

## **Crop Improvement**

#### Genetic Resources of Gossypium

- Fourteen perennials including nine of Gossypium barbadense and five of Gossypium arboreum were collected through exploration and collection surveys conducted in various districts of Maharashtra, Tamil Nadu and Andhra Pradesh.
- Among 838 accessions (including 38 exotic accessions) of *G. hirsutum* and 582 germplasm of *G. herbaceum* evaluated for economic and fibre quality traits, nine long linted exotic accessions with staple length 30.2–31.8 mm and 22 accessions for high fibre strength (26.3–28.4 g/tex) were identified and documented.
- One hundred and nineteen land races / perennials which include 80 Gossypium arboreum, 13 G. herbaceum and 26 Gossypium barbadense were evaluated for fibre quality. Morphological characterization including DUS characterization was completed for 19 germplasm accessions.
- Twenty four unique G. arboreum accessions were identified through diversity analysis using MEGA 6 software and established in the "Perennial Species Garden" at Panjari farm, ICAR-CICR, Nagpur.
- Seven thousand and fifty one (G. hirsutum 4400, G. arboreum – 2063, G. herbaceum – 569 and wild species – seeds and cuttings – 19), 14 perennial and landraces (Gossypium herbaceum) were distributed to breeders/ scientists of CICR, State Agricultural Universities for utilization.
- Twenty six wild species were conserved in wild species garden. One new species (EC 669583), genetically distinct from others flowered under field condition.
- Five genetic stocks viz; CNA 405 (INGR 15005: Narrow leaf lobed and brown linted), CNA 407 (INGR 15024: Narrow leaf lobed, spotted petal and light brown linted), CNA 407 (SLP) (INGR 15025: Spotless petals and brown linted), CNA 1051 (INGR 15016: Distinct yellow top leaves virescent mutant) and CNH 1102 (INGR 15015:

High ginning outturn, bacterial leaf blight resistance, long staple and high strength) were registered with ICAR – NBPGR, New Delhi.

- A set of 1100 accessions of *G. arboreum* evaluated for various morphological parameters.
  Principal Component Analysis (PCA) based on qualitative traits revealed traits *viz.*, boll shape, leaf shape and bract size distinguished all genotypes in higher order than the other traits.
- Three hundred and ten *G. barbadense* germplasm lines were maintained during 2015-16. Five new germplasm lines *viz.*, NGB-555, NGB-556, NGB-557, NGB-558 and NGB-559 were evaluated. NGB-556, a hairy line, is moderately resistant to sucking pest. Of the eleven hairy germplasm accessions evaluated for wax content, EC-18 had the maximum amount of wax (343 µg/cm<sup>2</sup>) followed by HAG-02 (305 µg/cm<sup>2</sup>).

#### **Genetic Improvement for Target Traits**

- Based on plant width and number of monopodia, N 170, Pratima, CSH 3178, Anjali, CNH 09-7, Arogya, DSC 99, CNH 1102 and AKH 8828 were identified as most promising for compact plant architecture. KC-3, G.Cot 20, CNH 2034, NISC 44, AKH 081, JK-4, CNH 409-9, CNH 07-34, ADB 532 and NH 545 were found promising for jassid tolerance scoring lowest jassid grade and lower nymph number. Based on per cent open bolls at 150 DAS, Pratima, CNH 409-9, CNHO 12, Sahana, IC 359478, Arogya, G.Cot 18, JK-4, CNH 2028 and F1378 were identified as promising for earliness.
- Germplasm lines with high fibre strength were selected for developing a 10 × 10 diallel crosses without reciprocals. General combining ability variance (GCA) was higher than specific combining ability (SCA) for upper half mean length, uniformity index, micronaire and fibre strength indicating the predominance of additive gene action in the inheritance of all these traits. CCH 7122 and Suraj exhibited significant GCA effects for all fibre traits except micronaire.
- Two crosses viz; CSH-3119-10-30-60 (2960 kg/ha seed cotton, 1067 kg/ha lint yield and fiber strength 27.4 g/tex in HVI mode) and MMO.3 (39-

## **ICAR-CICR**

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2-5)-3114-10-64 (2976 kg/ha seed cotton, 1020 kg/ha lint yield and fiber strength 26.7 g/tex) were early and yielded higher than the check H-1226.

