was observed with the application of 50% of N<sub>120</sub>P<sub>60</sub>K<sub>60</sub> (through soil + 50% N<sub>120</sub>P<sub>60</sub>K<sub>60</sub> through fertigation with zinc sulphate @ 10 kg/ha and bio-fertilizer (T4) followed by same treatment without bio-fertilizer (T3) (1780 kg/ha) over soil application of N<sub>120</sub>P<sub>60</sub>K<sub>60</sub> (1370 kg/ha). Similarly, higher gross return of Rs. 38,080/- was obtained in T4 treatment with an input cost of Rs. 6190/- per ha as compared to a gross return of Rs. 25,888/- in treatment N<sub>90</sub>P<sub>45</sub>K<sub>45</sub> where input cost was Rs. 2650/- per ha. Maximum nutrient use efficiency was recorded in 50% nutrient fertigation treatment compared to soil applied nutrient.

#### Integrated water management system for quality fibre production

Effect of rain water management through different agro-

techniques in cotton indicated that higher seed cotton yield was observed in intercropping system followed by moisture conservation treatment like opening of furrows in alternate row as compared to rainfed control treatment. During deficit rainfall years, green gram (570 kg/ha)-cotton (1300 kg/ha) was the best intercropping system. Highest gross return of Rs.39541 per hectare was obtained in green gram followed by soybean (Rs.38,690 per hectare) intercropping system.

Response of cotton to protective irrigation at different growth stages indicated that two life saving irrigations, one at flowering and the other at early boll development stage gave higher seed cotton yield (1400 kg/ha) as compared to irrigation at 0.8 IW/CPE (1310 kg/ha) and rain fed treatment (1180 kg/ha) in medium deep soil. When the availability of water is less, one irrigation at peak boll development stage was found economical. Pooled data of three years revealed that significant difference in seed cotton yield was not observed between two or three irrigations. However, one irrigation at peak boll development stage was on par with three irrigations given at different growth stages in obtaining significantly higher yields.

#### **Coimbatore**

#### **Rain water management through agro techniques**

Amongst the different treatments tested, highest seed cotton yield (15.3 q/ha) was recorded with the application of protective irrigation (three times) to protect the crop from continuous dry spell which occurred during the crop growth period. The result was on par with soil moisture conservation methods viz; furrow opening at each row (14.0 q/ha) and alternate rows (13.8 q/ha), tied hoeing (12.8 q/ha) and the combination of compartmental bunding + straw mulching @ 5t/ha + kaolin (1%) spray 30 days after last rain (12.4 q/ha). The soil moisture conservation methods registered significantly higher yield as compared to rainfed control and cotton based intercropping system (cotton + soybean, cotton + black gram and cotton + green gram). However, in terms of seed cotton equivalent yield, cotton + black gram system was equivalent of producing of 15.6 q/ha seed cotton yield, because of higher market price for black gram. None of the quality parameters were influenced significantly by rainwater management techniques as compared to rainfed control. Protective irrigation showed the highest nutrient uptake of nitrogen, phosphorus and potassium followed by soil

moisture conservation practices.

#### **Response of different critical growth stages of cotton to protective irrigation**

Significant response was observed with application of protective irrigation to rainfed cotton crop at critical crop growth periods. Providing a single irrigation at square formation or boll development stages, two irrigations at squaring and boll development stages were compared with scheduling of four irrigations as per the climatic needs (0.8 IW/CPE ratio) and rainfed control. The highest seed cotton yield (17.4 q/ha) was harvested with irrigation applied at 0.8 IW/CPE ratio and was comparable to two irrigations (17.1 q/ha) and single irrigation at boll development period (16.7 q/ha). The highest water use efficiency (WUE) of 5.6 kg/ha-mm and irrigation use efficiency (IUE) of 33.8 kg/ha were realized by providing single irrigation at boll development stage. Water use efficiency and irrigation use efficiency decreased with an increase in frequency of irrigation (1 to 4).

#### **Low cost drip irrigation system**

The highest net return (Rs. 24,404/ha) and benefit cost ratio (1.9) were obtained with poly tube (using 300 gauge thickness) drip system. The results revealed that an average increase of 13% seed cotton yield, higher irrigation use efficiency and water saving was recorded with the drip irrigation treatments than ridges and furrow method of irrigation.

#### **Yield maximization in ELS cotton**

The combination of chisel ploughing + drip fertigation + foliar spraying of speciality fertilizer (19:19:19 @ 1% at 75 and 105 DAS and 13:0:46 @ 1% at 90 DAS) yielded 2950 kg/ha seed cotton at 30 % increased in comparison with control. None of the yield maximization techniques had significant influence on quality parameters of ELS cotton.

#### **Water saving, growth, yield and quality of ELS cotton under poly mulching, drip and drip + poly mulching**

The growth of ELS cotton hybrid RCHB 708 Bt was influenced favourably due to poly mulching and drip + poly mulching and was found better than drip irrigated cotton. Poly mulch + Drip at 0.4 Etc recorded the highest seed cotton yield (5494 kg/ha), lesser water requirement (44.5 ha-cm) and the highest water use efficiency (123.5

kg/ha-cm). Water requirement was the highest (95.8 ha-cm) for conventionally irrigated cotton.

Fibre length was significantly influenced by poly mulching. Among the treatments poly mulch + drip 0.8 Etc and poly mulch without drip recorded higher fibre length of 36.5mm as compared to significantly lower fibre length of 34.2 and 34.6 mm respectively with drip 0.4 Etc and conventional method. The fibre strength is also numerically higher in poly mulch + drip at 0.4 Etc.

#### **Drip fertigation of major, secondary and micronutrients for enhancing the productivity of ELScotton**

Water conservation technique of poly mulch alongwith drip irrigation recorded significantly higher seed cotton yield (6257 kg/ha) followed by drip system without poly mulch (5246 kg/ha). The lowest seed cotton yield of 4272 kg/ha was recorded in the conventional method. Seed cotton yield increased by 22.8 % due to drip and 46.7 % due to Drip + poly mulching. Application of recommended dose of fertilizers (RDF) of (120-60-60 NPK kg/ha) resulted in the least seed cotton yield (4316 kg/ha) compared to RDF+ZnSO<sub>4</sub> (5066 kg/ha) or application boron (0.15%) foliar spray alongwith RDF (5389 kg/ha). Combined application of ZnSO<sub>4</sub>, MgSO<sub>4</sub> and B resulted in the highest seed cotton yield (5580 kg/ha) and was at par with RDF+B and significantly superior to RDF & RDF+ ZnSO<sub>4</sub>.

#### **Growth and yield performance of Bt and non Bt cotton under poly mulching and planting techniques**

The poly mulched Bt under double row planting recorded the highest yield of 6644 kg/ha as compared to 4463 kg/ha under non mulching. The non Bt genotype also responded significantly to poly mulching with 4530 kg/ha under double row planting as against 3694 kg/ha under non mulching. The triangular planting was on par with single row planting for seed cotton yield with an additional benefit of 5.5 t/ha of radish yield. Green gram grown all around the raised bed in irrigation channels yielded 426 kg of dried grains/ha.

#### **4.10: Conservation Tillage**

##### **Nagpur**

#### **Tillage and green manure effects on growth and yield of cotton and soil properties**

Field experiments were conducted to evaluate the green manure effects on growth and yield of Bt cotton. Seed cotton yield was not affected by tillage treatments. Conventional tillage treatment (1403 kg/ha) was at par with the reduced tillage treatments (1457-1564 kg/ha). Similarly, plant growth (dry matter) and yield attributes (boll numbers) were not affected by any of the treatments. Tillage effects were significant on the weed density and weed dry matter accumulation. Significantly fewer weeds were recorded in the reduced tillage plots as compared to the conventional tillage treatment. Consequently, weed dry matter was significantly lower in the reduced tillage plot (21.3-26.2 g/sq. m.) than the conventional till plot (55.5 g/sq. m.). Tillage effects did not influence the fibre quality parameters.

Green manure with 80 kg N/ha yielded at par with application of 100 kg N/ha indicating a potential for 20% N saving with *in-situ* green manure. Further reduction in N to 60 kg N/ha lowered seed cotton yields (1168 kg/ha) and was significantly lower than application of 80 kg N/ha (1515 kg/ha) and 100 kg N/ha (1641 kg/ha). This was because the plants accumulated significantly lesser dry matter at all the growth stages and produced fewer bolls per plant. The plots also had lower seed index (7.75 g) than the 80 kg N (8.37 g) and 100 kg N plots (8.94 g). Fibre quality parameters were not affected.

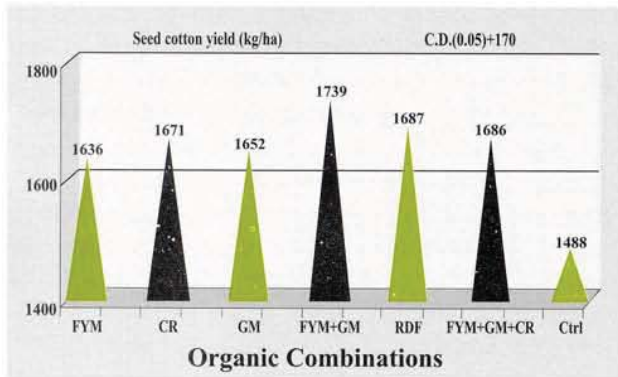
##### **Coimbatore**

#### **Assessment of organic residues along with *in-situ* incorporation of green manure on soil fertility dynamics and cotton productivity**

Simultaneous application of FYM (5 t/ha) and *in-situ* sun-hemp green manure buried at 45 days of planting (10t biomass) produced highest seed cotton yield (1739 kg/ha) and was significantly superior in fibre production efficiency and yield over control. In addition, the said combination was superior in respect of both gross and net return over RDF over the years.

The benefit cost ratios were 1.75 for cotton stalk (5 t/ha) incorporation, 1.67 for sunhemp *in-situ* green manure, followed by FYM application (1.52). Combination of green manure and cotton stalk also improved the B: C ratio (1.71) over green manure alone (1.67). FYM (or its combination with others) being a costly input especially when purchased from outside sources gave lowest return and B: C ratio (Fig.6).

**Fig.6: Effect of GM & other organic combinations on cotton productivity**



**Changes in the soil physico-chemical properties and crop productivity under various soil cover/incorporation of *ex-situ* plant wastes.**

Significant seed cotton yield was recorded under *itsit* (*Trianthema*) weeds (2291 kg/ha), *Cynodon dactylon* grass (2290 kg/ha) and FYM (2295 kg/ha) applied @ 5 t/ha (on dry weight basis) over others including RDF (2035 kg/ha) and control (1694 kg/ha). Shading of top soil surface under the semi-arid condition with mulch had an advantage in terms of moisture conservation, weed suppression and nutrient availability besides improving field performance of cotton crop.

Maximum economic yield advantages were obtained with easily decomposable bio-wastes. Soft plant tissues such as *Trianthema*, and dried grass were more remunerative than FYM.

**4.11: Cotton Based Cropping Systems**

**Nagpur**

**Strip Cropping Systems**

Cotton + Pigeonpea stripcropping hybrids 6:2 and *desi* cotton 12:2 were economical and profitable.

**Coimbatore**

**Long term effect of nutrients in fixed cotton based crop rotation**

Studies on cotton based cropping system revealed that introduction of grainjowar as a sequential crop in cotton fallows enhanced seed cotton yield by 21.6% (376 kg/ha) and total biomass yield at 1<sup>st</sup> picking by 32% (1446 kg/ha). In absolute terms, cotton productivity pooled over the years were 1061 and 1340 kg/ha for cotton-fallow and cotton-sorghum, respectively.

Application of NPK (60:13:25 kg/ha) recommended dose offertilizers (RDF) resulted in significantly higher yields than the control and crop residue treatments due to partial nutrient immobilization, during the current year.

**Cotton based cropping system for higher production and return**

Studies on intercropping multiple vegetables with different growth habits revealed that seed cotton yield was significantly influenced by different multi-tier cropping systems. Highest seed cotton yield 3520 kg/ha was harvested with the intercropping of cluster bean, vegetable cowpea and *Dolichos* between cotton rows. The positive effect of legume intercropping (all three intercrops are legumes) through N fixation, helped produce the highest seed cotton yield. Seed cotton yield was the least (2690 kg/ha) when cotton was intercropped with radish + beetroot + coriander system. These intercrops bring non legumes may have competed with cotton and reduce consequently the seed cotton yield. However the system recorded the highest net return of Rs. 85,644/ha and benefit cost ratio 2.9 because of higher performance of all three intercrops. The highest percentage of weed smothering efficiency, leaf area index (3.6 times as compared to sole cotton) and percentage of light interception (6.1 times compared to sole cotton) were arrived with cotton + cluster bean+ vegetable cowpea + *dolichos* system (Table 22).

**Table 22. Economics of multi-tier cropping system**

Multi tier cropping systems	Seed cotton yield (q/ha)	Total Gross return (Rs/ha)	Total cost of cultivation (Rs/ha)	Total Net return (Rs/ha)	Benefit cost ratio	Seed cotton equivalent yield (q/ha)
T 1. Sole cotton (90x60 cm)	26.9	53740	28500	25240	1.9	26.9
T2. Cotton + radish + Veg. cowpea + beetroot	30.4	127567	50281	77286	2.5	63.8
T3. Cotton + radish + cluster bean + beetroot	29.1	132942	52696	80246	2.5	66.5
T4. Cotton + radish + <i>dolichos</i> + beet root	30.6	114579	46413	68166	2.5	57.3
T5. Cotton + Coriander + veg.cowpea + cluster bean	32.3	115113	52569	62544	2.2	57.6
T6. Cotton + coriander + <i>dolichos</i> + cluster bean	35.1	113032	50422	62610	2.2	56.5
T7. Cotton + beet root + veg.cowpea + cluster bean	34.0	113191	51980	61211	2.2	56.6
T8. Cotton + cluster bean + veg.cowpea + <i>dolichos</i>	35.2	99761	52313	47448	1.9	49.9
T9. Cotton + radish + beet root + coriander	26.9	131392	45748	85644	2.9	65.7
nO.Sole cotton (120 x 45 cm)	31.5	62960	29883	33077	2.1	31.5
SEd	2.0					
CD 5%	4.2					

### Crop rotation

Preliminary pot culture experiment were planned to screen millets for off season green manuring. The crops ragi (*Eleusine coracana*), tenai (*Setaria italica*), v-ragu (*Paspalum scrobiculatum* L.), samar (*Panicum m. illhare*), kudhiraivalli (*Echinochloa colona*), panalvaragu (*panicum miliaceum*) and control (cotton-fallow) were raised in pot culture without disturbing the layout in off season and incorporated at 45 days of growth and subsequently cotton was raised in regular season. The rotation with samai and thenai registered significantly highest per plant yield of 22.1 and 20.0 g respectively, which were 46 and 32.5 % higher as compared to cotton-fallow.

