

# CROP IMPROVEMENT

## *Plant Breeding*

### Release of varieties

- The first successful interspecific hybrid variety Co 205 was released for cultivation way back in 1918 using the novel idea of using the wild species *Saccharum spontaneum* (as male with *S. officinarum* as female) in sugarcane breeding with the objectives of incorporating gene complexes for biotic and abiotic stresses and for high biomass production resulted in a tremendous level of useful genetic variability. This paved the way for revolutionising sugarcane agriculture and sugar industry not only in India, but also in most of the sugarcane growing countries of the world. This landmark achievement is unparalleled in the annals of crop breeding history. Most, if not all, of the present day varieties world-over have the Coimbatore bred canes in their genealogy.
- Further improvement was made by crossing Co 205 with other noble canes and their derivatives thereby succeeding in evolving a series of varieties with improved yield and quality through a programme called nobilisation.
- Co 312, released during 1928, became the most popular variety that dominated sugarcane agriculture in sub-tropical India for 3 decades and more.
- The spread of Co canes in foreign countries began when Co 285 was taken to Cuba and Florida for cultivation.
- Co 419 released in 1933 became the most popular variety in tropical India and was rightly hailed as the 'wonder cane' the world-over.
- Two outstanding varieties viz., Co 658 for Tamil Nadu and Co 740 for Maharashtra were released in 1940s. Co 740 is still a ruling variety in Maharashtra.
- Co 997 and Co 1148, released in 1950s, became the ruling varieties in Andhra Pradesh and North India respectively. Co 1148 remained the most predominant variety for over four decades in sub-tropical belt and was hailed the 'Wonder Cane of North India'.
- Co 62175 became the most sought after variety by jaggery farmers owing to its heavy yield.
- Co 6304, a heavy yielder, became the most important variety in Tamil Nadu replacing Co 419.
- Varietal evaluation conducted across seasons and different months of juice analysis helped in the identification of varieties with high sucrose levels such as; Co 7204, Co 7704, CoA 7601, CoC 671, Co 8336, Co 8338 etc.
- Performance of 67 cultivated varieties developed during different periods such as prior to 1960's, 1961-70, 1971-80, 1981-90 and 1991-2000 were studied and found a steady improvement for cane yield, CCS t/ha and sucrose % since the period from 1970-80, while sucrose % at 300 days showed improvement from the period prior to 1960s to 1991-2000. Mean sucrose % at 300 days of improved from 18.5% (varieties prior to 1960s) to 20.6% (varieties of 1991-2000).
- The National Hybridisation Garden established at the Institute resulted in development of varieties with location specific adaptation. CoC 671, CoJ 64, CoS 767, CoS 8436, CoLK 8001, CoLK 8102 and CoPant 84236 are some of the outstanding examples.

- A number of popular and promising varieties have been released by this Institute during the past and many promising ones are in the pipe-line.

### **Developing high sucrose genetic stocks**

- From a base population comprising of twenty Indian Co canes and fourteen commercial hybrids from other countries, improvement in sucrose content was obtained over two cycles of recurrent selection. The parental clones had an average sucrose of 19.40%. The cycle I hybrid progenies had juice sucrose values ranging from 17.0 % to 23.0% with a mean 21.6%. The cycle II progenies recorded average juice sucrose of 22.0% with 13.40% improvement over base population. After two cycles of selection, the progress made for sucrose content in comparison with the base population is substantial.

### **National Hybridization Garden**

- A large number of crosses have been carried out every year by participating centres. From the fluff supplied to them seedlings were raised, selections were made at different stages and promising clones and varieties have been identified through the final evaluation at All India Coordinated Project and Zonal Varietal Trails.
- Recently, an Elevated Hybridization Runways was installed in the field improved the efficiency of the crossing programme by simplifying the pollen collection and dusting operations. The scientists from the participating centres themselves could do the collection of male spikelets, effecting pollinations and collection of matured fluff, with limited assistance from other workers.

### **Botany**

- The work of Dr. Barber on the morphology and classification of Indian canes provided fundamental botanical knowledge necessary for cane breeding.
- The Institute pioneered in the description of sugarcane varieties under cultivation for easy identification. The publication was that of Dr.N.L.Dutt and J.Thuljaram Rao of 1947 and it was followed by a series of publications incorporating sugarcane varieties released for commercial cultivation from time to time

### **Genetics**

- The phenomenon of maternal inheritance with respect to gross appearance in habit and cane thickness was suggested, based on observations on reciprocal crosses involving *S. officinarum*, *S. spontaneum*, *S. robustum* and *S. barberi*. Maternal inheritance with respect to male sterility also was noticed in crosses involving *S. spontaneum* as pistil parents while reciprocals were male fertile.
- In *Sclerostachya x Narenga* hybrids some of the morphological characters of *Narenga* such as presence of sessile and pedicellate spikelets and circlet of hairs at nodes was found to be inherited as dominant character. The hybrids also were characterized by the *Sclerostachya* characters of absence of hairs on the leaf upper surface and nodal buds.
- The 4th glume of *Erianthus*, *Sclerostachya* and *Narenga* was observed to be inherited as a dominant factor in crosses with *S. officinarum*. Similarly in crosses of *S.*

*spontaneum* x *S. officinarum* and *S. spontaneum* x *S. robustum* the 4th glume of *S. spontaneum* was found to be inherited as a dominant character.

- A detailed investigation involving interspecific and intervarietal genetic stocks revealed the presence of both vertical (race specific) and horizontal (race non-specific) components for red rot resistance. It was also established that the level of horizontal resistance decreased with a decrease in the *S. spontaneum* chromosome complement present in the material.
- Study of large number of progenies involving resistant and susceptible parents established mendelian segregation for red rot resistance (conferring vertical resistance) in many of the crosses. It was found, however, that mendelian segregation could not explain fully the variability observed. Further investigations revealed that additive genetic variance (conferring horizontal resistance) accounted for nearly 50 % of the variation.

## Cyto-Genetics

- Transmission of 2n gametes from *Saccharum officinarum* (2n = 80) as female in crosses with *S. spontaneum* as male, but n + n transmission in the reciprocal cross was established beyond doubt by extensive cytological investigations at this Institute.
- Existence of polyploid series in *S. spontaneum* (2n = 48, 56, 64, 80, 96 and 112) was one of the most important findings of the Institute.
- It was established that there was en bloc elimination of chromosomes in some complex hybrids that accounted for the occurrence of progenies with unexpected chromosome numbers.
- Studies on the origin of *Saccharum* species postulated that North Indian canes have originated as a result of extensive hybridisation between *S. officinarum* and *S. spontaneum* in regions of Bengal, Bihar and Orissa. Detailed cytological investigations at the Institute suggested that the origin of *Saccharum*, *Narenga* and *Sclerostachya* could be from a common ancestor with 5 as the basic chromosome (x = 5).
- Monosomics were isolated from an intraspecific hybrid involving *S. spontaneum* Coimbatore (2n = 64) x *S. spontaneum* (2n = 64) from which several nullisomics and other aneuploids were produced. Triploid seedling of *S. officinarum* with 2n = 120 chromosomes was isolated through selfing.
- Intergeneric hybrids *Saccharum* x *Narenga* and *Saccharum* x *Sorghum* followed n + n transmission.
- Intergeneric hybrids involving *Saccharum* with *Sorghum*, *Imperata*, *Erianthus* and *Zea* were produced and their cytogenetic relationships were elucidated.
- Acidified Saffranin aniline blue was used as a selective stain for pollen tube growth inside the style.
- Three elegant techniques were standardised to study the Karyomorphology of *Saccharum* and related genera: a leaf squash, root squash and pollen grain squash techniques.
- Chromosome morphology was studied extensively and the evolution of the karyotype was elucidated.
- Chromosome numbers of 585 clones of *S. officinarum* from the World collection were examined. The clones with 2n = 80 were identified as typical forms of *S. officinarum* and the rest were classified as atypical clones.