- Based on the average performance of three years, seven progenies viz; P-68, 69, 70, 84, 139, 164 and 184 possessed GOT of more than 40%.
- Of the fourteen cultures tested, two genotypes CISA 10 and CISA-33-8 yielded more than 1700 kg/ha and have promise for spinning. CISA-6-350, CISA-6-295 and CISA-33-7 were having UHML (mm) >24.0 mm and strength >24.0 g/tex in HVI mode.
- Sixteen genotypes were evaluated under nonspinnable category CISA 310 and CISA 614, the seed cotton yield ranged from 2218 to 2641 kg/ha. Three genotypes CISA-6-2 (2641 kg/ha), CISA-33-3 (2593 kg/ha) and CISA-6-123 (2583 kg/ha) recorded higher seed cotton yield than local checks CISA 310 (2466 kg/ha) and CISA 614 (2570 kg/ha).
- From 15 stabilized advanced generation (BC<sub>3</sub>F<sub>9</sub>), four genotypes viz., CCB-51 (1633 kg/ha), CCB-64 (1572 kg/ha), CCB-66 (1541 kg/ha), and CCB-72 (1500 kg/ha) were significantly out yielded the check variety Suvin (910 kg/ha) and had better ELS fibre quality.
- Seven compact genotypes with super okra leaf were evaluated with three check varieties, Suraj, Anjali and Supriya under spacing of 30 cm x 10 cm. The culture Surabhi x MM02-19-1-8-2 was the highest yielder (2071 kg/ha). The culture MCU 13 x VNWH 1-7-2 was the best for fibre quality (2.5% span length of 33.1 mm and bundle strength of 23.0 g/tex in ICC mode).
- From F<sub>6</sub> generation, 50 single plants selected for further screening showed maximum boll weight of 5.4 g; similarly, 50 single plants selected for further screening showed the maximum lint index of 6.6 g. Apart from the above, 39 single plants selected for further screening showed maximum GOT of 47.1%.
- Based on the effect of drought stress on leaf temperature, photosynthetic rate, transpiration rate, NR activity, prolein, protein content, reducing sugar, phenol, amino acid and chlorophyll content, four genotypes namely DTS
  4, DTS 6, DTS 9 and NHP 3 were identified for drought tolerance.
- One hundred and fifty identified tolerant

accessions were evaluated for 48 days under waterlogged condition along with a control and morphological adaptations like lenticels and adventitious roots were prominently observed in tolerant accessions.

#### Heterosis Breeding

 Based on evaluation of six GMS G. arboreum hybrids for seed cotton yield with two check hybrids AAH 1 and CICR 2, three hybrids CISAA 15-38, CISAA 14-31 and CISAA 14-32 were identified for higher seed cotton yield.

#### Introgress Breeding

- In *G. arboreum* based introgressed derivatives, genotypes with fibre length from 23.2 to 26.5 mm and fibre strength from 20.0 to 23.2 g/tex were selected.
- Seed multiplication of colored cotton genotype MSH-53 (Vaidehi-95) was taken up and 650 kg seed was produced.
- Various breeding approaches were followed to develop populations / genotypes resistant to cotton leaf curl virus (CLCuV) viz; crossing of G. hirsutum lines with G. arboreum lines, colchicine treatment of G. arboreum and G. hirsutum genotypes, development of synthetic tetraploids of cultivated cotton by crossing G. arboreum and G. herbaceum with G. raimondii, crossing G. hirsutum lines with PAIG lines, crossing G. hirsutum lines with the registered genetic stock IS-376/14/21 reported to possess anatomical features of desi cotton in G. hirsutum background. Crossed bolls were harvested in all the approaches except G. herbaceum × G. raimondii crosses.

## Varietal Development and Multi-location Testing

- Identification proposal of CSH-3075 for cultivation in North Zone under HDPS and long staple high strength culture CCH 4474 for South Zone states under irrigated conditions were submitted.
- The G. hirsutum cultures CSH 2920 and CSH 2932 in Br 02(a) National trial, CSH 3232 and CSH 3088 in Br 03(a) Zonal trial, GMS based G. hirsutum hybrid CSHG 2911 in Br 05(a) National trial, G. hirsutum hybrid CSHH 2012 in Br 05(a) Zonal trial, compact cultures CSHH 3158, CSH 5038 and CSH 6109 in Br 06(a) National trial, compact cultures CSH 3113, CSH 3178 and



CSH 73 in Br 06(a) Zonal trial, *G. arboreum* culture CISA-6-123 in Br 22(a/b) National trial, CISA-6-2 in Br 24(a) zonal trial and *G. arboreum* GMS based hybrids CISAA 14-31 and CISAA 14-32 in Br 25(a/b) National trial were sponsored/promoted during 2015.

