

# 4. Research Achievements



## 4.1: Cotton Genetic Resources

### Nagpur

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

#### Exploration of land races of *des*; and perennial cotton

To preserve rich traditional diversity of Indian cotton and enrich cotton genetic resources, exploratory surveys were conducted in different regions of the country in the

International year of biodiversity.

One hundred and nineteen germ plasm (Table 1) including 82 perennials, 141 and races and 23 traditional cultures belonging to *G. arboreum* and *G. barbadense* were collected from states of Maharashtra, Madhya Pradesh, West Bengal, Andhra Pradesh, Tamil Nadu, Punjab, Meghalaya, Assam and Mizoram. Further characterization and establishment of these cotton land races / perennials in species garden is underway.

**Table 1: Perennials and landraces of cotton collected from different regions of India**

S.No	Districts	State	No. of Accessions	Species	Annual/Perennial/Landrace
1.	Bhandara	Maharashtra	11	<i>G. arboreum</i>	Perennials
2.	Wardha	Maharashtra	1	<i>G. arboreum</i>	Perennial
3.	Nagpur	Maharashtra	2	<i>G. barbadense</i>	Perennials
			1	<i>G. arboreum</i>	Perennial
4.	Washim and Parbhani	Maharashtra	9	<i>G. arboreum</i>	Perennials
			1	<i>G. barbadense</i>	Perennial
5.	Sehore	Madhya Pradesh	8	<i>G. arboreum</i>	Perennials
6.	Jalpaiguri	West Bengal	5	<i>G. barbadense</i>	Perennials
7.	East Godavari, West Godavari, Jammikunta & Srikakulam	Andhra Pradesh	20	<i>G. arboreum</i>	Perennials
8.	Srikakulam (Ponduru Cotton)	Andhra Pradesh	1	<i>G. arboreum</i> race 'indicum'	Landrace/Perennial
9.	Theni	Tamil Nadu	1	<i>G. arboreum</i>	Perennial
10.	Pondichery	Pondichery	1	<i>G. arboreum</i>	Perennial
11.	Talwadi	Punjab	1	<i>G. arboreum</i>	Perennial
12.	Faridkot	Punjab	1	<i>G. barbadense</i>	Perennial
13.	West Garo hills	Meghalaya	3	<i>G. arboreum</i>	Perennial
14.	Kaziranga National Park	Assam	1	<i>G. arboreum</i>	Perennial
15.	West Garo hills	Meghalaya	23	<i>G. arboreum</i> race 'cernuum'	Traditional cultivars/Annual
16.	Mizoram	Mizoram	16	<i>G. barbadense</i>	Perennials
17.	Mizoram	Mizoram	13	<i>G. arboreum</i> race 'cernuum'	Landraces/Annual
Total			119		



Perennial cotton in the backyard of Farmer's house in Bhandara district of Maharashtra







G. *arboreum* race cernuum collected from Meghalaya in bloom with typically elliptic bolls

observed in fibre quality traits like staple length (17.7-19.5 mm), micronaire value (5.2 - above scale) and fibre strength (15.3 -16.9 g/tex).

Twenty three exotic genotypes including 17 varieties of G. *hirsutum* and 6 varieties of G. *arboreum* were procured from Pakistan under Reciprocal Germplasm Exchange Agreement. These were evaluated at CICR Nagpur and in Regional Station Sirsa. Range of variability was recorded for seed cotton yield (22.4-83.5 g/plant), boll weight (2.8-3.1 g), ginning outturn (32.0-37.1%), staple length (23.2 - 28.2 mm), micronaire (2.2-5.7) and fibre strength (16.5-22.1 g/tex) in G. *hirsutum* cultures.

Registration of promising cultures and germplasms of cotton



CNH - CB 205: cluster boll bearing

tested in pre-release trial (Agronomy Trial).

Four genetic stocks of G. *hirsutum* viz; CNH-CB 215 (Dark Brown linted, pigmented & Cluster bearing habit), CNH-CB 211 (Okra leaf lobe and cluster bearing habit), CNH-CB 212 (Zero monopodia, Compact and cluster boll bearing habit) and CNH-CB 205 (Cluster boll bearing habit) and one promising inter-racial pigmented G. *arboreum* line (CNA 5) were identified for registration as unique genetic stocks.

#### Enrichment of germplasm

Twenty morphologically distinct variants of G. *arboreum* race cernuum were added to the germ plasm repository of the Institute and characterized for fiber quality traits. Highest boll weight was recorded in Comilla-22 (6.4 g), Comilla PR-2 (6.3 g) and Comilla 14 (6.3 g). The ginning outturn was higher in Comilla 1 (44.4%), Comilla 9 (44.2%) and Comilla 14 (43.5%), while a narrow range of variability was

observed in fibre quality traits like staple length (17.7-19.5 mm), micronaire value (5.2 - above scale) and fibre strength (15.3 -16.9 g/tex).

Twenty three exotic genotypes including 17 varieties of G. *hirsutum* and 6 varieties of G. *arboreum* were procured from Pakistan under Reciprocal Germplasm Exchange Agreement. These were evaluated at CICR Nagpur and in Regional Station Sirsa. Range of variability was recorded for seed cotton yield (22.4-83.5 g/plant), boll weight (2.8-3.1 g), ginning outturn (32.0-37.1%), staple length (23.2 - 28.2 mm), micronaire (2.2-5.7) and fibre strength (16.5-22.1 g/tex) in G. *hirsutum* cultures.

Registration of promising cultures and germplasms of cotton

One drought tolerant culture CNH 301 developed through pedigree breeding was registered by Plant Germplasm Registration Committee of Indian Council of Agricultural Research (INGR 11061; IC 0587405). The culture recorded 15 q/ha seed cotton yield in All India Coordinated Cotton Improvement programme (AICCIP) and was promoted and

tested in pre-release trial (Agronomy Trial).

Four genetic stocks of G. *hirsutum* viz; CNH-CB 215 (Dark Brown linted, pigmented & Cluster bearing habit), CNH-CB 211 (Okra leaf lobe and cluster bearing habit), CNH-CB 212 (Zero monopodia, Compact and cluster boll bearing habit) and CNH-CB 205 (Cluster boll bearing habit) and one promising inter-racial pigmented G. *arboreum* line (CNA 5) were identified for registration as unique genetic stocks.



CNA-5: one G. *arboreum* inter-racial pigmented square and flower



Evaluation of germ plasm for drought tolerance under rainfed conditions

Based on performance of seven thousand one hundred and eighty five (7185) G. *hirsutum* germplasm lines under rainfed conditions, 400 promising lines were identified. Further observation of 400 lines showed higher single plant yield (ranging from 40.0 to 67.3 g/plant) in at least 21 germplasm lines and higher epicuticular wax (ranging from 93 to 283  $\mu\text{g}/\text{cm}^2$ ) in at least 67 lines. Germplasm lines with high epicuticular wax are expected to possess high WUE, less transpiration rate, better tolerance to sucking pests and greater drought tolerance.

Assessment of gossypol content in germ plasm

To explore the possibility of using leaf gossypol content as a special test for varietal identification, gossypol content in cotyledonary leaves of 11 G. *hirsutum* germ plasm lines were estimated. Estimated gossypol content showed positive correlation with gossypol gland density which needs further confirmation. Assessment of seed gossypol content in 43 G. *arboreum* working collection of high yield group showed wide variability with gossypol content ranging between 0.44 - 0.99%.

Conservation of germ plasm and use in breeding programme

Seeds of 3000 accessions (G. *hirsutum*-2200 and G. *arboreum* - 800) were conserved in Medium Term Cold Storage Module at CICR, Nagpur. Passport data for 3145 germ plasm lines were prepared, compiled and sent to NBPGR, New Delhi for allotment of IC/EC Numbers.

Molecular characterization of cotton germplasm

Characterization of 235 working germ plasm accessions of G. *hirsutum* was carried out at CICR, Nagpur and NBPGR, New Delhi. A total of 44 SSR markers were employed to characterize 235 entries in the working collection.

Core collection of 94 accessions was characterized using 58 SSRs markers of which 54 were polymorphic. So far, 89 polymorphic SSRs, 5 TRAP, 13 SRAP and 10 RAPD (total 117 markers) were used for genotyping 94 core accessions.



## Distribution and use of germplasm in breeding programme

One hundred and thirty one elite germ plasm lines of *G. hirsutum* were selected in field condition by scientists for utilization in various research programmes.

## Maintenance and evaluation of wild species

Twenty-six wild species, 15 races and > 40 synthetic polyploids of *Gossypium* and related species was maintained in the Institute. The wild species were employed in introgression breeding for improvement of fibre quality traits and biotic stress tolerance. Twenty germplasm lines from Israel were grown in the pots and field all of which were found to be male sterile. These lines were received without the restorer lines.

Seeds of five wild species of *Gossypium* viz; *Gossypium africanum-A*, *Gossypium anoma/um-B1*, *Gossypium thurberi-D1*, *Gossypium soma/anse-E2* and *Gossypium bickii-G1* were supplied to Principal, Matreyi College, University of Delhi for research purpose under Material Transfer Agreement.

## Coimbatore

### Maintenance and evaluation of germ plasm

Two hundred and thirty five *G. hirsutum* germ plasm lines were grown in field besides cernuum race of *G. arboreum* that was characterized morphologically as per germ plasm index card.

One hundred and fifty five *G. barbadense* germ plasm lines including exotic accessions were grown in field for evaluation. Twenty-six exotic lines were evaluated in replicated yield trial with Suvin as check. Three lines viz., EC-617836 (1024 kg/ha), EC-617837 (988 kg/ha) and EC-617840 (714 kg) recorded significantly higher yield than the check Suvin (371 kg/ha). Three exotic genotypes EC-617836 (33%), EC-617840 (34.3%) and EC-617844 (31%) showed better GOT compared to Suvin (27%).

## Sirsa

### Collection, conservation and maintenance of genetic resources

Three thousand nine hundred and fifty four accessions were evaluated for their field performance in North zone. The yield / plant ranged from 16-209.3 g, boll weight from 2-4 g, boll number 5-64, plant height 35-182 cm with monopodia and sympodia ranging from 0-13 and 0-21, respectively.

Several compact genotypes amenable to High Density Planting System (HDPS) were identified with marker characters. Further, 2159 accessions were found free from CLCuD.

## 4.2 : Hybrid Cotton

### Nagpur

#### Maintenance of parental lines (male steriles and restorers)

One hundred and thirty seven *G. harknessii* based CMS, 15 *G. aridum* based CMS, 57 restorers and 20 GMS lines were maintained by crossing with their counterpart B lines, selfing and sibmating. Single plant to progeny rows of M<sub>3</sub> generation

were raised with 96, 48 and 212 plants for Rajat CMS *G. harknessii*, AK 32 CMS *G. aridum* and GMS line G 67. Fertile plants in case of GMS with comparatively bigger bolls were harvested for further studies. No distortion in fertility to sterility ratio in case of GMS line was observed.

Under GMS line development programme, four single plant to progenies were raised for PKV 081, AK 32 and L 147. They were tested for second year for confirmation of 1:1 segregation for male sterility and fertility. Forty-eight and twelve single plant progenies were raised for Suman and Sharda respectively, to identify progenies with 1:1 segregation and were maintained through sibmating for use in hybrid development programme.

#### TGMSsystem

The flowers of individual plants in TGMS line 1-1 sown during February (summer flowering) were observed for their fertility/sterility behavior. Complete male sterility was documented in line 1-1 during the month of May with no self boll-setting. Restoration of low levels of fertility (10-30%) was observed during the months of July, August and September while complete fertility was restored when minimum temperature was between 16-18 °C during November to January. Hence, exploitation of TGMS phenomenon during normal *kharif* season for hybrid seed production is not advisable.

Exploitation of male sterility in TGMS line during normal *kharif* season was attempted with application of chemicals known to induce sterility. Among the five sterilants studied at different concentrations, application of Maleic hydrazide @ 200 ppm resulted in flowers with maximum male sterility. Out of total of 110 flower buds treated with Maleic hydrazide, 60 produced male sterile flowers and 29 partially fertile flowers during the period when lines showed completely fertile flowers. Potential of chemical sterilants to induce male sterility need further standardization and validation.

#### EGMSsystem

Observations involving 8 EGMS lines showed that the phenomenon could not be fixed for exploitation for hybrid development even though complete sterility was observed in two EGMS lines 35 and 08093-1 OR in the month of May 2011 when temperature was above 40°C.

## Coimbatore

### Interspecific hybrids

Nine *G. hirsutum* x *G. barbadense* interspecific hybrids were found to be superior in yield over the check hybrid RCHB-708 Bt with highest seed cotton yield of 24.75 q/ha in CCHB 12. This hybrid also possessed highest bundle strength of 33.5 g/tex followed by CCHB-8 (33.4 g/tex) while the check RCHB-708Bt had 31.0 g/tex. Hybrid CCHB-11 had the highest span length of 38 mm followed by CCHB-6 (37.3 mm) and CCHB-13 (37.2 mm). CCHB-6 was found superior in yield as well as the fibre qualities. This hybrid had the highest ginning outturn of 35 per cent and highest micronaire value of 4.21J/inch.

Another set of 30 cross combinations were evaluated in a separate trial with check hybrid RCHB-708 Bt. Four hybrids showed yield superiority over the check hybrid RCHB-708 Bt. The hybrid CCHB-4 recorded highest yield of 27.50 q/ha with superior fibre quality traits.

Based upon superior performance over last five years, CCHB-4 was sponsored in multi location yield evaluation trial under AICCIP.

## Sirsa

Tetraploid Cotton

Maintenance of male sterile lines

The local adapted cultivars and parents of promising hybrids viz. CMS LRA5166, CMSJhorar, CMS RB281, CMS LH 1134, CMS Pusa 31, CMS HS 6, CMS K 34007, CMS F 505, CMS F 1183, CMS CSH 25 M and CMS SH 2379 having cytoplasm of *G. harkensii* were maintained through sibmating. Ten restorer lines were also maintained through selfing. Number of GMS lines viz., K 34, J 34, MCU 5 and GMS 13 were maintained through selfing. Crosses were affected between GMS lines and good combiner lines.

Identification and maintenance of restorer lines: Fertility restorer lines viz. CIR 8, CIR 12, CIR 15, CIR 23, CIR 26, CIR 32, CIR 38, CIR 47, CIR 70, CIR 72 were maintained through selfing. The new restorer lines CIR97P1, CIR97P3, CIR119P1, CIR119P3, CIR126P1, CIR526P1, CIR526P3, CIR 920 P1, CIR 926 P2, CIR 926 P3, CIR 1169 P1 and CIR 1169 P2 were developed through pedigree method of breeding.

Heterotic pools in *G. arboreum* and *G. hirsutum*

Evaluation of GMS hybrids

### *G. arboreum*

To develop heterotic pools of *G. arboreum*, 40 GMS hybrids along with parents were tested in randomized block design with three replications. The highest seed cotton yield was recorded in RED x CISA6-295 (1511 kg/ha) followed by CISA-2 x CISA 6-209 (1503 kg/ha) as against 1375 kg/ha of check hybrid CICR-2. Highest GOT of 39.4% was recorded in the hybrid DS 5 x CISA 6-165 while the highest 2.5% span length (25.3 mm) and tenacity (19.3 g/tex) was documented in hybrids CISA 2 x CISA 9 and GAK 413 A x CISA 6-123, respectively.

CISA-6-187, CISA 9 and CISA8 as male and DS 5 as female parents were found to be good general combiners for seed cotton yield. The cross combinations RED X CISA6-123, CISA 2 x CISA 6-209 were the high yielding hybrids which possessed significant specific combing ability effect.

### *G. hirsutum*

To develop heterotic pools of *G. hirsutum* cotton, 50 GMS hybrids along with their parents were tested in Randomized block design with three replications. The highest seed cotton yield was recorded in GMS-17 x CSH 3129 (1821 kg/ha) followed by GMS-27 x CSH 2907 and

GMS-26 x CSH 2912 (1728 kg/ha) as against 1688 kg/ha of conventional check hybrid CSHH 198. Maximum ginning outturn of 37.1% was recorded in hybrid GMS-27 x CSH 2907 DA. The highest 2.5% span length (29.6 mm) and tenacity (23.5 g/tex) was recorded in hybrids GMS-27 x 004 NAH and GMS-27 x 004 NAH, respectively.

In general of *G. hirsutum* cotton OK 2885, CSH 2912, CSH 3129, CSH 2907 as male parents and GMS-26, GMS-17, GMS-27 as female parents were found to be good general

combiners for seed cotton yield and GMS-17 for ginning percentage. The cross combinations GMS 20 x CNH 91 and GMS 17 x 004 NAH were high yielding hybrids possessing significant SCAeffect.

*Intra-hirsutum* GMS based hybrid CSHG 1862 identified

An *intra-hirsutum* GMS based hybrid CSHG 1862 recorded an average seed cotton yield of 21.02 q/ha over 21 locations as against 18.82 q/ha yield of CSHH 198 (zonal check) and 19.87 q/ha of local checks, registering an overall increase of 11.7 and 5.8 per cent over the two checks, respectively. This hybrid performed best in 23 out of 27 trials as against 15 out of 27 in case of common check CSHH 198. The hybrid documented GOT of 34.5% which was superior to that of zonal check CSHH 198 with 33.7% and local check with GOT of 34.1%. Hybrid CSHG 1862 recorded 2.5% span length of 27.8 mm, micronaire value of 4.2, uniformity ratio of 50% and fibre strength of 22.0 g/tex at par with common check CSHH 198 and superior than other check hybrids. Hybrid CSHG 1862 is a superior medium staple hybrid and possessed high fibre strength, capable of spinning at 40s counts. The hybrid was identified for release in irrigated tracts of north zone by the Central Varietal Identification Committee (AICCIP) during its Annual Group meeting held at Hisar.

## 4.3: Genetic Improvement

### Nagpur

A. *G. arboreum* (Diploid cotton)

Identification of promising cultures

Two promising cultures viz., CNA 389 and CNA 390 were identified for further evaluation and seed multiplication during the season.

Development of medium and long linted diploid cottons (*G. arboreum*)

Three cultures viz., PAIG-62 (22.4 g/tex), Dia-08-25 (22.1 g/tex) and Dia-08-26 (22.3 g/tex) recorded fibre strength above 22 g/tex in all the three trials. PAIG-358 recorded highest 2.5% span length of 29.4 mm. Most of the cultures tested were found to be superior in staple length with desirable micronaire value.

Thirty four high yielding long staple *G. arboreum* cultures with moderately high strength upto 21 g/tex were developed, that hold promise for fibre quality improvement in *G. arboreum*.

Promotion of *G. arboreum* and *G. hirsutum* cotton in Melghat region

Promotional activity to popularize public bred varieties in marginal soils of Melghat regions of Amravati districts in Vidarbha was undertaken. Seeds of *Desi* cotton (including CNA 1003, CAN 348, CAN 385, JA 505, JLA 794, AKA 5 and PA255) and *G. hirsutum* (CNHO 12, Suraj, AC 738 and CIHS-18) were distributed among the farmers. Encouraged with the benefits and additional income generated from their marginal lands number of farmers participating in this promotional activity swelled from 46 in the previous year to 100 during the current year, with 70 being tribal farmers.

A promising culture CNA 398 (*G. arboreum*) was identified based on highest seed cotton yield in National Varietal Evaluation trial of AICCIP 2011-12. It ranked first in central



zone and was promoted in the zone for further evaluation. *G. arboreum* culture CNA 1007 was retained in the zonal trial Br. 24(b) trial in south zone.

*G. hirsutum* (Tetraploid cotton)

## Nagpur

### Drought tolerance

Twelve F<sub>2</sub> crosses showed significant differences for seed cotton yield. Pusa 56-6 x 281 recorded highest seed cotton yield of 511.10 kg/ha with 69 per cent increase over the check. It was followed by 281x Pusa 56-4, 281x Pusa 56-6 with 62 and 54 per cent increase over the check Rajat. The parents 301,281 and 291 recorded upto 15 per cent increase which indicated that they were superior to the check for seed cotton yield. The crosses showed three fold increase in yield over their parents.

Advance generation for 64 single plant selections were tested in replicated trial with check LRA 5166 and Rajat. DTS 120 recorded highest seed cotton yield of 953.20 kg/ha with 68 and 39 per cent increase over the check LRA 5166 and Rajat respectively. It recorded fibre length of 27.6 mm, fibre strength of 18.1 g/tex, uniformity ratio of 50 per cent and micronaire value of 4.7 IJ/inch. DTS 114, DTS 102, DTS 126, DTS 131, DTS 134 were some of the other superior selections. DTS 111 recorded fibre length of 30.2 mm with fibre strength of 20.4 g/tex.

Out of seventeen single plant selections tested, the seed cotton yield ranged from 260-452 kg/ha. DTS 46-04 recorded highest yield of 452 kg/ha followed by DTS 31-14, DTS 31-12, DTS 31-13, DTS 41-02. Selection DTS 41-02 recorded fibre length of 27.6 and fibre strength of 22.2 g/tex while DTS 31-14 recorded fibre length of 31.0 mm, fibre strength of 23.8 g/tex, micronaire of 3.31IJ/inch and uniformity ratio of 48 per cent.