- B chromosomes were recorded in several clones of *Erianthus* and *S. spontaneum*. Transmission of B chromosomes was studied and directed non disjunction of B chromosomes reported in pollen grain mitosis.
- *Saccharum* x sweet Sorghum and *Saccharum* x *Erianthus* hybrids were produced. Hybrids involving *Erianthus* were found to be very promising for introducing many desirable traits for breeding new sugarcane varieties.

## **Biotechnology**

### **Tissue Culture**

- Tissue Culture studies were initiated in late 1970s and protocols were standardised for the tissue and meristem culture of sugarcane. Several somaclones were developed through tissue culture with improved productivity and eliminating certain defects like spines, leaf drying, disease susceptibility etc.
- Somaclones with smut resistance were developed from susceptible clones.
- Improvement in agronomic characters also have been reported in somaclones and some of the recent 'Co' releases like Co 92007, Co 92029, Co 93005, Co 94003, Co 94012, Co 94003, Co 95016, Co 99011 and Co 99012 are somaclones. Co 94012 has been released for cultivation in Maharashtra and is found to give high sugar recovery.
- Micropropagation techniques in sugarcane had been developed and transferred to the Industry for adoption and the technique is now widely used for the rapid multiplication of commercial varieties and is an important component of the sugarcane seed programmes at present.
- Micropropagation has made it possible to multiply new varieties very rapidly and make them available for cultivation in a very short time. Apart from this, micropropagation is also useful in the rejuvenation of the older varieties which shows varietal degeneration following years of continued cultivation.
- Elimination of sugarcane mosaic virus from infected clones was found to be effective through combination of heat therapy and meristem culture.
- Protocols for *in vitro* germplasm storage through meristem derived plants with normal root and shoot system maintained in liquid minimal medium were developed. This protocol required change of medium only once a year. Plants were stored for periods upto 3 years using this technique, with out any detectable cyto-morphological changes.

### **Molecular Biology**

- Molecular studies on *Saccharum* was initiated at Sugarcane Breeding Institute during the 1990s, with the objective of characterizing the *Saccharum* species, related genera and hybrids using molecular markers and to develop strategies for molecular breeding in sugarcane.
- Biochemical markers like isozymes were used for the study of genetic diversity among the *Saccharum* species and for the characterization of individual genotypes. Six hundred and ten samples of *S.spontaneum*, 256 samples of cultivated and breeding clones and 168 samples of *Erianthus* spp. were characterized for isoenzyme variation and a few duplicates in the collection identified. The data indicate a higher level of diversity among *S. spontaneum* and cultivated clones compared to *Erianthus* for the peroxidase, esterase and diaphorase enzyme systems studied. It was found that three

enzyme systems namely Esterase, Peroxidase and Diaphorase could differentiate individual accessions of *Saccharum spontaneum* and hybrid varieties of interspecific origin.

- Molecular diversity in *Saccharum* complex viz., *S. officinarum*, *S. robustum*, *S. spontaneum*, *S. barberi*, *S. sinense*, *Erianthus*, *Narenga* and *Sclerostachya* was studied using RAPD markers. Among the *Saccharum* species, *S. officinarum* showed a low level of genetic diversity while *S. sinense* was found to be more diverse. Six taxonomical groups were clearly resolved in the cluster analysis. *S. officinarum*, *S. robustum*, *S. spontaneum* and *Erianthus* spp. formed discrete groups. *S. barberi* and *S. sinense* formed a single cluster, so also *Narenga* and *Sclerostachya*. *S. officinarum* was found to be closer to *S. robustum* and distant from *S. spontaneum*. Among the related genera, *Sclerostachya* was closer to *Saccharum* while *Erianthus* was found to be highly divergent from all the *Saccharum* species. It is suggested that the *Erianthus* spp. can contribute substantially towards sugarcane varietal improvement.
- A detailed AFLP analysis for 30 clones belonging to *S. officinarum*, *S. robustum*, *S. spontaneum*, *S. barberi*, *S. sinense* and the related genera *Erianthus* was carried out. The phenetic tree of the species clones based on AFLP data was consistent with known taxonomical relationships and the results obtained with RAPD markers. The intraspecies similarity in both *S. barberi* and *S. sinense* was much higher than interspecies similarity suggesting a clear separation of the two, which are considered horticultural species. All the primers could identify markers that are specific to the different species and the genera *Erianthus*. Among the species, specific markers were highest in *S. spontaneum* followed by *S. robustum*, *S. barberi*, *S. officinarum* and *S. sinense*. *Erianthus* had a distinct profile with 30% of the total amplified fragments being specific to the genus that offers great scope for the identification of intergeneric hybrids which has proven to be very difficult using morphological traits.
- Genetic diversity in 28 prominent Indian sugarcane varieties cultivated under a wide range of agroclimatic conditions, was studied using 25 RAPD markers. The mean genetic distance among the 28 varieties was only 29.31%, implying that a large part of the genome is similar among the varieties.
- AFLP analysis was also carried out for commercial sugarcane varieties grown under tropical and subtropical regions of India. Cluster analysis based on the dissimilarity values clearly distinguished the tropical and subtropical varieties of India. Comparison of the AFLP profiles of the cultivars with that of their progenitor species *S. officinarum* and *S. spontaneum* indicated that 85.3% of the marker loci was contributed by *S. officinarum* and 14.7% by *S. spontaneum*. The study also showed high discrimination power of individual primer combinations suggesting their usefulness in varietal identification. Of the 12 primer combinations eleven could individually discriminate all the varieties from each other.
- **Cross transferability of maize microsatellites on sugarcane:** Evaluation of the cross transferability of maize microsatellite markers on sugarcane were studied on a set of *Saccharum* clones, *Erianthus* and commercial cultivars. Of the thirty four primer pairs obtained from maize genomic libraries, 14 showed repeatable amplifications in *Saccharum* species clones, commercial hybrids and the related genera *Erianthus*, accounting for 41.17% cross transferability. Complex banding patterns were encountered indicating the high polyploidy and heterozygosity existing in sugarcane. Higher level of divergence of *Erianthus* from *Saccharum* was also clearly established. The polymorphic primers when tested on a panel of 30 commercial sugarcane cultivars revealed a broad range (32.4 - 83.3%) of pair-wise similarity values indicating their ability to detect high levels of polymorphism.