- One *G. arboreum* GMS based hybrids CISAA 14-31 was promoted to zonal trial Br 25a and *G. arboreum* culture CISA-6-2 ranked 1<sup>st</sup> in zonal trial was further retained for large scale multilocation testing during 2015.
- The G. hirsutum hybrid CSHH 2012 was recommended for agronomy trial based on overall performance in AICRP on cotton trials during 2015.
- The culture Surabhi x M5Z2 18-5 (CCH 15-1) was tested in initial evaluation trial of *G. hirsutum* varieties under irrigated condition recorded an upper half mean length of 27.1 mm in north zone locations, 31.8 mm in central zone locations and 32.5 mm in south zone locations. It exhibited the highest bundle strength of 30.7 g/tex, 32.8 g/tex and 36.1 g/tex in HVI mode in north, central and south zone locations, respectively.
- The culture MM 03-40-4-3-1 (CCH 15-4) tested in initial evaluation trial of *G. hirsutum* varieties under rainfed condition in central and south zone locations recorded an upper half mean length of 31.2 mm in central zone locations and 30.9 mm in south zone locations with bundle strength of 30.5 g/tex and 31.5 g/tex in HVI mode respectively.
- Compact super okra culture Surabhi x M5Z2 4-2 (CCH 15-5) was tested in AICRP trials under irrigated conditions and cultures Surabhi x MM 02-16-5-2-3 (CCH 15-7) and MCU 13 X VNWH 1-7-2 (CCH 15-8) tested in rainfed situation were promoted for evaluation in coordinated varietal trial in central and south zone locations. All these cultures exhibited excellent fibre quality in the initial evaluation trial.
- An elite promising *barbadense* genotype CCB-29 ranked third and fourth in yield in central and south zone, respectively. With regards to quality parameters it ranked fourth in both the zones. In initial yield evaluation trial of AICRP (Br.12a), CCB-11a had ranked fourth in yield and third in fibre quality parameters.

#### **Molecular Breeding**

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

- 4417 SSR markers were screened for parental polymorphism. Seven hundred two were found to be polymorphic / informative which accounts for 15.9% polymorphism with the parental lines.
- Genomic DNA of 188 progenies was used for genotyping using 520 informative polymorphic SSRs. Finally, 227 anchor marker loci were used for making an integrated SSR-SNP genetic linkage map of AD genome (*G. hirsutum*).
- Genotyping of 172 RIL progenies with 2730 SNP markers done and data analyzed. The preliminary analysis showed 29 linkage groups with 1.31 cM distance between the consecutive markers.
- Genotyping data for 2730 SNPs (mapped on 29 linkage groups) were used for QTL mapping. Ten QTLs were identified for staple length, tenacity and micronaire. For staple length, two QTLs were identified each on LG 18 and LG\*4 with LOD score ranging from 3.72 5.15 and phenotypic variance (R<sup>2</sup>) from 8.7 10.6%. Similarly, for tenacity, total six QTLs were identified, two on LG 3 and one each on LG 10, LG 4, LG 5 and LG 8, respectively. For micronaire, two QTLs were identified on LG 18 and LG 4, respectively with LOD score for these two QTLs ranged from 4.1 to 5.8 and phenotypic variance (R<sup>2</sup>) from 9.8 to 14.3%.
- A set of 11 F<sub>1</sub> crosses were attempted among resistant/tolerant parents for gene pyramiding for CLCuD that includes CLCuD tolerant lines identified from the previous year and also tolerant Bt BGII hybrids.
- DNA was isolated from BC<sub>1</sub> plants and screened using SSR marker CIR 246. BC<sub>1</sub> plants were categorized as resistant and susceptible and compared with 101-102B which is universal resistant for BLB having 146 bp. Genomic DNA of individual F<sub>2</sub> and BC<sub>1</sub> plants was extracted and subjected to screening using SSR markers BNL 3279 and NAU 2152 for nematode resistant. The marker positive plants in BC<sub>1</sub> were backcrossed with recurrent parent Suraj to obtain BC<sub>2</sub> seeds.
- Sixty additional SSR markers screened for 48 public sector released tetraploid cotton varieties (*G. hirsutum* and *G. barbadense*) and 150



markers for 24 *G. arboreum* varieties. Twelve robust markers were obtained for *G. hirsutum*. Three markers found specific for *G. barbadense* varieties, Suvin and Sujatha, were further confirmed in 20 germplasm lines of *G. barbadense*.

The parents of five hybrids (4 G. hirsutum and 1 G. arboreum) and two G. arboreum varieties were screened using 117 SSR markers with high PIC values. Distinctly polymorphic markers were identified among male and female parent of each of the five hybrids.