Two cultures viz. DTS 123 and DTS 110 were tested in institute common trial and DTS 123 ranked fourth recording 473 kg/ha seed cotton yield, fibre length 28.1 mm, uniformity ratio 53 per cent, micronaire 3.8 IJ/inch. DTS 95 was tested under State Multilocation Varietal Trial and recorded 1013 kg/ha seed cotton yield with boll weight of 3.25 g and GOT of 38.89 per cent.

### Jassid tolerance

Fifty single plant progenies were selected based on tolerance to jassid (Grade I and II), earliness (130-140 days) and for yield potential in F<sub>5</sub> generation. These single plant selections were derived from 12 individual crosses involving promising lines with tolerance to drought, sucking pests and were inter-specific derivatives.

### Genetic enhancement

Under the programme on enhancement of genetic potential of cotton for yield and yield contributing traits, advanced 13 row bulk populations were evaluated in an unreplicated trial. CNH 2011 viz. (Pedigree - LRA-5166 x CIHS97-9) was found free from sucking pests. In the 16 advanced backcross populations in BC<sub>2</sub>F<sub>1</sub> generation, the backcross viz., LRK-516 x (LRK-516 x Deltapine-66) gave high yields with high boll number. There was an increase in boll weight upto 3.8 g in case of PKV-081 x (PKV-081 x PIL-8) and H-777 x (H-777 x Rex) over PKV 081 with 2.9 g boll weight.

In the advanced *intra-hirsutum* populations (F7-FB)' CNH 2056 viz., (Pedigree- LH-1134 x Reba- Pvt-9) was found to be high yielding and early with an average 18 bolls per plant compared to 15 bolls per plant in check variety NH 615. Also CNH 2013 in pedigree- Gcot-1 0 x Reba- Pvt 9) was observed uniform in maturity with 32 bolls per plant with boll weight of 3.2 g. These populations were grown under 60 x 30 cm spacing. A new culture CNH 2013, early maturing, with synchronized boll bursting has been identified as a promising entry.



CNH 2013, early maturing with synchronized boll bursting

In advanced cultures developed by crossing *G. hirsutum* x *G. barbadense* cotton, all five entries which involved Suvin as male parent were observed as high yielding with lustrous white cotton. The cultures on an average supported 15-18 bolls per plant with good sympodial plant types. Development of value added advanced populations, new cultures and elite trait specific gene pools for further improvement of cotton is available.

### Breeding short duration cotton suitable for HOPS

In *Khari*2010-11, 550 selective *G. hirsutum* germplasm lines with zero monopodia were evaluated at 30 x 20 cm spacing. Based on their performance 86 lines with one or more traits like short sympodia, high strength, bacterial blight resistance, leaf hopper resistance, high boll weight, early maturity and compactness, the traits critical for development of cotton amenable to high density planting system (HDPS) were selected. Out of 86 lines 34 accessions were evaluated in replicated trial at 45 x 15 cm spacing during the crop season. The selected plants showed dwarf habit and possessed short

sympodia and resistance against bacterial blight and leaf hopper. The below mentioned lines including one highly compact early maturing *G. hirsutum* germ plasm N-170, were selected for breeding cotton suitable for HDPS.

#### Short duration, compact *Gossypium hirsutum* cultures selected for developing cotton suitable for HOPS

S.No.	Name of germ plasm	Traits
1	IC-358080	Bacterial blight resistance
2	IC-358358	High strength and long staple
3	IC-358221	High strength
4	IC-358207	Dwarf type
5	IC-358771	High bolls per square metre area
6	IC-357126	High boll no. per square metre
7	N-170	Early maturing compact short sympodia as base female parent
8	Gcot-100	Semi-compact type as check

#### Population improvement

##### Random mating population in *G. arboreum* and *G. hirsutum*

The random mating population through conventional crossing was constituted and maintained by bulk harvesting one burst boll from each plant. The economic yield of the conventional *G. hirsutum* randomly mated (RM) population was compared with the base population and it was observed that population yield declined to the extent of 27.54% than that of base population. This may be due to fixation of the *segregants* heterozygotes in successive generations. The performance of diploids was very poor during the season, hence performance of the RM population could not be compared.

##### Use of GMS for developing random mating population

The fifth cycle of GMS based random mating population was completed in *G. hirsutum*. A composite population was grown on large plot area and all sterile plants in the population were tagged at flowering and allowed to open pollinate. The out crossed bolls from all the sterile plants were bulk harvested to be raised in the next crop season.

Similarly, sixth cycle of GMS based random mating was completed in *G. arboreum* during 2011-12 and the composite population shall be taken to the next cycle.

#### Single plant selection

During 2011-12, 458 single plant selections from random mating population have been evaluated in plant to row progeny plots. From the composite random mating population 492 plants were selected based on manual testing for fibre quality traits. About 506 superior single plants were reselected from the segregating single plant progenies. Several single plant selections were identified for big boll size, better fibre quality and compact plant type for further evaluation.

#### Entries sponsored under AICCIP

CCH 1111 *G. hirsutum* culture ranked second in the central zone has been promoted to zonal trial Br. 03(b) trial in central zone. Two *G. arboreum* cultures, CNA 1016 and CNA 1020

were sponsored to Br. 22(a/b) National trial of the AICCIP for the crop season 2012-13.

*G. hirsutum* culture CNH 14 registered promising higher yield in National Varietal Evaluation trial of AICCIP 2011-12 and ranked fourth in central zone. The culture has been promoted in the central zone for further evaluation.

Culture CNH 315 found promising in Institute trial was sponsored in Br02 (b) trial of AICCIP during 2011-12.

Similarly, CNH 1110 *G. hirsutum* culture was sponsored for Br. 02(b) National trial. Two more *G. hirsutum* cultures namely CNH 1105 and CNH 1109 were sponsored for compact plant type trial Br. 06 (b).

#### Development of heterotic pool for superior medium staple

From 140 single plant progeny rows, 30 most productive progeny rows were bulked at F<sub>6</sub>. The selections were made on the basis of earliness (120-140 days) and for superior medium staple (25.5 mm -27.0 mm) cotton coupled with yield potential. Crosses involved LRK 516 x Acala 1517, PKV Rajat x Acala 1517, LRK 516 x DHY 286 and PKV Rajat x DHY 286 in one group and LH 1948 x MCU 9, ACCLD 163 x H 1252, H 1252 x LH 1948, H 1252 x MCU 9 in other group. Thus, 30 breeding lines derived from these two heterotic groups will be evaluated for initial yield trials.

#### Coimbatore

##### Genetic improvement

The medium staple culture CCH 2623 was tested in various centres of All India Coordinated Cotton Improvement Project during the past four years in both central and south zones. It showed yield superiority in both the zones and recorded a mean seed cotton yield of 1739 kg/ha in central zone and 1798 kg/ha in south zone locations with 34 and 13 per cent increase over the zonal checks LRA 5166 and Surabhi respectively. The culture has been recommended for testing the agronomic requirements in both the zones.

From segregating generation (F<sub>6</sub>) 125 superior single plants were selected based on morphological characters. Some plant progenies combined both yield and quality. Progeny PI-32-1-2 recorded a seed cotton yield of 105 g/plant and also had a very good bundle strength of 24.6 g/tex with 29.5 mm 2.5% span length.

#### Sirsa

##### Genetic improvement for high productivity and superior quality

In *G. hirsutum*, two crosses viz. CSH-3119 x MM03-27-5-1-8 and MM22-1-2 x CSH-3047 out of 13 affected between long linted cultures from Coimbatore and high fibre strength cultures from Sirsa were early in maturity and high yielding.

CISA 6-165 yielded seed cotton yield of 1934.2 kg/ha with 40.30% increase over the local check HD-123. CISA 6-165 had fibre length of 22.3 mm and value less than 6.0. In the station trial of TMC genotypes CISA-8 significantly out yielded all the three local checks. As many as 17 out of 22 single plant progenies out yielded the checks CISA-31 0 (86.7 g/plant) and CISA-614 (83.0 g/plant).



#### Evaluation of CLCuV resistant cultures

Out of 20 cultures tested against CLCuV at Sirsa, 6 cultures viz., CSH 2808, 2810, 2811, 2813, 2836, and 2916) were found to be resistant to the virus, whereas in the hot spot area at Sriganganagar, only three cultures viz CSH 2907, 2908 and 2916 showed resistance. Highest seed cotton yield was recorded in the advance culture CSH 2833 (1821 kg/ha) followed by that in CSH 2811 and CSH 2844 with 1770 kg/ha compared to check variety RS 2013 recording only 1652 kg/ha seed cotton. Highest GOT of 35.7 per cent was recorded in the variety CSH 2916 while CSH 2907 recorded the highest 2.5% span length of 29.1 mm and bundle strength of 22.4 g/tex. In another trial with 16 cultures of *G. hirsutum* highest seed cotton yield was recorded in the culture CSH 2931 (2016 kg/ha) followed by CSH 2941 (1584 kg/ha). Maximum ginning out turn of 37.3% was recorded by CSH 2941 as compared to local check varieties 34.8% in RS 2013 and 34.7% in LH 2076. The culture CSH 2944 recorded the highest 2.5% span length (28.6 mm), whereas the highest bundle strength of 22.9 g/tex was observed in CSH 294.

#### 4.4: Genetic Diversity through Introgression

##### Nagpur

A new cross between *G. arboreum* and an African species *G. somalense* (E<sub>2</sub> genome; known for drought tolerance) and its reciprocal cross was harvested which will be advanced to next generation for drought tolerance studies.



*G. arboreum* x *G. somalense*     *G. somalense* x *G. arboreum*  
Reciprocal crosses between *G. arboreum* and *G. somalense*

*Thespesia lampas* - a related species of *Gossypium* known for possessing thick boll rind thereby conferring resistance to bollworm was established and crossed with all the six races of *G. arboreum*. A single F<sub>1</sub> boll was set and harvested only from the cross between *G. arboreum* race indicum and *Thespesia lampas* to transfer this trait of thick boll rind for further inheritance study.



*G. arboreum*  
Race indicum

F<sub>1</sub> boll harvested



*Thespesia lampas*, flower (Inset)

Cross ball (F<sub>1</sub>) of *G. arboreum* race indicum and  
*Thespesia lampas*

##### Introgression Breeding

Four hundred and twelve introgressed lines of cotton were evaluated for fiber quality traits based on which 29 lines with diverse fibre properties were selected. Lines CICR 1-29 showed diverse fiber traits with 2.5% SL ranging between 19.3 - 31.7 mm, strength ranging between 16.9 - 26.4 g/tex, micronaire ranging between 2.8 - 6.2 and uniformity ratio ranging between 40 - 56%.

#### 4.5: State Multi-Varietal Trial (SMVT)

##### Nagpur

A State Multi-location Varietal Trial (SMVT) consisting 20 genotypes of *G. arboreum* and 16 *G. hirsutum* were conducted at CICR, Nagpur, which stood vitiated due to continuous rains.

#### 4.6: Molecular Breeding

##### Nagpur

##### Mapping QTLs for fibre quality traits

In an effort to develop linkage map of diploid A genome in cotton, an interspecific F<sub>2</sub> mapping population (*G. arboreum* cv. KWAN-3 x *G. herbacum* cv. Jaydhar) was developed. During 2011-12, parental polymorphism studies were carried out using 625 SSR markers based on which 38 SSR polymorphic markers were identified (Fig. 1). Genotyping of mapping population with 76 informative markers (66 SSR, 9 SRAP and 1 RAPD) has been completed.



Fig. 1 : Survey for parental polymorphism using SSR markers in tetraploid and diploid cotton, 1-EL-958 (*G. hirsutum*), 2-UPA-5717 (*G. hirsutum*), 3- Jaydhar (*G. herbaceum*), 4- KWAN-3 (*G. arboreum*)

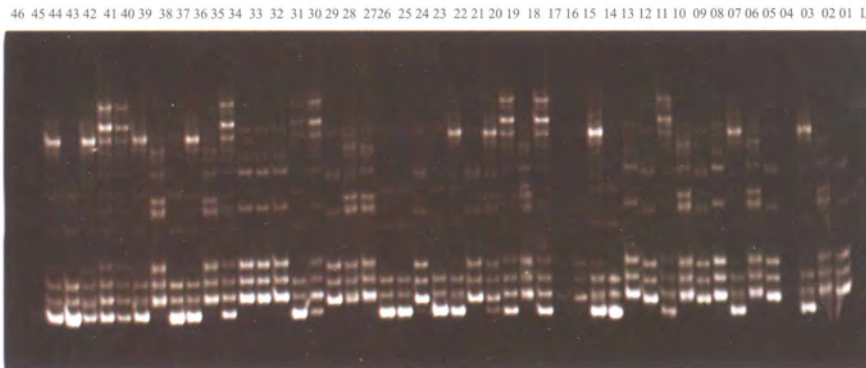


Fig. 2: Genotyping of F<sub>2</sub> mapping population with SSR marker BNL 3261 in tetraploid cotton

Development of mapping populations (RIL's) for fibre quality traits in diploid and tetraploid cotton

In diploids, 193 F<sub>2</sub> plant progenies were raised. One random plant from each progeny was selfed for further advancement to next generation. In few of the progenies, segregation for morphological characters was observed; hence, the progenies need to be further advanced for two more generations to attain uniformity. In *G. hirsutum* 273 F<sub>2</sub> boll to row progenies were grown, selfed bolls were obtained. The lint samples of each progeny are being evaluated for fibre quality traits using HVI. A set of 273 progenies of RILs are ready for mapping fibre quality traits.

Mapping bacterial blight resistance gene(s)

During 2011-12, a set of 335 SSR primers were surveyed to screen polymorphism between bacterial blight susceptible and resistant parents out of which 30 SSR markers were found to be polymorphic with the parental genotypes, Ganganagar Ageti and S295. A total of 73 informative markers were identified with the above parental genotypes. Fifty five informative markers were identified with Acala-44 and IM216. The extent of polymorphism ranged from 6.04 to 9.1 per cent among the contrasting parents, Acala 44 and 1M216, GA and 1M216, GA and S295, GA and 101-102B.

Inheritance of *Rhizoctonia* root rot in upland cotton

Five F<sub>2</sub> populations developed using root rot tolerant (Saubhagya, B.N., NISC-19, NISC-14 and Abhadita) and susceptible (LRA 5166) genotypes were screened against the

disease in *Rhizoctonia* root rot sick plots. The segregation in all five F<sub>2</sub> populations revealed that inheritance of *Rhizoctonia* root rot is digenic (two genes) with a ratio of 15 resistant: 1 susceptible and duplicate gene interaction. The susceptible variety LRA 5166 showed 100% susceptibility in the sick plot. The results obtained will facilitate development of resistant varieties in upland cotton.

Development of mapping population for drought tolerance

F<sub>2</sub> generation of three crosses Pusa 45-4 x 301, 281 x Pusa 56-4 and Pusa 56-4 x 291 were raised for advancing the generation. These crosses recorded high drought tolerant efficiency for two consecutive years.

#### 4.7: Development of Transgenic Cotton

Nagpur

Bollworm resistant transgenic cotton in tetraploid and diploid cotton

Confined field trial was conducted with 7 transformation events of Bt-cry1Ac gene (ILK Bt-77-1 to ILK Bt-77-7) to characterize and select effective events. Cry toxin expression was evaluated in all 7 events

from early bloom phase (50-55 DAS) to late maturity phase (155 DAS). Average concentration of Cry protein in leaves, squares and *lor* bolls in transgenic plants across the events was 0.52, 0.28 and 0.23  $\mu\text{g/gm}$  respectively, in 55 days old plants. After 155 days, concentration of Cry protein was 0.61 and 0.30  $\mu\text{g/gm}$  in leaves and bolls respectively. Three new events of *cry1Ac* in *G. hirsutum* variety Anjali (Anjali FBT-1 and 2) were also evaluated for Cry protein expression. Plants were raised in boll to row progenies and presence of transgenes was confirmed by PCR (Fig. 3). New transformation events were also generated using *cry1Ac* and *cry1F* genes in LRA 5166 and Suraj. Plant growth, seed oil content, seed cotton yield, besides fibre quality traits were recorded as per the RCGM recommendations. The original characteristics of the recipient host plants were not altered as a result of transformation with Bt genes.

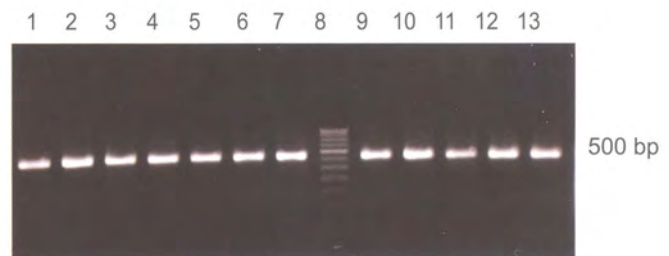


Fig. 3: PCR amplification of Bt *cry1F* gene in putative transformants of *G. hirsutum* cv. LRK 516. Lanes 1-7 and 9-13, PCR positive plants; Lane 8, 100 bp ladder





Putative transformants of *G. hirsutum* cv. Suraj carrying Bt *cry1Ac* gene

#### Bollworm resistant transgenic diploid cotton

##### Event selection trial

Confined field trial for event selection of PA 255 and RG 8 transgenic *G. arboreum* cotton containing *cry1Ac* gene was conducted at CICR, Nagpur. In all ten events including 4 events of Bt RG 8 (*Des*; Bt G-822-1 to *Des*; Bt G822-4) and six events of Bt PA 255 (CICR Bt *Des*; 1 to CICR Bt *Des*; 6) were characterized. Expression of Cry protein was estimated in leaves of 1350 transgenic plants of two varieties. Cry protein expression was upto 3.0 IJg/gm. PCR analysis of Bt-RG 8 events showed amplification of *cry1Ac* gene in 108 out of 180 plants tested for four events. Individual bolls from the Bt positive plants were harvested for further characterization.

##### Molecular characterization of new Bt-events

Twenty two new events of *G. arboreum* cvs. RG 8 and PA402 containing *cry1Ac* and *cry1F* genes were developed by *in planta* transformation. PCR carried out with 340 putatively transformed plants of new events of cv. RG 8 showed 166 PCR positive plants. While of 35 cv. PA 402 *cry1F* agro-inoculated plants five plants were PCR positive. These plants were also ELISA positive. The selfed bolls from PCR positive plants were harvested separately.

##### Molecular characterization of CICR transgenic events

Junction-specific primers used to amplify and sequence flanking regions in transgenic events generated previously, resulted in non-specific amplification. Therefore, genespecific primers based on the sequences of *np11* marker and *cry1Ac* transgene present in the binary plasmid pBinAR, were designed and synthesised to characterize the transgenic events.

##### Contained green house trial

Contained green house trials on Chitinase transgenic diploid cotton (3 events), RNAi mediated transgenic events (5 events with virus coat protein and *AC2* genes), *cry1Ac* (4 events) and *cry1F* (3 events) were approved by IBSC (Fig. 4).

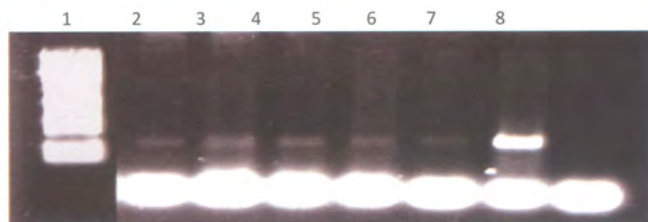


Fig. 4: PCR amplification of *cry1F* gene in putative cotton transformants of *G. arboreum* cv PA 402. Lanes:1, 1 kb ladder; Lane-2-7, PCR positive plants.

#### Transgenic cotton for leaf curl virus resistance

##### RNA interference approach

New transformants of *G. hirsutum* cv HS 6, F 846 and LH 2076 were generated with dsRNAi constructs viz., pBinAR-int-CP-SA (12.19 kb), pBin AR-int-BC4-SA (12.24 kb) and pBin AR-int-BV4-SA (12.17 kb). By shoot-tip and *in planta* transformation methods 8 events involving dsRNA of CP gene including two events in cv F 846, 3 in LH 2076 and three events in cv HS 6 were established. Out of 8 new events, five events were found to be PCR positive for the transgene. Seeds of individual bolls of PCR positive plants were harvested for evaluation against virus and screening of homozygous transgenic plants under contained green house trial at CICR RS, Sirsa (Fig. 5).

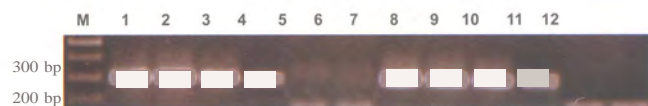


Fig. 5: PCR amplification of CP gene fragment used for creating dsRNAi construct in transgenic HS 6-CP plants. Amplification was done using two sets of primers P1 (F1 + R1) and P2 (F1 + R1) amplifying sense and antisense strands of the construct. M-100 bp; Lanes: 1-3, PCR amplification using primer set P1; 4, pBSK-CP-SA (plasmid); 5, cotton (wild type) HS 6; 6, PCR -ve sample; 7-9, Amplification using primer set 2; 10, pBSK-CP-SA plasmid; 11, HS6 cotton HS 6 (wild type); 12, PCR -ve sample.