- **Identification of intergeneric hybrids of sugarcane using molecular markers:** -To identify genuine intergeneric hybrids of *Saccharum* with *Erianthus*, *Sorghum* and *Zea*, RAPD and ISSR markers were used. In the three groups of hybrids examined, RAPD markers could precisely identify the true hybrids of *Saccharum* x *Sorghum* and *Saccharum* x *Zea*. Though *Erianthus* had a very distinct RAPD profile and as many as 107 *Erianthus*-specific bands were identified, a decisive profile that can identify all the hybrids could not be obtained. The strong molecular differentiation between *S. officinarum* and *Erianthus* was not adequately resolved in the RAPD profiles of their hybrid progenies which suggest that more refined molecular techniques will be required for the characterization of *Saccharum* - *Erianthus* hybrids. The phenogram of the hybrids and the four genera, based on 250 RAPD markers showed the hybrids to be closer to *Saccharum* than to the respective related genera.
- **Molecular markers for red rot resistance in sugarcane:** Progenies of crosses Co 7201 x Co 8208, Co 7314 x Co 87268 and somaclones of Co 7717 were screened for red rot markers. Bulk segregant analysis using 160 RAPD primers produced 12 bulk specific amplifications for resistance / susceptibility. They were tested on the individuals that constitute the respective bulks and one such marker was found to produce reproducible amplification in the bulk individuals also. This fragment had been isolated, purified and cloned for sequencing and developing specific primers.
- AFLP analysis of the parents and the two bulks was also carried out, using 64 primer combinations. Twelve primer combinations produced bulk specific amplification. Further screening of these primer combinations with the mapping population consisting of 90 individuals is in progress.
- **Development of Sugarcane specific microsatellites:** Attempts to develop sugarcane specific microsatellites also are in progress in collaboration with the Indian Agricultural Research Institute, New Delhi. A preliminary work was carried out on the development of microsatellite markers from publicly available databases in sugarcane. 1250 nucleotide databases of sugarcane hybrid and species were analyzed and 121 sequences containing microsatellite repeats were identified. From these sequences 51 SSR primers were designed, synthesized and validated in diverse sugarcane hybrids and *Saccharum* species. Thirty nine such STMS primers were tested on 24 commonly used parental clones . The Polymorphism Information Content (PIC) values of the primers ranged from 0.5 to 0.81 showing the discriminatory power of these markers in the molecular analysis of sugarcane.

## Sugarcane Genetic Transformation

- Transgenics in sugarcane started with the production of herbicide resistant plants. The commercial variety CoC 671 was transformed using the bar gene. The plants regenerated were confirmed by southern analysis. Herbicide resistant plants were multiplied and the third generation transgenics showed stable expression withstanding 0.4% spray of the herbicide Basta. This was followed by the production of transgenics for fungal resistance using the antifungal peptide DM-AMP1 gene and Rs-AFP2 gene. Sugarcane varieties CoC 92061 and CoC 671 were used for the transformation experiments. Transgenic plants were regenerated and confirmed by PCR and western blot analysis. Transgenics of CoC 671 showed resistance to red rot.
- Sugarcane variety CoC 92061 was also transformed using genes coding for chitinase,  $\beta$ -1,3-glucanase and Thaumatin like protein. Transgenics for insect resistance were also attempted. Varieties CoC 671, CoC 92061 and Co 86032 were bombarded with Cry 1A (b) gene and genes coding for Aprotinin and protease inhibitors. PCR analysis

of all the three varieties indicated the integration of genes coding for Aprotinin and Cry 1A (b). Screening for top borer resistance along with control plants showed significant reduction in weight of the larvae that fed the transgenics plants compared to the control plants.

### **Seed technology**

- Seed Technology Laboratory was set up at Sugarcane Breeding Institute, Coimbatore in 1991 to undertake research work on true seed and cane seed of sugarcane.
- Methodologies were standardised for seed drying in a wooden cabinet dryer or Dehumidifier Drying room.
- Techniques for fluff defuzzing, seed testing for germination and seed storage were standardized.
- A simple technology for taking sugarcane bud chips manually using a bud chipping machine, their treatment, packing, transport and raising of seedlings was standardised.

## **CROP PRODUCTION**

### **Agronomy**

- Integrated nutrient supply systems for sugarcane was evolved to arrest deterioration of soil quality and productivity.
- Various integrated nutrient supply systems for sugarcane viz., application of organics, chemical fertilizers and biofertilizers, raising leguminous intercrops and in situ incorporation and recycling of crop residues were tested. The treatment involving application of organics to supply 25% of the recommended dose of N + application of chemical fertilizers to supply 50% of the recommended dose of NPK + application of *Azospirillum* and *Phosphobacteria* each at 10 kg/ha in two equal split doses at 30 and 60 days after planting (DAP) + raising daincha as intercrop in the interspaces and *in situ* incorporation at 45 DAP resulted in higher productivity. The cane yield was 37.6% more compared to control (no manuring) and 16.0% higher compared to application of the recommended dose of nutrients entirely through chemical fertilizers.
- **Crop-weed competition in plant and ratoon sugarcane** - Studies carried out for two seasons at Sugarcane Breeding Institute, Coimbatore in plant and ratoon sugarcane indicated that in plant cane, the critical period of crop-weed competition under tropical-Indian conditions is from 30 to 45 days after planting.
- In ratoon crop, the study indicated that the critical period of crop-weed competition under tropical conditions is from 30 to 90 days after ratoon initiation .
- **Dual row planting technology**- To facilitate mechanisation in sugarcane cultivation, wide row planting adopting a spacing of 150 cm is becoming popular. To further improve the cane yield under wide rows, a new technology, 'dual row planting' has been developed. In this method, broad furrows are formed at a spacing of 150 cm and in the middle of the furrows sugarcane setts are planted in two rows adopting a spacing of 30 cm between them. In a comparative study of two different methods of wide row planting, the dual row system gave a cane yield of 136.3 t/ha compared to 126.7 t/ha recorded by the single row system. In plant crop, variety Co 94005 recorded the highest cane yield under dual row planting. Among the spacings, the dual row planting and the normal 90 cm were on par and were significantly better

than the other spacings. In the ratoon crop, variety Co 94005 was best for wide row spacing followed by Co 91010.

- **Inter-cropping in Sugarcane** Studies on intercropping sugarcane with soybean indicated that the grain yield of soybean was higher under wide row planting (150 cm) compared to normal rows (90 cm). Sugarcane yield under wide row spacing was not significantly affected by intercropping with soybean, irrigation regimes and reduction in sugarcane seed rate from 75,000 two budded setts to 60,000 setts per ha. Intercropping also offers scope for reducing the fertilizer nitrogen dose by recycling soybean crop residues.
- **Organi Sugarcane Production System** - The feasibility of sugarcane production on a sustainable basis entirely using organics was studied over a period of five years in comparison with application of nutrients exclusively through inorganic fertilizers and in combinations of organics and inorganics. The 5-year cropping sequence was sugarcane (plant) – sugarcane (ratoon) – finger millet – cotton - sugarcane (plant) – sugarcane (ratoon). During the first three years, application of 100% of recommended N through organics plus biofertilizers produced comparable yield of crops with that of application of recommended dose of nutrients only through chemical fertilizers. In the subsequent years, application of 100% of recommended N through organics plus biofertilizers was better than continuous application of chemical fertilizers only. The cane yield of first plant cane, cane yield of first ratoon cane, grain yield of finger millet, kapas (seed cotton) yield of cotton, cane yield of second plant cane and cane yield of second ratoon cane were 121.6 t/ha, 65.6 t/ha, 2961 kg/ha, 643 kg/ha, 107.4 t/ha and 65.5 t/ha respectively under the organic farming practice. The corresponding values were 116.6 t/ha, 70.1 t/ha, 3027 kg/ha, 620 kg/ha, 92.5 t/ha and 56.8 t/ha under the conventional practice. On completion of the 5-year cropping sequence, bulk density, organic carbon and microbial status of the soil improved favorably under organic farming system. The study has indicated that it is feasible to obtain sustainable sugarcane production by meeting its nutrients requirements entirely through organics.