### Biotechnology

- G. hirsutum cv. Coker 310 plants carrying Cry1Ac (Tg2E-13 event) were received from Delhi University after Memorandum of Understanding and Material Transfer Agreement.
- Six elite cotton varieties viz, AKH 081, Anjali, CISH 3178, LRA 5166, NH 615 and Suraj were used as recipients in marker assisted transgene introgression breeding and crossing between donor and recipients were attempted and hybrid seed of each cross was produced in the contained glasshouse facility.
- Analysis of real time data using geNORM, Norm finder and Best keeper algorithm aided in identification of RPL32 in *H. armigera* and Actin in *Gossypium arboreum* as suitable normalilzer for gene expression analysis of developmental stages.
- New event of Cry2Ab1Ac : Chitinase gene were generated by *in-planta* transformation methods. In all 111 T<sub>0</sub> bolls were harvested.
- Novel genes/transcription factors having probable functions in somatic embryogenesis were traced.
- 2831 sheet tip explants were subjected to transformation with CICR cry fusion gene. The 75 Kanamycin resistant *ex-plants* were regenerated into putative transformants by direct shoot organogenensis.
- Putative transformants for cotton leaf curl virus resistance using *G. hirsutum* cvs HS 6, H 777 and F 846 were screened for the presence of gene.
- The role of five identified candidate genes (Ghces AI, Ghces A3, Ghces A7, Ghcobl4 and Ghfla3) in fibre development were used to validate lintless mutant (MCU 5) and its

counterpart wild type cotton genotype. The candidate genes are radically expressed in wild type compare to mutant.

#### **DUS Characterization**

49 new candidate varieties of which 12 were VCK and one FV; 68 new candidate varieties of G. hirsutum for second year of testing and 19 each of EDV and Initial varieties were subjected to DUS characterization.

#### Seed Production and Quality Improvement

- Around 2235 kg seeds of various categories were produced. Resource of around Rs. 6 lakhs was generated through the sale of these seeds or its by-products.
- Exogenous application of plant growth hormones and other chemicals on seed yield and quality of cotton variety Suraj indicated that the total seed cotton yield was significantly higher in spermine (0.1 mM) followed by putrescine (4.0 mM) (22.15 q/ha). The fuzzy seed yield was highest in spermine (0.1 mM) (14.0 q/ha) followed by putrescine (4.0 mM) (13.92 q/ha) and spermidine (0.1 mM) (13.74 q/ha). Highest seed germination was recorded in spermine (0.1 mM) followed by salicylic acid (1.0 mM) while vigor in terms of seedling length was highest in 5-sulphosalicylic acid (0.5 mM) followed by putrescine (2.0 mM) and spermine (0.5 mM).
- To improve the seed and boll setting efficiency in cotton, highest boll setting of 85.5% was observed with hand pollination followed by treatment with CICR consortium (83.0%) and NPK 2% (82.3%). The seed setting efficiency (93.3%) and yield (3375 kg/ha) was also higher in hand pollination.

## **Crop Production**

### High Density Planting System (HDPS)

American and *desi* cotton varieties / cultures were evaluated under HDPS. Among the American cotton varieties, the top five early yielders (yield at 165 DAS) at 60 x 10 cm spacing were LRK 516, Suraj JT, CNH 1111, CSH 3075 and CNH 09-4 at Nagpur. At Sirsa, CSH 3075 at 67.5 x 20 cm yielded 47% more than the Bt hybrid in large plot trials. AKA 8, RG-540, CNA 375, Phule Dhanwantary, CNA 418, MDLABB and CISA 6-256 yielded more than 2000 kg/ha among the *desi* cotton varieties. Phule Dhanwantary was early and compact in nature.

Productivity of Phule Dhanwantary could be enhanced with closer row spacing of 30-45 cm and 10-15 cm intra plant spacing. In general, high density had no influence on pest and disease incidence. Clorantraniliprole 18.5 SC and Indoxacarb 14.5 SC were most effective against bollworm (*H. armigera*) in terms of lowest per cent of fruiting bodies damage under HDPS.

#### Weed Management

 Cover crops as an alternative weed management strategy was evaluated at Nagpur and Coimbatore. Effective cover crops identified were sunnhemp, sorghum, sesame, bajra and desmodium at Nagpur. At Coimbatore, forage cowpea effectively smothered weeds and had the least weed density.

#### **Nutrient Management**

 To address nutrient stress during the crop season, a gadget to detect N stress, nondestructively was developed. Field evaluation of the gadget indicated a correlation coefficient of 0.795 for N content and greenness values.

#### Soil Biology

Cry toxin was not in the detectable range in the rhizosphere soil samples of the Bt cotton hybrid plots. Microbial parameters such as beneficial micro flora, soil microbial, biomass carbon and enzyme activity (acid and alkaline phosphatase, urease, dehydrogenase, fluroscent di-acetate hydrolysis) peaked at flowering stage of the crop.