### 4.12: Bt Cotton Production

#### Nagpur

### Response of Bt cotton to soil and foliar application of nutrients

A field trial was conducted to study the effect of micronutrient applied as soil and foliar on yield and quality of Bt hybrid cotton (MECH 184) in medium deep soil. There was no significant increase in seed cotton yield by foliar application of zinc or boron. Highest yield (1150 kg/ha) was observed with soil applied Boron followed by foliar treatment of Boron @ 0.5 % (1100 kg/ha) with soil application of RD NPK.

#### Coimbatore

### Optimization of irrigation and nitrogen requirement for improving input use efficiency and productivity of Bt cotton

There was no significant difference among the irrigation



treatments with respect to seed cotton yield. However, N applied @ 90 kg/ha registered significantly higher seed cotton yield. Under water stress conditions between the irrigation events or after the withdrawal of rain, relatively higher soil moisture storage was maintained under no nitrogen control, which may be attributed to poor crop growth and less evapo-transpiration under this treatment. The water use efficiency (WUE) was the maximum under protected irrigation condition and it decreased with

the increase in the level of irrigation. The maximum water use efficiency of cotton was recorded when N was applied @ 90 kg/ha (Table 23). The partial factor productivity of N (PFPN) decreased with the increase in the N levels in all the irrigation treatments. Among the irrigation treatments the maximum PFPN was recorded under protective irrigation treatment. With the increase in the level of irrigation, the PFPN decreased (Table 24).

Table 23: Water use efficiency of cotton (kg/ha-cm) as influenced by irrigation and nitrogen management

Treatments	N <sub>0</sub>	N <sub>60</sub>	N <sub>90</sub>	N <sub>120</sub>	Mean
Control (Protective irrigation)	35.0	41.6	44.5	45.8	41.7 a
0.6 IW/CPE	31.6	33.4	34.4	32.8	33.0 b
0.8 IW/CPE	30.2	27.9	31.1	26.6	29.0 c
1.0 IW/CPE	22.3	24.5	27.4	23.2	24.3 d
Mean	29.8 b*	31.8 ab	34.4 a	32.1 ab	

\*Means followed by same numbers are not significantly different (P<0.05) as per DMRT

Table 24: Partial factor productivity of Nitrogen (PFPN) (kg seed cotton yield / kg N applied) as influenced by irrigation and nitrogen management

Treatments	N <sub>60</sub>	N <sub>90</sub>	N <sub>120</sub>	Mean
Control (Protective irrigation)	34.6	23.1	19.4	25.7
0.6 IW/CPE	33.7	22.5	17.1	24.4
0.8 IW/CPE	31.3	20.9	15.6	22.6
1.0 IW/CPE	32.1	21.4	15.8	23.1
Mean	32.9 a*	22.0b	17.0 c	

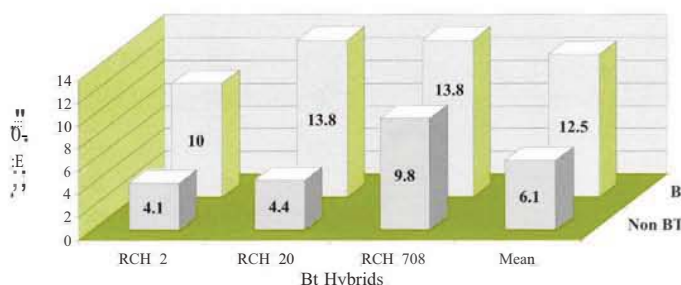
\*Means followed by same letters are not significantly different (P<0.05) as per DMRT

Performance of Bt cotton under scanty rainfall situation

Bt cotton hybrids under rain fed condition with sub optimum rainfall produced significantly higher mean yield of 1250 kg/ha, which was 105 % higher than their

non Bt counterparts (610 kg/ha). In Bt hybrids early formed bolls were protected by an in built resistance mechanism and converted as harvestable bolls and produced higher seed cotton yield (Fig. 7).

Fig. 7 : Seed cotton yield of Bt and isogenic non Bt hybrids under scanty rainfall situation



### 4.13: Ergonomically Efficient Implements for Cotton Production

#### Nagpur

#### Field evaluation of battery operated sprayer

A Battery operated knapsack sprayer has been developed. A twelve volt DC rechargeable battery and motor was used to generate pressure for the automization of spray fluid. The performance details of the new spray is given below:

#### Performance details of battery operated sprayer

1. Refilling time, (minutes)	:	2.5
2. Capacity, acre/day	:	3.6
3. Labour-hrs requirement	:	16
4. Effective working width, cm	:	90



**View of interculture tool for cotton**

#### Evaluation of Trifali (local weeding and interculture tool)

A locally available trifali (weeding and interculture tool) was tested in cotton crop for inter culture operation with the conventional *bakhar* in cotton field. The weeding efficiency of trifali is found to be 68 % as against 70% as against in the conventional *bakhar*. But it covers more area (1.62 acres) per day than the conventional *bakhar* (1.3 acres).

#### Testing of mechanical cotton picker

Studies on use of mechanical cotton picker (John, Deere 9935) for harvesting of seed cotton in CNH 120 MB and

CNH 36 showed that the picker efficiency was 89 % in CNH 120 MB and 84 % in CNH 36. The effective field capacity was found to be 2 hrs per hectare. The harvesting loss incurred was 5-10 %.

### 4.14: Production Physiology

#### Nagpur

#### Effect of plant growth regulators

Cotton cultivars NHH 44, MECH 184 Bt and Bunny grown under rainfed conditions were given foliar sprays of Gibberellic acid (100 ppm), Naphthalene acetic acid (0.45 ml/litre of water), Muriate of potash (1%), Single Super phosphate (1%) and Urea (1%). The results indicated that the treatment and interaction effects were not significant, whereas significant genotype differences were noticed with regard to most of the plant traits. With regard to yield response, the study showed that urea and single super phosphate 1% foliar spray during flowering increased seed cotton yield in NHH 44.

#### Effect of soil depth on productivity of *G. hirsutum* and *G. arboreum* cultivars

Seven genotypes belonging to *G. hirsutum* and seven to *G. arboreum* were grown in shallow, medium and deep soil conditions. Relative water content in leaf remained high in cultivars belonging to both the species grown under shallow soil condition. Medium soil type showed significant increase in root and shoot length, nodes, leaves, squares and dry matter production. Available soil moisture remained low in shallow soil whereas it was on par in both medium and deep soils. Root and shoot length remained high in *G. arboreum* genotypes in all soil types. Cultivars belonging to *G. hirsutum* had better leaf production in deep soil condition and had more dry matter production in both medium and deep soils. They possessed higher relative water content in leaf in shallow soil condition. Cultivars with better growth responses with regard to the soil types were identified.

#### Coimbatore

#### Physiological and molecular elucidation of fibre development process in cotton for enhancing fibre yield

#### Protein analysis in lint and lintless mutants

MCU 5 had a band of size 43.4 KDa on a 9% SDS PAGE gel which was not seen in the MCU 5 LL.

Studies on polymorphism in MCU 5 and its corresponding lint less mutant using PCR reactions:

Of the 100 primers studied, 20 were selected for the RAPD analysis. Totally 122 amplicons bands were seen of which 120 amplicons were found to be polymorphic. There was a difference in the band pattern in the primers sequence GAGAGGCTCC, in which the MCU 5 had two extra bands in them which were absent in MCU 5LL. The band size was about 2767 bp and 503 bp (Fig.8).

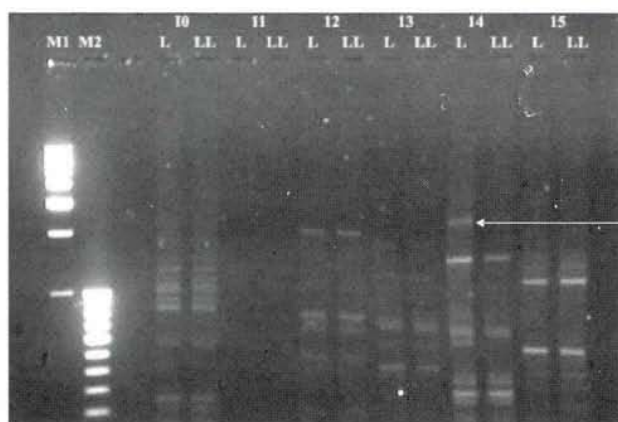


Fig. 8

- M1 - 1Kb ladder DNA (Genei Bangalore)
- M2 - 100 bp ladder DNA (Genei Bangalore)
- L - MCU5
- LL - MCU5LL
- - The arrow indicates the extra bands of size 2767 bp and 503 bp in the MCU 5 with the primer sequence 'GAGAGGCTCC'.

### Crop Modeling and Yield Prediction

#### Nagpur

In view of the rapid expansion of the Bt cotton hybrid area in the country, attempts were made to refine the INFOCROP model (validated earlier for varieties and traditional hybrids) to account for the changed phenology and other related attributes of Bt cotton, including root growth attributes. Accordingly, experiments were conducted at different project centres and the model was fine tuned by incorporating the requisite changes. The refined model was found to perform reasonably well at

different centres even though some more refinement is still needed. At the macro level, the integrated approach developed in the project was tested across the four cotton growing districts (viz. Nagpur, Bharuch, Dharwad and Sirsa), belonging to all the three cotton growing regions of the country. The area, production and productivity estimates through the integrated approach incorporating remote sensed data on crop acreage, geographical information based soil and weather maps and crop model for the year 2005-06 were found to be quite satisfactory in respect of Sirsa and Bharuch districts; while the values for Nagpur and Dharwad districts were on higher side specifically in respect of production and productivity.

### Source-sink relationship in cotton

#### Nagpur

In order to delay square initiation, ethrel was sprayed at a very low concentration of 0.01% and 0.1% at 54 DAS in LRA 5166 and DHY 286. Plant height, number of nodes, monopodia, sympodia, squares and weight of different plant parts did not show significant difference with the above treatments. However, yield increased marginally in LRA 5166 at 0.01% spray of ethrel. Studies on sink activity of Bt hybrids were taken up. In the absence of insect damage there was no difference in temporal and spatial distribution of fruiting forms between Bt and Non Bt hybrids. However, with insect attack most of the early formed fruiting points were lost in Non Bt in lower sympodia while, in Bt they were retained. This differential sink activity between Bt and Non Bt leads to various phenological and physiological changes.

#### Coimbatore

Foliar application of ethrel at 40 DAS showed a significant change in the biochemical constituents with increase in NR activity, enhanced accumulation of reducing sugars, proline and protein. The partitioning of the biomass was initially more to the vegetative parts like stem and roots. The stem girth doubled over the control plant. With the delayed initiation of reproductive growth, the shift in partitioning changed. There was a total shift and more than 80 per cent went to the fruiting parts and very little for the maintenance of stem and root. There was a synchronous flowering and boll development with ethrel application leading to uniform boll bursting. Application of ethrel @ 45 ppm at 40 DAS resulted in an enhanced yield to an extent of 26-46% (Table 25).

**Table 25: Effect of Ethrel (@45 ppm) on *kapas* yield**

Treatment	Yield in control plot (kg/ha)	Yield due to ethrel spray @ 45 ppm (kg/ha)	Per cent increase
RCH 2 Bt	2265	3320	46.6
RCH2NBt	2190	2940	34.2
BunnyBt	2300	2900	26.0
BunnyNBt	1975	2545	28.9

#### 4.15: Stress Physiology

##### Nagpur

##### Drought tolerance

The response to waterstress was studied at the terminal stage (100 DAS) in 12 drought tolerant genotypes of *G. hirsutum*, *G. arboreum* and *G. herbaceum*. Ambient grown plants were exposed to 100 %, 50 % and 25% available soil moisture for a period of 1 month.. The leaf water potential measured during the stress period showed lower water potential in *G. arboreum* and *G. herbaceum* varieties rather than in *G. hirsutum* varieties. Similar was the case with photosynthesis.

During the corresponding period, field grown plants were also experiencing stress under field condition. Leaf water potential of rain grown cotton corresponded with that of pot grown plants with 25 % ASM. The genotypic differences seen for tolerance were mainly attributed to root growth. Plants with shallow root experienced more stress as compared to deep rooted genotypes.

##### Study of drought tolerance during high temperature and low humidity condition

Bunny and LRA 5166 were grown in micro-plots during summer and drought treatment was imposed at flowering. The study revealed that development of water stress at elevated temperature and low humidity conditions led to marked increase in stomatal resistance and decrease in transpiration rate. Dry matter distribution remained lower in stressed plants. Leaf relative water content, growth and biomass production during recovery in stressed plants mostly reached to the level of control.

##### Screening of cotton genotypes for drought tolerance

The leaf water potential remained significantly higher in

*G. hirsutum* genotypes with a similar trend both in control and stressed plants as compared to *G. arboreum* lines. Leaf solute concentration on the other hand remained high in *G. arboreum* genotypes indicating a trend towards higher osmotic adjustment during drought condition. Stomatal resistance was found to be higher in *G. hirsutum* genotypes particularly under stressed condition. This resulted in the maintenance of higher leaf water status, whereas *G. arboreum* genotypes had relatively lower stomatal resistance under drought condition which led to higher transpiration rates and higher leaf cooling trends. The biomass production decreased under drought environment and it remained higher in genotypes belonging to *G. hirsutum*. Root-shoot ratio increased under drought stress and the ratio was maintained higher in *G. arboreum* as compared to *G. hirsutum* genotypes. Seed-cotton yield remained high in *G. arboreum* genotypes with high yield stability.