##### Antisense approach

The T<sub>3</sub> generations of F 846 and H 777 transgenic cotton with one event each with *ARep*, *ACP* and *SCP* genes were grown for multiplication of the seeds in the confined field trials. New events were also generated and the T<sub>1</sub> generation of RS 875 was screened against CLCuV in green house at Sirsa. The resistant plants were selfed and seeds were harvested.

##### Genetic engineering for abiotic stress tolerance

Putative transformants of *G. hirsutum* cvs. LRA5166 (2 events each with *DREB 1A* and *ZF 1A*) and LRK 516 (one event each with *DREB 1A* and *ZF 1A*) were grown for multiplication in the confined field trials with the approval of RCGM.

Eighty T<sub>1</sub> plants of each of the six transgenic events alongwith 20 control plants were screened for presence of transgene as well as the marker gene *np11*. Out of 160 T<sub>1</sub> plants of two events of OREB 1A gene in LRA5166, 46 plants were positive for the marker as well as transgene while 55 out of 160 T<sub>1</sub> plants of two events of OREB 1A in LRK 516 were positive for both the genes. In one event each of BcZF1 in LRA 5166 and LRK 516, 30 and 28 plants respectively, were found positive for the marker as well as transgene. New events were

developed with *DREB1A* and *ZF1* in LRA 5166 and LRK 516 with transformation frequency of 4.2 to 4.8%, respectively.

Physiological and biochemical characterization showed that the transgenic plants exhibited higher relative water content, leafwater potential and proline content compared to wild type cotton.

Development of inverted repeat construct of *~ cadinene synthase* gene

The 0 *cadinene synthase* gene was PCR amplified in sense and antisense orientations (Fig. 6 & 7) with suitable restriction sites and cloned in inverted repeat generating plasmid pBSK-int (HQ 343203). The cloned product was further confirmed by PCR and Restriction enzyme analysis.

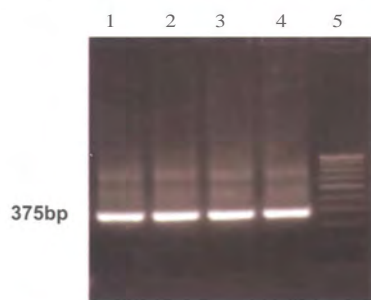


Fig. 6: PCR amplification of 15 *cadinene synthase* gene for cloning in sense and antisense orientations. Lanes 1-2, sense strand; lanes 3-4, antisense strand; 5, 100 bp ladder.

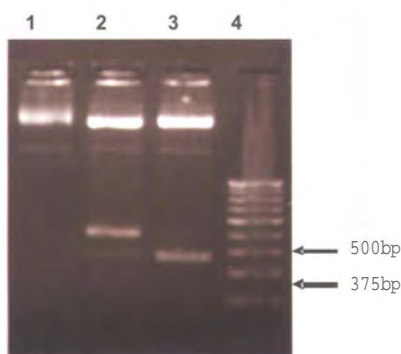


Fig. 7: RNAi construct of 15 *cadinene synthase* gene in plasmid pBSK-int. Lanes 1, pBSK-int (undigested); 2, *Bam*HI and *Kpn*I digestion resulted in release of 500bp product containing *Cadinene synthase* gene (375 bp) and Chitinase intron (125 bp); 3. *Kpn*I and *Eco*RI digestion resulted in release of *Cadinene synthase* (375bp) gene

Isolation of *~ Cadinene hydroxylase*

Another candidate gene for RNAi, 0 *Cadinene hydroxylase* was PCR amplified using cDNA synthesised from mature embryo as template and sequenced.

#### 4.8: Seed Production and Seed Quality Improvement

Nagpur

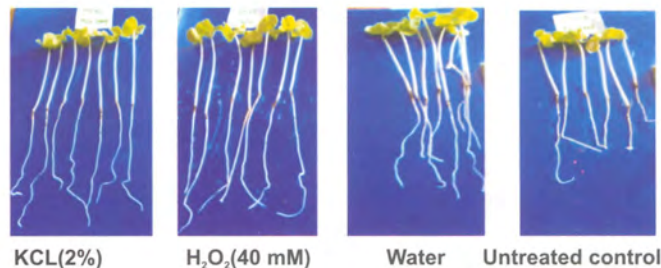
Screening for moisture stress tolerance and determination of effective seed treatments

Tolerance of 25 working collections each of *G. arboreum* and *G. hirsutum* to seed germination in PEG osmotic medium constituted to exert variable osmotic pressures revealed one

*G. arboreum* line (6565-M11-AC-65) to be highly tolerant (more than 70% germination) to high osmotic pressure of -1.5 mPa. In general, *G. arboreum* cotton were more tolerant to PEG stress with superior seed germination than *G. hirsutum*.

Seed treatments with 2% Potassium Chloride and 40 mM Hydrogen peroxide showed superior seedling growth compared to untreated and water treated controls. Furthermore, spray application of chemicals compared to seed soaking was more effective in promoting higher seedling growth. Seedlings raised from H<sub>2</sub>O<sub>2</sub> sprayed seeds demonstrated better stand and better growth under moisture stress compared to untreated controls.

Enhanced growth of cotton seedlings as a result of seed treatment with KCl and H<sub>2</sub>O<sub>2</sub>



Seedling stand of *G. hirsutum* cv NH 615 (pots) at soil moisture level of 60% field capacity



DUS characterization

Ten candidate varieties/hybrids from private sector were DUS characterised with the help of four reference varieties of *G. hirsutum* and one reference variety of *G. barbadense*.

In addition to the characters observed as per the National Test Guidelines, variation for gossypol gland density was also studied. Unit sections (0.2 mm<sup>2</sup>) from seed cotyledons and 7 days old cotyledonary leaves of the same variety under Light-Stereo microscope revealed variation in gossypol gland density. In general, gossypol gland density per unit area was higher in seed cotyledonary sections compared to that in the cotyledonary leaf sections. Genotypes differ in frequency of their gland density in cotyledonary leaf section. While density of gossypol gland was high in Surabhi and Khandwa 2, Vikas, MCU 5 and MCU 10 showed less density with scattered glands. However, feasibility of using gossypol gland density as



a special DUS test in varietal identification would depend upon its reproducibility.



Surabhi

MCU 5

#### Variation for gossypol gland density in cotyledonary section

Sample size for assessment of Bt seed purity

In an experiment with simulated seed lots having different percentage of Bt cotton seeds, sample sizes of more than 60 seeds drawn from a seed pack of 450 g could provide a near accurate measure of Bt seed purity. Per cent Bt seeds in seed lots was tested based on estimation of *cry1Ac* protein. It was concluded that, the existing norm of assessing Bt seed purity on the basis of 10 seeds will not hold good to assess purity of the entire seed lot.

Coimbatore

Implementation of PVP legislation, 2001

During 2011-12, Plant Variety registration certificate for 21 extant cotton varieties have been obtained from PPV&FRA. Fifteen *G. hirsutum* genotypes were sown for maintenance and seed multiplication. A total of 28 *G. arboreum* and 10 *G. herbaceum* varieties were seed multiplied, purified and being maintained at CICR, Nagpur for reference seed supply.

Characterization of germ plasm lines for DUS

Working germplasm lines (333) received from CICR, Nagpur were characterized for DUS (distinctiveness, uniformity and stability) adopting test procedures of national test guidelines for tetraploid cotton. Wide variability was observed in growth habit and reproductive traits (Table 2). Germplasm lines such as XAB 5 X TANGUIS -126 -DH, LYY, 21-1-1-4-5, 62 -2- (S) 2-3, BMCOT 95 BLL, B 4 EMPIRE, SAJAR 314, SOBHAGYA, TEXAS- 1050, CSH - 911, Arizona super okra leaf (green), MDH 89, MDH 90, B 56-181, KEKCHI(RED), 101-102 Bwere identified for early, compact, dwarf types with high yielding ability and good fiber traits.

Establishment of genetic purity of hybrid seeds through bio molecular profile

Table 2 : Variability observed in working germplasm

Characters	Min.	Max.
Plant: Growth habit (cm)	8.0	46.0
Plant: Height (cm)	37.0	107.0
Days to 50% flowering	49.0	71.0
Boll Weight (g)	1.86	5.49
Number of sympodia	7.0	27.3
Number of bolls	2.7	23.0
Seed cotton yield (g/plant )	20.3	133.85
Seed Index (g)	6.63	14.9
Ginning %	14.06	42.96
Fibre: Length 2.5% Span (mm)	22.1	34.9
Fibre: Strength (g/tex)	16.6	26.0
Fibre: Fineness (~glinch)	2.1	6.5
Fibre: Uniformity	42.0	51.0
Elongation Percentage	3.8	7.1

Seed proteins were estimated using Tris Soluble Proteins, Salt Soluble Globulins and Methanol precipitated fraction and separated by SDS-PAGE electrophoresis in CSHH-243 and its parents CSH 2013 and CSH 43. Presence of specific protein bands of defined mobility and intensity were observed in all the samples which will be useful for identification of hybrids and parental seeds after characterisation and assessment of reproducibility.

Standardization of seed coating with synthetic polymers and additives

The results on seed coating treatments revealed that Polymer coating (Polykote @ 3 ml/kg of seed), diluted with 5 ml of water with insecticide (Imidacloprid @ 6 ml/kg of seed) or Polymer + Royal flow 40 SC @ 2.4 ml/kg seed + insecticide (Imidacloprid @ 6 ml/kg of seed) was found better in retaining the seed viability under ambient storage condition.

#### Seed Production

Nagpur

In Nagpur, 4.37 quintal TFL fuzzy seeds of 40 *G. hirsutum* and *G. arboreum* cultures were produced under Mega Seed Project. In addition, 1.78 quintals of certified seeds of Gram (Vijay) and Kabuli Chana was also produced under the programme. A revenue of 13.0 lakhs has been generated through sale of these seeds and their byproducts. Protective irrigation was provided to the seed production plots from the farm pond.



Farm ponds with conserved rainwater for protective irrigation of cotton

Seed production of *G. arboreum* race *cernuum* collected from Meghalaya was taken up in the Institute. Plants with upto 14 bolls of 7-8 gms were recorded.

#### Coimbatore

Breeder seed production of cotton was undertaken at CICR Coimbatore under National Seed Project. In all, 336 kg of breeder seed of cultivars Suraj, LRK 516 and LRA 5166 was produced during the season. Under Mega Seed Project, 39.5 quintals of TFL seeds including Sorghum (C01), hybrid Maize (C06) and its male parent of C06 were produced.

#### Sirsa

Ninety kg seeds of male and female parents of four popular cotton hybrids and 82 kg seeds of two *Desi* cotton varieties were produced during the season under Mega Seed Project. In addition, 320 quintals seeds of wheat variety PBW 550 was also produced.

### 4.9: Nutrient Management

#### Nagpur

Performance of American (*G. hirsutum*) and *Desi* (*G. arboreum*) cotton varieties was evaluated under rainfed organic conditions. Among the species, *Desi* cottons yielded less than the *hirsutum* varieties due to greater shedding of fruiting parts. Out of the 13 *hirsutum* varieties evaluated, seed cotton yield in the range of 11.0 to 14.9 q/ha was recorded in NH 615, Suraj, PKV 081, LRK 516 and AKH 8828. High value of bundle strength and staple length was observed in the variety of Suraj (24.1 *gtex*, 30.7 mm), followed by LRK 516 (22.6 *gtex*, 28.1 mm) and PKV 081 (18.9 *gtex*, 28.1 mm). Among the *Desi* varieties, JLA 794, CNA 347 and AKA 7 recorded higher seed cotton yield ranging from 8.0 to 9.7 q/ha. These varieties also possessed good staple length of 26.2 to 27.9mm.

Total microbial population, total microbial biomass, nitrogen and carbon were found to be higher in the surface soil as compared to sub surface soil.

On farm trials were conducted at Nandura and Loni villages in Yeotmal district on different nutrient management production technologies in Bt cotton. Results indicate that highest seed cotton yield was obtained under recommended package of practices with INM technology (NPK: 80:40:40 +Zn 10+1t/ha FYM+PSB +2%DAP foliar spray) + along with a protective irrigation (26.25 q/ha). This yield was 147% higher over the Farmer's practice.

Potassium silicate and Calcium peroxide - as growth enhancer of cotton plants

Potassium silicate is known to alleviate nutrient imbalance, impart resistance to abiotic stress, sucking pests and improve crop vigour and quality. Calcium peroxide is an oxygen releasing compound which supplies O<sub>2</sub> to the roots constantly and continuously and adjusts rhizosphere pH making nutrients available to the plants. A field experiment was conducted during 2010 to evaluate the growth promoting effect of potassium silicate and calcium peroxide (60%) in cotton (Bunny Bt) plants. Potassium silicate is a liquid fertilizer containing 8-10% K<sub>2</sub>O and 20-24% SiO<sub>2</sub>.

Seeds of Bunny Bt cotton were soaked overnight in 2% or 4% calcium peroxide slurry and 1001:1 or 5001:1 potassium silicate solutions and sown. Seeds soaked overnight in water were sown as control. The results indicated that among the potassium silicate seed treatments, 500 ppm was found to be effective in increasing the number of leaves (75.3), bolls (16) and single plant yield (43 g/plant) over control with 66 leaves, 12 bolls and 30.16 g of seed cotton yield per plant. Seed treatment with 2% calcium peroxide slurry showed better seedling vigour, plant height and shiny green leaves compared to control.

#### Alleviation of moisture stress

Foliar application of potassium silicate (40, 60, 80 g/ha) was done at 30 and 60 DAS to evaluate its effect on leaf water relations and lipid peroxidation under irrigated and water stress conditions in cotton var. Bunny Bt 2. Potassium silicate treatments positively influenced the leaf water relations in terms of relative water content (RWC) and leaf water potential. Under stressed conditions, plants sprayed with 40 g/ha potassium silicate were found to exhibit higher RWC compared to control. Cell membrane integrity was also higher in potassium silicate treatments than control and hence the membrane injury was lower in plants treated with potassium silicate both under irrigated and stressed conditions. Under stressed conditions, potassium silicate treatments recorded higher proline content and lower levels of lipid peroxidation than control.

#### Hydroponic studies

Hydroponic studies was conducted to understand the effect of potassium silicate as growth supplement in varying concentrations viz., 20, 40, 60, 80, 100 and 120 g/ha. The supplement of 60 g/ha potassium silicate was found to be optimum induced better root growth and also vigour in seedlings. There was no negative effect at higher doses of even 120 g/ha potassium silicate.

### 4.10: Cropping Systems

#### Nagpur

##### Innovative Bt cotton based cropping systems

Averaged over three seasons (2008-11) on medium deep soils, Bt hybrid cotton intercropping with roselle, soybean and marigold produced similar cotton equivalent yields and net returns and significantly greater than sole cotton. Recommended dose of fertilizer along with soil application of MgSO<sub>4</sub> (25 kg ha<sup>-1</sup>) and Borax (10 kg ha<sup>-1</sup>) produced significantly lower seed cotton and cotton equivalent yields, net returns and B: C ratio than higher dose of fertilizer or foliar application of N treatments in a deficit rainfall year.

#### Coimbatore

##### Strategies for maximizing productivity of Bt cotton based intercropping systems

New plant geometry was studied (120 x 45 cm) in comparison with the normal recommended geometry of 90 x 60 cm for the Bt cotton + coriander, Bt cotton + radish and sole cropping system. Modified geometry of 120 x 45 cm was better than the normal spacing of 90 x 60 cm.



## Sirsa

Studies on soil plant narrations in intercropped kharif legumes with Bt cotton under irrigated condition

Feasibility and profitability of mung bean as an intercrop between the paired row lines with Bt hybrid MRC 7017 and Bioseed 6488 was evaluated. The check included sole cotton and paired row sole cotton. The seed cotton yield of sole cotton (28.0 to 31.6 q/ha) was significantly higher than the paired row sole cotton (26.3 to 30.2 q/ha) and intercropping systems. Inclusion of mungbean in Bt cotton hybrid was not a profitable option in the irrigated north zone conditions at Sirsa.

## 4.11: High Density Planting Systems (HDPS) for Maximizing Productivity

### Nagpur

Identification of compact and zero monopodia genotypes for HDPS for maximizing productivity of rainfed cotton

Thirty four germ plasm lines were identified on the basis of

- Compact plant body and short sympodia
- Shorter internode
- Development of fruiting units closer to main axis
- Perfect locule opening pattern

for their likely superiority under HDPS. All these germplasm lines showed determinate growth of lateral branches, reduction in boll numbers (6-8 bolls/plant), boll weight (2.5 g-3.1 g) and perfect locule opening. Among them higher retention of mature and open bolls (6-11) was observed in IC 358660, IC 358655, and IC 359710. Higher seed cotton yield was recorded in IC 357444 (28.3 g/plant), IC 357126 (30.0 g/plant), IC 358434 (30.8 g/plant), IC 356774 (31.8 g/plant) and IC 132026 (34.3 g/plant). The fibre quality range was recorded for staple length (22.2-29.1 mm), uniformity (45-52), micronaire (2.6-5.2) and fibre strength (14.6-20.7). The promising lines with better fibre quality namely G.cot 100, IC 358205, IC 359649 and IC 357106 were identified.

Eight genotypes were evaluated under two spacing (60 x 15 cm and 45 x 15 cm) and among them RS 875 was found to be most suitable for high density planting.

High Density Planting Systems (HDPS) for maximizing productivity

Ten genotypes of *G. hirsutum* (LH 900, SURAJ, PKV 081, NH 630, CNH 2, CNH 1109, CNH28 I, CNH 1108, ADB 39 and DSC 115) were evaluated at 60 x 30 cm (normal density) with RDF (M1), 45 x 15 cm (high density) with RDF (M2) and 45 x 15 cm with 125% RDF (M3) on rainfed Vertic Inceptisols of Nagpur representing sub-humid dry agro-eco-sub-region. Averaged over genotypes, the mean yield in HDPS (M2) was 1409 kg/ha as against 1088 kg/ha under normal planting (M1) indicating a 29% advantage with HDPS. Under HDPS (45 x 15 cm, 148000 plants/ha), genotypes Suraj, ADB 39, PKV 081 and 281 were found promising in terms of yield (Fig.8), morphology, earliness and nutrient use efficiency. The N response ratio or partial factor productivity of N ranged from 11.7 in DSC 115 to 30.8 in Suraj. The N utilization efficiency was higher in ADB 39 (18.6), PKV 081 (15.8) and Suraj (14.5) compared to the other genotypes.



Genotypes - PKV-081, Suraj and LH -900 under HDPS

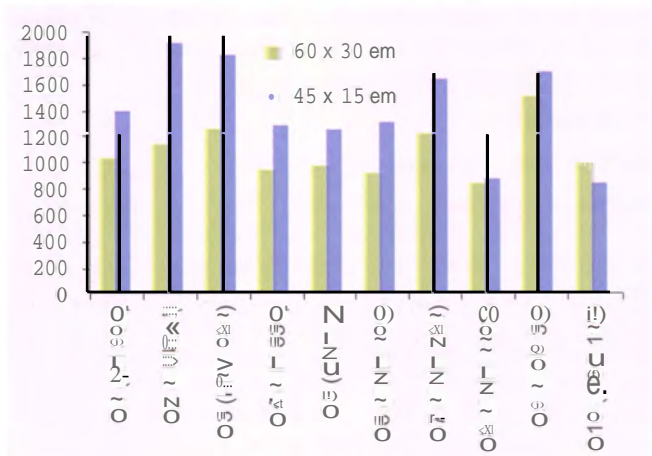


Fig. 8: Seed cotton yield (kg/ha) in *G. hirsutum* genotypes at normal (60 x 30 em, 55000 plants/ha) and high density planting (45 x 15 em, 148000 plants/ha)

Similarly, among the 8 genotypes of *G. arboreum* (AKA 07, CINA 404, JK 5, HD 123, PA 183, JLA 794, JLA 802 and JLA 505) evaluated, on the basis of yield, plant type and growth characteristics CINA404, HD123, and JLA505 performed well under high density planting (222000 plants/ha). The N response ratio or partial factor productivity of N ranged from 13.3 in PA 183 to 35.4 in CINA404. The N utilization efficiency was higher in JLA 794 (14.2) and JLA 505 (13.6) compared to the other genotypes.

Pest and disease incidence under HDPS

The mean incidence of leaf hopper population (number of nymphs/3 leaves/plant) on the 10 *hirsutum* genotypes were similar under normal and HDPS. The mean population was well below the economic threshold level (6 nymphs/3 leaves/plant). Among the 10 genotypes of *G. hirsutum*, the least leaf hopper population was observed on LH-900 and CNH-1108.