## **Micro Biology**

- Application of different biofertilizers viz., *Azotobacter*, *Azospirillum* and *Gluconacetobacter* in combination with different levels of fertilizer nitrogen indicated that application of *Azospirillum* could significantly improve the sugarcane and sugar yields of different sugarcane varieties followed by *Gluconacetobacter* compared to *Azotobacter* and uninoculated control. The response of sugarcane varieties was more pronounced to *Azospirillum* application in terms of cane yield under lower fertilizer level compared to normal fertilizer level.
- Application of PSB, *Gluconacetobacter* and VAM individually and in combination, in two soil types, at 0, 50 and 100% of the recommended dosage of P<sub>2</sub>O<sub>5</sub>/ha indicated the beneficial effects of all the microbial combinations. There was very good response to VAM in terms of increase in cane yield.
- *Gluconacetobacter diazotrophicus* is an endophytic diazotroph associated with sugarcane. Ten local isolates of *G. diazotrophicus* typical to type strain *G. diazotrophicus* Pal 5 were isolated from sugarcane and their identity was confirmed by polymerase chain reaction (PCR) using species-specific primers. Besides nitrogen fixation, these isolates were found to possess phosphate solubilizing potential.
- **In vitro phosphate solubilization by of *G. diazotrophicus*** - Compared to *B. megaterium*, the *G. diazotrophicus* isolates were found efficient in solubilizing



tricalcium phosphate under *in vitro* conditions. Quantitative solubilization indicated that the local isolate, SBI-AD-5 solubilized 80 per cent of tricalcium phosphate added in the medium, while *B. megaterium* solubilized only 46 percent in 7 days.

- Identifying efficient biofertilizer strains to supplement sugarcane nutrition and for utilization of sugarcane wastes and sugar industry byproducts.
- Inoculation of *Acetobacter* and *Azospirillum* significantly improved sugarcane biomass in pot culture experiments.
- Efficient isolates of *Acetobacter*, *Azospirillum*, *Herbaspirillum* and *Phosphobacteria* have been isolated from sugarcane rhizosphere.
- Technique for mass multiplication of fungi used in nematode biocontrol by using sugarcane wastes has been perfected.

## **Plant Physiology**

- In sugarcane breeding, crossing between some of the desirable parents is not possible due to their non-flowering nature or non-synchronous flowering. By manipulating the photoperiod, temperature and humidity, flowering of such parents can be regulated. In the field condition, incandescent lamps were installed and used for altering the photoperiod. A modern photoperiodic facility with provision to control the above factors is also available at Sugarcane Breeding Institute, Coimbatore. These facilities are being utilized to artificially induce flowering in non-flowering parents, delaying flowering in early flowering parents and advancing flowering in late flowering parents. This facilitated crossing between non-flowering and non-synchronous flowering parents in the breeding programme.
- **On-farm control of flowering** - Flowering is very common in peninsular India where sugarcane fields would be in full bloom during October-November. Chemical control of flowering is more feasible in sugarcane crop. Promising results were obtained with ethephon (2-chloroethyl phosphonic acid), an ethylene-releasing compound. Large-scale demonstration trials using ethrel in areas conducive to flowering have shown positive results of both prevention of flowering and improvement in yield and sucrose content in variety Co 62175. On-farm trials were conducted in different areas of tropical states Tamil Nadu and Karnataka indicated that flowering was inhibited by 55 to 75 % in 2002 season and by 23 to 86 % in 2003 season.
- **Drought** -Drought stress is a major environmental factor affecting the sugarcane productivity in all agro-climatic zones of India. Drought in combination with high temperature during summer months is known to suppress the cane yields in both tropical and sub-tropical climates. Hence experiments were conducted to understand the physiological basis of drought resistance and the response of sugarcane varieties to the imposed moisture stress. Formative growth stage (60-150 days) was identified as the critical water demand period and any amount of stress at this early growth phase had a direct influence on the cane yield and juice quality and yield reduction up to 50 % has been recorded in a typical drought year. Moisture stress at formative phase reduced juice quality, while at maturity the moisture stress improved juice quality. Variety x drought treatment interaction was significant only during the years of severe drought.
- The first formed sett roots, which reached a maximum length of about 15 cm, were replaced by a potential shoot root system mostly concentrated in the upper surface (50 cm depth). Variety Co 8021, a drought susceptible type, has shown identical root mass with a surge to penetrate deeper as the stress intensity became severe. Variety

Co 85061 showed root spread at superficial layers under normal irrigation, while under stress, a clear tendency was visible towards penetrating root system.

- The same phenomenon was quite evident in Co 86032 where in majority of the roots started penetrating deeper for extracting more moisture at lower strata of the soil. In Co 95020 and Co 88006, the root mass production itself was affected under drought suggesting the inhibitory influence of drought on root growth. In an inter-specific hybrid ISH-111, the roots have shown a unique nature of horizontal spread and penetration under normal condition. The drought plants also showed a pronounced nature of vertical movement in response to drought.
- In sugarcane, cane height, juice quality, and NMC are the potent traits contributing to yield and stress during the formative growth phase has reduced the cane yield and juice quality.

## **Soil Science**

- Application of nitrogen in three equal splits at 30, 60 & 90 days after planting gave highest cane yield in early maturing varieties as against two equal splits at 45 and 90th day. Different levels of potassium had no significant influence on cane yield, sugar yield, juice quality and fibre content of sugarcane grown in potassium rich soil. Available potassium content was marginally improved by potassium application.
- Application of ferrous sulphate both as foliar spray (1.0%) thrice and soil application @ 150 kg/ha improved cane and sugar yield of Co 62175 grown in highly calcareous soil deficient in iron. The efficiency of ferrous sulphate was improved when applied as FYM enriched with FeSO<sub>4</sub>. Soil application of zinc sulphate @ 25 kg/ha coupled with 0.5% ZnSO<sub>4</sub> foliar spray thrice resulted in higher cane yield of CoC 92061 grown in zinc deficient clay soil.
- Co 86032, Co 86249 and Co 8021 were identified as tolerant varieties to iron deficiency while Co 87025 was found highly susceptible to this malady.
- CoC 771 and G 85335 were found suitable for growing in tannery effluent affected areas. Further the yield and quality were improved by coir pith and cane trash mulching

## **Agri. Chemistry**

- Covering the harvested cane with trash and sprinkling with water both under sun and shade reduced the moisture loss and prevented quality deterioration. Among the varieties studied, maximum loss of moisture (5.9%) was recorded by the variety Co 86249 while the varieties Co 94008 and Co 85019 have registered minimum loss of moisture (4.7%).
- **Particle board from bagasse** -Pulverised sugarcane fibre is treated with either urea-formaldehyde or phenol-formaldehyde resins (15% on weight of fibre) and pressed in a hot press at 150°C to make particle board, which have superior strength properties similar to particle board made from wood chips.
- **Improving the quality of jaggery** -Usefulness of lemon juice as an organic clarificant in jaggery making with the variety Co 86032 was studied. Lemon juice @ 8 ml per 2 litre of juice improved the jaggery colour. However the Reducing Sugar (RS) content of jaggery was found to increase slightly due to use of lemon juice. The keeping qualities of these jaggery samples indicated no appreciable drop in jaggery sucrose, purity but only an increase in RS content of the jaggery at 6 months of

storage. The colour of jaggery was found to be slightly darker compared to what it was at the time preparation. There was no appreciable drop in overall quality with lemon juice clarified jaggery as compared to jaggery prepared using lime solution.

## **CROP PROTECTION**

### ***Plant Pathology***

#### **Identification of resistant clones**

- Since not much information is so far available on the inheritance and genetics of disease resistance in sugarcane the parents for hybridization programmes have been chosen on the basis of observed disease reaction of the clones. Using these approach red rot resistant varieties such as Co 86010, Co 86011, Co 86249, Co 93009 and Co 94008 have been developed. Sugarcane varieties resistant to red rot are being evolved every year using the identified resistance sources. The varieties resistant to red rot suitable for different states in the country are listed below. Tropical region Co 8021, Co 86010, Co 86011, Co 86249, Co 93009. Sub Tropical region BO 91, Co 89003, Co 96015, Co 97009, CoPant 94211.
- In addition to commercial varieties major sources of disease resistance have been identified in sugarcane germplasm materials maintained by the Institute at Cannanore. Resistance sources were identified in genotypes of *Saccharum officinarum*, *S. barberi*, *S. sinense*, *S. robustum* and *S. spontaneum* as well as related genera such as *Erianthus*, *Sclerostachya* etc. Among these several clones of *S. spontaneum* are highly resistant to red rot and are suitable donors of red rot resistance.