#### Abiotic Stress Management

- Leaf reddening : DNA laddering studies conclusively indicate that leaf reddening is not a death signal. Leaf reddening could be managed by spray of ICAR-CICR Nutrient Consortia, IBA at square initiation or 2 % DAP at 80 and 110 days after sowing.
- Drought : Phenotyping of 104 germplasm was done during summer of which 14 were identified as drought tolerant and 7 as susceptible. IC 357406 and Nagpur 9 were highly tolerant to drought.
- Water logging : Phenotyping of 2700 germplasm lines was done and 211 were short listed as tolerant.

#### **Cropping Systems**

 Twenty legumes were evaluated as intercrops for compatibility and N fixation. Rice bean and cluster bean were ideal intercrops in Suraj, an American cotton variety. In the *desi* cotton inter cropped with groundnut, cluster bean, soybean, alfalfa and mothbean had highest productivity. At Coimbatore, legume intercrops except vegetable cowpea and Dolichos, enhanced seed cotton yield.

#### Water Management

 Structured water irrigated plots had higher cotton yield than the bore well irrigated plots.

#### Paper Tube Nursery

 Cotton seedlings raised in paper tube rolls when transplanted in the field established quickly and yielded more than the direct sown cotton.

#### **Cotton Mechanization**

 Cotton harvester developed in collaboration ICAR-CICR-CIRCOT and Mahindra & Mahindra was field tested. The machine had a picking efficiency of 98% with a header loss of 11.5% to 12.5%. Field capacity of the machine was 2h / ha.

#### e-Kapas

 More than 28,000 farmers were registered by ICAR-CICR in 2015-16 under e-Kapas. With more than 10.5 lakh voice messages sent in Marathi, the local language with delivery success of 72%.

## **Crop Protection**

#### **Pest Dynamics**

- Seasonal pest population dynamics data was generated under protected and pesticide free conditions on DCH 32. At Nagpur, hot spots of jassid, thrips and whitefly infestation were identified in >20% villages during the season. In Sirsa, whiteflies infestation were above ETL throughout the season on the 4 genotypes studied. 23 alternate hosts of whitefly were recorded during off-season.
- All the three bollworms viz., Helicoverpa armigera, Erias insulana, Pectinophora gossypiella were seen to damage cotton. Unusual early incidence of pink bollworm was recorded this year.

#### **Novel Technologies**

- Gossypol detoxifying gene cyp6ae14 in T/A cloning vector was successfully subcloned in to bacterial expression vector pET28c and confirmed through restriction digestion.
- Cotton seed cake (deoiled) treatment with

No.

*Enterobacter cloacae* subspp. *cloacae* was found effective in reducing total gossypol content from 1.24% in control to 0.9% with treatment.

- Data regarding the suction efficacy of the ICAR CICR Whitefly Adult Suction Trap developed for whitefly management was generated and, the reduction in whitefly adults was up to 40% during the peak period of activity of whitefly adults. This suction trap is power operated, shoulder mounted portable and adjustable and helps in suction of whitefly adults available on the underside of the cotton leaves without any harm to the natural enemy flora and cotton crop itself. The trap is in the process of patenting.
- Application of ethylene inhibitors has no significant effect on pest population and cotton yield. Wilted plants were found emit significantly higher ethylene than healthy plants and treatments involving Cobalt chloride and Bavistin were not effective in recovery of wilted plants.
- Protocol was developed for production of Bt toxin on novel low cost solid medium, in cooperation with the Ginning Training Centre, Nagpur.
- Reverse Transcription loop mediated Isothermal amplification (RT-LAMP) protocol was developed and standardised for diagnosis of Tobacco Streak Virus of Cotton and Soybean.
- Five sprays of 4 exogenous waxes along with guar gum applied helped in 1.38-12.17% reduction in whitefly incidence.

#### **Biological Control**

- During surveys conducted to record diversity of Mealy bugs in Maharashtra and Madhya Pradesh, 6 mealy bug species viz., P. solenopsis, N. viridis, M. hirsutus, F. virgata and P. marginatus, M. hirsutus belonging to Pseudococcidae family of order Hemiptera were recorded. P. solenopsis was the dominant species.
- Microcapsulation of *Bacillus thuringiensis* was attempted for improving efficiency as a biopesticides.
- Spraying of talc based formulation of Lecanicillium lecanii and Metarhizium anisopliae at the rate of 10 g/l significantly reduced the population of aphids, jassids and whitefly under field condition. Field evaluation of two talc based formulation of L. lecanii and M. anisopliae under AICRP on cotton centres revealed that they were safer to natural enemies (spiders and

coccinnellids) and reduced the sucking pest population.