On the basis of the incidence of bacterial blight disease, *Myrothecium* leaf spot and *A/ternaria* leaf spot disease, Suraj showed the highest susceptibility followed by PKV 081 and NH 630. Continuous dry spell from September to harvesting, resulted in negligible incidence of grey mildew in *G. hirsutum* varieties. CNH 1108 and LH 900 showing resistance to most of the diseases under field conditions. The early incidence of grey mildew was observed in all the *G. arboreum* varieties



except HO-123 but due to longer dry spell during September till the harvesting of cotton further spread of the disease was not observed. The incidence of bacterial blight and other fungal leaf spot diseases were more with a spacing of 45 x 15 in comparison to 60 x 30 spacing in *G. hirsutum* varieties.

Compatibility studies of various plant protection inputs for HOPS cotton

Compatibility between seed treatment chemicals (pesticides and fungicides) and bio-control agents (microbial consortia consisting of ten bacterial isolates and *T. viride*) was evaluated *in-vitro*. Acephate and Imidacloprid were compatible with microbial consortia and *T. viride*. Carbendazim and Thiram were compatible with microbial consortia but not with *T. viride*. Imidacloprid was compatible with individual isolates of microbial consortia and Acephate was incompatible with isolate no. 4, 7 and 10. Based on these observations a seed treatment schedule for protection against pests and diseases under HOPS was worked out.

### Coimbatore

#### Identification of genotypes for HOPS

Ten genotypes (Anjali, C 1412, CCH 724-5, TCH 1608, KC-3, F-2383, NH 615, MCU 7, SVPR-3, PKV 081) were screened for suitability under HOPS under irrigated condition under high density planting system at spacing of 45 x 15 cm and compared with RCH 2 Bt planted at 90 x 60 cm. Results indicated that KC-3 recorded higher seed cotton yield (2655 kg/ha) followed by PKV 081 (2253 kg/ha), Anjali (2215 kg/ha) and NH 615 (2121 kg/ha) RCH 2 Bt planted at 90 x 60 cm registered seed cotton yield 1596 kg/ha.

The highest gross return (Rs. 79,650/ha) was observed with KC-3. However, Anjali registered the highest net return (Rs. 39,805/ha) and benefit cost ratio (2.1) because of higher market price (Table 3)



Anjali under HDPS

#### Sowing by inclined plate planter

Sowing under high density planting system is labour intensive. An alternate strategy of sowing using tractor drawn inclined plate planter was attempted. Tractor drawn inclined plate planter could not maintain plant to plant spacing (15 cm). However, yields (1918 kg/ha) were similar to manual method of sowing (2003 kg/ha) While, gross return were maximum with manual method of sowing (Rs. 70,105/ha) the increased cost associated with manual method lowered the net profit (Rs. 32,992/ha) as compared to tractor drawn inclined plate planter (Rs. 34,883/ha).

Table 3: Seed cotton yield and economics as influenced by genotypes planted under high density planting system

Genotypes	Seed cotton (kg/ha)	Cost of cultivation (Rs/ha)	Gross Return (Rs/ha)	Net Return (Rs/ha)
T1. Anjali	2215	37720	77525	39805
T2. C1412	1645	33160	49350	16190
T3. CCH7245	2079	36632	72765	36133
T4. TCH1608	1823	34580	63788	29208
T5. KC-3	2655	41240	79650	38410
T6. TCH 1705	1904	35230	66631	31401
T7. NH615	2121	36970	70001	33031
T8. MCU7	1293	30340	38775	8435
T9. SVPR-3	1385	31080	41550	10470
T10. PKV081	2253	38020	67575	29555
T11. LH 900	1711	33690	51338	17648
T12. RCH2 Bt*	1596	32768	60648	27880
SED	107			
CD	221			
CV(%)	14			

\* Planted at recommended spacing of 90 x 30 cm.

#### Genotypes x nutrient interaction under HOPS

Genotypes (Anjali, C1412 & CCH 7245) and their interaction with nutrient levels (75, 100, 125 & 150% ROF) under high density planting system (planted at 45 x 15 cm) were compared with RCH 2 Bt. RCH 2 Bt was planted at 90 x 60 cm 18,519 plant/ha with 100 per cent ROF recorded the lowest seed cotton yield of 14.7 q/ha. High density planting system (45 x 15 cm) recorded additional yield of 2.2 to 8.7 q/ha over that of RCH 2 Bt. Seed cotton yield was not affected by levels of nutrient application. However, genotypes by nutrient level interaction was significant. Anjali planted at 45 x 15 cm and applied with 150% ROF registered significantly highest seed cotton yield (24.91 q/ha), gross return (Rs. 94,658/ha), net return (Rs. 54,730/ha) and benefit cost ratio (2.37), which were 69.5, 60.9, 102 and 28 per cent respectively higher than RCH 2 Bt applied with 100 percent of ROF (Fig. 9).

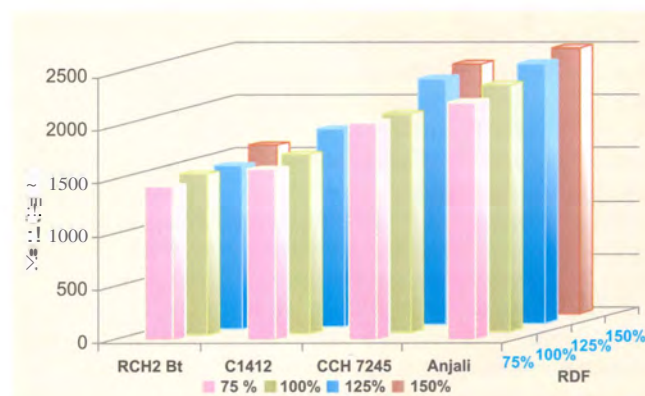


Fig. 9: Yield (kg/ha) under HOPS (Anjali, CCH 7245 & C 1412) and conventional (RCH 2 Bt) one with nutrient levels



## Sirsa

### Identification of genotypes for HOPS

*G. arboreum* genotypes i.e. HD-123, RG-542, HD-432, CISA-310, CISA-111 were evaluated under three spacing (67.5 x 10, 67.5 x 20 and 67.5 x 30 cm). Higher yield in general was recorded for 67.5 x 20 cm spacing followed by 67.5 x 30 cm. Only CISA-310 performed well under 67.5 x 10 cm spacing. In 67.5 x 20 cm spacing an increase of 3-5 q/ha in yield was obtained over 67.5 x 30 cm.

### 4.12: Weed Management

#### Nagpur

Herbicide weed management strategy of pendimethalin (1 kg a.i./ha) as pre emergence) and pyriithiobac Na (70 g a.i./ha) as early post emergence) provided timely and effective weed control.

Herbicides were evaluated 0.5 to 2.5 times the recommended dose and allowing sufficient time for toxicity expression. Resistant weeds were noted. *Cynodon dactylon* and *Digera arvensis* with reported tolerant to *Glyphosate* while *Merremia emarginata* and *Acalypha indica* were susceptible. *Tridax procumbense* and *Cyperus rotendus* weeds were susceptible to Pyriithiobac. Imzethapyr herbicides were noted. No tolerant weeds were observed for Quizalofop ethyl.

#### Coimbatore

##### Stale seed bed technique of weed control



Stale seed bed weed control (pendimethalin 1.0 kg and glyphosate 1.0 kg per ha applied one week before sowing) resulted in the highest weed control efficiency (83.7%) due to broad spectrum weed control. The germinated weeds were killed by glyphosate and the germinating weeds by the residual herbicide, pendimethalin. This technique also resulted in maximizing seed cotton yields.

##### Weeding by post emergence

Weed control by application of residual chemical, pendimethalin (1.25 kg a.i./ha as pre emergence) followed by post emergence application of quizalofop-ethyl (50 g a.i./ha) and pyriithiobac sodium (75 g a.i./ha at 30 & 35 DAS) respectively was similar to pendimethalin (1.25 kg a.i./ha)

followed by post emergence application of tank mix of quizalofop-ethyl (50 g a.i./ha) and pyriithiobac sodium (75 g a.i./ha at 30 DAS) and pre emergence application of pendimethalin (1.25 kg a.i./ha) + with one hand weeding 30 DAS.

##### Herbigation with herbicide/mixture/herbicide rotation for efficient weed control in cotton and its effects on succeeding pulse crop

Among the combinations of rotation herbicides pendimethalin (1.0 kg a.i./ha) as pre emergence herbicide on third day followed by hand weeding 35 - 40 DAS and pyriithiobac sodium 50 g + quizalofop-P-ethyl 50 g on 60 DAS recorded the highest seed cotton yield, net return and B:C ratio. It was on par with Pendimethalin followed by hand weeding and pyriithiobac sodium 50 g + fenoxoprop-P-ethyl 50 g on 60 DAS and hand weeding thrice (20, 40 and 60 DAS).

### 4.13: Soil Biology and Biochemistry

#### Nagpur

##### Developing efficient carrier based microbial delivery system for cotton nutrition and soil health

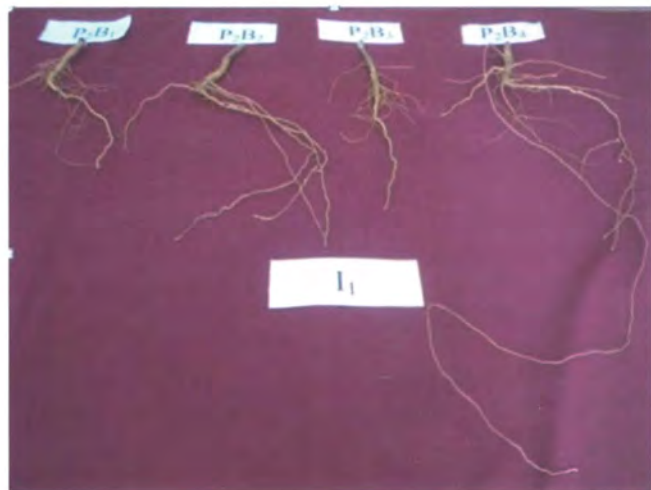
Two field experiments were taken up during the 2011-12 season. In the first experiment, microbial delivery methods were compared with different levels of nutrient on cotton growth and productivity, while in the second experiment, microbial delivery methods were tested on one *G. hirsutum* variety NH 615, one conventional hybrid NHH 44 and 2 Bt hybrids viz. Bunny and Rasi 138.

Microbial consortia as carrier based delivery system recorded significantly higher (27%) seed cotton yield compared to absolute control (no nutrient and no microbial cons), followed by microbial consortia delivered through seed treatment (13%) and soil treatment (11%). The interaction between variety/hybrid and delivery system was not significant.

Among microbial delivery methods, significantly higher mean seed cotton yield was obtained with seed treatment (511 kg/ha) followed by 100% RDF (483 kg/ha) and carrier based microbial delivery (454 kg/ha)

#### Coimbatore

##### Evaluation of arbuscular mycorrhizae and phosphorus



## **solubilising bacteria for enhancing phosphorus acquisition and mitigation of water stress in Bt cotton**

Combined application of arbuscular mycorrhizae (AM) and phosphorus solubilising bacteria (PSB) followed by sole application of AM and PSB enhanced the germination percentage and plant growth. Under irrigated conditions, all the above parameters were improved by sole inoculation of AM compared to sole application of PSB. Recommended dose of phosphorus, nitrogen and potassium with combined bioinoculants, viz., AM and PSB enhanced the agronomical, physiological and biochemical characteristics in Bt cotton as compared to 50 per cent recommended dose of P and bioinoculants.

## **Geo-referenced Soil Information System for Land Use Planning and Monitoring Soil and Land Quality for Agriculture**

A survey on established benchmark (BM) soil series in different agro-ecological sub regions (AESR) of Black Soil Regions (BSR) of India was done to investigate the impacts of bio-climates, cropping systems, soil sub groups, land use, and management practices on the soil biological attributes such urease, dehydrogenase (DHA), microbial biomass carbon (MBC), culturable microbial population, and microbial diversity indices in BSR. All the soil biological attributes declined with soil depth and the maximum activity was observed in top 30 cm soil. Cropping systems and bio-climates significantly ( $p < 0.01$ ) influenced urease activity. The average urease activity in different bio-climates were in decreasing order of arid > sub humid (moist) > sub humid (dry) > semi-arid (dry). Among cropping systems, cotton based systems had lesser activity than the legume or sugarcane or cereals based cropping systems. Urease activity was higher in irrigated compared to the rainfed systems. Cropping systems and bio-climates significantly ( $p < 0.01$ ) influence the DHA in soil. Significantly higher ( $p < 0.01$ ) DHA was recorded in sub humid moist bio-climate (2.45 IJg TPF  $g^{-1}$ ) followed by sub arid dry (2.00 IJg TPF  $g^{-1}$ ) and the least DHA was recorded in arid bio-climate (1.62 IJg TPF  $g^{-1}$ ). Legume-based cropping system recorded significantly ( $p < 0.01$ ) higher DHA (2.32- 2.88 IJg TPF  $g^{-1}$ ) among the cropping systems. Significantly higher ( $p < 0.001$ ) MBC was recorded in Sub humid dry bio-climate (267 IJg  $g^{-1}$ ) followed by sub humid moist (179 IJg  $g^{-1}$ ) and the least MBC was recorded in arid bio-climate (97.5 IJg  $g^{-1}$ ). Significantly higher ( $p < 0.01$ ) microbial population was recorded in sub humid moist bio-climate (6.26  $\log_{10}$  cfu  $g^{-1}$ ) followed by sub humid dry (6.21  $\log_{10}$  cfu  $g^{-1}$ ) and the least microbial population was recorded in arid bio-climate (6.14  $\log_{10}$  cfu  $g^{-1}$ ). Legume-based cropping system recorded significantly ( $p < 0.01$ ) higher microbial population (6.23  $\log_{10}$  cfu  $g^{-1}$ ) followed by cereal based cropping system (6.23  $\log_{10}$  cfu  $g^{-1}$ ). Significantly ( $p < 0.01$ ) higher microbial diversity indices was recorded in sub humid moist and sub humid dry compared to semi arid dry and arid bio climates. Among the cropping systems, except Shannon diversity index ( $H'$ ) which was similar in both legume-based and cereal-based cropping system, all other diversity indices (Simpsons index (D), Simpsons reciprocal index (1/D), and Simpson evenness (E)) were found to be significantly ( $p < 0.05$ ) higher in cereal-based

cropping system followed by legume-based system. The pooled analysis of diversity indices indicated a significant difference ( $p < 0.01$ ) between the land use types (irrigated and rainfed agro ecosystems). In management, BM spots which adopted high management practices recorded significantly ( $p < 0.05$ ) higher Shannon diversity index ( $H'$ ) ( $H' = 2.48$ ) compared to low management ( $H' = 2.45$ ). The microbial diversity indices found to significantly ( $p < 0.01$ ) decline in all the BM spots with increase in soil depth. The maximum diversity indices were recorded in the surface soil (0-15 cm) and nearly 50% of diversity was found to be restricted within 0-30 cm soil depth.

## **4.14: Cotton Mechanization**

### **Nagpur**

#### **Development of Wick Applicator**

A Wick Applicator was developed to smear the weeds with herbicide solution in between the rows of cotton plants.

This was developed especially for HDPS system since spraying such chemicals has the risk of killing nearby cotton plants by drift of the weedicides. The wick applicator has a cylindrical tank of 10 lit. capacity, one conveying pipe from tank to wicks at the end of a long handle. It is shoulder mounted, the fluid is injected by gravity from the small openings of the wicks. The brush width is 24 cm, below the tank one cock is provided which regulates the flow.

The wick applicator was tested in the laboratory and calibrated in field. The field capacity of wick applicator was found to be 100 lit./ha to 550 lit./ha which could be changed depending upon the density and age of weeds.



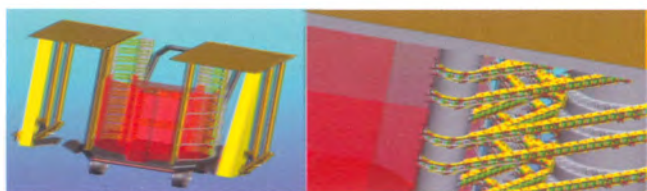
**Herbicide Wick Applicator**

#### **Development of trolley mounted chain type cotton picker**

Conceptual design of a cotton picking machine to be operated by a pair of bullocks or a person behind the machine was prepared in CAD. Various parameters like machine



parameters, plant parameters and soil parameters were analysed to design the cotton picking machine. The list of parameters considered for the design of cotton picking machine were plant parameters: crop height, lateral spread, lowermost boll height, percent compression of crop; Machine parameters: forward speed of machine, weight (approx.), height, width of machine, power required to operate picking mechanism, power required for forward travel of the machine. The machine is divided into mainly three components i.e. 1. picker trolley 2. crop guider and compressing mechanism and 3. picking mechanism. GAD drawing and 3D solid model of the picker was made. Fabrication of all the components was completed after testing the assembly for its strength and stability in the standing crop of cotton at each stage of fabrication, and suitable modifications thereof.



Chain type cotton picker

#### 4.15: Morphoframe / Boll Load Management

Nagpur

Physiological manipulation of Bt plant morphoframe

Effect of manipulation of Bt plant morphoframe (NGS-145 Bt and Mallika Bt vs NGS-14 nBt) using plant growth regulators and mechanical methods on crop productivity was evaluated. Among the treatments, foliar application of maleic hydrazide (500 ppm) at 85 DAS recorded highest plant height, leaf area and Leaf Area Index (LAI) of 82.3, 6964.0 em<sup>2</sup> and 1.3 respectively and produced significantly higher yield than detopping main stem at 95 DAS and sympodial meristem at 105 DAS.

Physiological manipulation of cotton morphoframe by spraying Ethrel

Foliar application of ethrel (5.7mM ==0.75ml/litre) at 39 DAS in cotton hybrid Jai Bt increased the photo-synthetic leaf area



MH Treated cotton plants

and leaf area index of the cotton plants than control. Ethrel treated plants recorded more bolls and resulted in greater seed cotton yield than control (Table 4).

Table 4: Effect of Ethrel and growth and yield of cotton hybrid Jai Bt

Treatments	Plant height (em)	Height to node ratio	No. of Sympodia	No. of bolls	No. of leaves (em <sup>2</sup> )	Leaf area	LAI	Yield (kg/ha)
Control	72.0	1.3	15.8	13.4	66.2	3298.4	0.92	890
Ethrel (0.75 mill)	87.7	1.4	18.0	24.7	125.0	8003.2	2.22	1116

#### 4.16: Studies on Abiotic Stress

Nagpur

Leaf reddening

Different combinations of nutrients, growth retardants, reflectants and insecticides were tried to ameliorate leaf reddening in the hybrid RGH2Bt. Only a low degree of reddening was observed and these symptoms appeared towards the end of the crop growth period. During that period, plants treated with 3% KNO<sub>3</sub>, 2% DAP, Lime 0.5% at 60 DAS and 1% at 80 DAS, Lihocin (GGG) 50 ppm, 1% potash, Acephate (1g/litre), Monocrotophos (3 ml/litre) appeared healthy and green except for lihocin treated plants which appeared greenish but the plants were extremely stunted. Higher N content was observed in plants treated with 60 ppm potassium silicate, Kaolin and 50 ppm Lihocin (GGG) - than control. P content was higher in plants treated with 50 ppm Lihocin (GGG) - (0.388%), 1% KOH (0.360%) and Kaolin (2 and 3%) (0.304%) overcontrol (0.181 %). 1%KOH(3.71%), and insecticides such as acephate (3.71%), confider (3.66%) and monocrotophos (3.39%) recorded higher K content over control (2.33%). Yield was significantly higher in the potash, DAP and lime, potassium silicate and KNO<sub>3</sub> (924 to 1046 kg/ha) treated plants overcontrol.

Leaf total chlorophyll content varied from 0.54 to 0.82 mg/g fresh weight of leaf at 76 DAS and 0.65 to 0.9 mg/g at 97 DAS. Higher values were observed in GGG (chloromepiquat chloride) 50 ppm and 2,4 dinitrophenol treatments at both the stages. Leaf chlorophyll content was high as plants did not experience any severe leaf reddening stress. The same is also true for leaf anthocyanin content which was in the range 0.0055 to 0.0033 mole/em<sup>2</sup>. Since leaf reddening incidence was low, anthocyanin concentration was also low. The role of anthocyanin for its protective role during plant stress could not be ascertained. Further, leaf peroxidase activity was not affected during the season as the enzyme activity was not triggered to combat the oxidative stress.

Physiology of drought resistant transgenic cotton

Thirty transformed plants for DREB and ZAT 12 genes were subjected to different soil moisture levels under pot culture. Five transgenic plants along with one non transgenic plant were subjected to different moisture levels (100, 75, 60, 45, and 30%). Few transgenic plants performed better than the control by exhibiting high leaf water potential, relative water content, cell membrane stability and proline content. Transgenic plants that performed well under 30 and 45%

moisture levels can be considered as highly drought tolerant when compared to non transgenic plants.