#### **Breeding for red rot resistance**

- Biparental crossing has been generally adopted for development of clones with disease resistance. Proven parental combinations have been identified for generating red rot resistant materials in the Institute. In addition back cross method of breeding has also been used to develop disease resistant sugarcane clones

#### **Screening and evaluation of disease resistance**

- To assess red rot resistance the clones are inoculated with the pathogen culture when the crop is about 8 months old by plug method. The reaction of the clones is evaluated as resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS) to red rot based on 0-9 scale developed at the Institute. The resistance assessed by this method is referred as protoplasmic resistance or physiological resistance. In another method of disease evaluation the nodal method where the pathogen inoculum is applied at the nodal region between the stalk and leaf sheath. In this method, mechanical resistance in the genotypes to the pathogen in being evaluated. However, high levels of field disease escapes were noticed in the field due to lack of desired environmental parameters. A new technique for evaluation of red rot resistance in sugarcane seedlings was developed. This technique enabled to eliminate the disease susceptible seedlings at an early stage.
- To overcome environment-induced variability in red rot disease reaction and to rapidly screen sugarcane genotypes for red rot a controlled condition testing (CCT)

was developed at this Institute. In this method the pathogen is applied by nodal swabbing in the cane tops of 6 to 8 months old cane and kept in a temperature and humidity controlled chamber. A new 0-9 scale was developed to assess disease resistance as R, MR, MS, S and HS in the clones. This method of testing was found to be precise, rapid and reliable as compared to plug and nodal methods to assess red rot resistance. The new of testing showed less HS/S types and more R/MR types as compared to plug method. When compared with nodal method this method of testing showed lesser R/MR types indicating disease escape in the former.

## Red Rot Resistance markers

- The red rot pigments (RRPs) synthesized in sugarcane tissues at the site of host pathogen interaction were purified and characterized. The results revealed that in resistant interaction about seven pigment compounds were identified whereas, in susceptible interaction three of them were absent. Later studies with HPLC established that the RRP's have 3-deoxyanthocyanidin phytoalexins such as luteolinidin, apigeninidin and caffeic ester of 5-O-arabinosyl apigeninidin. Studies with a set of differential hosts and *C. falcatum* pathotypes showed that these phytoalexins were accumulated in incompatible interactions whereas in susceptible interactions the compounds are not triggered in the host. The *C. falcatum* toxin also triggers phytoalexin accumulation in sugarcane cultivars. In addition, quantification of anthocyanin pigment compounds in the red rot inoculated canes has been found as useful marker in identifying red rot resistance.
- The newly developed rapid screening method for red rot resistance was found compatible for applying histological, serological and biochemical markers for disease resistance. Further works on other biochemical indices revealed that peroxidase and polyphenol oxidase play a role in red rot resistance. The enzymes accumulate in higher levels quickly at cellular level in resistant cultivars. In response to *C. falcatum* its toxin treatment, similar observation was established. The sugarcane varieties were screened for their reaction to electrolyte leakage in response to *C. falcatum* toxin treatment and the results showed that the disease susceptible cultivars show high electrolyte leakage as compared to resistant varieties.

## Characterization of pathogen variation

- Commercial varieties developed with high levels of resistance to red rot have maintained their resistance for several years. However it is observed that in due course, this resistance breaks down and these varieties become susceptible. This is mainly due to development of new pathotypes.
- **Variation in cultural characteristics of *Colletotrichum falcatum* pathotypes-** Existence of different pathotypes/races of the red rot pathogen is known in the recent years. The pathotypes exhibit limited variation in cultural and morphological features. Hence studies are being conducted to identify differential host cultivars which selectively react to different pathotypes. Based on the similarity or variation in these disease reactions produced by various pathogenic isolates on different host clones the isolates are grouped and identified as pathotypes. Detailed studies involving tropical and sub-tropical pathotypes indicated that the isolates from tropical region of the country were found to be more virulent than the sub-tropical ones. RAPD technique has been standardized to characterize pathogen variation at molecular level. Recently

vegetative compatibility grouping has been successfully attempted to group the pathotypes based on VCG patterns.

### **Biological control/ Induced systemic resistance**

- Different antagonistic strains were screened against various *C. falcatum* pathotypes to select more effective biocontrol strain. Recent studies also indicated that native strains of *Pseudomonas fluorescens* were effective against red rot pathogen.
- These antagonistic strains were found to induce systemic resistance in the disease susceptible varieties. In addition certain systemic signal molecules like bion (CGA 245704) were found induce systemic resistance in red rot susceptible sugarcane varieties. Production of anti-fungal chitinases in the medium by the antagonists was established. Production of certain secondary metabolites by fluorescent pseudomonads such as pyocyanine, phenazine and 2,4-diacetyl phloroglucinol was found. A microtitre-plate based assay was developed to test the anti-fungal activities of the enzymes and metabolites. Studies are in progress to establish the efficacy of these strains against the disease under different endemic locations.

### **Smut**

- Similar to red rot, smut also disease severity increases with increase in the levels of sett-borne inoculum. Dormant smut mycelium, which is in the seed setts, grows along with the sprouting shoot and reaches the growing point of the cane and subsequently converts the top of the cane into a smut sorus. The smut spore dispersal increases with increase in wind velocity and temperature has no effect on spore dispersal or spore production. Studies on smut resistance have established the role of morphological factors like number of bud scales and presence of bud-grooves and the role of physiological and biochemical factors like nature of germination - apical or subapical, involvement of total and free amino acids, phenols and bud scales diffusates in conditioning the resistance against smut.
- Breeding for smut resistance yielded many disease resistant varieties. The resistance to smut appeared to be favoured by two dominant genes (S1 and S2), whose action was greatly modified by inhibitor and anti-inhibitor genes. Sources of resistance for sugarcane smut have been identified in *S. spontaneum* and *S. officinarum* genotypes. Many somaclones were developed utilizing somaclonal variation. Some of the somaclones released as 'Co' canes possessed high levels of resistance to sugarcane smut.
- To screen sugarcane clones for smut resistance, artificial inoculation of the pathogen is being done in the field. The screening procedure consist of dipping the seed sett in heavy spore suspension of smut fungus and planting in the field. The periodical observation of smut incidence is recorded and on the basis of cumulative final percentage of disease incidence varieties are graded as R, MR , MS S, and HS. Recently dikaryotic mycelial culture was established under laboratory. A simple technique was developed to inoculate sugarcane setts using dikaryotic cultures. Smut pathogen colonization was assessed by trypan blue staining later. This method ensures rapid screening for smut disease and diseases escapes can be minimized. A technique for inoculating the true seeds of sugarcane with smut spore suspension and screening out the seedlings periodically up to 120 days after germination based on whip formation was standardized to screen large population of seedlings for smut resistance. Polyclonal antiserum raised against dikaryotic mycelium was useful in

detecting smut colonization in sugarcane tissues. An indirect ELISA technique was standardized to detect smut pathogen infection in smut affected sugarcane varieties.