Nitrate Reductase activity showed a trend of decline due to water stress except in few genotypes. Peroxidase activity on the other hand increased under water stress particularly in *G. hirsutum* genotypes. Protein profile determined through PAGE indicated accumulation of protein under water stress in AC 6755.

Based on drought related plant traits, five lines viz., CAT 3640, CAT 3874, CAT 1058, AC 7602 and AC 7185 were identified as drought tolerant lines.

##### Salinity tolerance

##### Physiological and biochemical basis of salinity

Cotton cultivars belonging to *G. hirsutum*, *G. arboreum*, *G. herbaceum* and germplasm lines were screened for their salinity tolerance in petriplates, pots, microplots and under field condition. Twenty eight lines were exposed to 15 EC salinity in petriplates. The germination decline over control ranged from 17 to 75 %. The seedling weight

decline was from 6 to 41 %. The protein utilization in the seeds was delayed with salinity treatment. Thus, the seed protein content at germination had a very high correlation with decline in seedling weight.

Leaf protein on the other hand declined and osmotic compounds such as proline accumulated with salinity. Wide variability was seen in proline accumulation across species and genotypes. RWC in Leaf and water potential declined with salinity. However, it did not affect either the chlorophyll or leaf photosynthesis.

The K content of root and stem declined significantly with salinity while in leaf and fruiting parts it was on par with the control. On the other hand, the Na content steeply increased in root, stem and leaves while fruiting parts were totally free from any additional accumulation of Na. Across the genotypes K content remained almost on par between 0 and 10 EC while Na content increased by nearly 3 fold. LRA5166 showed the least accumulation of Na while it was highest in AK 32.

The genotypes selected for their salinity tolerance showed wide variability in yield when they were grown under saline soils of Gangavati, Kamataka. Seed cotton yield ranged from 300 to 880 kg/ha. The highest yield was recorded in LRA 5166.

### Physiological and biochemical basis of waterlogging

Contrasting genotypes viz. MECH 184 Bt wilt sensitive and RCH2 Bt wilt tolerant were raised in brick structure till flowering. At 90 DAS the brick structure was dismantled and the intact root was excavated. MECH 184 Bt had 30 % less root length, 25 % less root weight and nearly 50 % less root volume. The above ground biomass was almost same. This suggested that the genotypes with shallow root system are more prone to wilting. Waterlogging depletes the oxygen in the top layer very fast and hence, genotypes with a shallow root system are subjected to anaerobic condition which leads to damage of root hairs. This restricts the uptake of water and leads to wilting of plants.

### Coimbatore

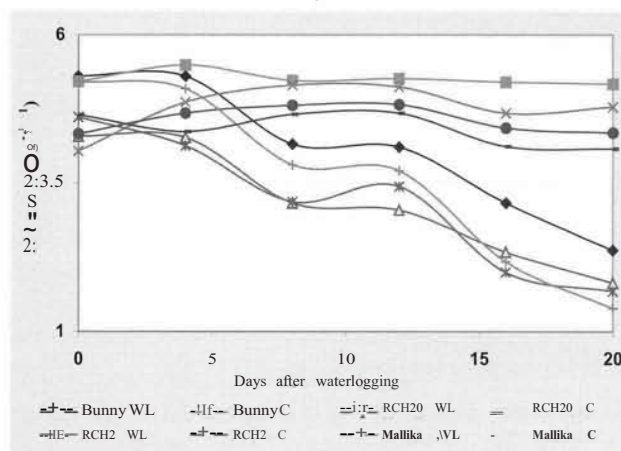
#### Impact of water logging on Bt cotton hybrids

Response of Bt cotton to water logging stress (5, 10, and 20 days) was evaluated in pot culture experiments at squaring phase of the crop. Water logging significantly reduced plant height, sympodia, leaf, boll, boll weight and yield beyond 5 days, irrespective of the Bt cotton cultivars.

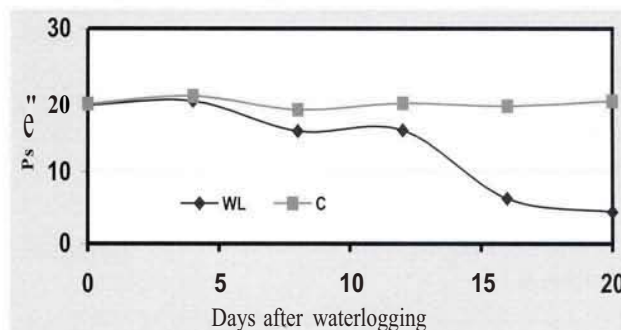
Bt cotton hybrids Bunny and Mallika were found more tolerant to water logging stress than RCH-2 and RCH 20 Bt. Reduction of only 22 and 29 % was recorded for Bunny and Mallika while RCH 20 and RCH 2 Bt suffered a loss of 45 and 33 % respectively when water logging was extended to 10 days. Further water logging for 20 days brought about a significant reduction in morphological, physiological and yield attributes studied with yield loss of 61 to 68 %. Chlorophyll content, nitrate reductase activity and photosynthetic rate started declining from fourth day of water logging irrespective of the hybrids (Fig. 9 & 10).

Among the hybrids Bunny and Mallika recorded a better photosynthetic rate, nitrate reductase activity with more chlorophyll content. Lenticel formation started earlier in Bunny covering the entire circumference of the stem just below the waterlogged level by tenth day. Other hybrids took 15 days for the process to be completed indicating that adaptation mechanism was rapid more in Bunny.

**Fig. 9: Water logging impact on Nitrate reductase activity**



**Fig. 10: Water logging impact on Photosynthesis (mean effect)**



#### 4.16: Social Dynamics of Cotton Production

##### Coimbatore

##### Present status, constraints and future strategies of cottonseed production in Tamil Nadu

SWOT analysis indicated additional returns to cotton farmers who enter into seed production and regulated flow of quality seed through public seed companies as strength, umpteen number of private seed companies and varieties/ hybrids, weak marketing channel, poor infrastructure facilities, higher rates from private seed companies and labour problems in hybrid seed production as weakness, emphasis on seed village and good quality seeds can be obtained as opportunities and admixture of seeds poor quality in cotton and International price Vs Domestic price as threats.

##### Gender role in cotton - role of women in cotton based cropping systems

More than 80 % of the respondents reported that the major activities performed by the farmwomen in cotton are stubble removal, sowing, gap filling, intercropping, thinning, weeding, fetching water for pesticide application, de-topping, labour supervision, harvesting of crop and intercrop and cotton grading. The major health hazards causing farm activities as reported by the farm women (85%) are mixing concentrated chemicals with water and filling spray tanks, mixing and subsequently working in the fields, working in a recently sprayed field and applying pesticides. The major signs and symptoms of acute pesticide poisoning reported by them (87 %) were burning eyes and nose, tearing, difficulties in breathing, sweating, excess salivation, headache, body ache, running nose, skin rashes, blurred vision, muscle cramps, nausea, tremors, vomiting, drowsiness and seizure. Regarding the gender division of labour in cotton, women comprise 72% of the labour force. A high share of work was done by women at peak times (weeding and harvest). Fetching water for spraying was reported as predominantly female task and required 20 hours labour per hectare.

##### Evaluation of technologies and economic viability

Seven technological interventions were assessed through 70 on-farm trials. Varieties, Surabhi and Sumangala performed well with 1:1.58 and 1:1.81 cost-benefit ratio respectively. Seed treatment and correct date of sowing intervention offered 25 % increase in the seed cotton yield. Intercropping with vegetables viz., beetroot, radish and cluster bean resulted in 1:2.25 cost-benefit ratio.

Integrated nutrient management based on soil test gave net returns of Rs. 27150/- as against applying huge amount of nitrogenous and complex fertilizers (Rs. 17925/-). Integrated weed management (IWM) yielded 11.11 % more yield than manual weeding. Integrated pest management (IPM) reduced the cost of production by Rs. 225/ quintal seed cotton. Integrated Disease Management proved to be a successful technology with C: B ratio of 1:1.86 as against fungicides application (1: 1.63).

##### Accessibility to mass media and information technology

##### Nagpur

A study was conducted in Wardha and Nagpur District to assess the accessibility of the latest information on cotton cultivation by the extension workers. As many as 82 % extension workers had regular access to vernacular print media and 44% to the printed news item from SAUs/ ICAR Institutes. As regards electronic media it was seen that 55 % extension workers regularly watched the agricultural programmes in Radio and 45 % in television. Toll free kisan call centres set up by the Central and State Governments was also made use of by 55 % extension workers. About 20% of the extension workers use the mobile phones to get information from the experts and 15 % of them have even access to websites to get first hand technical information. The data clearly indicated that there is a huge potential for quality extension service.

#### 4.17: Cotton Economics and Marketing

##### Coimbatore

##### Farm level economic benefits of Bt cotton in Tamil Nadu

Most of the cotton cultivators (85%), opined higher yield as a reason adopting Bt cotton coupled with less cost of cultivation, marketing facilities and early maturity. Bt hybrids performed better under irrigated conditions when compared to non irrigated conditions. The relative yield advantage was offset by lower prices for Bt cotton when compared to non Bt fields. The B:C ratio was almost in the range of 1.4 to 2.4 in Bt cotton under irrigated conditions and 1.2 to 1.8 under rainfed conditions. In case of non Bt, B:C ratio was around 1.3 to 2.4. Non Bt was more economical than Bt under rainfed conditions.

##### Economic analysis of contract farming in cotton in Tamil Nadu

The total cost of cultivation was higher in case of non

contract farming when compared to contract farming by a difference of Rs.2200/- which was due to higher labour use in the former case. B:C ratio over total cost and cost of production per quintal was remunerative under contract farming (1.53; Rs.1356/q) compared to non contract farming (1.18; Rs.2016/q) in cotton. The main constraint faced by the farmers is that the output price is not realised as promised by the contractor. Similarly when the market price is higher than the price quoted by the contractor, farmers sell their produce in the outside market.

### Information system on cotton

Large volume of data as well as documents on all aspect of cotton from different sources were collected, digitized and suitable databases were created. User-friendly information retrieval system using Visual Basic.NET as well as ASP.NET was developed. The software can be distributed both CD version as well as ready to float at web site portal. Key word search tool has been developed through which the user can download the desired information from the document base.

CICR web site ([www.cicr.gov.in](http://www.cicr.gov.in)) is being maintained with about 350 pages of information in the form of HTML, .doc, PDF format and about 450 images in the image gallery.

### 4.18: Pest Scenario

#### Nagpur

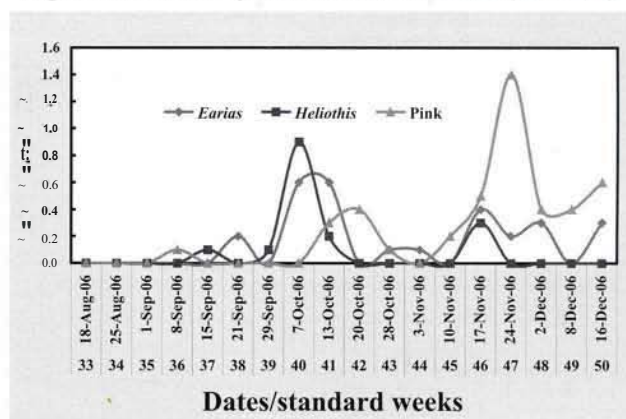
**Seasonal dynamics of insect pests:** Seven species of Hemipterans- one of Lygaeidae, two of Miridae and four of Pentatomidae were documented in the cotton ecosystem during the season. While the pentatomids viz., *Nezara viridula* (Linnaeus) var. *torquata* (Fabricius) and *Nezara viridula* (Linnaeus) var. *smargdula* (Fabricius), and mirid, *Badozorus* sp. belong to pest category, lygaeid, *Geocoris ochropterus* (Fieber) and mirid, *Zanichius* sp. are predatory. Two pentatomids *Plautia fimbriata* (Fabricius) and *Piezodorus rubrofasciatus* (Fabricius) are yet to be characterized for their relative importance and association in cotton ecosystem.

Jassids were associated with the crop from the end of July until harvest with a peak during second fortnight of August. While aphid incidence was minimal, thrips had a single moderate peak during first week of September. Mirid populations were found to infest from the last week of August until crop harvest,

*Helicoverpa armigera* larval incidence from the first oviposition peak was higher than the second. Larval peaks coincided with first and third weeks of September, and the first week of October. The first and second peaks

of *Earias vittella* coincided with those of *H. armigera*. Overall, the incidence of all lepidopteran insects including bollworms, was minimal during the season. Moth catches of *H. armigera* in pheromone traps indicated surge in population levels from mid December subsequent to increased population levels on pigeonpea. The damage to cotton fruiting structures was higher due to *H. armigera* between mid- September up to October first week. The combined damage due to *H. armigera* and *E. vittella* during October to mid November caused greater than 10% damage. Beyond mid November, the damage was due to *E. vittella* and *Pectinophora gossypiella* (Fig. II).

Fig.11: Seasonal dynamics of bollworms (2006-07)



**Seasonal dynamics of natural enemies:** Among the native predators, coccinellid and chrysopid populations were few during the season. Spider populations started to appear from August onwards, attaining a peak during mid October. *E.vittella* parasitisation by *Rogas aligarhensis*, *H. armigera* on red gram by *Campoletis chlorideae*, *P gossypiella* by *Apantelis angaleti* and *A.jlava* by tachinid *Palxorista laxa* was 19.2%, 25.4%, 11.1% and 50%, respectively.

**Multispecies associations:** While the sap feeders viz., jassids, thrips and mirids had significant temporal associations, spiders were associated with jassid adults and mirids. Jassid nymphs and thrips had significant simultaneous occurrence with *H. armigera* larvae and *Anomis flava*. The period of oviposition by *H. armigera* coincided with the occurrence of *Anomisjlava* larvae.

#### Coimbatore

**Jassids:** No significant difference was observed in the level of population of jassids between the Bt and NBt hybrids. Except the H<sup>nd</sup> fortnight of October and

December, during the other periods jassid population crossed ETL in RCHB 708 and MRC6918 hybrids. Among the hybrids RCHB 708 and MRC 6918 recorded higher population levels and minimum population was recorded in MECH 184.