- Safety of biopesticides to natural enemies was studied. Spraying of Coccinellid beetle, *Cheilomenes sexmaculata* with spore suspension of *Lecanicillium lecanii* at 10<sup>6</sup>-10<sup>8</sup> spores/ml in direct and indirect (residual toxicity) method was found to be safe for grubs and adults under lab and field condition. Soil application of *L. lecanii* at field even at ten times of field recommended dose was found safe to earth worm.
- Spraying of biopesticide formulation of *L. lecanii* with corn oil and skimmed milk powder recorded maximum persistence of spores on cotton leaves upto four days. Among 14 UV protectants tested, Starch, Tinopal and skimmed milk powder recorded more than 95 percent germination at 24 hours after inoculation.

#### **Host Plant Resistance**

- Single plant selections were made and raised as boll to row for development of Jassid tolerant Suraj. Jassid tolerant Suraj had similar fibre properties as Suraj.
- Inter-specific variation in volatile emission in 3 species of Gossypium in response to leaf hopper, caterpillar and mechanical damage was investigated. Ten genes, ERF 1, 2, 3. TPS 1,2,3 Alpha pinene, Lipoxygense 1, Allene oxide synthase 6, Methyl jasmonate transferase, involved in the signal transduction pathway were characterized using RT PCR to understand their role in volatile emissions that reportedly play a well defined role in inter plant communication in cotton.
- G. arboreum is tolerant to leaf hoppers by virtue of up-regulation of the methyl transferase gene (that governs conversion of jasmonic acid to methyl jasmonate) and this mechanism is absent in G. hirsutum.
- Volatiles emitted from pink bollworm damaged flower vis a vis undamaged flower were studied. Relative abundance of β Caryophyllene, methyl ester of pentadecanoic acid and linolenic acid, the precursor of jasmonic recorded was higher in PBW infested flowers than normal flowers.
- Role of epicuticular wax on whitefly and CLCuD incidence was investigated. Germplasm No. IC357886 exhibited higher wax content throughout the canopy and on both upper and

lower surfaces and recorded lowest whitefly incidence. Germplasm No. IC358823 had very low wax content across the canopy on both adaxial and abaxial sides and this line recorded highest whitefly incidence.

#### **Resistance Monitoring**

- Protocol was standardised for bioassays with pink bollworm using green bolls. Observations on exit holes, mines on epicarp, number of larvae and per cent locule damage were recorded 21 days after release. Neonates of resistant culture caused 67-100% loculi damage in non Bt control bolls while 25-50% loculi damage was caused on BGII green bolls.
- Insecticide resistance monitoring carried out against eight leaf hopper populations from Central and South India with four insecticides namely Flonicamid, Acetamiprid, Thiamethoxam and Imidacloprid. The resistance ratios of Flonicamid, Monocrotophos, Acephate, Imidaclorpid, Acetamiprid, Thiamethoxam ranged from 1-16, 1-7.7, 1-8.9, 1-19.6, 1-331 fold, respectively.
- Flubendiamide was the most effective insecticide (0.005 mg/L and 0.004 mg/L) against Nagpur and Wardha populations of *H. armigera*. Cypermethrin was the least effective.
- Cry toxin resistance monitoring recorded a variability of 50 fold and 78 fold in the LC<sub>50</sub> and EC<sub>50</sub> of Cry1Ac against *H. armigera* populations of Maharashtra and Andhra Pradesh.
- Cry2Ab did not cause significant dose dependent mortality on one day old larvae of *H. armigera*. A variability of 247 fold was recorded in the EC<sub>50</sub> values of *H. armigera* populations from Maharashtra and Andhra Pradesh to Cry2Ab alone.
- ICAR-CICR has been monitoring bollworm resistance development to Bt cotton during the past 15 years. During 2015-16, resistance monitoring was carried out with pink bollworm larvae collected from 46 districts of cotton growing states across the country. Bioassays were conducted with pink bollworm populations collected from 39 districts. Results showed that pink bollworm populations in 15 districts have developed resistance to Bollgard-II (Cry1 Ac+ Cry2Ab); 20 populations were resistant to Bollgard (Cry1Ac) and 18 populations were resistant to Cry2Ab.