### Coimbatore

Nutrient foliar spray, under moisture stress condition increased boll number in Bt cotton. Significant increase in boll weight was also recorded irrespective of genotypes resulting in a significant increase in yield compared to the control (Fig. 10). Irrespective of genotypes and Bt cotton, Nitrate reductase activity was significantly more in nutrient sprayed plants. The photosynthetic activity was higher in Bt cotton (24.7 to 27.2  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) than the genotypes (22.8 to 25.8  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ).

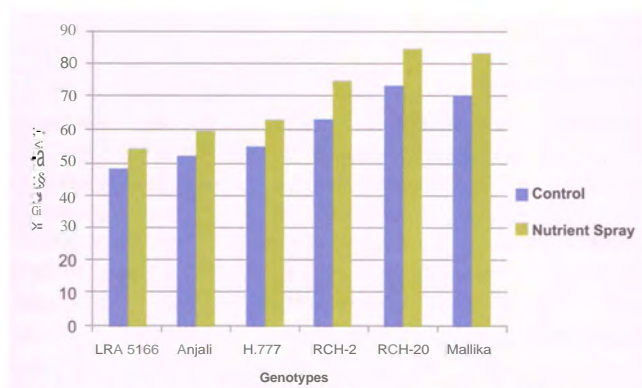


Fig. 10 : Effect of Nutrient spray on yield

### Alleviation of water logging by nutrient consortia spray

Waterlogging reduced the boll number from 18 - 23 to 12 - 15 per plant. In general, boll weight was not significantly affected due to water logging and only a marginal improvement was discernible in plants that received nutrient spray. A drastic reduction in yield was observed due to water logging irrespective of genotypes and Bt cotton. The reduction in yield was significant in Bt cotton (30 - 37 %) than genotypes (15 - 24 %) (Table 5). This reduction in yield per plant could be improved by 5-9% in genotypes while the improvement in yield was 29 to 46% in Bt cotton through nutrient consortia spray. Nutrient foliar sprays were effective in alleviate waterlogging stress to a greater extent in the Bt cotton than the genotypes.

Table 5: Seed cotton yield (g/plant) under normal (control) and waterlogged conditions with and without nutrient spray.

Genotypes Bt hybrids yield / plant (g)	Control	Water Logged (No spray)	Water Logged (Nutrient spray)
LRA 5166	62.0	47.0	51.4
Anjali	64.8	51.5	54.3
H 777	70.1	60.7	60.9
RCH 2	86.7	61.2	79.1
RCH 20	109.7	69.0	100.8
Mallika	90.4	58.7	77.0
CD at 5% (VXT) = 8.8			

## 4.17: Socio Economic Dimensions of Cotton Farming

Analysis of yield gap and constraints in cotton production

Central zone (Gujarat and Madhya Pradesh)



Meeting with farmers to prioritize constraints in cotton production

Identification and prioritization of constraints and yield gap in cotton production was done in Gujarat and Madhya Pradesh. This study was conducted in Bharuch, Vadodara, Surendranagar, Ahmadabad and Surat districts of Gujarat and Khandwa, Khargone and Barwani districts of Madhya Pradesh. Yield gap in Gujarat ranged between 8 to 25 q/ha while it ranged from 1.2 to 20 q/ha in Madhya Pradesh. It was found that in Gujarat incidence of sucking pests was the major constraint in cotton production followed by non availability of labour, weed infestation and high cost and shortage of genuine seeds. In Madhya Pradesh incidence of sucking pests, weed infestation, leaf reddening and shortage of fertilizer were identified as major constraints affecting cotton production.

Besides, excess rains during initial crop growth period, late sowing, shortage of capital for purchasing inputs, mealy bug infestation, price fluctuations, drought during later crop growth period, pink bollworm, labour shortage/high wage, shortage of irrigation/electricity, cloudy weather/rains during flowering and non availability of seeds are causing considerable yield loss. These constraints need to be addressed on priority basis to bridge the yield gap and to improve average productivity in all three districts.

North zone (Haryana and Rajasthan)

In Haryana, Sirsa and Hisar districts selected for the study had a yield gap of 8.15 q/ha and 7.07 q/ha, respectively. In Haryana state, damage caused due to sucking pests, dry spell during flowering stage and lack of knowledge about recommended practices were major constraints affecting cotton production. Beside these, lack of availability of genuine plant protection chemicals, incidence of cotton leaf curl viral disease, lack of credit, deficiency of micro nutrients were other constraints affecting productivity in this state. Delayed sowing due to non-availability of canal water in Haryana, that caused considerable yield loss was the added constraint. In Rajasthan, Sri Ganganagar and Hanumangarh districts, incidence and damage caused due to sucking pests, and delayed sowing



due to non-availability of canal water were the major constraints. Besides these, higher degree of use of non-certified seed, dry spell during flowering stage and incidence of cotton leaf curl viral disease caused considerable cotton loss.

#### South zone (A.P. and Karnataka)

In A.P., Guntur district has a yield gap of 11.0 q/ha. Weed infestation, low price of farm produce at the time of harvesting, non availability of canal water, delayed payment by the marketing agencies, lack of storage facilities, reduced use of organic manures, leaf reddening were major constraints faced by the cotton farmers with an average yield loss of 4 to 6 quintals per ha. In Karnataka, Dharwad and Haveri districts had yield gap ranging from 9.2 to 10.2 q/ha. Non availability of labour, weed infestation, unable to take proper care due to non availability as well as high cost of human labour, incidence of sucking pests and sub optimal use of inputs were important constraints in this zone.

#### Assessment of cotton based intercropping system

Adoption behaviour and perception of cotton intercropping by farmers of Gujarat and Tamil Nadu was studied with focus on Surendranagar district in Gujarat and Coimbatore and Vellore districts in Tamil Nadu.

Majority of the respondents (72%) were aware about cotton intercropping techniques and more than half of the respondents were aware about the varieties suitable for intercropping, and about the benefits of intercropping such as economic gains, minimizing the soil erosion and increasing soil fertility. However, adoption levels was medium to low (52.5%). The major problems experienced by farmers in adoption of intercropping were labour scarcity followed by difficulty in intercultural operations, fear of more insect pest attack, fear of low yield and the complex nature of technology.

Farmer-to-farmer technology dissemination model was assessed and studied in Wardha, Maharashtra for popularisation of intercropping technology. Results indicated adoption of intercropping specially cotton + soyabean increased among young and educated farmers. Further, farmer to farmer approach also improved adoption of intercropping packages. Mean score of adoption prior to launch of programme was 28.6 which increased to 66.4 during implementation of the farmer to farmer dissemination. These farmer innovators are likely to represent important sources of information amongst other local farmers and for technology dissemination.

#### Agrarian distress among cotton farmers

The study was conducted in Gujarat and Madhya Pradesh cotton belt to investigate socio- psychological and economic factors and distress like situations if any faced by the cotton growers. In the irrigated areas, there was no distress like situation among cotton growers. However, several social, psychological, economical, situational, technical and marketing problems played an indispensable role in cotton production in progressive and backward regions. The major socio- psychological factors perceived by farmers in non- progressive and progressive areas of selected districts were low income from agriculture as compared to high expenditure

for livelihood. Other problems perceived were high cost of fertilizers, insecticides and implements, non-availability of labour, absence of quality based price policy and insufficient income from farming. Heavy incidence of sucking pests, electricity and water shortage, climatic conditions, non-availability of good seed at proper time, lack of awareness about improved technology were prominent situational and technical factors perceived by the farmers engaged in cotton farming.



Interaction with farmer on agrarian distress among cotton farmers

#### Coimbatore

##### Implications of trade openness on Indian cotton economy

Secondary data on world cotton production, consumption, exports - quantity and value, percentage of exports by the trading partners - share of world cotton fibre imports were collected. Nominal Protection Co-efficient (NPC) and Effective Protection Co-efficient (EFC) were estimated to be 0.83 and 0.67. Domestic Resource Cost (DRC) and Social Cost Benefit (SCB) estimated around 0.78 and 0.62, respectively, indicating comparative advantage of domestic versus the international prices. It is observed that the domestic markets of cotton were strongly integrated with central market in the post-WTO compared to pre - WTO period. Among different cotton markets, Tiruppur, Mumbai and Ahmedabad markets were highly integrated with international markets. Residual trend linear regressions estimated for international prices revealed a positive and significant trend for cotton in the post-liberalisation period. It was observed that the international price variability was almost equal or less than the domestic price variability during the reform period. Moreover, variability in both international and domestic price declined during the post-liberalisation period. The co-efficient of cross product trend for cotton was significant at 10 per cent level of probability indicating that movements in international prices affect the domestic prices.

##### Identification and prioritization of constraints in the Bt cotton scenario

In the north zone, Total Factor Productivity (TFP) growth rate showed a positive trend in all the three States viz., Punjab, Haryana and Rajasthan indicating the better perspectives of adoption of Bt cotton (Table 6). In the central zone, the trend was similar in Gujarat and Maharashtra but in case of Madhya Pradesh, it showed a negative trend due to the non significant growth in output index and positive growth in input index.

**Table 6: State wise Total Factor Productivity of Cotton in India (1981-2009)**

States	Total input index (TII)				Total output index (TOI)				Total Factor Productivity Index (TFPI)			
	1981-90	1990-00	2000-09	1981-2009	1981-90	1990-00	2000-09	1981-2009	1981-90	1990-00	2000-09	1981-2009
<b>North Zone</b>												
Punjab	1.02	0.99	-4.65	0.71	10.58	-10.91	11.75	0.81	9.45	-11.79	17.20	0.10
Haryana	-0.24	-0.11	1.85	0.54	1.67	-6.25	12.49	0.42	1.91	-6.14	10.45	-0.12
Rajasthan*	-	1.67	1.97	0.97	-	-2.18	4.11	1.65	-	-3.78	2.10	0.68
<b>Central Zone</b>												
Maharashtra	4.56	-4.66	0.63	0.25	4.41	6.49	13.37	5.00	-0.14	11.69	12.65	4.74
Gujarat	-1.81	-1.71	1.41	-0.27	-0.76	6.43	12.20	5.44	1.05	8.14	10.79	5.71
M.P.	9.30	1.43	7.27	4.39	15.47	8.20	0.89	8.20	5.64	6.68	-5.95	3.65
<b>South Zone</b>												
A.P.	-2.97	-0.17	-0.74	-0.17	-4.90	3.07	6.67	4.10	-1.99	3.25	7.46	4.28
Karnataka	-3.64	-1.53	-1.81	-1.53	5.22	0.95	7.28	4.24	9.19	2.51	9.25	5.86
Tamil Nadu	0.60	-2.25	-2.44	-1.07	5.07	2.13	6.63	4.29	4.45	4.48	9.29	5.41

In the south zone too, the TFP growth rate showed a positive trend in A.P., Karnataka and Tamil Nadu.

**Comparative analysis of conventional, biotech and organic cotton production systems in India**

The study revealed that more than 80 per cent of the 8t cotton growers had positive attitude towards 8t cotton cultivation, more than half of the organic cotton growers had positive attitude towards organic cotton cultivation and less than one third of the conventional cotton growers had favorable attitude towards their practices. The major factors *motivated* 8t cotton growers to adopt the technology were financial motives and quality of output related motives. The factors viz., soil health oriented and environmental motives prompted the organic cotton growers. The analysis on knowledge profile of the growers in three different production systems revealed that the organic cotton growers had high level of knowledge profile than the growers in other two systems. The analysis on adoption profile of the growers in different production system revealed that the organic cotton growers had the high level of adoption followed by the 8t cotton growers and the conventional cotton growers. Poor adoption rate of refugia, unavailability of organic cotton seeds and high production cost were top most threats observed respectively in biotech,

organic and conventional production systems.

**Gender and labour issues in hybrid cotton seed production sector in India**

The survey among the labourers working in hybrid seed cotton farms revealed that more than 82% of the labour was spent for crossing works and majority of them were female workers belonging to middle age group and had 25 per capita man days per hectare per season. On an *average*, they earned Rs. 3000-4000 per season through wages. Inward migration behavior for other works within the village was observed among majority of the female workers due to *Government* schemes. Less wages, lack of organizations for collective bargaining, drudgeries and health hazards faced in the agricultural works, lack of specific development schemes for agricultural labourers, lack of supportive farm machineries, lack of training programs especially for agricultural labourers and lack of subsidies were the major problems expressed by the agricultural labourers. Focus groups discussions among cotton seed producers indicated that limited availability of labour, their cost, their less technical know-how and work efficiency were major threats faced by the cotton seed producing industries.





## 4.18: Seasonal Dynamics of Insect Pests and Diseases

### Nagpur

#### Seasonal dynamics of cotton sucking pests and bollworms

Two major peaks were recorded for mirid (*Campylomma livida*) population, first on 17<sup>th</sup> Sept., 2011 (3.56 mirids/plant) and another on 8<sup>th</sup> October 2011 (2.72 mirids/plant). During the season leafhopper (*Empoasca devastans*) population was above 3.28 leafhopper/ 3 leaves while highest leafhopper population was noticed on 10<sup>th</sup> September (Fig.11) Thrips (*Thrips* sp.) population was highest in the initial phase of crop growth and thereafter gradually decreased and was negligible after 17 Sept., 2011. Mealybugs *Phenacoccus solenopsis* and *Nipaecoccus viridis* were recorded in some fields with negligible population during late season of crop. Negligible population of all the three bollworms and *Spodoptera litura* was recorded.

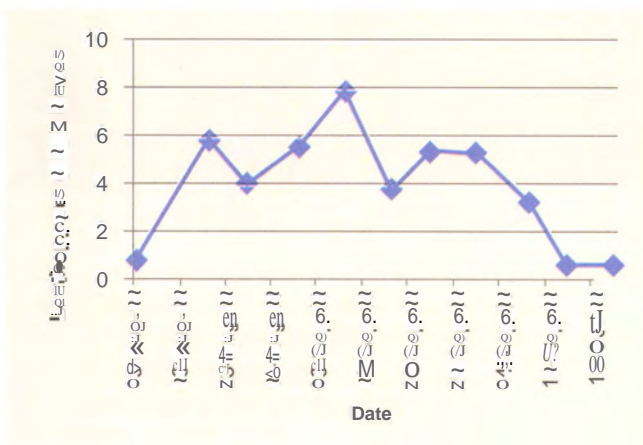
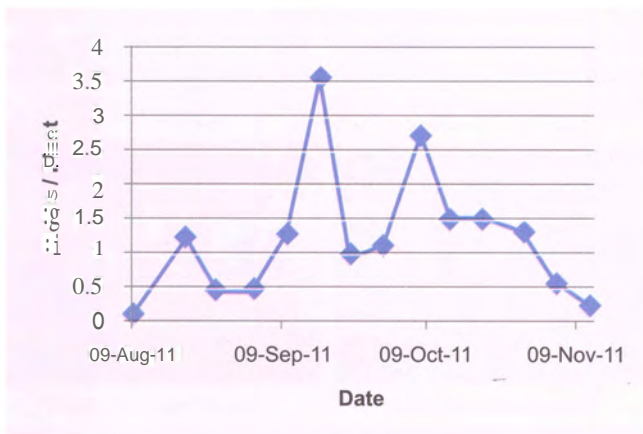


Fig.11 : Mirid and leafhopper population on cotton in Nagpur

MRC-6301 Bt (Mon 531), Fusion Bt (Nath event), Dr Brent BG-II (Mon 15985) and Ajeet-155BG-11 (Mon 15985) hybrids exhibited zero pink bollworm larval population, nil exit holes and zero per cent locule damage throughout the crop growth period as compared to JK Bt (Event I) and the corresponding non Bt hybrids in central India. Larval population was highest



on AKH - 8828 and PA-255 which are non Bt hybrids.

Moth catches of pink bollworm were recorded from 14/07/2011 to 09/02/2012 and the lowest moth catches was on 20/10/2011 with 1.53 moths/trap/week. The incidence of pink bollworm moths was high in the third week of January with 15.53/trap/week.

Incidence of pink bollworm was absent on all the Bt hybrids evaluated at 100 DAS, 115 DAS, 130 DAS, 145 DAS and 160 DAS.

### Coimbatore

#### Population dynamics of mirid bug (*Creontiades biseratense*)

Nymph and adult population of mirid bug *Creontiades biseratense* (Distant) build up was observed from the second week of December, lowest and highest nymphal population was recorded during 50<sup>th</sup> std week (0.36 nymphs / plant) and 1<sup>st</sup> std week (4.17 nymphs/ plant) respectively. Significantly lowest and highest adult population was recorded during 50<sup>th</sup> standard week (0.14 adults /plant) and 2<sup>nd</sup> std week (1.39 adults / plant) respectively. The percentage of green boll and square damage were increasing from 1<sup>st</sup> standard week to the 5<sup>th</sup> standard week and the percentage of green boll damage and square damage varied from 1.85 to 17.14 % and 2.37 to 18.55 %, respectively. Buildup of coccinellids and spiders were recorded corresponding with the increase in mirid nymph and adult population. Negligible population of spider was recorded.

#### Population dynamics of mealybugs

No mealybug infestation (*Paracoccus marginatus* and *Phenacoccus solenopsis*) was observed throughout September - February. Population of mealybug was recorded as nil on the weed hosts identified earlier - *Trianthema portulacastrum*, *Achyranthus aspera*, *Celosia argentea*, *Parthenium hysterophorus*, *Tridax procumbense*, *Cleome viscosa*, *Euphorbia hirta*, *Phyllanthus niruri* and *Leucas aspera*.

#### Occurrence and seasonal dynamics of emerging pests and their predators in Tiruppur district

Mealybug infestation was not observed in any of the villages throughout the season. The mean infestation of mirid bug ranged from 0 to 1.6 per cent and the nymphal population

ranged from 1.3 to 4.5 per 50 squares. The predominant predators were coccinellids and spiders. The former ranged from 7.17 to 25.17 and the latter from 0.33 to 2.83 per 50 plants.

Pest scenario in IRM and non IRM fields at project village

a) Sucking pests viz., aphids, leafhoppers, thrips and whiteflies population were below threshold level and averaged 7.64, 2.86, 2.24 and 0.31 and 11.20, 4.10, 3.37 and 0.53/3 leaves in IRM and non IRM fields, respectively. The mirid and mealybug populations were recorded in all the project villages as 1.62 and 2.03 and grade 0 and grade I in IRM and non IRM fields, respectively. IRM fields showed reduction in population of aphids (31.00 %), leafhopper (46.00 %), thrips (43.00%), whiteflies (50.00%) and miridbug (25.00%) when compared to non IRM fields.

b) Bollworms

Incidence of bollworms was nil.

c) Natural enemies

Natural enemies viz., coccinellids, spiders and *Chrysopa* averaged at 0.89, 0.84 and 0.17 per plant, respectively in IRM fields, whereas it was 0.61, 0.60 and 0.12 in non participatory fields.

## Sirsa

Field dynamics of mealybug revealed that late initiation (October) of infestation of mealybug was observed at 3 locations and severity grade did not reach 4 at any locations. A total of 71 alternate hosts of mealybug have been recorded till date.

Parasitisation of cotton mealybug

Maximum parasitization (%) due to the dominant parasitoid, *A. bambawa/ei* was 83% per cent in 2011, whereas mean parasitization (%) recorded was 14.26 and 5.25 at farmers fields. Data were also recorded from ten new locations at the end of season where mealybug incidence was available. The average incidence (%), severity index and per cent parasitization due to *A. bambawa/ei* reported were 27.53, 1.58 and 24.92, respectively.

Cotton leaf curl disease

Survey on the incidence of CLCuD was carried out in major cotton growing districts of North India (Haryana, Punjab and Rajasthan) in August, 2011 and the incidence was recorded as low to moderate PDI (5.0-25.0%) in few places like Abohar and Fazilka regions of Punjab and Hisar of Haryana in an isolated manner, while it was absent in most parts of north zone.

## 4.19: Biological Diversity of Insect Pests and Pathogens

Nagpur

Biological diversity of mealybug in central India

Diversity of four mealybug species viz, *Phenacoccus so/enopsis*, *Nipaeococcus viridis*, *Ferrisia virgata* and one unidentified mealybug species (from Saoner, Nagpur) (Homoptera: Pseudococcidae) was recorded. They were found infesting cotton with Grade- IV infestation in cotton+

pigeonpea-fallow cropping system of central India. However all the mealybug populations were under control.



*Ferrisia virgata*



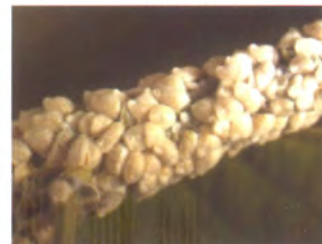
Unidentified mealybug

Other insect biodiversity

Weevil *Tanymecus proneps* (Faust) (Coleoptera: Curculionidae) was recorded infesting non-Bt cotton during off-season. Mirid bug *Creontiades biseratense* which was mostly predominant in southern cotton growing states was observed in Central India in very small number. Mirid bug *Campyomma livida* which is mostly prevalent in central India also showed its presence as usual. One mealybug species *Coccidohystrix insolita* Green and scale insect *Orepanococcus cajani* (Homoptera: Pseudococcidae) have been recorded on pigeonpea, a preferred intercrop crop in cotton.