**Aphids:** The Bt and NBt hybrids recorded no significant difference in the level of population of aphid. However, maximum and minimum number of aphids was recorded in RCHB 708 NBt and RCH 2 NBt respectively when compared to the other hybrids.

**Whitefly:** There were no difference in the population density of white fly in all the Bt and NBt hybrids.

**Pink Bollworm:** The incidence of PBW was significantly lower on Bt hybrid than the non Bt hybrids. Among the three different aged bolls 40 days old bolls recorded maximum percentage of incidence followed by 30 and 10 days old bolls. The incidence of PBW on Bt hybrid varied from 0-17.5 % and on NBt the incidence varied from 0-65.73%.

**Bollworm damage:** Significant difference in the bollworm damage percentage was recorded between the Bt and NBt hybrids. The Bt hybrids were superior than the NBt hybrids by recording minimum percentage of boll damage, with no incidence on MRC 6918 Bt and ACHII Bt during October and November. Bollworm population crossed ETL in NHH44, ACH-II NBt, MECH 184 NBt during October, November and December respectively.

**Pest status during the season in IRM Project villages**

Sucking pests viz., jassid, aphids, thrips and whiteflies population were below threshold level and averaged 0.43, 0.66, 0.15 and 0.28 per leaf respectively for the IRM villages in Salem District and 0.50, 0.50, 0.22 and 0.23 per leaf in Theni District. The low population of the bollworms resulted in lower damage.

**Population dynamics of cotton pests and their natural enemies**

Investigations made on the population of insects pests of cotton revealed that the incidence of aphids was negligible throughout the cropping period, both in Bt and N Bt RCH 2 cotton, raised under protected and unprotected conditions. A maximum jassid population of 10-12/plant was recorded during the middle of November. Mirid bug incidence was recorded during the first week of January both in protected and unprotected conditions in Bt and NBt cotton. About 80-90 % plants were observed to have mealy bug incidence both in Bt

and NBt cotton. Bt cotton both in protected and unprotected conditions recorded nil incidence of bollworm particularly *Helicoverpa armigera*. In general, the infestation on non Bt cotton was very low. An incidence of 8-10% was recorded during the middle of January.

**Disease survey**

During the winter cotton season of 2006 -07, grey mildew was noticed in severe form throughout Tamil Nadu and all four cultivated *Gossypium* spp. were affected. Sporadic incidences of *Alternaria* leaf spot were noticed.

#### 4.19: Resistance to Insect Pests and Diseases in *Gossypium* Spp

**Insect Pests**

**Nagpur**

Influence of jasmine perfume on plant resistance to insect pests: Jasmine perfume is an induced volatile chemical triggered by injury in plants. A synthetic analogue, commonly used as a perfume, was obtained commercially and tested on NHH 44 (sucking pest resistant) and RASI 2 (sucking pest susceptible). NHH 44 and RASI 2 responded to jasmine perfume by showing a significant reduction in the jassid nymph population at its peak infestation. Jasmine perfume also induced significant earliness in boll opening in RASI 2 and NHH 44 by almost 20-25 days as compared to the untreated plots. There were significant differences in the yield between jasmine perfume treated and untreated plots.

Introgression of trypsin inhibitor (Ti) gene into native varieties for bollworm resistance: A trypsin inhibitor (Ti) gene is being introgressed from an exotic germplasm PeeDee 0695, into elite Indian varieties, Bikaneri Narma and G-Cot-10. One hundred and fifty plants each of parents and BC<sub>1</sub>F<sub>1</sub> crosses were evaluated for their tolerance to sucking pests and bollworms under unprotected conditions. Progeny of crosses were tolerant to jassids as compared to the donor parent Pee Dee 0695. Bollworm incidence and damage was low in the season and thus comparative assessment could not be made. Yield of high trypsin inhibitor plants of Bikaneri Narma x Pee Dee 0695, and G. Cot 10 x Pee Dee 0695 was 57.6 ± 8.8 g and 42.5 ± 3.2 g per plant, respectively. The low Ti plants yielded 45.6 ± 4.0 g and 32.0 ± 3.2 g respectively in each of the two progenies. Forty healthy plants from each of the crosses were chosen to study their trypsin inhibitory properties using *in-vitro* enzyme assays with BAPNA as substrate. In the two crosses out of the 40 plants tested, 17 and 20 plants, each of the two crosses,



demonstrated trypsin inhibitory properties thus correlating well with the expected ratio for a monogenic trait in a backcross for chi-square test. Flowers from the high Ti plants were used for further introgression. BC<sub>2</sub>F<sub>2</sub> boll to row material of two crosses under introgression was evaluated for their pest tolerance under unprotected conditions and for their trypsin inhibitory properties. High Ti and no Ti plants (and plants with intermediary trypsin inhibitory properties) were identified and selfed to generate BC<sub>2</sub>F<sub>3</sub> for further identification of

homozygous plants.

Evaluation of BC<sub>2</sub>F<sub>2</sub> progeny for in vivo trypsin inhibition. Bioassays Wyre carried out with liquid nitrogen crushed boll rind powder of Bikaneri Nerma x Pee Dee 0695 BC<sub>2</sub>F<sub>2</sub> using early second instar larvae of *H. armigera*. Boll rind was diet incorporated and larvae were allowed to feed for 5 days with the diet changed twice. Larval mortality and growth regulating effects are reported in Table 26.

**Table 26 : *H. armigera* bioassays on BC<sub>2</sub>F<sub>2</sub> BN X Pee Dee 0695**

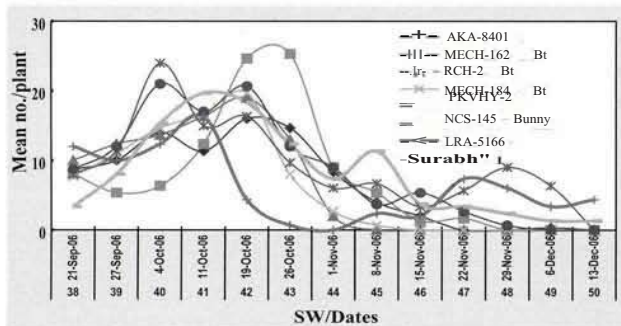
Boll to row	Ti UIIO Ilg boll protein	% mortality	Wt. of surviving larvae mg + S.E	%Ti
Br6	8.86	30	22.0 + 1.5	81.75
Br6	17.72	50	10.0 + 2.8	81.75
Br18	7.94	50	6.0+1.1	71.86
Br25	6.90	50	3.0 + 0.3	75.28
Br25	13.80	90	< 1,0	75.28
Br52	5.66	40	2.7 + 0.1	71.58
Br 52	11.33	30	10.0 + 1,1	71.58
Br22	5.95	20	2,1 + 0.2	89.16
8r22	11,89	50	< 1,0	89.16
Br 60	3.81	50	< 1,0	80.04
Control	nil	5	33.2 + 1,5	0.00

Development of insect resistant cultures and hybrids During the 2006-07 season, a total of 729 entries comprising 35,127,28,89,40,34, 16,135,185 and 40 F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, F<sub>4</sub>, F<sub>5</sub>, F<sub>6</sub>, F<sub>7</sub> single plant selections, backcrosses and germplasm selections were field evaluated for comprehensive pest tolerance under unprotected conditions. 184 selections from F<sub>2</sub>, 23 from F<sub>1</sub>, 74 from F<sub>4</sub>, 32 from F<sub>5</sub>, 29 from F<sub>6</sub>, 11 from F<sub>7</sub>, 169 from pure lines and 392 from backcrosses were made through selfing and/or single plant selections. 58 crosses were made using the lines developed for bollworm tolerance. Five of F<sub>7</sub>s, three of F<sub>6</sub> and four of F<sub>5</sub>s were assessed for the features of compensation and bulk harvested. Ten of the hybrids were superior the check hybrid NHH 44 with two of the hybrids having significantly lower bollworm damage in open bolls. Two of the hybrids viz., CIPT HHI and CIPT HH2 developed involving three parents Ambassador (2), LII (A) 5(A) and Raj performed better with yield levels of 1627 and 1631 Kg/ha, respectively under rainfed conditions.

Cultivar-insect pest interactions: Eight cultivars viz., AKA-8401, MECH-162 Bt, MECH-184 Bt, RCH-2 Bt, Bunny, PKV HY-2, Surabhi and LRA-5166 grown in demonstration plots were studied for cultivar pest interactions for the consecutive year. Late onset of squaring during the season vis a vis simultaneous damage to squares and bolls during mid October allowed only little time for cultivars to compensate actively. Differential phenology and response to fruiting structure loss determined the genotypes' yield potential (Fig.12).

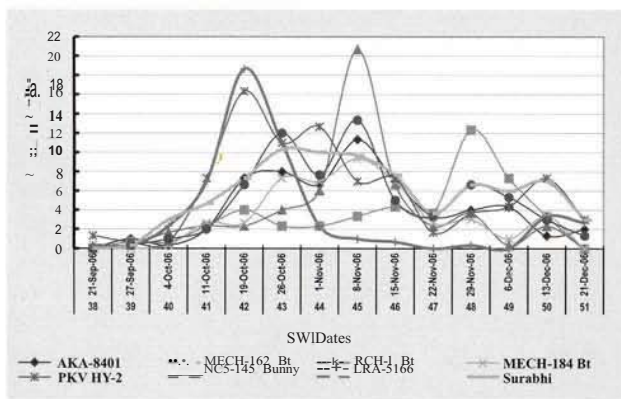
However, the cultivars had regulated passively to retain and develop bolls that could result in attainable yields during the given season. While the transgenics have contributed to yield due to the input trait of *cry-IaC*, the conventional hybrids made up for yield levels due to their ability to respond for damage through passive compensation. Bunny had the highest yield of all the cultivars and was on par with the three Bt-cotton hybrids. All the varieties were on par with each other for yield levels.

**Fig.12: Phenology of Square production**



The crop growth, fruiting and setting pattern of four parents (BN 1, AC 738, AK 32, DHY 286-1) of two hybrids viz., NHH44 and PKV HY2 revealed that the fixing up measures of compensation for bollworm damage using the end season crop growth and developmental estimates was not possible when damage occurred during early and late, as well as only during late seasons (Fig.13).

**Fig.13: Phenology of total fruiting structure loss**



**Resistance to *Rhizoctonia* root rot in tetraploid and *Fusarium* wilt in diploid cotton:** Out of the advanced cultures (66 of *G. hirsutum* and 7 of *G. arboreum*), screened through blotter, agar gel test, pot experiments and field evaluation in sick plot, seven lines of *G. hirsutum* and six strains of *G. arboreum* respectively were found to be resistant/tolerant against *Rhizoctonia* root rot and *Fusarium* wilt. Seven *G. hirsutum* cultures exhibited susceptibility against root rot. These were, SVLS-97-3, Sahana, NISC-26, NISC-29, NISC-16, NISC-1 and NISC-10. Out of the *G. arboreum* cultures, only CINA-316 exhibited susceptibility to *Fusarium* wilt, especially under low average temperature of 21.4°C and insufficient rainfall condition of 0.3 to 25.44 mm from July to December, 2006.

**Utilization of resistant sources:** Two hundred and sixty nine single plant selections with resistance to bacterial blight were made from progeny of resistant donor parents crossed with susceptible cultivars. Seed cotton yield of these selected plants varied from 53.4 - 87.8 gm/plant with an average of 17.3-23.4 bolls/plant and boll weight of 2.96-4.03 gm/boll. Seventeen resistant selections were identified for their superior parameters. The average boll number of these selections varied from 17.54 - 29.79 per plant with an average boll weight of 3.14-3.82 gm/boll. The average yield of seed cotton varied from 63.2-79.7 gm/plant with the plant height of 87.3-139.5 cm/plant.

### Coimbatore

#### Identification of resistant lines against Jassids

About 100 genotypes were screened for their reaction to jassids. Among these, six lines viz., GSHV-153, NH 630, CCH LS4, AKH 05-5, CNH-1101 and CNH 1102 recorded resistant reaction to jassid.

#### Screening of genotypes for resistance to insect pests

Promising advanced genotypes of 88 cultivars were screened for their reaction to major sucking pest, jassid and bollworms under field conditions. Twenty two cultivars showed tolerant reaction to jassid while twelve cultivars showed moderate level of tolerance to bollworm complex. The jassid tolerant cultivars recorded 0.9 to 1.9 jassid per leaf as compared to 4.0 in susceptible cultivars-VRS x V 112-3214-4 while the bollworm tolerant cultivars recorded 5.65-9.68 % bollworm damage as compared to 40.21 % in susceptible check variety MCU 5 VT.

Derivatives of tolerant introgressed lines possessed higher levels of terpenoid metabolites and phenols in young squares and developing bolls. Introgressed lines viz., IRH series and select conventional hybrids like LS 3 x LS 13 were found less susceptible to bollworm damage and possessed better biochemical attributes.

#### Development of resistant lines for foliar diseases

Eighteen advanced lines having resistance to grey mildew (GMR-6 nos.), alternaria leafspot (ALR -4 nos.), bacterial leaf blight (CBR -4 nos.) and MAR lines (4 nos.) were evaluated in the field for yield in comparison with LRA 5166 and Sumangala through randomized blocks design with three replications. Four lines viz. ALR- 20 (13.39 q/ha), GMR- 13 (13.74 q/ha), CBR -29 (13.26 q/ha) and MAR-8 (14.18 q/ha) had better seed cotton yield than LRA 5166 (11.73 q/ha) and Sumangala (7.80

q/ha).

Evaluation of AICCIP entries and breeder's lines for resistance to grey mildew

The AICCIP entries (333 nos.) consisting of National (124), Central Zone (119) and South Zone (90) were screened under poly house conditions separately for their reactions against grey mildew. After the development of the symptoms the plants were assessed for disease development. Eight lines viz RG 459, GAM 67, GAM 141, HLSa 802, KWA 225, AKA 9703 and ARBHA 35 (*G. arboreum*) and CCHB 727 were found to have resistance to grey mildew and 58 moderate resistances. Breeder's lines (77 nos.) were screened for their reaction against grey mildew and eleven lines exhibited moderate resistance to grey mildew.