- Detection of profuse growth of *Ustilago scitaminea* inside the meristematic tissues of smut disease susceptible clones. In resistant clones no such fungal growth is observed

### **Thermotherapy in sugarcane disease management –**

- Thermotherapy is a process by which seed cane is subjected to high temperature for a specific period of time. This inactivates some of the sett borne pathogens of sugarcane which are sensitive to heat. Aerated steam therapy was developed in this Institute to treat the setts with a mixture of hot air and steam.
- **Aerated steam therapy unit with treating chamber and control panel** -Treatment of setts for 1 hr at 50 degree C eliminates the pathogens causing smut , grassy shoot and ratoon stunting disease in the setts. Based on this method of sugarcane sett treatment, a three-tier seed nursery programme was recommended for each sugar factory. This method ensures supply of disease free seed canes to sugar factory areas once in 5 years. In addition, hot water treatment is being followed to treat the sugarcane setts in a limited scale especially in quarantine programmes. In such cases fungicides viz. carbendazim or tridimefon @ 0.1% is mixed with the water for elimination of fungal propagules in the seed cane. Such treatments eliminate smut and other fungal colonization in the seed canes. However, for large scale treatment of seed canes aerated steam therapy is recommended.
- **Managing seedling rot in nurseries** -Several species of *Pythium* viz. *P. graminicolum*, *P. aphanidermatum* and *P. catenulatum* were implicated with root rot and seedling diseases of sugarcane, *P. graminicolum* being the most frequent. Satisfactory control of the disease could be obtained either by autoclave steaming of pot mixture at 20 lbs for 3 hrs by heating the soil in a semi-moist condition for 1 hr in an open pan before sowing and by post-emergence drenching of Captan I. Studies conducted at the institute showed success of biological control agent *Trichoderma viride* against *Pythium graminicolum* causing seedling root rot in the nursery. The *T. viride* cultures are mass multiplied and routinely applied in the seedling pans every year to reduce death of seedlings due to the disease. Recent studies also indicated that strains of fluorescent pseudomonads are also effective against pathogens causing seedling rot.

### **Bacterial diseases**

- Leaf scald disease caused by *Xanthomonas albilineans* was recorded recently in Tamil Nadu on varieties such as CoSi 86071 and CoC 90063. The pathogen was found to affect both yield and quality of sugarcane. The causative bacterium was isolated on modified Wilbrink's medium. Polyclonal antiserum against the bacterium was developed for diagnosing the disease in the host tissues. Serological techniques such as ELISA and dot-blot were sensitive in diagnosing the bacterium causing ratoon stunting disease *Leifsonia xyli* subsp. *xyli*.

### **Virus and phytoplasma diseases**

- Sugarcane mosaic virus (SCMV) belonging to *Potyviridae* affects the crop growth in certain locations. Presence of different viral strains was recorded in different parts of the country. The viral strains exhibit different type of symptoms in sugarcane

cultivars. The major symptoms of the virus can be grouped as streaks, blotchy mosaics and mottling. An indirect ELISA technique was standardized for diagnosis was found highly sensitive to detect the virus in the suspected host.

- Presence of sugarcane bacilliform virus (SCBV) belonging to *Pararetro viridae* has been reported recently in sugarcane germplasm materials. Certain genotypes of noble canes and foreign hybrids are infected by the virus severely as compared to the Indian hybrids and *S. spontaneum* clones. The presence of the virus was detected by ELISA and ISEM techniques. Presence of sugarcane yellow leaf syndrome (YLS) was recorded recently in different sugarcane cultivars. Detailed symptomatology of the syndrome at Coimbatore has been recorded. The disease causes yellowing of mid ribs in matured crop and in severe cases it causes drying of leaves/canes. Association of sugarcane yellow leaf virus (ScYLV) with the syndrome was confirmed by DAS-ELISA.
- Characteristic grassy shoot symptoms with chlorotic tillers in sugarcane Yellow discolouration of laminar region alongwith mid rib in YLS affected sugarcane.
- The grassy shoot disease (GSD) caused by phytoplasmas has been recorded in all sugarcane growing areas. Work done based on electron microscopy and tetracycline antibiotics treatment conclusively proved that the disease is caused by phytoplasmas. The severity of the disease increase in the ratoon crops. Sugarcane varieties vary in their susceptibility to the disease. Serological techniques such as ELISA and indirect immuno-fluorescence were standardized for the detection of the phytoplasmas in sugarcane tissues.

## Quarantine

- Infected seed cane is the primary means of introduction of most of the sugarcane diseases into areas where they are not usually prevalent. This makes it necessary to follow proper quarantine measures and if possible legislative measures also to prevent introduction of diseases. All seed crops which are proposed to be transported to disease free locations are to be systematically examined for prevalence of major diseases and only after such verification and freedom from diseases, seed treatment and certification such seed material should be permitted to be transported to disease free areas. Before distributing the cane from such seed material, nursery crops should be raised in carefully monitored quarantine areas and only after confirming that such crops are free from all seed transmitted diseases these crops can be used as seed cane for general cultivation.
- Currently for purpose of research and developmental activities there is considerable exchange of sugarcane seed material both nationally and internationally. All such seed cane should be subjected to strict quarantine measures both during despatch and receipt. Such quarantine measures are essential to prevent the possible introduction of serious diseases like Fiji disease, severe forms of mosaic etc. that are not prevalent in India.
- Recently advanced serological techniques such as ELISA, dot-blot and ISEM have been standardized for the diagnosis of sugarcane pathogens causing red rot, smut, ratoon stunting and leaf scald, sugarcane mosaic virus, grassy shoot phytoplasmas, sugarcane yellow leaf virus and sugarcane bacilliform virus. These sensitive techniques are useful for diagnosing suspected sugarcane samples for pathogen infection. These highly sensitive techniques are very much essential in quarantine since many of the pathogens especially viruses do not exhibit clear disease symptoms are they possess dormant infections in the cane pieces.

## Plant Pathology- Current Research Activities

### Studies on the early events in red rot pathogen-sugarcane interaction

1. Identification of specific PR-proteins and its utilization as marker(s) for red rot resistance - Studies are in progress on the identification of pathogenesis related (PR) - proteins chitinase,  $\beta$ -1,3-glucanase and thaumatin-like proteins (TLPs) in sugarcane varieties differing in resistance to *Colletotrichum falcatum* causing red rot disease. The pathogen is inoculated in leaf and stalk tissues of sugarcane varieties and induction of these PR-proteins are assayed at different time intervals by Western blot. In resistant variety showed induction of four chitinase proteins after pathogen inoculation. The intensity of these proteins increased with time from 6 to 42 hr after inoculation. In susceptible variety induction of a chitinase protein was recorded. Uninoculated tissues of both resistant and susceptible varieties showed presence of a different chitinase protein. In stalk samples comparatively lesser number of chitinases were induced. In leaves induction of 43.0 kDa TLP was found in response to pathogen inoculation. In stalk samples, presence of two TLPs of 43.0 kDa and 37.5 kDa were observed in the 1-8 days after pathogen inoculation in resistant variety. In susceptible variety such induction was less intense and could be seen in the 8th and 9th day samples.
2. Molecular basis of pathogen recognition, signal transduction and PR-protein(s) gene activation
3. A controlled condition testing system [ CCT ] have been developed to rapidly and precisely evaluate sugarcane clones for red rot resistance. The method has the following advantages.
  - Rapid, reliable and reproducible
  - Minimizes disease escape by providing ideal environmental conditions.
  - Large number of clones can be tested simultaneously.
  - The testing can be carried out throughout the year.
  - Testing against various races of the pathogen can be carried out simultaneously under uniform condition.
  - It is compatible with other physiological/biochemical indices.
  - Less resources in terms of land, labour and capital
4. So far we have evaluated more than 12000 clones from different sources such as PZVT, ZVT, ISH, germplasm, ID clones, CD clones, Recurrent selection seedling, progenies from various crosses at different stages of selection .Using this technique R and MR clones identified. for use in breeding programme.