- In Gujarat, pink bollworm infestation was observed in flowers of different Bollgard-II hybrids ranging from 0 to 67.0 % in different districts of Gujarat. In November 2015, pink bollworm infestation was 72.0% in Surat, 64.0 to 92.0 % in Bharuch, 48.0 to 96.0 % in Vadodara, 50.0% in Ahmedabad, 56.0 to 84.0 % in Bhavnagar, 60.0 to 72.0 % in Amreli, 76.0 to 88.0 % in Junagadh and 60.0 to 64.0 % in Rajkot. In the third week of January 2016, the per cent infestation on Bollgard–II in Vadodara 72.0 %, Surat 92.0%, Anand 82.0% Surendranagar 92.0% Ahmedabad 100%; Amreli 100%, Rajkot 64.0%; Bhavnagar 56.0% and Junagadh 100%.
- In Maharashtra, the intensity of pink bollworm was more in irrigated tracts of Khandesh region as compared to Vidarbha. Infestation of pink bollworm in green bolls of third-fourth picking on Bollgard-II cotton was 100% in Khandwa. In Andhra Pradesh, all the Bt hybrids were found to be susceptible to the pink bollworm. In Telangana by the second week of December 2015, the crop was terminated in 90% of the area in the state. Pink bollworm infestation in green bolls of the 2-3<sup>rd</sup> picking in residual crop in Adilabad was 52.0%-96.0%, Khamam 32.0%-100%, Warangal 60.0%-100% and Karimnagar 68.0%-96.0% respectively. Pink bollworm infestation in green bolls of Bollgard-II was 20.0% in Coimbatore and 24.0% in Srivilliputtur. The infestation of pink bollworm in green bolls of Bollgard-II in Raichur was 48.0%-92.0% while in Dharwad it was 8.0%.
- Monitoring for pink bollworm (PBW) resistance was done in North Zone during 2015-16 where 5 districts (Faridkot and Bhatinda in Punjab; Sriganganagar in Rajasthan; Hisar and Sirsa in Haryana) were monitored for recovery of PBW larvae through dissection of green bolls collected at various stage of crop growth. PBW larvae were not recovered in RCH 650 BGII from any location. But in non Bt genotypes, average recovery(%) of PBW larvae ranged from 8-20.0, 10-20.8, 11.7-25.8 and 13-44.0 at 120, 140, 160 and 170 days after sowing, respectively, indicating pink bollworm activity in North India.
- The most commonly used insecticides with label claim for whitefly on cotton were studied for their resistance status in whitefly adult populations during 2015-16 from the four different locations. Out of the four locations studied (Sirsa, Sriganganagar, Hisar, Mansa), the Hisar

population was found highly resistant. The population from Nagpur was taken as susceptible population since it was not exposed to these insecticides during the season.

- The whitefly has acquired resistance to almost all insecticides and the resistance ratio varied from 98-1400 folds for Bifenthrin 10EC, 14-137 for Dinotefuran 20SG, 60-131 for Acephate 75SP, 21-331 for Acetamiprid 20SP, 153-340 for Fipronil 5SC, 371-2237 for Triazophos 40EC, 51-706 for Buprofezin 25SC, 9-512 for Imidacloprid 17.8SL, 40-347 for Diafenthiuron, 2-19 for Chlorpyriphos 20EC, 1-2 for Thiamethoxam 30FS, 2-7 for Clothianidin 50WDG, 2-23 for Pyriproxifen and 1-6 for Flonicamid.
- Cry toxin resistance monitoring was carried against S. litura. LC<sub>50</sub> of Cry1C ranged from 14.90 to 19.19 ug/ml of diet for Spodoptera exigua population with highest C50 recorded with for population from Hisar (19.19 ug/ml of diet).
- Five hundred single pair matings of *H. armigera* populations collected from red gram/ chickpea in Maharashtra were set for Cry1Ac and 270 single pair matings were set up for Cry2Ab. In the F<sub>3</sub> generation 16 colonies demonstrated resistance to Cry1Ac and none of the colonies demonstrated larval mortality when exposed to the highest concentration of Cry2Ab.
- Single pair matings were set up with three hundred and thirty moths of which only 4 colonies demonstrated resistance to Cry1Ac in the F<sub>3</sub> generation in populations of *H. armigera* collected on redgram in Gujarat. Isofemale lines (70) from *Earias insulana* population of Mansa were screened at Sirsa for presence of rare resistance allele for Cry toxins.

#### **Novel Genes**

- For work on identification of new gene sources for pest management, full length *cyp6ae14* gene initially cloned in T/A cloning vector (pGEM-T easy vector) was further sub cloned in to bacterial expression vector pET28c and transformed in to *E.Coli* (αDH5) cells.
- The Enterobacter cloacae subspp. cloacae identified for glyphosate degradation was found to reduce total gossypol from 1.25 % (control) to 0.9% of treated cotton seed cake.
- Suitable reference genes were identified for quantitative real-time PCR normalization in *Helicoverpa armigera* and *Gossypium*

*arboreum.* RPL32 in *H. armigera* and Actin in *Gossypium arboreum* were identified as suitable normalizer for gene expression analysis of developmental stages.