Reaction of Bt hybrids to insect pests

Sucking pest-Jassid

Seven Bt hybrids which were released for commercial cultivation in Southern Zone showed differential response to jassid. The seed treatment with imidacloprid gave good protection upto 62 days after sowing (DAS) in RCH 2 Bt, MECH 184 Bt, Bunny Bt and Mallika Bt, while RCH 20 Bt, RCH 708 Bt and MRC 6918 Bt suffered and recorded significantly higher population (2.0 to 3.6 jassid per leaf) 62 DAS. Insecticidal spray (Confidor FS) brought down the population to a greater extent 70 to 85.3 % in all the Bt hybrids except MECH 184 Bt which had meagre population before spray as well as after spray. The natural reduction (by natural enemies and other causes) in unprotected field was 0-31.30 % in all the Bt hybrids except MECH 184 Bt which had a slightly higher population from 0.50 to 0.73 per leaf after three days in the post count.

Relative susceptibility of Bt hybrids to Mirid bug

Mirid bug, *Creontiodes biseratense* (Distant) damage leading to shedding of tender bolls revealed a loss of seed cotton yield by 134,95, 91, 87, 73, 59 and 51 Kg / ha in MECH 184 Bt, RCH 20 Bt, MRC 6918, RCH 708 Bt, Bunny Bt, Mallika Bt and RCH 2 Bt, respectively.

#### 4.20: Variability in Insect Pests and Pathogens

Nagpur

Intra specific variation in bollworm: Based on the partial CO-I and CO- II sequences, 19 haplotypes of *Helicoverpa* were identified in the country. The frequency and distribution of the haplotypes is presented in Table.27.

The haplotype nucleotide sequences are available on the NCBI site (National Center for Biotechnological Information, USA). Haplotype 17 was the founder haplotype and was distributed in moth populations collected from North, South and Central India. Using PCR- RFLP with Bst 2 UI as the restriction enzyme, a molecular diagnostic kit was developed and validated to identify haplotype 17 from the other existing haplotypes. This is also a pointer to the fact that intra-specific variation is present, whose functional significance is not known, in the mitochondrial genome of the insect.

Pathogenic variability of *Xanthomonas axonopodis* pv. *malvacearum* (*Xam*) races: Bacterial blight infected leaves were collected from five susceptible cultivars viz. Ganganagar ageti, LRA 5166, LRK 516, PKV 081 and Rajat having varying degree of susceptibility to know the pathogenic variability in *X a. pv. malvacearum*. Five races viz. 3, 7,10,15 and 18 of *Xam* were identified from the isolates made from these cultivars. The maximum

Table 27: Sampling populations, sampling localities and the haplotypes found in particular population along with their frequencies

Population	Sampling localities	Haplotype (frequency)
North	Abohar, Fatehabad, Shriganganagar, Sangaria, Bhatinda, Mansa, Sirsa	2(1),4(1), 7(1), 11(1), 12(5), 14(4),17(3)
Central	Jalgaon, Hingoli, Aurangabad, Amaravati, Nagpur, Yeotmal, Wardha	3(1),5(3),6(1),8(2),9(1), 15(2), 16(1), 17(10)
South	Warangal, Coimbatore, Guntur, Dharwad, Mehbubabad,	1(1), 2(2), 5(3), 6(4), 10(2), 13(1), 14(1), 15(1), 17(9)

isolates (73.33-90.00) per cent belonged to race 18 that indicated its predominance.

Bacterial blight infected leaves were collected from cotton growing areas of Maharashtra for monitoring the prevalence of races of *Xam*. Four races viz. 4, 7, 15 and 18 of *Xam* were identified from 64 isolates made from the infected leaves. Ninety four per cent isolates were of race 18 that indicated its predominance.

Races 5 and 18 of *Xam* having virulence against two major genes BIn, BN and five major genes B7, B2, BIn, BN, B4, respectively were inoculated for knowing their virulence specificity after passing through resistant S 295 and susceptible Stoneville 20 hosts. Race 18 was able to maintain its specific virulent nature when re-isolated from hypersensitive reactions of S 295 inoculated with race 18. Inoculation of resistant host with race 5 improved its virulence from two genes (Bin, BN) to three genes (B2, BIn, BN) of race 7. Race 5 was able to maintain its specific virulent nature up to 92 per cent when re-isolated from susceptible reactions of Stoneville 20 inoculated with race 5. However, dilution of virulence from two genes to single gene (Bin) of race 3 and five genes to three genes (B2, BIn, BN) of race 7 was observed with inoculation of susceptible host with races 5 and 18. Increase or dilution of virulence of races is associated with susceptible or resistant hosts.

**Characterization of variability in grey mildew pathogen *Ramularia areola*:** From freshly infected leaves of *G. herbaceum*, *G. arboreum* and *G. hirsutum*, nine isolates were made with the method of inoculation of healthy leaf tissue using the newly formulated synthetic media to study the pathogenic variability in *R. areola*. The isolates from the cultivars of *G. arboreum* and *G. herbaceum* were fast in growth as compared to the isolates from the varieties/hybrids of *G. hirsutum* on new synthetic media/broth. The size of conidiophores of *R. areola* from freshly infected leaves of cultivars/germplasm lines of *G. herbaceum* and *G. arboreum* was comparatively smaller than the conidiophores from the varieties/hybrids of *G. hirsutum*.

Twenty six different cultivars/germplasm lines four cultivated species of *Gossypium* viz. *G. arboreum*, *G. herbaceum*, *G. hirsutum* and *G. barbadense* were used for cross inoculation with nine isolates of *R. areola*. Isolates from *G. arboreum* and *G. herbaceum* produced the typical areolate symptoms on susceptible cultivars /germplasm lines of *G. arboreum* and *G. herbaceum*

besides that on *G. hirsutum*. Isolates from *G. hirsutum* were able to infect more prominently the susceptible cultivars /germplasm lines and differently with hypersensitive reaction to *G. arboreum* and *G. herbaceum* cultivars /germplasm lines. Isolates from *G. arboreum*, *G. herbaceum* and *G. hirsutum*, infected susceptible cultivars of their respective species but failed to infect the *G. barbadense* cultivars /germplasm lines. Development of infection and lesser or more pronounced appearance of symptoms clearly indicate difference in virulence of the pathogen. Isolates from *G. arboreum* and *G. herbaceum* appeared more virulent in comparison to isolates from *G. hirsutum*. Variation in specific host reactions, cultural and morphological characters indicates the presence of races in *R. areola*.

**Characterization of variability in *Fusarium oxysporum* f. sp. *vasinfectum*:** *Fusarium* wilt affected plants were collected from cotton growing areas of Maharashtra and Gujarat and eleven isolates of *F. o. f. sp. vasinfectum* were made for knowing the variability in the pathogen. Cultural characteristic i. e. growth rate showed variation in cultures of *F. o. f. sp. vasinfectum* isolated from different location of the cotton growing areas. Slow to rapid growth with raised to smooth surface and regular to irregular margin was observed in different cultures. Highly variable pigmentation i. e. dark violet, violet, pink, pinkish white and white were recorded in different isolates on potato dextrose broth, boiled rice and sorghum grains. Distinct variability was also observed in the isolates for their salt tolerance capacity. Pathogenic variability of these cultures was tested on susceptible *G. arboreum* cultivar G-27 under pot culture test. The mortality varied between 45.47 - 95.65 per cent due to various isolates after 30 days of seed germination.

Variability in growth pattern, influence of salt tolerance on growth, pigmentation, pathogenic host response in initiation of the disease as well as molecular approach indicated the presence of various strains in *F. o. f. sp. vasinfectum* having different pathogenic ability.

**Variability among strains of cotton leaf curl virus**

Six different symptoms types of cotton leaf curl virus disease viz., 1, upward and 2, downward curling of lamina; 3, severe (clone 4.1) and 4, mild curling; 5, vein-thickening (clone 7.1) and 6, enation (clone 5.1), were documented based on survey of disease in the states of Punjab, Haryana and Rajasthan. The leaf curl viral genome from each of the symptoms types were cloned to

determine variability in the strains of virus existing in North India and their role in causing different grades of severity and symptoms, if any. Six overlapping sets of primer were designed to amplify the whole genome comprising of 2.7 kb DNA- A and 1.3 kb DNA-~ components of CLCuV. Comparison of nucleotide sequence of DNA-A components did not reveal significant variation in the sequenced regions. However, the sequences of the cloned ~ DNA components of the strains isolated from three out of six different symptoms types vis-a-vis were prominently variable.

CLCuV strain 4.1 that was associated with severe leaf curling was remarkably different from other strains. Unique stretches of nucleotides make this particular strain different from other strains. The consistency in DNA sequence variability and its precise correlation with specific symptoms or severity of disease would indicate existence of multiple strains of this virus in North Indian states.

#### Immunodiagnosis of CLCuV

In order to facilitate development of antibodies against virus, the coat protein gene of CLCuV was cloned and sequenced. The gene was successfully expressed in prokaryotic expression vectors pET28b and pCALn (Stratagene). The protein was purified and out-sourced for immunization of rabbits and to raise polyclonal antibodies.

#### Coimbatore

The variability among the populations of *H. armigera* collected from Redgram (2 locations), Sunflower, Chick-pea and cotton (4 locations) of Coimbatore, Nandyal, Theni, Annur and Salem was assessed through PCR studies using SSR markers. The results clearly indicate that there is specific variation among the populations collected from other crops and cotton also from different locations.

#### Interaction between various *Gossypium* spp. and the weed host and isolates of *Ramularia areola*

Based on the past three years experiments, selected genotypes of the four *Gossypium* spp. along with the weed host, *Euphorbia heterophylla* were inoculated with *Ramularia areola* isolates collected from *G. herbaceum*, *G. hirsutum*, *G. barbadense* and *E. heterophylla*. Since the *herbaceum* and *arboreum* isolates behaved almost identical on the hosts, only the *herbaceum* isolate was

selected for the study.

Among the hosts, the line G. 27 (*G. arboreum*), Jayadhar (*G. herbaceum*), LRA 5166 (*G. hirsutum*) and the weed (*E. heterophylla*) were highly susceptible to all isolates of *R. areola*.

The *G. hirsutum* hosts viz. IC 629 and IC 1017 exhibited resistance to *herbaceum*, *hirsutum* and weed isolates where as GMR 9 and GMR 5 were resistant to only *herbaceum* isolate.

The *G. barbadense* hosts viz. Suvin, GB 124, GB 119, GB 23 and ERB 13758 showed differential response to the four isolates tested with all of them exhibiting resistance to the weed isolate. GB 124 was resistant to all the isolates.

#### 4.21: Development of Molecular Tools

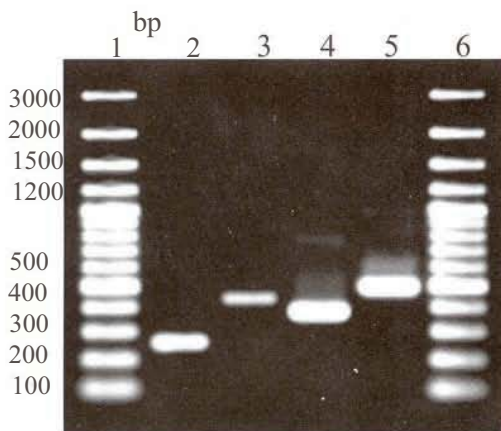
##### Nagpur

##### Diagnostic tools for fungal pathogens

Diagnostic tools for detection and identification of four major foliar fungal pathogens of cotton viz., *R. areola*, *R. bataticola*, *R. solani* and *A. macrospora* were developed and improvised. PCR based protocols were developed for specific detection of each of these four pathogens. Primers were developed based on variable nucleotide sequences in the Internal transcribed spacer sequences of the ribosomal RNA genes of these pathogens. Five sets of primers capable of differentially detecting these four pathogens were designed (Table 28).

**Table 28: The fungal pathogens, diagnostic primers and sizes of the amplified products**

Sr. No	Species	Primers	Ampli - con (bp)
1	<i>Rhizoctonia solani</i>	pRhi	280
	<i>Rhizctonia bataticola</i>		311
2	<i>Rhizoctonia solani</i>	pSol	255
3	<i>Rhizctonia bataticola</i>	pBat	400
4	<i>Ramularia areola</i>	pRare	372
5	<i>Alternaria macrospora</i>	pAmac	542



A common set of primer pRhiz could diagnose strains of *Rhizoctonia* irrespective of its two species that infect cotton. In case of *R. solani* the primer generates an amplicon of 280 bp while for *R. bataticola* the same primer generated a DNA fragment of 311 bp. Primers pRSol and pRbat were specific to strains of *R. solani* and *R. bataticola* and supported amplifications of rDNA fragments of 255 and 400 bp, respectively. Primer pRare indiscriminately detected four strains of *R. areola* isolated from each of the only four cultivated species of cotton by supporting amplification of an universal amplicon of 372 bp. Strains of *A. macrospora* could be

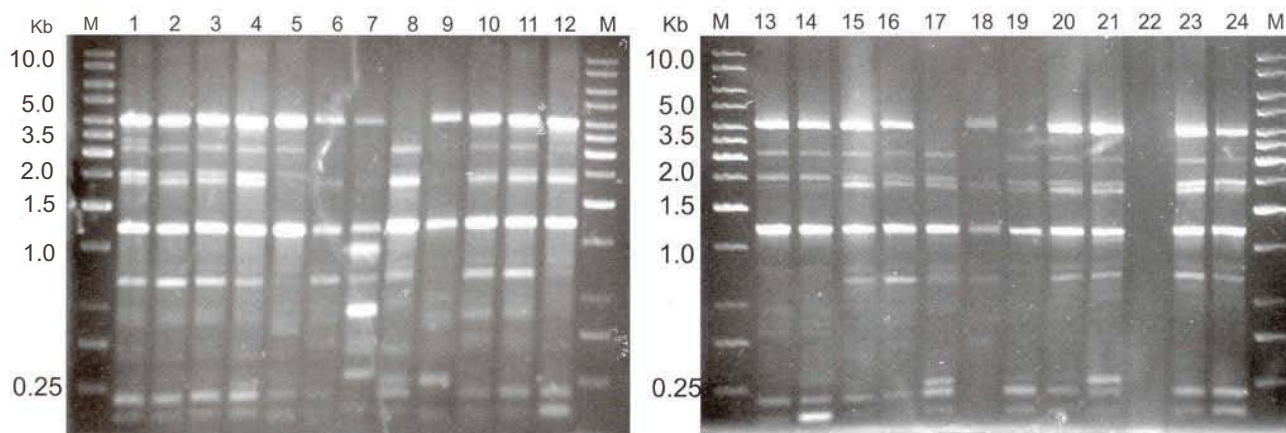
identified by amplification of a DNA fragment of 542 bp using primer pAmac and differentiated from other species of *Altemaria* by PCR-RFLP of the rDNA product with BanII, MseI and HaeIII restriction endonucleases.