*Tanymecus proneps*



*Drepanococcus cajani*



*Creontiades biseratense*



*Coccidohystrix insolita*

Cropping system based population dynamics for mirid (*C. livida*), leafhopper (*E. devastans*) and spider in cotton + pigeon pea - fallow cropping system

Highest population of mirid (*C. livida*) was recorded in unprotected condition followed by cotton field adjacent to soybean. Cropping systems viz., cotton field along roadside, cotton field adjacent to water canal, cotton field adjacent to cotton, cotton field adjacent to fallow land indicated no distinct trend with reference to mirid infestation. Leafhopper nymphs were highest in unprotected conditions followed by cotton field adjacent to soybean. Comparatively higher sucking pest population was recorded in cotton field adjacent to soybean. Identical numbers of nymphs were recorded in cotton field adjacent to water canal, cotton field adjacent to cotton and cotton field adjacent to fallow land. Least spider population



was recorded in cotton field adjacent to fallow land followed by cotton field adjacent to soybean and were statistically different from each other. Spider populations were same in three cropping systems viz., cotton field along roadside, cotton field adjacent to water canal, cotton field adjacent to cotton. It has been seen from the data that overall pest and spider population was higher in unprotected condition than protected.

#### Genetic diversity of thrips

Thrips were collected from north India (Sriganganagar of Rajasthan, Bhatinda, Faridkot and Mansa of Punjab and Hisar, Fatehabad and Sirsa of Haryana), central India (Nagpur, Akola, and Buldhana of Maharashtra) and south India (Adilabad of Andhra Pradesh) from cotton. DNA was isolated from individual insect using a refined protocol for extraction of DNA from museum specimens.



**Thrips species**

The sequence analysis of cytochrome oxidase I (CO I) of *Thrips sp.* from different locations revealed that a mixed population of thrips occurs on cotton across India comprising mainly of three different species viz. *Thrips palmi*, *Scirtothrips dorsalis* and *Scirtothrips oligocheatus*. The tree derived from the bootstrap analysis produced two main clusters. Clade A was represented by genus *Thrips* and clade B was represented by genera *Scirtothrips* and *Thrips*. Cluster A was strictly represented by *Thrips palmi* species. Some of the samples that showed identities with *Thrips palmi* were also included in Cluster B. Cluster B was divided into two main sub-clusters C and D, where sub-cluster D was represented by only *Thrips palmi* and cluster C was represented by the genus *Scirtothrips*. Again sub-cluster C was divided into two sub-clusters C1 and C2 representing *Scirtothrips dorsalis* and *Scirtothrips oligocheatus* respectively. *Thrips palmi* is predominant in north India especially Haryana during the early vegetative stage while *Scirtothrips dorsalis* is dominant in Maharashtra during early reproductive stage.

#### Genetic diversity of leafhoppers

Effect of nucleotide changes on the COI amino acid sequence of leafhopper was examined. Translation of COI sequences of

leafhopper from different regions of India showed little variation. The changes that occurred in the protein sequence were not significant and did not result in changes in protein structure. Graphical analysis of the representative protein sequence showed that the changed amino acids were mainly located in the  $\alpha$ -helix region of the protein.  $\alpha$ -helix region of the protein is membrane integrated and is not involved in the reaction of the electron transport chain. Sequence analysis revealed one conserved domain of 28 amino acids from amino acid numbers 70 to 97 that were located mainly outside the  $\alpha$ -helix region.

#### Identification and characterization of viral diseases of cotton in India

Very low incidence of cotton leaf roll dwarf virus, CLRDV was observed in Nagpur, Akola and Warangal. Artificial methods of transmission of CLRDV from cotton to chickpea was successful but transmission from chickpea to cotton could not be established. Sequences of the PCR amplicon (Fig. 12) for CLRDV and Chickpea stunt disease associated virus, ChPSDaV showed more than 95% similarity indicating the possibility of their same origin.

No genetic differences were found between upward and downward curling of cotton leaf curl virus, CLCuV. Primers designed on the basis of consensus sequences for amplifications of DNA-A, B and coat protein gene could be used for molecular diagnostics of CLCuV. Late incidence of tobacco streak virus, TSV was observed in cotton growing districts of Andhra Pradesh except Adilabad. The incidence was highest in Guntur district (3-25%) followed by Warangal (0.1 to 7%) and Karimnagar (up to 5%).

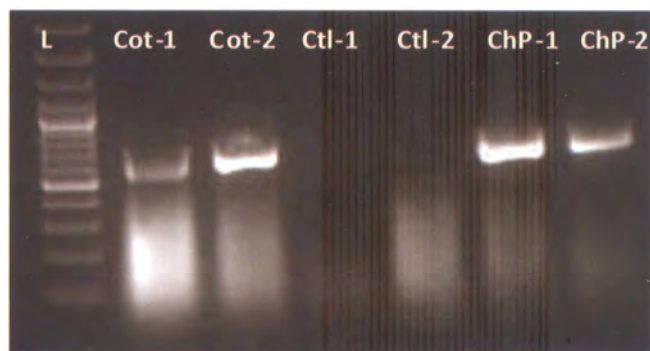
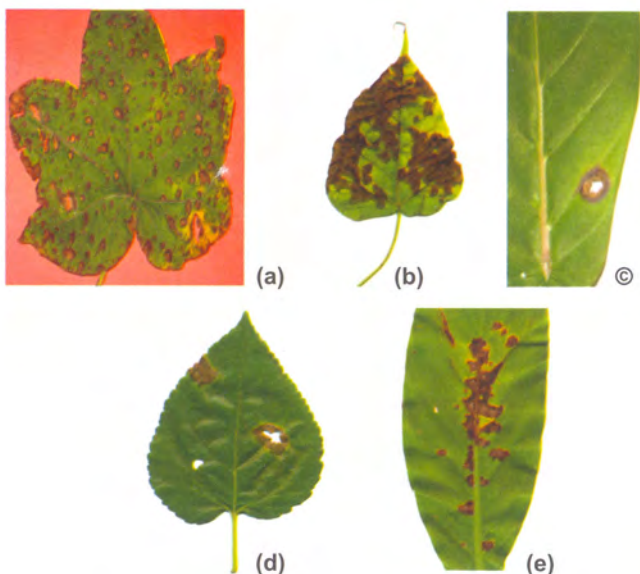


Fig. 12: Detection of CLRDV and ChPSDaV from infected cotton (Cot-1 & Cot-2) and infected chickpea (ChP-1 & ChP-2) respectively. Ctl-1 is uninfected cotton and Ctl-2 is uninfected chickpea.

#### Genetic diversity of *Alternaria*

Differences among three species of *Alternaria* infecting cotton were recorded using spore morphology and molecular markers. *Myrothecium* leaf spot is emerging as one of the major emerging diseases alone or in combination with *Alternaria* leaf spot. The incidence of grey mildew was observed in the AICCIP trial of Bhawanipatna of Odisha. Higher incidence of *Alternaria* leaf spot in combination with *Myrothecium* leaf spot in Bt cotton was observed in the farmers' field of Guntur district of Andhra Pradesh. Five collateral hosts of *Myrothecium rorridum* have been identified. Bacterial boll rot is also one of the emerging diseases in the farmers' field.



*Myrothecium* leaf spot on (a) Cotton, (b) Peepal, (c) Calitropis, (d) Mulberry and (e) Cana leaf.

Determination of emerging pests and natural enemies associated with Bt hybrids

Twelve under-release Bt hybrids belonging to 5 seed companies with 4 events were evaluated. Out of these, GK-228 BGII and PCH-66 were tolerant to aphids; PCH-44 and KDCHH-553 BGII to leafhoppers and PCH-55 & PCH-22 to whiteflies. No significant difference was observed with respect to thrips, mirid and spider population on Bt-hybrids. Highest seed cotton yield (917 kg ha<sup>-1</sup>) was observed in PCH-44Bt under rainfed conditions.

Developmental studies of *P. so/enopsis* on alternate hosts

Developmental studies of *P. so/enopsis* was carried out at constant temperature of 27°C and RH 60% under laboratory conditions on four different alternate hosts viz. okra (*Abelmoschus esculentus*), congress grass (*Parthenium hysterophorus*), hibiscus (*Hibiscus rosasinensis*), tomato (*Lycopersicon esculentum*). Highest fecundity was observed on congress grass (268 eggs) followed by hibiscus (239 eggs), okra (200 eggs) and tomato (186 eggs).

Intensity of pink bollworm infestation in India

Incidence of pink bollworm on BG, BG-II and non Bt cotton fields was monitored across India. In North India, 9 districts from three states (Hisar, Fatehabad and Sirsa of Haryana, Mansa, Abohar, Bathinda and Faridkot of Punjab, Sriganaganar and Hanumangarh of Rajasthan), 23 districts of Central India namely Wardha, Yavatmal, Washim, Hingoli, Nanded, Parbhani, Aurangabad, Buldana, Akola, Amravati, Nandurbar, Dhule and Jalgaon districts of Maharashtra; Surat, Bharuch, Vadaodara, Anand, Ahmedabad, Bhavnagar, Amrelli, Junagadh, Rajkot and Surendranagar districts of Gujarat and 7 districts of Andhra Pradesh namely Guntur, Nandyal, Krishna, Khammam, Warangal, Karimnagar and Adilabad were monitored.

The intensity of pink bollworm infestation on non Bt cotton

(Table 7) was maximum in Karnataka (83.64%, Dharwad), Rahuri, Nanded and Akola in Maharashtra recorded pink bollworm incidence on non Bt cotton while there was no incidence of pink bollworm on Bt cotton in these district.. Non Bt cotton was not found in Hingoli at the time of collection. Larval intensity on extended crop of BG from Hingoli (4.70%), Nandurbar (26.14%), Dhule (19.80%) and Jalgaon (18.18%) was recorded. The corresponding larval intensity on non Bt was 0.45% (*Desi* cotton) 6.92% (*Hirsutum*) and 25.89% (*Desi* cotton) from Nandurbar, Dhule and Jalgaon, respectively. Gujarat (3.97% specifically in Surat, Bharuch, Vadodara and Bhavnagar) recorded minimal incidence of pink bollworm. In BG-II the per cent larval recovery was nil from all the districts monitored both at 140 and 160 DAS.

Table 7 : Intensity of Pink bollworm infestation in different state of India

Sr. No	State	Larval incidence % in green bolls		
		NBt	BGI	BG II
1	Gujarat	23.22	3.97	0.00
2	Maharashtra	17.30	20.58	0.00
3	Andhra Pradesh	10.96	0.00	0.00
4	Tamil Nadu	13.66	0.00	0.00
5	Karnataka	83.64	0.00	0.00
6	Punjab	13.70	0.00	0.00
7	Rajasthan	16.73	0.00	0.00
8	Haryana	10.71	0.00	0.00

## Coimbatore

Infestation of *P. gossypiella* recorded from different centres of south zone

Recovery of *P. gossypiella* larvae was significantly higher in NBt hybrids than Bt hybrids collected from different centres from Tamil nadu, Karnataka and Andhra Pradesh. Significantly, higher number of exit holes were recorded from Dharwad and Coimbatore followed by Haveri and Srivilliputhur. Mean recovery of *P. gossypiella* on NBt hybrids was maximum from Dharwad (77.21 %) followed by Coimbatore (25.26%), Srivilliputhur (18.00%) and Nandyal (12.33%).

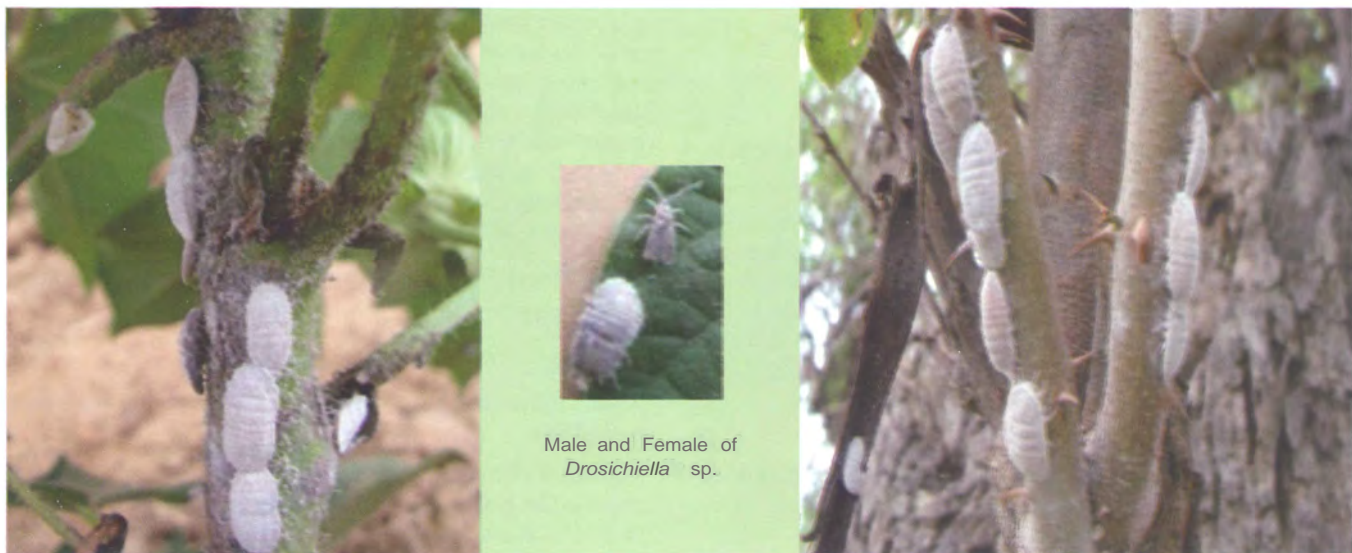
Isolation of cotton foliar pathogens

Isolations from Bunny Bt - BG yielded cotton leaf spot pathogen, *Alternaria* sp., whereas isolations from LRA 5166 yielded *Alternaria* spp., *Drechslera* spp., *Fusarium* sp., *Colletotrichum* sp. and *Curvularia* sp. At present there are 10 isolates of *Alternaria* spp., four isolates of *Drechslera* spp., three isolates of *Fusarium* sp., two isolates of *Colletotrichum* sp., five isolates of *Curvularia* sp., and one isolate of *Rhizoctonia* in our culture collection.

## Sirsa

A single species i.e, *Phenacoccus solenopsis* Tinsley was observed on cotton in north India. At four locations *Drosichiella* spp. of mealybug was recorded, that did not cause significant economic damage.





Male and Female of *Drosichiella* sp.

Cotton plant infested with *Drosichiella* sp.

Ber tree infested with *Drosichiella* sp.

*Rodolia fumida* (Mulsant) (Coleoptera: Coccinellidae) was a predator observed on *Drosichiella* spp.

#### 4.20 : Isolation and Identification of New Genes and Gene Sources for Pest Management :

Nagpur

Lectins from novel sources

Using bioinformatics, three full length genes encoding man nose-specific lectins have been identified in the *Trichoderma virens* genome.

RNAi for *Helicoverpa* management

For generating dsRNA, using gene sequences of same and related species, primers were designed and amplified to obtain fragments of the desired gene of interest in *Helicoverpa armigera*. Amplicons of expected sizes were obtained for Juvenile Hormone Esterase, Trehalose Phosphate Synthase and Helicostatin using cDNA and genomic DNA of *Helicoverpa* as template. *In vitro* transcription for generating dsRNA of Chitin synthase A, Juvenile Hormone Esterase, Trehalose Phosphate Synthase, and Helicostatin was found effective in terms of desired yield. The sequences showed homology to the target sequences of the organism from which primers were designed. Surface coating bioassay method was standardized with dsRNA that demonstrated an effect on two day old larvae. Chitin synthase A and Trehalose Phosphate Synthase were found to be promising in terms of their mortality and growth regulating effects on *Helicoverpa*.

Cloning and characterisation of potent toxin gene from heat tolerant isolate developed of *Heterorhabditis indica*, an entomopathogenic nematode

Bacterial symbionts associated with heat tolerant entomopathogenic nematodes (EPN) were isolated and characterized. Apart from entomopathogenic nematodes a number of bacterial flora were found associated with EPN. For molecular characterization, 16sRNA was amplified and sequenced and bacterial flora belonged to *Photorhabdus*, *Paeni-*

*bacillus* and species of *Xenorhabdus* as *Xpoinarii*, *Xindica* and species of *Bacillus* as *B.cereus*, *B. subtilis*, *B.nealsonii* etc.

Primers were designed for toxin genes viz. *tcaA*, *tcaB*, *tcaC*, *tcaZ*, *tccA*, *tccB*, *tccC*, *tccZ*, *tcdA*, *tcdA2*, *tcdB*, *tcdAB*, *txp40*, *W14tccA*, *W14tccB*, *W14tccC*, *W14tccZ*, *W14tcbA*, *Pae tca*, *Pae TcaB2*, *Pae tcaC*, *Pae tccC* and *Pae cry*. Genomic DNA of all the bacteria were extracted and used for amplification reactions with these primer sets.

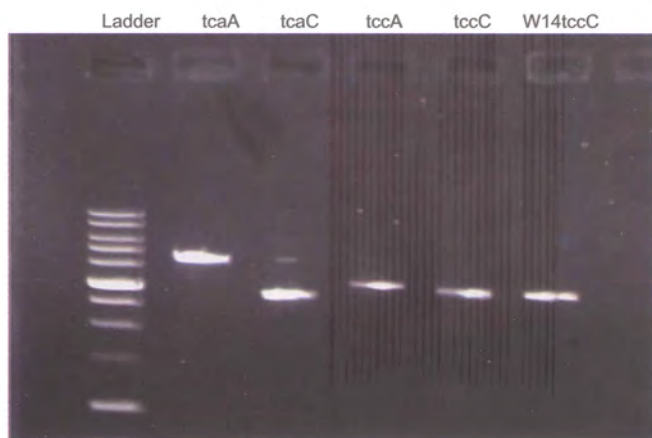


Fig. 13 : Amplification of toxin genes from bacterial symbionts of EPN

From *Photorhabdus* *tcaA* (4.5kb), *tcaC* (2.5kb), *tccA* (3kb), *tccC* (around 3kb) and *W14tccC* (around 3kb) could be amplified using long range Taq from Promega (Fig. 13). *tcaA*, *tcaC*, *tccA* were amplified from *Xenorhabdus indica* and *X. poinarii* isolates. These were cloned in pGEM-T vector and sequenced. Sequencing of *tccA*, *tccC* and *W14tccC* has been carried out and shows 95-97% similarity to *Photorhabdus* genes. *Tcc* Genes from *Paenibacillus* sp. is significant as this appears to be first report of a *Paenibacillus* species, strain, or protein having toxicity to lepidopterans.

## 4.21: Development of New Methods, Tools and Protocols

### Nagpur

#### Validation of sampling method and sample size for field population of mirid *Campyomma livida*

Validation of sampling methodology with respect to visual sampling method and sample size for mirid bug *Campyomma livida* was done on Jai Bt BG II. The results showed variation in nymph and adult counts in different plant portions; however for ease in observation, top 1/3, dportion was found to be ideal. A sample size of 15 plants/ acre was optimum. Sampling from top 1/3, dplant canopy with 15 plants /acre was found to be optimum for assessing field population of *C. livida*.

#### Standardisation of a robust replicable bioassay protocol for evaluating the direct and indirect toxicity of lectins to *Chrysoperla sps* and *Mallada boniensis*

Direct toxicity was measured by providing LC<sub>0</sub>, LC<sub>50</sub>, LC<sub>75</sub> and LC<sub>90</sub> doses of each of the three lectins (CEA, AMTL and Banana lectin) that were most effective against sucking pests. Lectin concentration (100 µg/ml) was dispensed in PCR tubes with a cotton wick passing through its lid and placed in separate containers into which neonate or 4 day *Chrysoperla* grubs were released. Fresh lectins were provided each day. The bioassays were continued up to pupation. A slight modification in the feeding device was made with the adults.

Bioassays were standardized to quantify the indirect toxicity of lectins by exposing aphids across different time periods and concentrations to lectin laced diet and subsequently feeding *Chrysoperla* grubs with both live and dead aphids, separately. Protocols to identify and quantify the lectin in the prey as well as predator through lectin ELISA were standardized.

### Coimbatore

Cotton seed based cost effective artificial diet was standardized for continuous rearing of pink bollworm *Pectinophora gossypiella*. The basic ingredients are cotton seed flour (processed) and chick pea flour with carbohydrate, protein, fat sources, multi vitamin, antimicrobial agents and agar as thickening agent are used as other ingredients. Micro centrifuge tubes were used as rearing containers. Individual neonate larvae were released on each piece of the diet inside the micro centrifuge tube and the lids were closed tightly. This

prevented larval escape thus retaining them inside the tubes and also prevented diet dehydration. The per cent recovery of the insect reared on diet was recorded as 95.56 per cent. Egg hatchability, adult emergence are 100 per cent and pupal malformation was nil. Eggs, larval and pupal periods were recorded as 4.8 ± 0.632, 25.10 ± 0.994 and 7.9 ± 0.88 days, respectively. Larval and pupal weight were recorded as 21.40 mg ± 3.63, 18.00 mg ± 2.73, respectively.