### Screening for field tolerance against red rot utilising primary infection process.

- Red rot infected cane debris and pathogen culture multiplied on sorghum grain were compared to evaluate field tolerance against red rot. Red rot culture multiplied on sorghum grains was found to be more suitable to evaluate field tolerance. However the results are reliable only when ideal environmental condition are ensured.
- Sorghum grain at 150 g for 3 m row at monthly interval is applied from the time of planting till the completion of tillering phase. It was found that many clones moderately susceptible by plug or nodal method remained free of red rot infection following this method of inoculation indicating their field tolerance.



## Characterisation of red rot pathotypes

- **Molecular characterization of pathotypes** - Genomic DNA of *C. falcatum* cultures (numbering 29 isolates collected from different geographical locations viz. Tamil Nadu, Andhra Pradesh, Kerala, Haryana and Bihar) were isolated, purified and was analyzed . Analytical protocol was standardized to precisely purify and quantify genomic DNA from major pathotypes of *C falcatum* and this proved effective for PCR amplification as a method to compare genetic variability among the isolate. For this purpose isolates(comprising of Cf 997,Cf 419,Cf 7717,CfJ 64,Cf 671,Cf 90063,Cf 92061,Cf 7514 and two mutants of *C. falcatum* ) representing different geographical regions were taken up. Optimal conditions for PCR amplifications were standardized Cf 671,Cf 90063,Cf 92061,Cf 7514 and two mutants of *C. falcatum* were subjected to RAPD-PCR analysis. Diferrent primer combinations were used . The results revealed that 7 primers produced good amplification and considerable level of polymorphism for all the ten cultures used for the study.
- **Pathogenicity determinants** - Based on the results obtained from earlier studies on VCG among 9 pathotypes (Cf671, Cf90063, Cf92061, Cf419, Cf997, Cf1148, Cf7717, Cf767 and Cf64) work has been initiated to study the nature of compatibility among different isolates of single pathotype. Mutants of nine isolates of Cf671 were generated on minimal medium and they were tested for compatibility at all possible combinations (81). Results showed that there was heterokaryon formation in all the combinations, which indicates compatibility nature among different isolates of single pathotype. VCG results on incompatibility of Cf1148 and Cf7717 have correlation with pathogenicity and RAPD analysis.
- **Pathogenic variability** -Differential interaction of 9 pathotypes Cf419, Cf997, Cf1148, Cf7717, Cf671, Cf90063, Cf92061, Cf767, Cf64 on Co 419, Co 997, Co 1148, Co 7717, CoC 671, CoC 90063, CoC 92061, CoJ 64 and CoS 767 was studied. The results indicated that Cf92061 (tropical) and Cf7717 (sub tropical) were found to be highly virulent types. While Cf64 and Cf767 were least virulent types among all the pathotypes. This confirms the earlier studies on differential interaction with susceptible and resistant clones of various *Saccharum* spp.
- **Pathogen metabolites** -Correlation between disease expression and production of secondary metabolites viz., toxin and enzymes by the pathogen has been established. This was confirmed by inoculating *C. falcatum* conidial suspension and its toxin on cut leaves under in vitro conditions. The conidia in the droplets on leaf lamina were germinated, developed mycelial mat and produced typical necrotic symptom with yellowing. Correspondingly they found to produce cellulolytic (cellulase) and pectinolytic (exo and endo polygalacturonases-PG, pectin methyl esterases-PME) enzymes. Reduction in symptom production was observed with the microbial inactivation of enzymes. Addition of biocontrol agents with the conidial suspension inhibited its germination, which lead to reduced enzyme production. Similarly microbial detoxification with reduced symptom was also observed.
- **Management of red rot disease using thiophanate methyl and bio-control agents** - The systemic fungicide Thiophanate methyl (TM) was found to be effective against red rot both under pot and green house condition. Sett dipping with Thiophanate methyl at a concentration of 0.25% with Carboxy Methyl Cellulose [CMC] as sticker effectively reduced primary infection of red rot up to 90 days. The biocontrol agent *P. fluorescens* was found to be compatible with Thiophanate methyl and can be used in combination with this fungicide.

- **Identification of anti-fungal genes** - Isolation and characterization of anti-fungal genes from antagonistic microbes for management of red rot disease. From among Forty-nine endophytic bacterial strains isolated from different cultivated sugarcane varieties, clones of *Saccharum spontaneum* and *Erianthus* species. three native rhizosphere bacterial strains seven isolates were identified to be effective against the red rot pathogen *Colletotrichum falcatum* . Incorporation of chitin in the media increased the biocontrol efficacy of the antagonists. Biochemical characterization of the efficient antagonistic endophytic strains revealed that three endophytes were strains of *Pseudomonas aeruginosa*. Other three endophytes were strains of *Pseudomonas fluorescens* and remaining one endophyte was *Pseudomonas putida*. Three rhizosphere strains belonged to *P. fluorescens*.
- **Selection of chitinolytic strains** - Detailed studies were undertaken to establish the role of lytic enzymes produced by antagonistic microbes in the inhibition of *C. falcatum*. Among all (mentioned above) the tested strains, four strains of fluorescent pseudomonads, viz. AFG2, AFG4, FP7 and VPT4 produced larger size of clearing zones in chitin amended media indicating efficient production of chitinases.
- Selection of chitinolytic strains of antagonistic bacteria in chitin agar medium
- Production of mycolytic enzymes and their antifungal activities
- Characterization of microbial enzymes
- Cloning of antifungal genes

### Bacterial and Phytoplasmal diseases

- Polymerase chain reaction (PCR) technique was standardized for the detection of phytoplasmas causing grassy shoot disease (GSD) in sugarcane. Two sets of forward and reverse primers of 16S rRNA /23S rRNA gene were used for the detection. DNA fragments of 1.80 kb and 1.35 kb encoding for the 16/23S spacer region and 16S of rRNA were specifically amplified by PCR respectively using these universal primers from DNA of GSD-affected plants. The primer set I 5' AAG AGT TTG ATC CTG GCT CAG GATT3' (Forward)/ 5' CGT CCT TCA TCG GCT CTT 3' (Reverse) amplified a 1.80 kb DNA product from infected samples and from healthy samples no such amplification was found. Similarly Primer set –II 5' AAG AGT TTG ATC CTG GCT CAG GATT3' (Forward)/5' AAC CCC GAG AAG GTA TTC ACC 3' (Reverse) amplified a 1.35 kb DNA product from GSD infected leaf samples. The results indicate that PCR based diagnosis can be used for the detection of GSD phytoplasmas in sugarcane.
- Tissue blot immunoassay was standardized for the diagnosis of RSD infection in sugarcane. In this assay suspected cane stalks were blotted directly on the nitrocellulose membranes and infection of different vascular bundles were detected using the polyclonal antisera. In the studies infected vascular bundles showed characteristic colour development on the membranes to alkaline phosphate substrate and bacterium-free bundles remained colourless indicating that this is a useful technique to detect RSD infected seed canes under field conditions.