#### **Molecular characterization of variability within race 18 of *Xanthomonas malvacearum***

Fifty six race 18 isolates were collected from different regions of Maharashtra and MP to study genetic variability and documentation of virulent biotypes within the race. All isolates were subjected to molecular characterization using number of DNA markers including randomly amplified polymorphic DNA (RAPD), IS 112, Enterobacterial repetitive intragenic consensus sequences (ERIC) and restriction fragmentation length polymorphism (RFLP) markers.

Fifty six race 18 strains were subjected to RAPD analysis using arbitrary primer OPA13 (Operon Tech, USA). More than 560 amplicons were generated several of which were polymorphic (Fig. 14 a&b). Dendrogram generated based on unpaired group mean average analysis of RAPD data grouped the strains in at least 10 clusters. Majority of the strains (36%) belonged to cluster I with amplicons ranging from 0.3- 4.0 kb size. This was followed by cluster II which comprised of 18% of the strains. Many strains exhibited unique RAPD profiles.

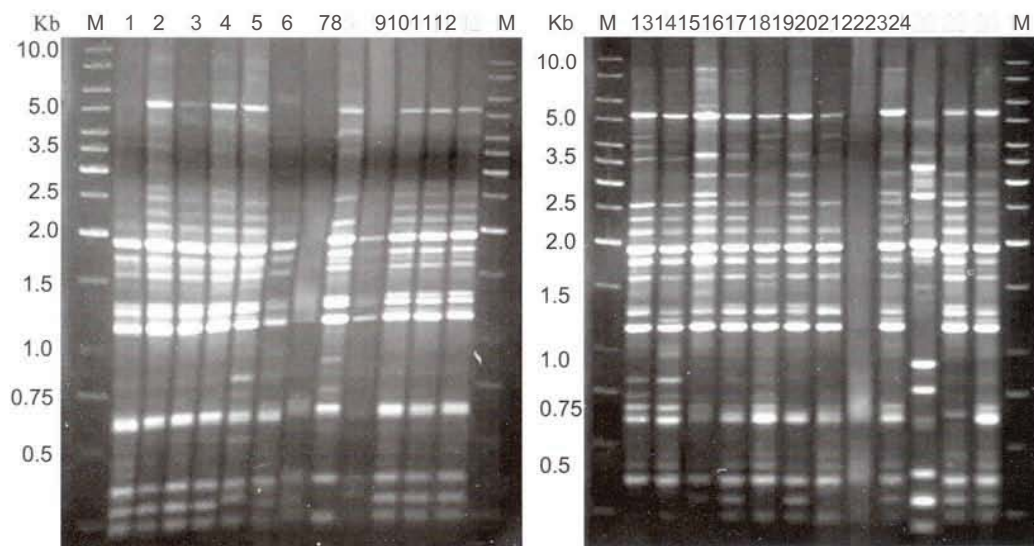
**Fig. 14 a, b: RAPD fingerprinting of race 18 strains of *Xanthomonas malvacearum***



Isolates were also characterized using IS 112 element marker which delineated distinct genetic variability among race 18 strains (Fig.15 a&b). The marker

generated more number of amplicons than the arbitrary primers. Based on the IS 112 elements, the strains were grouped in 9 clusters.

**Fig. 15 a,b : IS112 element fingerprinting of race 18 strains of *Xanthomonas malvacearum***

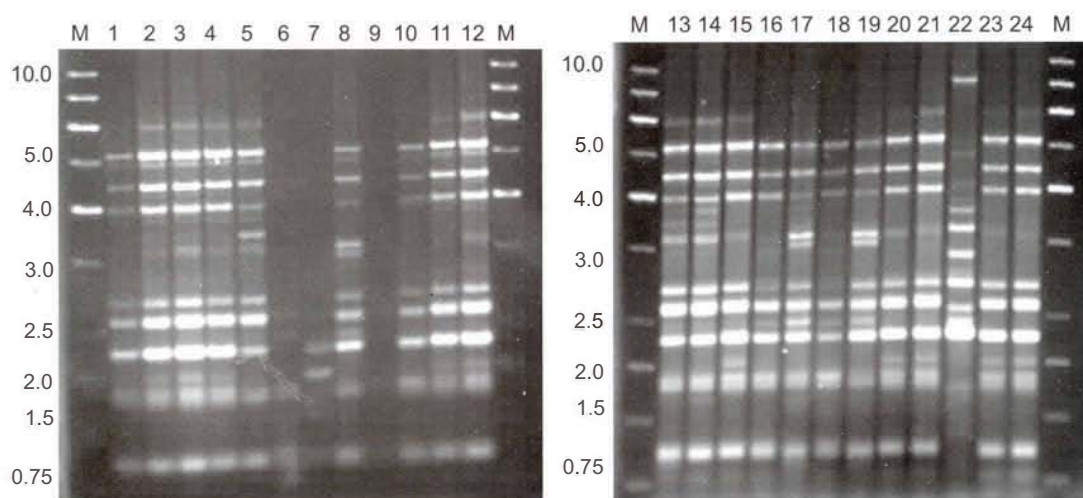


Based on ERIC primed PCR DNA fingerprinting, The 56 strains found 10 clusters. Some members however exhibited unique banding pattern (Fig. 16a&b).

Southern hybridization of race 18 isolates was done using pathogenicity gene *pthN* (AFO16221) as DNA probe. The race 18 isolates in general did not possess *pthN* hybridizing bands between 5-14 kb while the less

virulent races possessed several bands between these race 18 specific markers of 5 and 14 kb. Based on RFLP analysis, 56 race 18 variants were clustered in ten groups. The variability existed due to slight variation in size of the RFLP marker of 5 kb or existence of one or two different markers above 14kb size.

**Fig. 16 a, b: ERIC-PCR fingerprinting of race 18 strains of *Xanthomonas malvacearum***



**Commercialization and patent filing for *Xanthomonas malvacearum* PCR detection kit**

Application to patent ready-to-use PCR kit for detection of strains of *Xanthomonas axonopodis* pv. *malvacearum* is approved by the Institute. Evaluation of shelf-life of the kit showed that PCR-mix was able to support amplification of 0.75 kb diagnostic fragment without any loss in efficacy even after 12 months of storage at -20°C. The kit has been validated successfully by 7 research labs of ICAR Institutes and SAD's.

**Biochemical and molecular characterization of antagonists**

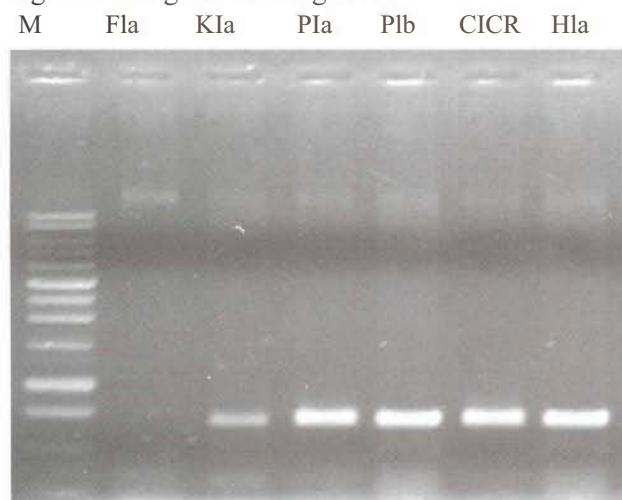
Nine potential antagonists with eight species of fluorescent and non-fluorescent *Pseudomonas* and one species of *Bacillus firmus* isolated from rhizosphere and phylloplane of cotton that provided effective inhibition of *Xam* as well as other fungal pathogens earlier *in-Vitro*, were further characterized for their beneficial attributes and also the determinants of antagonism.



Inhibition of *Xanthomonas malvacearum* (1) and *Alternaria macrospora* (2) by strain H1a of *Pseudomonas fluorescens*

The effective strains of *Pseudomonas* expressed 2-4 diacetyl phloroglucinol activity (pHI). pHI gene fragment of 0.75 kb was amplified from strains of *Pseudomonas* by PCR (Fig. 17), cloned, sequenced and used for RFLP analysis to decipher genetic diversity among different strains.

Fig. 17: **Phi** gene in antagonist



A replicated experiment to test the efficacy of *Pseudomonas fluorescens* (Pt) strain H1a in plant growth promotion was conducted in pot house. The growth of the *Pseudomonas* treated plants was improved significantly. Talc formulation containing 108 cfu per gram of powder of the PGPR strain H1a of *Pseudomonas fluorescens* was prepared. Treatment of cotton seeds with Talc formulation improved germination by 21 per cent.

**Sirs a**

**Development of diagnostic tools**

Diagnostic tools for the detection and differentiation of *Rhizoctonia bataticola* the causal organism of root rot disease of cotton have been developed. In these studies amplification of Internal Transcribed Spacer (ITS) region of isolates of *Rhizoctonia* species (six isolates of *R. bataticola* and four isolates of *R. solani*) was done. The amplified fragments were cloned, sequenced and the sequences were published in gene bank, (DQ 222238-41, DQ 223780-82, DQ 408294, DQ 212767, DQ 218056). Based on sequence information primers specific to *R. bataticola* have been developed. The primers were further validated with other soil borne plant pathogens



like *Fusarium* spp isolated from other hosts and *R. solani* isolated from cotton to see their specificity. The primers did not show any amplification in case of these fungi (Fig. 18 & 19).

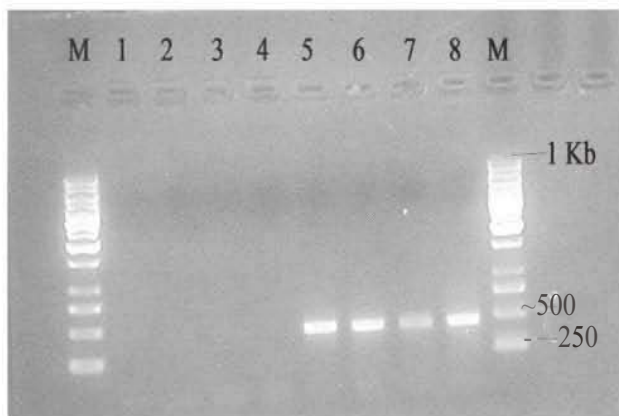


Fig.18: Specificity of the PCR assay (ITS-Rb-F and ITS-Rb-R primer pairs) with genomic DNA from different isolates of *R. bataticola* and *Fusarium* species. Lanes 1-4 No DNA amplified from *Fusarium* spp isolates, Lanes 5-8 DNA amplification from *R. bataticola* species; M: 1 kb molecular weight marker.

The primers were also validated with *R. bataticola* isolated from hosts other than cotton and showed amplification when *R. bataticola* isolated from other hosts were tested.



Fig.19: Specificity of the PCR assay (ITS-Rb-F and ITS-Rb-R primer pairs) with genomic DNA from different isolates of *R. bataticola* and *R. sikabu*. Lanes 1-10 DNA amplified from *R. bataticola* isolates; Lane 11: No amplification with DNA *R. solani*; M: 1 kb molecular weight marker.

4.22: Epidemiology

Nagpur

Prediction of onset and severity of *H. armigera* Calendar based accumulated degree days of 2424 was used to predict the onset of *H. armigera* oviposition on cotton. The criteria of direct proportionality of degree of incidence on cotton to that on pigeonpea did not validate

positively, probably due to the changing population dynamics (or host shift?) of *H. armigera* than due to the weather influence.

Prediction of *P.gossypiella* severity: During 2006, the severity of pink bollworm was less with maximum and minimum temperatures and the morning relative humidity conditions not congenial for the population development.

Weather variables & Standard week	Criteria predicting severity	Goodness of fit durin22006	Observed severity
Maximum temperature (40)	> 33°C	31.5	Less
Morning relative humidity (41)	<70%	71	
Evenin2 relative humidity (43)	>40%	46.7	
Minimum temperature (48)	< 12°C	18.9	
Minimum temperature (49)		16.5	

Coimbatore

Assessment of yield loss due to sucking pests in Bt cotton hybrids

There was no significant difference in seed cotton yield

between protected and unprotected fields for sucking pests in MECH 184 Bt while three hybrids viz., RCH2 Bt, MRC 6918 Bt and Bunny Bt suffered minor losses upto 2.2, 5.9 and 7.8 % respectively. The other three hybrids viz., Mallika Bt, RCH 20 Bt and RCH 708 Bt suffered

substantial losses to the extent of 13.9, 14.2 and 16.4 % respectively.

#### Yield loss assessment due to grey mildew

Spore inoculum of *Ramularia areola* was applied on cotton cv. LRA 5166 in the field on 35 and 50 DAS followed by continuous spray through sprinkler to create epiphytotic conditions. The spray of the fungicide Carbendazim 50 WP @ 0.1% between 35DAS and 95 DAS at fortnightly intervals greatly reduced the incidence of grey mildew (44.7 PDI) when compared with single spray at 35 DAS(83.9 PDI) or 95 DAS(66.3PDI) or no spray (88.8 PDI) and averted 26 % yield loss in seed cotton.