## 4.22: Host-Plant Resistance to Insect Pests and Diseases

### Nagpur

#### RNAi mediated crop protection against root-knot nematode

Root-knot nematode genes especially genes that encode esophageal gland proteins were taken up for their amenability to RNAi. Based on sequences of esophageal parasitism genes in data bases, primers were synthesized for ten more parasitism genes. Evaluation of dsRNA for parasitism genes against root-knot juveniles and reniform preadults was carried out *in-vitro*.

dsRNA for parasitism genes was synthesized using Ambion megascript kit. dsRNA for genes including MjTis11, ADF 1-4 were evaluated for penetration and reproduction of root-knot and reniform nematode. Out of these MjTis11 reduced penetration of rootknot nematode by 58% while the effect on reniform nematode penetration was significantly less at 28%. However, cessation of nematode reproduction was not recorded with these genes, indicating the need for screening of other parasitism genes for use in RNAi for nematode management.

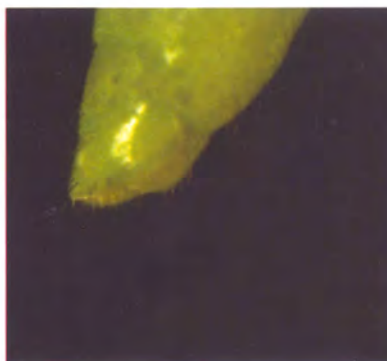
### Coimbatore

#### Monitoring for development of resistance against Bt hybrids in *Pectinophora gossypiella* during different growth period of the crop

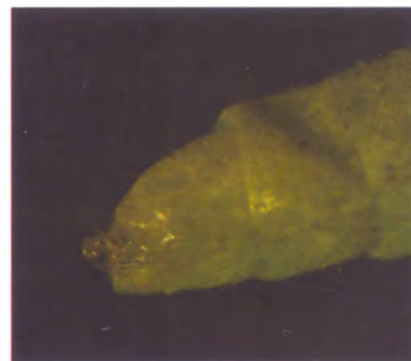
Bt hybrids namely, RCH 2 Bt, RCH 20 Bt and RCH 2 NBt were monitored under field conditions for the development of resistance if any against *P. gossypiella*. Number of exit holes was maximum in RCH 2 NBt followed by RCH 20 Bt. Damaged locules/5bolls were significantly maximum in RCH 2 NBt hybrid (0.63/5 bolls). The overall seasonal mean of percentage of locules damage was higher in RCH 2 NBt



Rearing of sucking pests on artificial diet



Female *Chrysoperla*



Male *Chrysoperla*





Stages of Pink bollworm

(73.12%), minimum in RCH 2 Bt (1.12%) and RCH 20 Bt (12.58%). Mines on the epicarp were minimum in Bt as compared to NBt. No significant difference was recorded in the number of warts observed in the epicarp of the bolls of different Bt and NBt hybrids. Number of surviving larvae recorded on NBt hybrid (RCH 2) was maximum as compared to the larvae present in the RCH 2 and RCH 20 Bt hybrids. Mean number of dead larvae of 0.07, 0.025 and 0.04 were recorded in RCH 2, RCH 20 and RCH 2 NBt hybrids, respectively.

#### Insecticide resistance monitoring against sucking pests

Leafhoppers (*Amrasca devastans* Oist.) were assessed for their resistance levels to insecticides such as Imidacloprid (Confidor & Victor), Thiamethoxam (Actara), Acephate (Asataf) and Monocrotophos through leaf dip bioassay. Among the insecticides tested Victor registered the minimum LD<sub>50</sub> value (0.0035 mL) followed by Confidor (0.0050 mL), Monocrotophos (0.0054 mL), Thiamethoxam (0.0130 mL) and Acephate (0.0184 mL).

#### Monitoring for resistance against Cry toxins

Bioassay with *cry2Ab* against *P. gossypiella* from Oharwad recorded LO<sub>50</sub> and LO<sub>90</sub> values as 0.002 ppm and 0.087 ppm respectively and Coimbatore population recorded LO<sub>50</sub> and LO<sub>90</sub> values of 0.0623 ppm and 0.095 ppm, respectively. Bioassay with *cry1C* indicated that the population collected from Oharwad recorded LO<sub>50</sub> and LO<sub>90</sub> values of 0.0312 ppm and 0.507 ppm respectively and Coimbatore population recorded LO<sub>50</sub> and LO<sub>90</sub> values of 0.0001 ppm and 0.630 ppm, respectively.

#### Sirsa

##### Determining cultivar association with emerging pests

A total of 31 Bt cotton cultivars were grown to study their association with emerging and key pests. No mealybug incidence was observed in any of the hybrids. The mean incidence of leafhopper / 3 leaves on the basis of 7 fortnightly observations varied between 2.79 (MRC-6025 Bt) to 5.26 (SWCH-4713 BG-II). The maximum population of leafhopper (12.60/3 leaves) was recorded in hybrid NCS-905 Bt during 32 SW of the season and minimum population (0.00) was recorded in MRC-6025 Bt during 36 standard week of the season. The mean incidence of whitefly / 3 leaves on the basis of 7 fortnightly observations varied between 4.26 whitefly/ 3 leaves to 6.39 whitefly/ 3 leaves. The maximum population on the basis of 7 fortnightly observation was recorded on RCH-569 BG-II (6.39 whitefly/ 3 leaves), Bioseed 6488 BG-II (6.13 whitefly/ 3 leaves) and VICH-309 BG-II (6.11 whitefly/ 3 leaves) where as the lowest population was recorded in case of SWCH-4713 BG-II (4.26 whitefly/ 3 leaves) followed by SP-7007 Bt (4.73 whitefly/ 3 leaves). The peak whitefly population was recorded during the 42 Sw.. The mean thrips population / 3 leaves based on 7 observations recorded ranged between 7.39 (MRC-6304 Bt) to 15.01 (Shakti 9 Bt).

Minimum CLCuO incidence (0.84%) was recorded in VICH309BG-11 and maximum (10.53%) was in RCH134 BG-II.. In case of root rot the minimum (0.00%) and maximum (29.50%) disease incidence was recorded in RCH134 BG-II and RCH605 BG-II, respectively.

#### Resistance monitoring in sucking pests to commonly used insecticides

In case of the leafhopper population collected from Kot Samir (Bathinda) under sprayed condition, the lowest LO<sub>50</sub> value (0.000003 ppm) was recorded for BYI 02960 and highest LO<sub>50</sub> value (0.0058 ppm) was obtained in dimethoate. RR ranged from 1933.33 (dimethoate) to 33.00 (victor) fold when compared with the highly susceptible population.

#### 4.23: Identification of Germplasm Sources of Resistance to Insect Pests and Diseases

##### Sirsa

Out of 3954 germplasm accessions evaluated under north zone, 2159 lines were found free from CLCuO.

#### 4.24: Biological Control

##### Nagpur

##### Parasitoid diversity

Six new parasitoids of *P. solenopsis* were recorded out of which two parasitoids viz., *Prochiloncurus albifuniculus* and *Prochiloncurus pulchellus* showed parasitisation up to 4 and 2 % respectively. One parasitoid *Aprostocetus* sp having distinct red eye have been recorded from Vidarbha and Marathwada region while *Promuscidea unifasciiventris* recorded 1-35% parasitization. Two parasitoids have been recorded from mealybug population collected from Gujarat. Cumulative 16 parasitoids and 9 predators have been recorded on mealybugs infesting cotton in three cotton growing zones of India.

A new *Trichoderma* strain naturally parasitizing a pathogenic *Ganoderma basidiocarp* has been isolated and purified.

Development, validation, utilization and/or commercialization of biopesticides and bioinoculants

### DNA finger printing of bio-pesticides

DNA finger printing of native bio-pesticides shortlisted as effective against cotton insect pests was done for registration with Central Insecticidal Board (CIB). Molecular characterization of bacterial and fungal antagonists was done by amplification of 16sRNA and 18sRNA region respectively. Amplicon size for 16sRNA amplification of bacterial antagonists identified as belonging to *Bacillus cereus*, *B. nea/sonii*, *B. subtilis*, *B. nea/sonii*, *Xenorhabdus indica*, *X. poinarii*, *Brevibacterium* and *Photorhabdus spp.* was about 1.5Kb (Fig. 14). The amplicons were cloned in pGEMTvector and sequenced. The sequences have been submitted to NCBI.

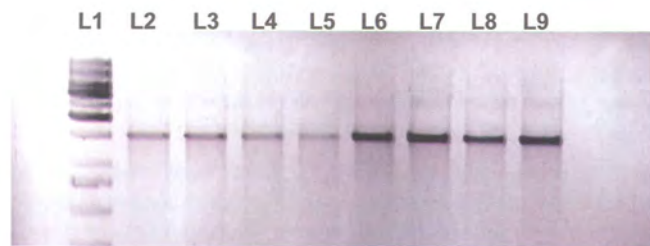


Fig. 14: Amplification of 16sRNA gene of bacterial antagonists. Lane 1, DNA ladder, Lane 2-9- *Bacillus cereus* CICR X1, *B. cereus* CICR X2, *B. nealsoni* CICR PKV, *Xenorhabdus poinarii* CICR WR, *X.indica* CICR WG, *Brevibacterium epidermidis* CICR G1, *X.indica* CICR WGR1

Amplicon size for fungal antagonists as *Fusarium incarnatum* (*Fusarium pallidoroseum*), *Metarrhizium anisopliae* (Ma4), *Lecanicillium lecanii* (VL-5 & Coimbatore) isolates ranged between 552-569 bp. The sequences (*F. incarnatum* Ace. No. JN206645, *M. anisopliae* Ma4 Ace. No. JN206646, *L. lecanii* VL-5 Ace. No. JN206647 & *L. lecanii* Coimbatore Ace. No. JN982329) have been deposited in NCBI gene bank.

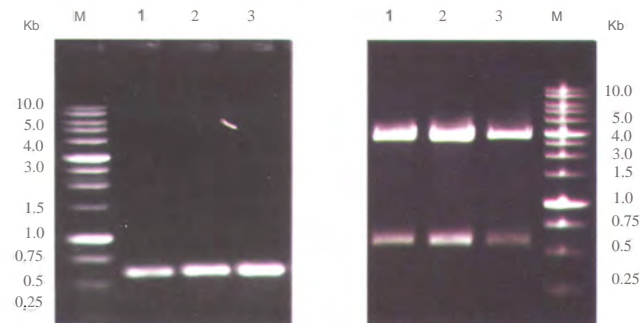


Fig. 15: Cloning and sequencing of rRNA genes. Lane 1, *Lecanicillium lecanii*, 2, *Metarrhizium anisopliae*; *Fusarium pallidoroseum*

### Biochemical and molecular characterization of bacterial isolates

Biochemical and molecular characterization was carried out for six isolates (GV1, GV2, GV4, GV5, BB and BW of bacteria *Xenorhabdus* and *Photorhabdus* symbiotically associated with Entomopathogenic nematodes and which had been found effective against sucking pests as mealy bug. The biochemical parameters taken up were Colony Morphology on

Nutrient Agar, Gram Stain, Pigmentation, Levan production, Methyl Red, Voges-Proskauer Test, starch hydrolysis, oxygen requirement, H<sub>2</sub>S production, indole production, nitrate reduction, Urease test, ADH test, citrate, catalase, gelatinase, motility, tyrosinase, Galactosidase. Carbohydrate fermentation studies were carried out for 21 carbohydrates.

Molecular characterization of these bacterial isolates was done by amplification of 16sRNA. Amplicon of 1.5 kb was cloned in pGE-T vector and sequenced. Using OPA primers delineation of similarity and dissimilarity of bacterial was carried out and dendrogram made. Further analysis is underway. Restriction enzyme profiling of 16sRNA amplicon of bacterial isolates was done with five restriction enzymes MSP1, Alu1, Dde1, HaeIII and Hinf1.

### peR Multiplex for detection of cotton pathogens

Multiplex PCR protocol was standardized for detection of cotton pathogens. PCR conditions and primers were standardized to achieve amplification of all cotton pathogens in one PCR reaction. This technique can be used for quick detection and identification of cotton pathogens in soil as well as plantsample (Fig.16).

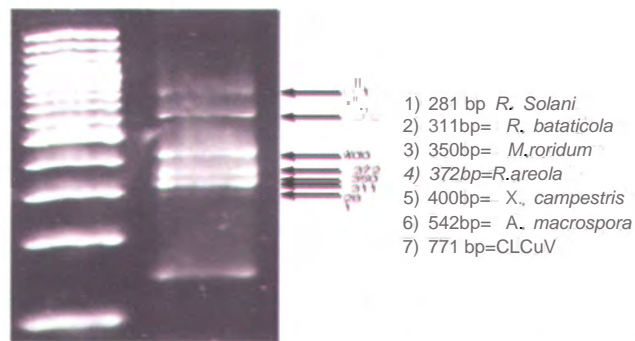


Fig. 16 : Multiplex PCR for detection of cotton pathogens

### Development of nanoparticles based biocontrol formulation for the management of major cotton pests and diseases

Silver nanoparticles have been synthesized through 0.01 and 0.001 M silver nitrate solution using boiled fresh cotton leaf extract as reducing agent in green method.

### Coimbatore

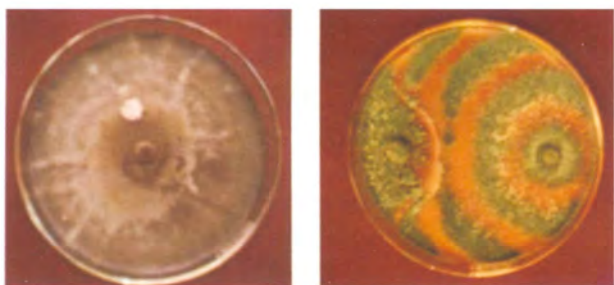
Testing the compatibility of efficient bioagents for mutual antagonism

On dual culturing of bioagents isolated from soils of experimental field, it was observed that there was no mutual antagonism between the two bioagents viz., isolates of *Trichoderma spp.* and *Pseudomonas*.

Assessing the antagonistic property of bioagents against leaf spot pathogen, *Alternaria sp.*

Dual culture testing of bioagents, for their antagonistic activity, against cotton foliar pathogen, *Alternaria spp.* was done with all available bioagents. Two isolates of *Trichoderma spp.* and an isolate of *Pseudomonas* (from soil samples) were found effective against *Alternaria spp.*





*Alternaria* sp.

*Alternaria* sp. X *Trichoderma* Sp.

### Evaluation of solid substrates for mass multiplication of bioagents

Grains of sorghum and finger millet were used as solid substrates for growing isolates of *Trichoderma*, *Drechslera* and *Alternaria*; finger millet supported more spore growth in all the isolates.

### Molecular characterization of native entomopathogenic fungi from mealybug

Molecular characterization of five native entomopathogenic fungi isolated from mealybug from 2009 to 2011 were carried out and submitted to Gene Bank. The fungi identified were *L. attenuatum* (Gene Bank Accession No. JQ327150), *M. anisopliae*-ARSEF-9613 (Gene Bank Accession No. JQ062986), *M. anisopliae*-ARSEF-9612 (Gene Bank Accession No. JN712743), *L. araneicola* (Gene Bank Accession No. JN255572) and *L. fusisporum* (Gene Bank Accession No. JF 427909).

### Mass multiplication of a native entomopathogenic fungus, *L. ecanii*

A total of six nitrogen sources (Peptone, Ammonium Nitrate, Sodium Nitrate, Beef extract, Urea and Thio Urea) were tested for the mass multiplication of *L. ecanii*. Among them Ammonium Nitrate and Thio Urea supported maximum growth and sporulation.

### Standardization of temperature optima for multiplication of native entomopathogenic fungi

Effect of temperature on growth of six native entomopathogenic fungi (*L. ecanii*, *L. aphanocladii*, *M. anisopliae* ARSEF - 9612, *M. anisopliae* ARSEF-9613 and *V. ecanii*), were studied at wide range of temperature from 10 to 40°C. Though all fungi were able to grow at a wide range of temperature from 15-30°C 20-30°C was found to be optimum for growth and sporulation. Two isolates *M. anisopliae* (ARSEF 9612 and 9613) were able to grow well at 35°C.

### Standardisation of methods to increase the virulence of *L. ecanii*

Media supplemented with insect extract was found to increase the growth, sporulation and virulence of *L. ecanii*. High activity of pathogenesis related enzymes was also observed.

### Development of formulation of promising entomopathogenic fungi and testing

Talc and oil based formulations of *L. ecanii* and *M. anisopliae* (ARSEF-9612) were developed and tested for the efficacy under pot culture condition. Among two formulations tested, oil based formulation of *L. ecanii* recorded significantly higher mortality of aphids under pot culture condition.

### Development of talc based formulation of a native entomopathogenic fungus, *L. ecanii*

Three talc based formulations of *L. ecanii* were developed and stored at room temperature and refrigerator. Spore viability and virulence were tested for six months. *L. ecanii* multiplied in SDAY Broth and formulated in talc supported maximum spore viability of 70 per cent at the end of six months storage under refrigerated condition. The same result was recorded for two years.

Production of insecticidal toxin by *Xenorhabdus stockiae*, a bacterial symbiont of a native entomopathogenic nematode, *Steinernema siamkayai* was proved. Mortality of *H. armigera* due to different aliquots ranged from 66 to 100 per cent 24 h after inoculation.

### Pathogenicity of bacterial symbiont of native entomopathogenic nematode

Pathogenicity of *X. stockiae* to *Aphis gossypii* Grover was studied under lab condition. The bacterial cells and their cell free supernatant of both primary and secondary phase of *X. stockiae* were tested against *A. gossypii*. Cell suspension and cell free supernatant of *X. stockiae* were found to be highly pathogenic to *A. gossypii* when compared to control. The results revealed primary phase of *X. stockiae* was found to be more toxic than secondary phase and cell free supernatant of both phases were more virulent than bacterial cells alone. Spraying of primary and secondary phases of bacteria alone recorded a maximum of 87 and 82% mortality whereas cell free supernatant of both primary and secondary phase recorded 100 and 99% mortality at 48 hours after treatment.

### Sirsa

Among various insecticides and biopesticides, imidacloprid (57.17 %) resulted in maximum reduction of leafhopper population and VL-5 resulted in maximum (51.06%) reduction of whitefly population. Thrips population was effectively reduced by biopesticide, Pest guard L 50EC (56.97%). The biopesticides and entomopathogens were found safer to generalist predators.

### Entomopathogens for mealybug management

Based on a three year study of off-season survey (2007-08 to 2009-10) of mealy bug cadaver collection in hot spots of north zone followed by isolation of fungi, their identification and proving of Koch's postulates, *Fusarium pallidoroseum* was identified as an entomopathogen of mealybug (*Phenacoccus so/enopsis*). The efficacy of this entomopathogen was then established against mealybug under *in-vitro* and field conditions (Talc based preparation @ 1-2%) at Sirsa and other AICCIP centres i.e. Faridkot, Coimbatore and Rahuri. During 2011-12, there was increased mortality of mealybug *P. so/enopsis* with increased concentration of *F. pallidoroseum* in first spray whereas it remained at par in case of second spray. Maximum mortality of 88.1 % was recorded after two sprays of entomopathogen @ 2% talc based preparation.

The effect of *F. pallidoroseum* was also studied on other sucking pests and beneficial insects and it was effective against jassids (59.3% mortality) and white fly (34.6% mortality). It did not cause any harmful effect on beneficial insects. Colony forming units (CFUs) of *Fusarium pallidoro-*

seum were observed above required threshold after 9<sup>th</sup> month of storage under room temperature.

#### 4.25: Integrated Pest Management

##### Nagpur

Evaluation and validation of IPM/IRM strategies for Bt-cotton against sucking pest complex

An experiment was carried out on development and validation of IPM/IRM strategies against sucking pests. Results indicated that significant higher population of aphid, whiteflies and mirid was recorded in IPM as compared to RPP over the season. Whereas, no significant difference in population of leafhoppers and thrips was observed. Spider population was significantly higher in IPM while coccinellids were at par. Statistically there was no difference in yield.

Evaluation of ecofriendly biopesticides

Evaluation of Pest Guard L, Pest Guard J and Pest Guard M in multi location trials

(carried out in network mode with CICR as Lead centre)

North India: Sirsa, Faridkot, Sriganganagar, Hisar

Pest guard J 50 EC, Pest guard L 50 EC, *Fusarium pallidoroseum*, VL-5, MA-4, neem oil + Nirma powder, Buprofezin, *Beauveria bassiana*, Imidacloprid, Cactus extract and control with water. The latter two were at Sirsa alone while the rest were evaluated at 4 centres of north India on RCH 134 BGII/Mist. The total number of sprays ranged from 1-4 and the trial was replicated thrice. As the incidence of sucking pests was noticed, 4 applications of treatments were repeated at an interval of fifteen days at Sirsa. Among various treatments for leafhopper, the differences in the reduction of leafhopper populations between treatments were non-significant. Maximum reduction in whitefly population was due to VL-5 (51.06%) followed by Pest Guard L and the reduction was statistically superior over other treatments.