### Viral diseases

- PCR amplification was standardized for sugarcane bacilliform virus (SCBV) from the infected sugarcane clones with two sets of primers (Badna virus group primers & SCBV specific primers). The sequence of badna virus group primer was 5' TAY ATH GAY GAY ATH YT 3' (forward) and 5' CCC CAT RCA NCC RTC NGT YTC 3'

(reverse). The sequence of SCBV specific primer was 5' TCA AAG TTT GAT TTG AAG AGC GGG 3' (forward) and 5' CTC CGA GAA AAC CAA TAT GTC ATC 3' (reverse). The PCR conditions used were 94°C for 7min, 3cycles of 94°C for 30 sec, 50°C for 30 sec, 72°C for 1min, 37 cycles of 94°C for 30sec, 55°C for 30sec, 72°C for 1min followed by a 72°C extension for 7min. No amplification was occurred with Badna virus group primers in different SCBV suspected samples. However with SCBV specific primers, a 221bp DNA fragment was amplified and in healthy samples taken from Coimbatore, no amplification occurred. The samples that revealed positive results are Badila Java, Boetatoe bilatoe, Black Tanna, Caledonia Amarilla, Guam A, Iscambine, Listada, Mungo 252, Negros purple, Pepuca Nor, B 46-199, D 1135, D 1135 striped, IK 76-68. In few samples, a non-specific DNA fragments were also seen and the amplification conditions are being modified to overcome this.

## Entomology

### Biological Control

- *Cotesia flavipes* parasitization rates in the laboratory were positively influenced by proportion of males in parent batch and negatively affected by larval number and number of larvae per female parasitoid.
- In the laboratory, *Trichogramma chilonis* derived either from *Corcyra cephalonica* or internode borer (INB), parasitised 100% freshly laid eggs.
- In field studies on dispersal of *T. chilonis* using trap-cards, the parasitoid was trapped up to a distance of 1-10 m in different directions from the point of release.
- The whitefly parasitoid *Amitus minerva* showed superparasitisation of the host. However, the multiplication rate of this parasitoid was found to be high varying from 60 to 90 times.
- The Granulosis Virus of shoot borer stored at 40C for 16 years caused considerable mortality of borer larvae in the laboratory.
- A granulosis virus (GV) was identified on top borer larvae collected at Coimbatore.
- Formulations of *Bacillus thuringiensis* were effective against neonate shoot borer larvae in pot culture experiments.
- The fungus *Beauveria brongniartii* caused significant mortality of different stages of the white grub *Holotrichia serrata* in laboratory and pot culture experiments. The fungus *Metarhizium anisopliae* was more infective to eggs of the white grub.
- In the field, *B. brongniartii* applied at 1013 – 1014 spores/ha caused 46% infection of third stage white grubs as against zero in control.
- *Beauveria brongniartii* was mass cultured on a low-cost molasses-based medium and formulated using press mud or lignite as carriers.
- The fungus *B. brongniartii* applied to the soil was found to survive in the soil for more than four years.

### Behavioural studies

- *Cotesia* multiplied continuously on shoot borer showed increased response to the borer in terms of time taken for oviposition and parasitisation.
- 'Y' tube olfactometer studies indicated that more number of *Cotesia flavipes* were attracted towards frass and damaged plants than their respective controls.

- *Cotesia flavipes* showed greater attraction towards a plant harbouring an unparasitised larva than a plant holding a parasitised larva.
- Three mg indigenous synthetic sex pheromone lures of early shoot borer, internode borer and top borer, were found effective for mass trapping of adult males.
- Development of low cost pheromone traps for mass trapping of internode borer male moths is currently under progress

### Host plant resistance

- Genotypes showing resistant to individual pests and all the 21 combinations of two pests, 24 out of 35 combinations three pests, 10 out of 35 combinations of four pests, 5 out of 21 combinations of five pests were identified. Resistant sources were available for single pest and some of the two pests combinations in all the species of *Saccharum* and *Erianthus*.
- *Saccharum robustum* and *Erianthus* contributed the highest source of resistance of 102 and 58 accessions respectively to various pest combinations.
- Plant morphology in terms of plant height, girth, internode shape, alignment and waxiness, leaf length and width, nature of leaf sheath and nature of ligular process and the phytochemical parameter lignin did not show any relationship with resistance to internode borer in the clones of *Erianthus* spp., *Saccharum robustum*, *S. sinense* and *S. barberi*.
- Seven foreign hybrids were found tolerant to white grub *Holotrichia serrata* in preliminary studies.

### Chemical Control

- Regent 3G @ 75g a.i/ha was found to be effective against shoot borer.
- Among the various leaf extracts tested, tobacco was the best and it acted as antifeedant and contact poison for shoot borer. Among kernels, *Thevitia nerifolia* gave the highest mortality to shoot borer and scale insect. In oil formulations, *Pongamea glabra* was the best as contact and stomach poison. Tobacco and *T. nerifolia* extracts had synergistic action when combined together. All the plant extracts were toxic to the adult parasitoids, *T.chilonis* and *Cotesia flavipes* and inhibited per cent parasitisation.

### Other studies

- In ecological studies of internode borer involving edaphic and nutritional factors, intensity of the borer was found to be higher in plots subjected to drought than in plots that received normal irrigation.
- Rearing methodology for mass production of shoot borer on artificial diet has been developed.
- Extensive studies on chemical, cultural and biological control of Sugarcane White Wolly Aphid (*Ceratovacuna lanigera* Zehntner (Homoptera: Aphididae)

### Plant parasitic nematodes

- **Nematode Survey & Biodiversity** -Sugarcane growing areas in tropical and subtropical parts of India were surveyed and abundance and distribution patterns of

important genera of plant parasitic nematodes were determined. About 48 species belong to 23 genera of plant parasitic nematodes were recorded from sugarcane ecosystem.

- Lesion nematode *Pratylenchus zae* was identified as the most widespread and economically important nematode infecting sugarcane. Other important nematode genera infecting sugarcane were *Meloidogyne*, *Hoplolaimus*, *Tylenchorhynchus* and *Helicotylenchus*.
- **Biology, Pathogenicity & Crop Loss** - Detailed investigations on the taxonomy, biology, pathogenicity, population dynamics and management of two most predominant nematodes viz., *Pratylenchus zae* and *Meloidogyne* spp. were completed.
- Crop loss caused by nematodes in sugarcane was estimated to be 10-20%. One nematode/gram of soil was found as the economic threshold level for *Pratylenchus zae*.
- Host Plant Resistance - About 400 sugarcane clones were screened for resistance to lesion nematode *Pratylenchus* spp. in pot culture and 10 resistant clones were identified. Three varieties viz., CoT8201, Co7717 and MS68/47 were found to be resistant to *P. zae* under field conditions.
- Biochemical basis of resistance in sugarcane against *P. zae* was elucidated and potential isozyme markers were identified for screening for resistance.
- **Biological control** - Nematode antagonistic fungi viz., *Paecilomyces lilacinus*, *Trichoderma viride* and *Trichoderma harzianum* were found to be effective in suppressing root-knot and lesion nematodes.
- Plant growth promoting rhizobacterial (PGPR) isolates with nematode suppressive ability against root-knot and lesion nematodes were identified
- Fungal and rhizobacterial isolates with nematode suppression and phosphate solubilization ability were identified.
- Low cost technologies based on locally available substrates were developed for mass production of nematode biocontrol agents.
- **Integrated Nematode Management** - An Integrated Nematode Management (INM) package based on eco-friendly components such as organic amendments, intercrops, biocontrol agents and host plant resistance was developed for reducing nematode damage and improving soil health in sugarcane.

## Entomopathogenic nematodes

- Sugarcane fields in different parts of India were surveyed for natural occurrence of entomopathogenic nematodes (EPN). About 40 isolates of EPN belonging to the genera *Heterorhabditis* and *Steinernema* were isolated. Bioecological and molecular characterization of these isolates is in progress.
- *Heterorhabditis indica*, a new species described from this institute and *H. bacteriophora* showed promise in controlling sugarcane white grub *Holotrichia serrata*.
- *In vivo* and *in vitro* mass production techniques have been standardized for *H. indica*, *H. bacteriophora* and *Steinernema carpocapsae*.