#### 4.23: Management of Pests

##### Nagpur

##### Testing of new molecules

S-1812+Fenprothrin from Sumitomo: A new molecule S1812 was tested alone and in combination with fenvalerate and fenprothrin, at three sprays on NHH 44. The treatments were 1. S1812 75 gm a.i./ha., 2. Fenvalerate 100 g a.i./ha., 3. Fenprothrin 100 g a.i./ha., 4. S1812-fenprothrin 60+80 gm a.i./ha., 5. S1812-fenprothrin 67.5+90 gm a.i./ha., 6. S1812-fenprothrin 75+100 gm a.i./ha., 7. S1812-fenvalerate 60+80 gm a.i./ha., 8. S1812-fenvalerate 67.5+90 gm a.i./ha., 9. S1812-fenvalerate 75+100 gm a.i./ha., 10. profenophos + cypermethrin 500+50 gm a.i./ha and 11. control. Results after the first spray indicated that S 1812- Fenvalerate 60 + 80 g a.i./ha was the most effective on jassid nymph populations, followed by profenophos + ypermethrin and fenprothrin 100 g a.i. /ha. The other treatments were on par or inferior to control.

Diseases incidence during seedling and boll development stage : Cotyledonary leaves and boll infection isolations in the varieties/hybrids/germplasm lines revealed the presence of pathogenic fungi viz. *Alternaria macrospora* (AKA-5, AKA 7, AKA 27, AKH 4, Buri 1007, Chandrolla, DB 3-12, F 414, G 27, JKHY1, LD 327, Maljari, MDCH-201, Suman), *Colletotrichum indicum* (AKA 8401, GMT-I, GMT-2, GMT-3, RG 8), *Macrophomina phaseolina* (AKA 5, AKA 7, AKA 53, DB 3-12, G 1, G 27, H 777, MDCH 201, NHH 44, Surabhi), *Myrothecium roridum* (AKA 7, AKA 53, AKH 4, Chandrolla, DHY 286, Jayadhar, GMT -1, GMT-2, GMT-3, G 46, H 6, L 147, MCU 5, SIMA-I), *Phoma exigua* (AKH 4, Jayadhar, GMT-I, GMT-2, GMT-3, G 27, G 46) and *X. a.pv.Malvacearum*.

Isolations from cotyledonary leaf infections from Bt cotton hybrids revealed the presence of Anthracnose pathogen *Colletotrichum indicum* (2 entries), *Cercospora* leaf spot pathogen *Cercospora gossypina* (5 entries), *Macrophomina* stem break/root rot pathogen *Macrophomina phaseolina* (8 entries) and *Fusarium* wilt pathogen *Fusarium oxysporum* (1 entry).

Detection of pathogenic infections (seed samples) Cotton seed samples belonging to germplasm lines/varieties/hybrids (F<sub>2</sub>) of the previous crop season were examined for seed discoloration, fungus fructifications and yellow slime of bacteria. Infection ranging between 1 to 6% was observed due to *Alternaria macrospora*, *Colletotrichum indicum*, *Macrophomina phaseolina*, *Myrothecium roridum*, *Phoma exigua* and *Xa. pv. malvacearum*. Association of other secondary nature of fungi viz., *Curvularia lunata*, *Epicoccum pUipurascens*, *Fusarium moniliforme*, *F oxysporum*, *F semitectum* and *Nigrospora oryzae* was also observed.

Evaluation of storage fungi : Higher incidence of storage fungi especially that of *Aspergillus flavus* and *A. nidulans* was observed. Incidence of lint spoiling fungus *Nigrospora oryzae* was more in *G. arboreum* cultivars. Unusual occurrence of wheat grain pink discoloration fungus *Fusarium graminearum* was noticed in seeds of Jayadhar.

##### Coimbatore

##### Evaluation of newer insecticides against sucking pests

Seed treating chemicals thiomethoxam 500FS @ 5 ml and 7.5 ml/kg of seeds were effective up to 35 days in reducing jassids and aphids. Seed treatment with thiomethoxam resulted in seed cotton yield of 2390 kg/ha as compared to 1890 kg/ha in untreated check.

##### Evaluation of insecticides against *R. gossypiella*

Two initial sprays, did not exhibit any significant difference among the treatments. The results of third spray indicated minimum number of larvae in Triazophos 0.05% which was superior to Cypermethrin 0.007%, Chlorpyrifos 0.05%, Thiodicarb 0.075%, Lambda cyhalothrin 0.002 % , and Deltamethrin 0.005% wherein the larval population ranged from 1.47-2.23/boll. Minimum locule damage was recorded in the third spray with Triazophos (5.17%) being superior to all other treatments with maximum damage in Lambda cyhalothrin (35.77) and Neem oil (33.00) which were on par with the control (40.17).

## Evaluation of TERI (The Energy and Resources Institute) molecules in cotton

### Effect on sucking pests and predators

Three days after application of TERI I, TERI V and TERI III treatments brought a reduction 001.1, 66.7 and 64.5 % jassid population respectively while the reduction for aphid ranged from 42.6 to 92.1 % in the various TERI treatments. TERI III formulation recorded an increase of 47.80% predator population, while other TERI compounds did not show any significant adverse effect on predator activity.

### Effect on bollworms and productivity

Seven days after application revealed that TERI formulations could bring down larval damage upto 10.8% in the first spray and upto 29.3 % in the second spray. TERI V followed by Neemazal, TERI III, TERI I and TERI II recorded an increase of seed cotton yield by 25.0, 15.5, 8.9, 7.1 and 3.2 % respectively over control.

### Effect of imidacloprid spray on mirid bug in Bt hybrids

A reduction of 44.1 to 60.9 % population of mirid bugs was observed after application of imidacloprid in seven Bt cotton hybrids viz., RCH 2 Bt, RCH 20 Bt, MRCH 184 Bt, Bunny Bt, Mallika Bt, RCH 708 Bt and MRC 6918 Bt.

### Evaluation of new molecules of insecticides on mirid bug damage and on predator activity

New insecticides E 2Y45 at 40 g a.i / ha followed by Spinosad 75 g a.i. / ha were moderately effective against mirid bug infestation and brought down the damage by 31.7 and 20.2 % respectively over control. Predators (Coccinellids, Chrysopids, Syrphids and Spiders) activity was reduced by 54.2 % in Spinosad and 22.8 % in E2Y45 (at 40 g a.i. / ha) treatments.

### Evaluation of new insecticides against bollworms in cotton

New insecticides E2Y45 and Flubendiamide + Thiacloprid were effective in reducing bollworm damage by 68 to 85% and 78 to 81% respectively as compared to Indoxacarb (86%) and Spinosad (50%) over control.

The new insecticide E2Y45 was effective in reducing the boll and locule damage significantly over control and contributed to significantly higher yield by 70 to 80 % over control.

### Testing of new molecule- Propineb 70 WP against

grey mildew

The Fungicide-Antracol (Propineb) 70 WP was assessed in the field at two doses viz. 1050 g. a.i./ha and 1400 g. a.i./ha in comparison with standard fungicide Propiconazole @ 0.1% against grey mildew. The plants in the field were inoculated with spore suspensions of *Ramularia areola*. Immediately after the appearance of the grey mildew symptoms, the first spray of the fungicides was given and this was repeated twice at fortnightly intervals. Leaf samples were taken at periodical intervals and the disease development was assessed. The test fungicide Propineb 70 WP at both doses (24.1 and 11.7 PDI) were as effective as the standard Propiconazole (18.1 PDI) in the control of grey mildew (check 53.6 PDI).

## Bio Control Studies

### Nagpur

#### Identification of efficient strains of bio-control:

Out of thirteen rhizosphere bacterial isolates, four were found to exhibit maximum inhibition and disease suppression of *Fusarium* wilt pathogen *Fusarium oxysporum* f. sp. *vasinfectum* and dry root rot pathogen *Macrophomina phaseolina* apart from promoting seedling vigour. The most promising bacteria belonged to *Pseudomonas fluorescence* and *Bacillus* spp.

**Genetic improvement of EPN isolates:** Studies were carried out on per cent increase in temperature tolerance in successive selection cycles of different entomopathogenic nematode (EPN) and variation in host finding ability (vertical and horizontal). Ten infective juveniles (IJ) per *H. armigera* larva were found to be effective dose. Inoculum levels of 1 billion nematodes per ha was sprayed in the evening with addition of 1% sticker and glycerine as phagostimulant. Results indicate that two sprays at an interval of 4-5 days were able to reduce bollworm population by 58%. Semilooper larvae were more susceptible and reduction in their population was to extent of 61%. Promising control of *H. armigera* larvae on chickpea crop was also obtained. On chickpea, single spray of 1 billion nematodes per ha was found to reduce insect infestation by 50%.

**Development of bioagent mixtures:** A new combination suspension comprising of EPN (*H. indica*), *Metarhizium* and *Beauveria* was developed. The combination comprised of sublethal concentration for all three components wherein each component alone was not lethal to bollworm larvae. Five isolates of rhizobacteria were found to have antagonistic effect on

nematodes. *Gluconacetobacter diazotrophicus*, a diazotrophic acetic acid bacterium was evaluated for antagonistic activity against reniform nematode. Culture supernatant was found to reduce egg hatching and caused considerable mortality of pre adult infective stages of reniform nematode. Seed treatment of IOS-cell ml inoculum was found to reduce penetration of reniform nematode by about 50%. Bacterial symbiont of one isolate of *Heterorhabditis indica* was found to be promising as a potent bio-agent for management of sucking pests.

**Testing a novel botanical product:** A botanical from TERI, was evaluated as 6 formulations- Teri I, Teri II, Teri III a, Teri III b, Teri V a, Teri V b, at CICR, Nagpur for the second year (2006-07). The molecule was evaluated singly and compared with Neem (0.5%) for its efficacy against cotton insect pests on NHH 44 under rainfed situation. No significant differences in yield were observed.

## Coimbatore

### Survey for the occurrence of entomopathogenic nematodes

Surveys conducted to assess the prevalence and distribution of entomopathogenic nematodes revealed the presence of entomopathogenic nematodes (*Heterorhabditis indica* and *Steinernema siamkayai* and ~n unidentified species of *Steinernema*) in 8-10 % of the samples in cotton ecosystem.

### Pathogenicity of entomopathogenic nematodes against *H. armigera*

The pathogenicity of two native entomopathogenic nematodes viz., *Heterorhabditis indica* and *Steinernema glaseri* against different stages (egg, larva and pupa) of *H. armigera* was studied. Eggs were not found to be infected by both nematodes. No marked difference in egg hatchability was observed between treatments and control. The median lethal concentration (LC 50) and associated statistics could not be estimated for first and second instar larvae because complete mortality was recorded at even lowest dose of 2 IJ (Infective Juveniles) / larva within 48 hours after inoculation. Among the remaining stages, third instar larva was highly susceptible to nematode infection. LC50 varied from 3.69 to 84.67 IJ to third instar larvae to pupae for *H. indica* whereas for *S. glaseri* it was 2.43 to 91.46 IJ for third instar to pupa respectively. Adults emerged from treated pupae were malformed and 50 % of them died immediately after emergence. When the cadavers were

dissected they harbored nematodes.

### Identification of bacterial symbionts of entomopathogenic nematodes

The cultural characteristics of primary and secondary form produced by the bacterial symbionts were studied. The thermal death point for the bacterial symbionts was 60°C for 10 minutes. All the bacterial symbionts associated with entomopathogenic nematodes were gram negative rods, which absorbed blue colour from Bromothymol blue, produced antibiotics in culture, insecticidal in nature and grew well in Mac Conkeys Agar. They produced yellow colour pigment, highly motile, phase variation was present and grew at 40, 50 and 60°C. The difference between *Photorhabdus* and *Xenorhabdus* was that *Photorhabdus* was bioluminescent and catalase negative whereas *Xenorhabdus* was not bioluminescent and catalase positive.

The media, pH and duration for mass multiplication of *Photorhabdus* and *Xenorhabdus* primary forms were standardised. Both bacterial symbionts multiplied well in Nutrient Broth at all the pH (5-9) studied. Maximum growth was achieved at 24-36 hours thereafter the primary forms were slowly converted into secondary form.

### Pathogenicity of bacterial symbionts of entomopathogenic nematodes against *H. armigera*

Pathogenicity of bacterial symbionts of two entomopathogenic nematodes viz., *Xenorhabdus* sp and *Photorhabdus* sp. alone and their cell free extract against third instar larvae of *H. armigera* was tested by diet incorporation method. A maximum of 100 % mortality was observed when the larvae were treated with *Xenorhabdus* sp. (cell free extract), *Photorhabdus* sp. (bacterial cell alone and cell free extract). The larval mortality was found to be significantly increased with increase in exposure period. No insect mortality was recorded in untreated check (sterile water) whereas in treated check (nutrient broth) 2.78 % mortality was recorded. The experimental results clearly indicated that both bacterial cells alone and their secretions caused mortality of *H. armigera* larvae and also the secretion of insecticidal toxin by the bacteria.

### Prevalence and distribution of plant parasitic nematodes

Soil and root samples were collected from different places in Tamil Nadu (Coimbatore, Salem, Thiruppattur,

and Theni) and Kamataka (Dharwad). Community analysis of different nematodes was carried out. Based on prominence value *Rotylenchulus reniformis* was identified as key nematode pest of cotton. Application of Farm Yard Manure (FYM) and INM ( $\frac{1}{2}$  N + FYM + P + K) significantly reduced reniform nematode population. But application of FYM significantly increased the beneficial nematode (Bacterial Feeder) population. Application of INM (Crop residue) was found to be on par with FYM in supporting the beneficial nematode population.

**Biology of *R. reniformis* on cotton var. Suvin**

Life table of *R. reniformis* on var. Suvin was studied under green house condition. The immature female feeds as semi-endoparasitic with about one third of its body inside the root tissue. The female continue to enlarge and attain the typical reniform shape on fourth or fifth days after infection and starts secreting the matrix. The nematode starts laying eggs on 24<sup>th</sup> day. Peak egg lying was occurred on 25<sup>th</sup> and 26<sup>th</sup> day then decreased. After 27<sup>th</sup> day egg lying was completed and female was assumed dead. Survival fraction (Ix) remained constant from 24 days onward due to low adult mortality.

The total number of female birth amounted to a net reproductive rate (Ro) of 14.32 female /female / generation in approximate time (Tc) of 25.45 days. The innate capacity for natural increase was 0.1046. The true intrinsic rate of natural increase (rm) was 0.1005 female /female /day. The calculated finite rate of increase showed that the population of *R. reniformis* would increase by 1.01057 female /female / day. At this rate, the time required to double the population was computed as 6.8 days.