Maximum reduction (%) in thrips population was reported in biopesticides Pest Guard L 50 EC and Pest Guard J 50 EC with 56.97 and 56.36 respectively and was statistically superior over other treatments. The lowest per cent population reduction was observed in MA-4 (31.32%).

The population of natural enemies was also numerically affected by different insecticides, bio-pesticide and entomopathogens. However, treatment differences were non-significant. Highest yield was obtained in Imidacloprid treatment (32.25 q/ha). Yields between treatments when compared were statistically insignificant.

At Sriganganagar, maximum reduction of leafhoppers was brought about by Imidacloprid and Pest Guard series. Maximum reduction in whitefly population was observed with Pest Guard series and *F pallidoroseum*. Pest Guard series and Buprofezin were most effective against thrips. Yields were highest with Pest Guard Land neem oil,

At Hisar leafhoppers were reduced effectively by Pest Guard O that was on par with Buprofezin followed by *Fusarium pallidoroseum*. Buprofezin was on par with Pest Guard O against whiteflies and thrips.

At Faridkot, based on the pre and post-count data it was

concluded that Pest guard O 50 EC, Pest guard L 50 EC and neem oil + Nirma powder proved statistically superior against whitefly and jassid as compared to the control however, their efficacy was significantly low as compared to the standard control Buprofezin and Imidacloprid. The biopesticides *Fusarium pallidoroseum*, *Verticillium lecani*, and *Beauveria bassiana* proved to be ineffective and were at par with control. The predator population was at par among all the treatments, however slightly low population was recorded in the Imidacloprid and Buprofezin after the 1<sup>st</sup> spray. In terms of the yield on whole plot basis the maximum yield was recorded in imidacloprid (7.2 kg/plot (44.44 q/ha)) and this was statistically at par with all the other biopesticides except *Verticillium lecani*, and *Beauveria bassiana*.

South India: Guntur

Bio-agents like *Verticillium lecani*@ 2500 g/ha and 5000 g/ha, *Metarrhizium anisopliae* @ 2500 g/ha and 5000 g/ha not effective against leafhoppers, which is evident from the data on per cent reduction of leafhopper population over control at three, seven and ten days after spray. On the other hand, compounds like NSKE @ 5% and Surf powder were comparatively better over bio-agents. In these treatments, per cent reduction over control ranged from 33.30 to 43.3% at three days after spray. Highest reduction was recorded in surf powder @ 5000 g/ha. Non-significant differences were recorded among treatments at seven and ten days after spray. Seed cotton yield ranged from 967.2 to 642.2 kg/ha with non-significant differences among the treatments. Pest Guard, L, M and O were not tested at this centre.

Central India: Khandwa

After 1<sup>st</sup>, 2<sup>d</sup> and 3<sup>d</sup> spray, aphids and leafhopper were minimum in Pest Guard J 50 EC 600 g/ha. Minimum open boll damage was observed in Pest Guard J 50 EC and it was at par with Pest Guard L 50 EC and Fish oil rosin soap. The next better treatments were *Verticillium lecani*, Pest Guard O 50 EC, NBAII *Lecanicillium lecanii*-5, L1, *Fusarium pallidoroseum*, Ma4 (NBA II), neem oil 1% and Mealy Quit. Minimum locule damage was observed in Pest guard J 50 EC and it was at par with Pest Guard L 50 EC Fish Oil Rosin Soap, *Fusarium pallidoroseum*, LI and Ma 4 (NBAII). Maximum seed cotton yield was recorded in Pest Guard J 50 EC which was at par with seven other treatments while rest of the treatments was found superior to control.

##### Coimbatore

The effectiveness of bio-pesticides on the mirid bug was low and there were no significant differences among the treatments. *Verticillium lecanii* (NBAII, Bangalore) followed by *Metarrhizium anisopliae* (CICR, Coimbatore Strain) and neem oil brought about minor reduction of 29.96, 27.4, and 23.12% respectively as against 18.08 and 0% in unsprayed and water sprayed controls respectively. When coccinellid predators were affected by the pesticide treatments, spiders survived.

##### Sirsa

Innovative interventions for leaf curl management

The following treatments ie T1- Whey protein @ 5%, T2- Cow urine @6.6%, T3- Neem oil @1%, T4- Mustard oil @3%, T5- Kaolin @ 2%, T6- Calcium nitrate @ 0.5%, T7- Potassium



nitrate @ 0.5% T8- Paraffin liquid @ 2% T9- Strobilurin @ 0.1%, T10- Acephate @ 0.4% and T11- Control were sown with three replications under RBD to study their effect on the cotton leaf curl virus disease control. Six sprays starting from appearance of disease were given at fortnightly interval. Pre and post spray data (weekly) on CLCuD incidence were recorded. Data on whitefly incidence was recorded at weekly interval.

Among different treatments whey protein @ 5% found most effective in managing disease (9.8% incidence, PDI-3.8) followed by Calcium nitrate @ 0.5% (10.1%, 5.4) neem oil @ 1% (12.5%, 5.5) and strobilurin @ 0.1% (13.5%, 6.4) as compared to control (31.5%, 14.3). White fly reduction was observed only in case of neem oil and Acephate.

**Pink bollworm larval recovery from north zone**

Monitoring for PBW incidence in Bt cotton was conducted in North Zone (Faridkot, Srigananagr, Sirsa and Hirsar) through examinations of 150 green bolls, thrice at 140,160 and 175 DAS.

Recovery of pink bollworm larvae from the non Bt cotton bolls received from north zone ranged from 4.17% in Faridkot 124 to

7.44 % in H-1098 Hisar at 160 DAS. But no larval recovery was observed from these locations from the bolls received of Bt cotton at 160 DAS.

Recovery of pink bollworm larvae from the non Bt cotton bolls received from north zone ranged from 6.67% in Faridkot 124 to 20.37% in non Bt Des; at Sirsa at 175 DAS. But no larval recovery was observed from these locations from the bolls received of Bt cotton at 160 DAS.

**Adult moth catch of cotton bollworms in pheromone traps**

Pheromone traps were installed in the experimental area. The lures were changed after each 15 days. Pheromone trap catches reveal mean male moth catch per trap per week during the 2011 were 3.58 for pink bollworm (PBW), 2.43 for American boll worm (ABW), 3.79 for spotted boll worm (SPW) and 14.22 for tobacco caterpillar (TC). The peak catch for pink bollworm was during 43<sup>th</sup> SMW (21.67/trap/week), for American boll worm 14<sup>th</sup> SMW (19.10/trap/week), for spotted boll worm was during 45 SMW (19.29/trap/week) and for tobacco caterpillar was during 39 SMW (51.43/trap/week) (Fig. 17 and 18).

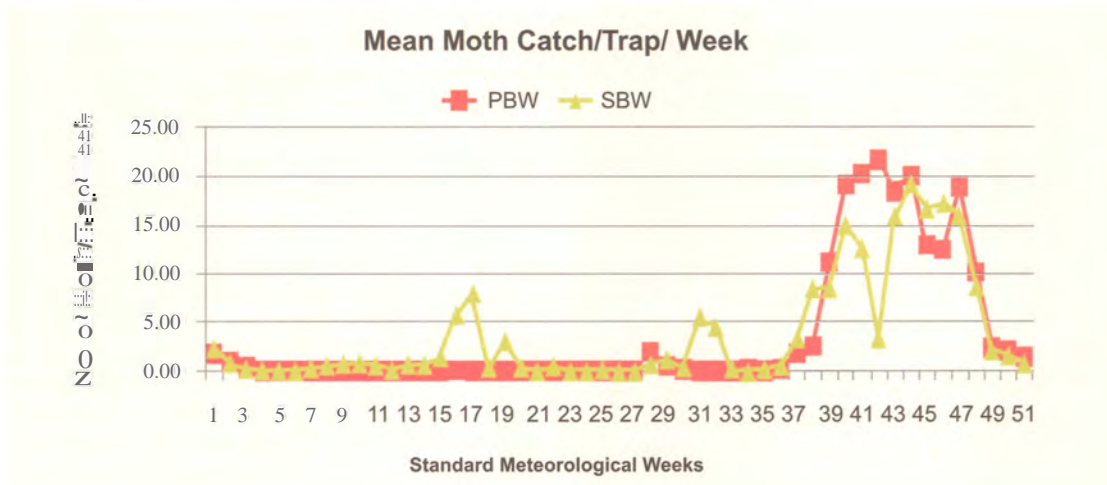


Fig. 17: Pheromone monitoring data at Sirsa (2011-12)

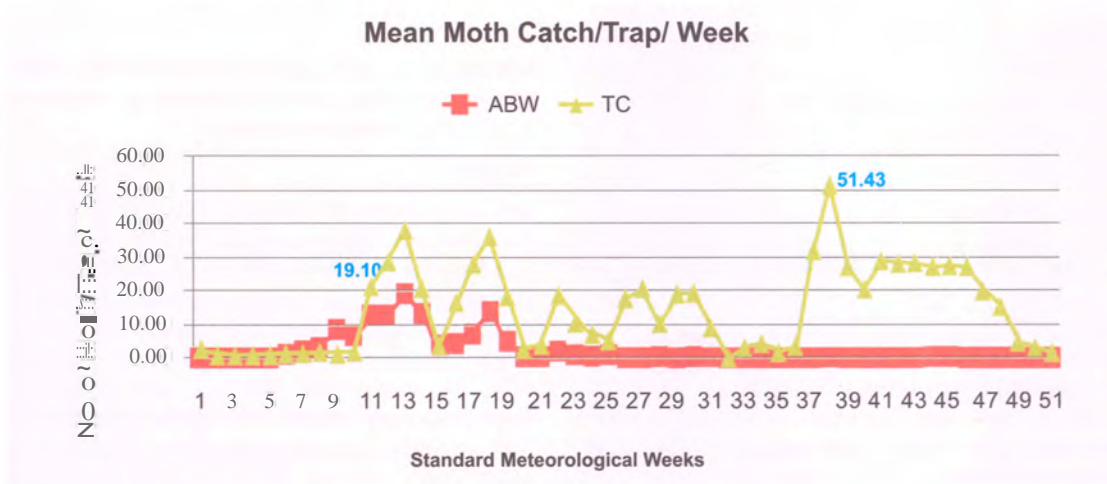


Fig. 18 : Pheromone monitoring data at Sirsa (2011-12)

## Nagpur

Insecticide resistance monitoring of 5 insecticides against leaf hoppers (carried out in network mode)

Leaf hopper samples from 10 locations were subjected to resistance monitoring studies. LC<sub>50</sub> to Imidacloprid ranged from 0.00012 mg/L (Bhatinda) to 0.128 mg/L (Buldana). LC<sub>50</sub>s of thiamethoxam ranged from 0.00013 mg/L (Bhatinda) to 0.145 mg/L (Buldana). Seven populations of leaf hoppers were subjected to bioassays with acephate and monocrotophos and its LC<sub>50</sub>s ranged from 0.0008 mg/L to (Bhatinda) 0.1622 mg/L (Nagpur) and 0.008 (Bhatinda) to 0.0779 mg/L (Jalna) respectively. Bhatinda populations were at least 100 fold more susceptible than the populations that showed the highest LC<sub>50</sub>. Bioassays with population from 8 locations were carried out at CICR, Nagpur while the rest were carried out at NAU Navsari (populations from Gujarat) and CICR RS Sirsa (populations from Bhatinda).

Monitoring of changes in baseline susceptibility of Cry toxins in 4 bollworms (carried out in network mode)

Bioassays with *H. armigera* collected from 16 districts from crops other than cotton and reared to F<sub>1</sub> were carried out with *cry1Ac*. LC<sub>50</sub> values were derived from 11,200 larvae tested, from 16 districts, ranged from 0.245 J-*lg cry1Ac/ml* in Dhule strain to 5.10 J-*lg cry1Ac/ml* in Bharuch strain with a low value of 0.026 J-*lg cry1Ac/ml* of diet in the reference susceptible strain. The variability in the field strains was about 22-fold. The LC<sub>50</sub> values ranged from 0.003 in the reference susceptible strain and 0.036 *~g/ml* in Akola and Mansa populations and 0.363 *~g cry1Ac/ml* of diet in Surat population with 10 fold variability between the field strains. *Cry2Ab* bioassays were carried out on F<sub>1</sub> *H. armigera* obtained from populations collected across 14 districts and the EC<sub>50</sub> ranged from 1.33 *μl/ml* (Guntur) to 8.58 J-*lg/ml* of diet (Amrelli). Bioassays on F<sub>1</sub> from 10 populations were carried out with *cry1Ac + cry2Ab* in wherein the ratio of *cry1Ac* to *cry2Ab* was maintained at 1:4. *S. litura* populations were collected from 8 locations and bioassays with *cry1Ac*, *cry2Ab* revealed the LC<sub>50</sub> values to range from 5.0 (Bhatinda) -18.9 (Sriganganagar) *ug/ml* of diet, 2.41 (Abohar) -6.03 (Sirsa) J-*lg/ml* of diet. EC<sub>50</sub> values ranged from 1.19 (Bhatinda) - 26.4 J-*lg/ml* (Baruch) of diet for *cry1Ac*. Log dose probit assays with *cry1Ac* indicated that LC<sub>50</sub> against pink bollworms ranged from 0.02 J-*lg/ml* (Jalgaon) to 0.2 J-*lg/ml* (Dhule).

Diagnostic dose bioassays with *cry1Ac* were conducted for populations of pink bollworm collected from different locations of India on Bt and non Bt cotton fields using diagnostic doses viz. 10 ppm, 1 ppm and 0.1 ppm. Cent per cent mortality was recorded over the control except for population from Jalgaon. Jalgaon non-Bt population at 10 ppm and more than 50 per cent mortality at 1 ppm in non Bt population but PBW populations collected from Dhule, Nandurbar and Jalgaon on extended BG less than 100.00 per cent mortality over control was recorded at the diagnostic dose of 10 ppm (Table 8).

Table 8: *Cry1Ac* diagnostic dose bioassays with F<sub>1</sub> generation collected on Non Bt

States	% Corrected mortality		
	10 ppm	1 ppm	0.1 ppm
Punjab	100.00	78.65	45.32
Haryana	100.00	78.57	7.14
Rajasthan	100.00	100.00	83.97
Gujarat	100.00	100.00	15.38
Maharashtra	90.00	61.86	16.71

## Sirsa

Dissemination of insecticide resistance management programme

The program of dissemination of insecticide resistance management was taken up in Haryana in the districts of Sirsa and Hisar. Fifteen villages were selected in each district. A total of 2540 ha and 5215 ha area was covered. This was followed by engagement and training of field workers about insecticide resistance management strategies. The weekly data on insects, diseases and beneficials was recorded in villages and used for decision making interventions. Major emphasis was given on the management of resistance in sucking pests against insecticides and bollworms against cry toxins and farmers were encouraged to grow refugia around Bt cotton hybrids. This was followed by collecting information on insecticide consumption and number of sprays. Information on the seed cotton yield and cost of cultivation was also gathered to arrive at Cost Benefit ratios of IRM and non-IRM farmers.

There were 3.59 and 2.38 sprays by IRM farmers as compared to 4.22 & 3.82 sprays in non IRM farmers fields. Insecticide consumption was reduced upto 27.1 and 31.4% respectively in IRM farmers of Sirsa and Hisar. There was saving from insecticides by around Rs. 1100/- per ha and an improvement in yield by around four q/ha of IRM farmers showing an overall C:B ratio of 1:3.72 and 1:3.65 over 1:3.00 and 1:2.93 at Sirsa and Hisar respectively. Net profit in IRM was Rs 84087 per ha and in non IRM is Rs. 64038 per ha in Sirsa. The net profit per ha of IRM farmers over non IRM was Rs. 20049. Whereas net profit in IRM was Rs. 84747 /ha and in non IRM is Rs. 64294 /ha in Hisar. The net profit per ha of IRM farmers over non IRM was Rs. 20453.

Validation of efficacy of neonicotinoid seed treatment against sucking pests of cotton in multilocation trials (carried out in network mode)

## Nagpur

Jassid incidence did not cross ETL upto 50 DAS. There were no significant differences between the treatments at 15 DAS. Although the pest did not cross ETL at 25 DAS, Gaucho and Cruiser untreated plots harbored statistically significant higher leafhopper nymphs than the treated plots especially in the susceptible Bt genotype RCH 2. This was not true of RCH 20 BGII (a moderately tolerant genotypes) wherein the treatments were on par. The treated plots 40 DAS in RCH 2 BGII and RCH 2 non Bt continued to be statistically superior. Leafhoppers crossed ETLs 50 DAS and the treated and untreated plots were on par with each other, genotype wise.



The genotypes tested would have done well in the absence of seed treatment this year as the pest did not cross ETL till 50 DAS. Neonicotinoids as seed treatment was effective upto 50 DAS in central India as far as leafhopper nymph numbers were concerned.

#### Gujarat

Cotton leaf hoppers did not cross ETLs upto 56 DAS in any of the treatments. Neonicotinoid (both Imidacloprid and Thiamethoxam) treatment conferred protection to RCH 2 BGII and RCH 20 BGII upto 56 DAS as can be observed from the data. There were no statistically significant differences 63DAS between the treatments despite the leafhopper population having crossed ETLs. There were statistically no significant differences between the incidence of aphids and whiteflies between the treatments and their numbers were low numerically.

When yields were compared across genotypes Bts clearly out yielded their counterparts by about 300 kg/ha and seed treatment gave an advantage of approximately of 307 kg/ha and 222 kg/ha with Imidacloprid and Thiamethoxam respectively. In Gujarat seed treatment still is advantageous to Bt cotton for protection against sucking pests.

#### Guntur

Leafhopper nymphs did not cross ETLs till 45 DAS. Observations on leaf hopper incidence were recorded up to 80 DAS. The incidence of leafhopper nymphs on treated genotypes was significantly lower than on the untreated in the susceptible genotype RCH 2BGII. The treated and untreated genotypes were on par with each other just 10 DAS. Treated plots harbored statistically significant lower numbers of nymphs although they did not cross ETL upto 37 DAS as compared to untreated plots. However at 45 DAS treated and untreated plots were statistically on par with each other with the pest having crossed ETL. The pest crossed ETL at 45 DAS at Guntur and neonicotinoids were ineffective in checking the outbreak. The jassid injury grade ranged between 1-2 in the treated and untreated plots of susceptible, moderately susceptible and highly susceptible genotypes upto 45 DAS. Only seed treatment did not contribute to yield increase in any genotype as the crop succumbed to leaf hopper damage in

treated and untreated plots 45 DAS.

#### Sirsa

Development and validation of IPMIIRM strategies for Bt cotton hybrids carrying different events against sucking pest complex, observations the population of leafhoppers ranged between 2.78 to 3.18 average whitefly ranged between 4.64 to 6.36 and average thrips population recorded in different hybrids was 4.09 to 5.84 per 3 leaves under IPM practices. Under RPP (Recommended package of practices) the leafhopper population recorded between 2.84 to 4.11, whitefly between 5.11 to 5.89 and thrips between 4.58 to 7.73 per 3 leaves in various hybrids sown. The net profit gained was Rs. 26865 in IPM where as it was Rs. 21656 in RPP with a cost: benefit ratio of 1: 2.00 and 1:1.56 respectively in IPM and RPP plots, respectively. Increase in net profit in IPM over RPP was 19.39%.

#### Bioassay with *cry1Ac*, *cry2Ab* and *cry1C* to *Earias vitella*

During 2011 a total of 42 bioassays were conducted. Bioassay conducted with *cry1Ac* indicated that larval population of spotted bollworm collected from Sirsa on 13/07/11 was recorded with highest LC<sub>50</sub> value of 2.026 IJg/ml and larval population collected from Sriganaganagar on 17/08/11 was with lowest LC<sub>50</sub> value of 0.125 IJg/ml. Bioassay with *cry2Ab* recorded that larval population of spotted bollworm collected on 13/11/11 from Sriganaganagar demonstrated highest LC<sub>50</sub> value of 712.596 IJg/ml and larval population collected from Sirsa on 13/07/11 was with lowest LC<sub>50</sub> value of 0.36 IJg/ml. Bioassays with *cry1Ac*, *cry2Ab* and *cry1C* on *Helicoverpa* collected from Sirsa on 24/4/11 showed a LC<sub>50</sub> value of 1.001 IJg/ml, 168.223IJg/ml and 3.729IJg/ml respectively.

#### Insecticide induced resurgence

The experiment conducted for insecticides induced resurgence for commonly used insecticides against sucking pests revealed that in cumulative of 10 sprays no resurgence in population leaf hopper, whitefly and thrips was recorded, but in some of the treatments like Fipronil (at recommended dose) resurgence of whitefly was recorded after individual spray applications.