### **Endophytes and Endosymbiots**

- Seventeen bacteria were isolated as endophytes from stem and leaf parts of cotton plant and based on its virulence, nine isolates were selected for field evaluation. Application of *Bacillus subtilis, Bacillus* sp. E13 and *Bacillus cereus* B1 recorded low population of sucking pests.
- Out of eight *Beauveria bassiana* fungal endophytes evaluated, isolates, Bb2, Bb4, Bb3, Bb1 and Bb8 recorded low population of sucking pests.

## Non-compliance of Refugia Guidelines

 Poor compliance of regulatory guidelines was recorded this year with some refuge seed packets carrying the Bt toxin.

### **Pest Disease and Nematode Management**

- Natural enemies, parasites and parasitoids of cotton pests was recorded. Endoparasitoid *Bracon lefroyi* was recorded on pink bollworm larvae collected on RCH 2 BGII of Surat district.
- Four sprays of the formulation containing Curcumin 1% was most effective in reducing reniform nematode population by as much as 80 % and doubling seed cotton yield as compared to control, in field studies.
- Lysnibacillus sphaericus was found to induce systemic acquired resistance against nematode Rotylenchulus reniformis.
- Soil application of talc based formulation of a native nematode antagonistic fungus; *Purpureocillium lilacinus* significantly reduced the reniform nematode population in soil and root and increased plant growth of cotton.

#### **Yield Modelling**

Software for cotton yield calculator was built using Java as the backend code and HTML as the front end code. The software is designed to run on all browsers present in Windows and Linux based operating systems. To integrate the backend resources with the browser, TOMCAT server installation which is an Open Source System was used. Software used in the development of this application have been Open Source Softwares. ECLIPSE was used to design the



Java code.

#### **Price Forecasting**

Price modeling for price forecasting was tried using Artificial Neural Network with EXCEL and SPSS software. The forecasted price using Excel for 2016 was Rs. 4200 to Rs. 4800 in the North, Central and South cotton growing zones. The forecast accuracy was to the tune of 91 to 96 per cent in all the cotton growing states except in Madhya Pradesh, where it was 89 per cent. Alternative model ANN through SPSS showed better accuracy to the tune of 92 to 99 per cent.

#### Dissemination of Pest Management Strategies

- IRM strategies were disseminated to 9849 farmers in 26011 acres in a total of 345 villages of 19 districts from 12 different states across India. In IRM fields, farmers sprayed an average of 3.86 sprays/ha as compared to 6.07 sprays/ha by non-IRM lames. Average yield of IRM and non-IRM fields was 16.75 and 15.12 q/ha. Implementation of the IRM resulted in yield increases estimated at a net additional benefit of Rs 6.76 crores and a saving on reduction in insecticide use accounting for Rs 1.89 crores, thus adding up to a total additional benefit of Rs 8.65 crores due to the project.
- A total of 1375 acre was covered for the implementation of IRM strategies in four villages

of Sirsa. The average yields obtained under IRM and Non IRM was 15.10 q/ha and 12.25 q/ha respectively. Reduction in number of sprays applied by IRM farmers was 20.27% recorded over Non IRM and 17.24% reduction in pesticide consumption was recorded.

- IRM strategies were successfully in eight districts of Tamilnadu namely Virudhunagar, Coimbatore, Vellore, Erode, Dharmapuri, Tirunelveli, Perambalur and Madurai districts covering 2000 acres and were successfully adopted by 667 farmers. Net profit of Rs. 51,525 and Rs. 38,026 was recorded in IRM and Non IRM fields respectively.
- Impact analysis of the dissemination of IRM strategies in eight districts of Tamilnadu indicated that an average number of sprays of 2.6 in IRM farmers fields was resorted to when compared 4.6 in Non IRM farmers' fields.
- Under Online Pest Monitoring and Advisory Services (NFSM-OPMAS) advisories were issued to the 1333 farmers belonging to 9 villages of district Sirsa and Fatehabad. Based on the data recording for pests and diseases from random and fixed locations advisories were issued to the farmers through E-Kapas network.
- Imidacloprid when used as seed treatment residues were nonelected in pollen and guttation fluid.



## Glimpses of Achievements



Variety CSH-3129 (*G. hirsutum*) notified for irrigated north zone



Variety CCH 2623 (G. hirsutum) notified for irrigated south zone



Tractor mounted cotton harvester



A gadget to detect Nitrogen deficiency in leaves



**ICAR-CICR Whitefly Adult Suction Trap** 



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