**Biological control for the management of grey mildew**

Talc formulations of three fungal bio-agents viz., *Trichoderma viride*, *T harzianum* and *T virens* and two bacterial bioagents - *Pseudomonas fluorescens* Pfl and combinations of the above fungal bioagents with *P. fluorescens* Pfl strain and the standard fungicides Propiconazole and Carbendazim @ 0.1% were sprayed in the field at 10 and 15 days intervals following the appearance of grey mildew on cv. Sumangala. Assessments on the effectiveness of the treatments were made at frequent intervals by collecting leaf samples.

The fungicide treatments with Propiconazole @ 0.1% (2.5 PDI) and Carbendazim @ 0.1% (14.6 PDI) were found to be the most effective treatments for the

management of the disease. Among the bio-agents, the combination spray of *T harzianum* + *P fluorescens* Pfl and *T virens* + Pfl were effective in reducing the disease to limited extent when sprayed at 10 day intervals.

**Integrated Pest Management**

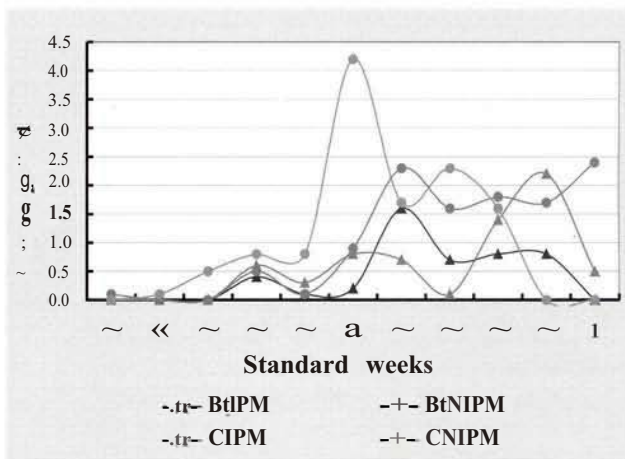
**Nagpur**

**Bt cotton IPM in farmer fields**

Ankur 651-Bt and Ankur 651 Non-Bt were used under IPM and non-IPM (NIPM) modes to demonstrate the efficacy of Bt cultivar against bollworms and the need for IPM on Bt in farmers' fields. While all the farms of BtIPM and CIPM were sprayed once for sucking pest management at pre squaring period (mid August), the latter was sprayed for pink bollworms during third week of November. On the other hand the Bt NIPM farms were sprayed twice for sucking pests (including insecticide mixtures) and once for bollworms. CNIPM farm had two sprays of insecticide mixtures during mid August and mid September, respectively.

The NBtNIPM farms had significantly higher green boll damage due to *H. armigera* and bollworm damage on open boll and loculi basis indicated significantly higher damage in Bt NIPM farms. The Bt-cotton hybrids were also found to be susceptible to pink bollworm infestation (Fig.20). Input output ratios for the Bt and NBt cotton cultivation under IPM and NIPM situations were of the order: BtIPM (2.22) > BtNIPM (2.03) > NBtIPM (1.86) > NBtNIPM (1.71). The inclination of farmers to use insecticidal mixtures with overall reduction in number of sprays on cotton was seen as a changing practice.

**Fig. 20: Dynamics of pink bollworm incidence**



**Coimbatore**

IPM at village level to produce cost effective quality fibre

Location specific IPM Module was developed and evaluated in 81 ha. of 124 farmers' fields covering eight villages in Avinashi and Annur Blocks of Coimbatore District, The IPM strategies helped to reduce insecticide usage significantly besides increasing seed cotton yield by 8 %. The cost benefit ratio was also higher 1:2.04 to the IPM farmers as compared to 1:1.57 to the non IPM farmers.

**Sirsa**

Integrated Pest Management (IPM) at village level to produce cost effective quality fibre

Integrated pest management strategies were demonstrated in Rangri village (nucleus village) in 25 acres. The four clusters of villages covering 100 acres area with 20 farmers also participated in this demonstration programme. Jassid, whitefly and Aphid population was less in both IPM and non IPM fields. Thrips were noticed more in IPM village than in non IPM village. Fruiting bodies damage was more in non IPM compared to IPM plots. Average yields recorded was 3000 kg/ha for hybrids and 2500 kg/hectare for varieties in IPM villages as against 2500 kg/ha and 2100 kg/ha, respectively, in the control village. The cost benefit ratio was more in IPM village (1:3.9 for hybrids and 1:3.75 for varieties) than in control villages (1:2.53 and 1:2.38, respectively) (Table 29).

Table 29:. Details of spray, yield, C: B ratio and Net profit in IPM and Non IPM in conventional cotton (2006-07)

Sr. No.	Details	Hybrids		American	
		IPM	Non-IPM	IPM	Non-IPM
1	Average yield (q/ha)	30	25	25	21
2	No. of spray	6*	8**	6	8
3	Cost of spray	2989	7226	2989	7226
4	Reduced cost over Non-IPM	4237		4237	
5	Net profit	43511	29524	35761	23724
6	C: B ratio	1: 3.90	1: 2.53	1: 3.75	1: 2.38
7	% increase in net profit over Non-IPM	47.37		50.73	

**Insecticide Resistance Management**

**Nagpur**

Insecticide Resistance Management: Bollworm resistance to Cry toxins

Monitoring *H. armigera* resistance to cry toxins: Bollworm resistance to cry (crystal) toxins of *Bacillus thuringiensis* was monitored through bioassays carried out on *H. armigera* larvae collected from 30 cotton growing districts (8 from north, 16 from central and 6 of south) of the country. Three cry toxins, *cryIAC*-MAHYCO, *cryIAC*-JK and *cry2Ab2* were used in surface coating and diet incorporation bioassays. The results did not show resistance thus far to any of the three toxins tested. However a significant reduction in variability between populations was observed.

Estimating the initial frequency *cryIAC* resistant alleles in *Helicoverpa armigera* populations in India: The frequency of *cryIAC* resistant alleles in *H. armigera* were estimated using a method called 'F<sub>2</sub> screen' The method is

based on performing sib-mating amongst progeny of individual isofemale lines and examining the survival of the F<sub>2</sub> progeny with diagnostic dose to enable the detection of any resistant allele that is initially present in field populations. At least one out of every sixteen larvae tested are expected to be homozygous for the major resistant allele and hence show resistance.

The F<sub>2</sub> screen test was conducted with 198,330 and 165 isofemale lines of *H. armigera* collected from north, central and southern parts of the country. We detected only one *cryIAC* resistance conferring allele in the central Indian population. A Bayesian analysis of the data indicated that the frequency of resistance alleles was 0.00125, 0.0015 and 0.00149 in south, central and north India, with 95% probability, and a detection probability of >85%. The results suggest that the initial frequency of resistant alleles is probably not adequately rare enough in India for the high-dose plus refuge strategy to delay resistance to *cryIAC* Bt-cotton (Table 30).

**Table 30 : Frequency of *cry1Ac* resistance alleles in field populations of *H. armigera*.**

Zone	n	E(q)	Variance	95% CI	Genotype frequency	Number
North	198	0.00125	6.21113E -06	0.013	0.0025	1/400
South	165	0.001497	8.89741E-06	0.015	0.002994	1/334
Central	330	0.001506	4.51578E-06	0.012	0.003012	1/332

Development of a molecular kit to detect *cry1Ac* resistant *H. armigera*: A total number of 120 primers were tested on *cry1Ac* susceptible and NIL (Near isogenic lines) to identify random primers that would be able to distinguish resistant insects from susceptibles. Two primers (S-4 and S-18) were identified for their capability to discriminate the resistant genotypes. The primers amplified certain specific amplicons from the resistant insects, which were rarely (1-3% frequency) amplified in susceptible insects. One such unique amplicon (1.18 Kb) was cloned and sequenced. Based on the sequences obtained a set of three primers was

designed to specifically amplify two different amplicons from the resistant (1.18 Kb) and susceptible (1.06 Kb). The primer set enables the characterization of unknown samples with respect to their susceptible / resistant properties towards *cry1Ac*. The sequence of the two random primers is as follows:

S-4 5'-GGACTGGAGT-3'

S-18 5'-CCACAGCAGT-3'

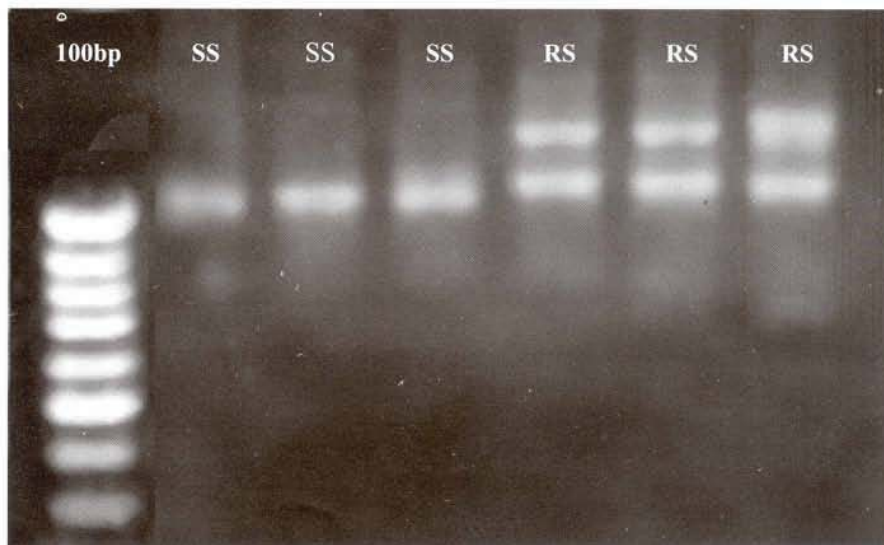
Primer set to distinguish *cry1Ac* resistant insects

S4-SP6-F, 5'-GGACTGGAGTCCTGTCT-3'

S4-SP6-F 5'-ATGGGCATCACTATGGCGAGCTTGCG-3'

S4-C 5'-GGACAGCAGC-3'

Validation of the SCAR marker on mapping population backcross progeny of RS x SS



Implementation of IRM strategies in 1062 villages across India: During the 2006-07 cropping season, the IRM strategies were disseminated by 72,783 farmers in 1.38 lakh hectares in a total of 1062 villages in of 33 districts across India. A total of 33,085 farmers of 430 villages implemented the programme in 1.02 lakh hectares in the North Indian states of Punjab, Haryana and Rajasthan. In Central India (Gujarat, Maharashtra and MP) 26,605 farmers implemented the programme in

an area of 21,376 hectares in 372 villages. In West Bengal and South India (Andhra Pradesh, Kamataka, and Tamilnadu) the programme was implemented in 14,496 hectares of 13,093 farmers in 260 villages. Implementation of the programme resulted in yield increases estimated at a net additional benefit of Rs 48.4 crores and a saving on reduction in insecticide use accounting for Rs 27.4 crores, thus adding up to a total additional benefit of Rs 75.8 crores due to the project.

Zone	States	Villages	Area(ha)	Farmers
North	Punjab, Haryana & Rajasthan	430	102265	33085
Central	Gujarat, Maharashtra & MP	372	21376	26605
South	AP, Kamataka, TN and WB	260	14496	13093
<b>Total</b>		<b>1062</b>	<b>138137</b>	<b>72783</b>

### Coimbatore

#### Dissemination of IRM strategies in Salem and Theni districts.

The various components of IRM viz., Seed treatment, maintenance of crop diversity, agronomic practices, cultural practices, monitoring of pests, use of botanicals and biopesticides, assessment of ETL and need based application of insecticides were disseminated to the farmers through group meetings and training facilitators at the project villages of Salem and Theni districts.

#### Resistance monitoring for insecticides against *H. armigera*

Discriminating dose bio-assay tests were carried out for assessing the resistance levels to different insecticides. The resistance levels were very high to fenvalerate (90%) and low level to Chlorpyrifos (3.33%).

**Impact of IRM dissemination:** Implementation of IRM strategies in the project villages resulted in the reduction of number of sprays by 67.12% and 36.64% in Salem and Theni, respectively and the plant protection cost from Rs.2,384 to 1228 and 2,942 to 1,180 in Salem and Theni respectively. Besides an increase in yield by 36.56% and 51.95% over non IRM villages was observed in Salem and Theni, respectively. Additional income of Rs. 609 and 2,385/ha was realized in Salem and Theni, respectively by growing intercrop (cowpea, black gram, chilies and tomato) by the project farmers and obtained a net profit Rs 38,880 and 17,680/ha.

#### Sirsa

In Non Bt cotton fields, there was 32.72 % reduction in

pesticide load and 39.49 % reduction in cost of plant protection in IRM villages compared to non IRM villages. In the case of Bt cotton hybrids, the reduction was of the order of 24.3 % and 36.5%, respectively.

By following IRM strategies 22.6 to 39.5 % reduction in number of sprays was achieved in IRM villages, which has contributed to Rs.1065 to 3168 per hectare reduction in cost of cultivation over non-IRM villages in non-Bt cultivars. The average seed cotton yield recorded ranged from 1897 to 2185 kg / ha in IRM villages compared to that of 1804 to 1995 kg / ha in non-IRM villages. This has led to increase in C:B ratio which subsequently yielded Rs.2433 to 5233 more net profit per hectare in IRM villages over non-IRM villages. In Bt hybrids, by implementing IRM strategies around 16.10 to 18.47 % reduction in number of sprays was achieved in IRM villages, which has contributed to Rs. 255 to 592 /hectare reduction in cost of cultivation over non-IRM villages. The average seed cotton yield ranged from 2278 to 2364 kg / ha in IRM villages compared to that of 2097 to 2141 kg/ ha in non-IRM villages. This has led to increase in C:B ratio which subsequently yielded Rs. 3251 to 5595 more net profit per hectare in IRM villages over non-IRM villages.

A resistance level of 5% for endosulphan and 4% for spinosad was noticed in the population of *H. armigera*. In the case of fenvalerate, 100% resistance was recorded. In the case of chlorpyrifos and cypermethrin 67% and 74% resistance, respectively, were recorded. In Fatehabad the resistance level ranged from 33.3% against Endosulfan to 87.5 % against Cypermethrin and Fenvalerate.

