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PROGRESS IN SUGARCANE VIRUS RESEARCH IN INDIA VIS-À-VIS DEVELOPMENTS IN MOLECULAR DIAGNOSTIC TOOLS

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Few decades ago, virus diseases in sugarcane were considered as minor or not so important in India. There were also reports that virus diseases do not cause any loss to sugarcane. Although the virus diseases seriously affected sugarcane productivity through varietal degeneration, it was not realized for many years. Major reason for the failure to notice such impact could be lack of precise diagnostic techniques, non-availability of virus genomic information and also due to lack of clarity in symptoms caused by different viral diseases.

In the absence of precise diagnostic techniques, scientists relied on differential hosts to establish variability in virus strains and serological techniques for the diagnosis. Although serological assays were supportive in diagnosis, large scale cross reactions were ignored especially in members of *Potyviridae* infecting sugarcane and related hosts. Mosaic was the only reported virus disease in sugarcane for many decades. During 1990s suspected occurrence of *Sugarcane bacilliform virus* (SCBV) was reported in *Saccharum officinarum* and other *Saccharum spp* clones. Though foliar symptoms indicated the suspected virus, it was not clear until confirmation by ISEM studies (Viswanathan *et al*., 1996). These studies gave authentic confirmation on SCBV infection in sugarcane from India. The virus exhibited enormous variation in symptoms on different genotypes of *Saccharum spp, Pennisetum sp* and cultivated varieties. ELISA assays were helpful to detect the virus suspected clones; however, genomic variation in the causative virus could not be brought out. Recently, molecular studies conducted at SBI clearly demarcated five different SCBV species infecting sugarcane and SCBV genome ranged from 7553 to 7884 nucleotides in size. The Indian SCBV isolates share identities of 69-85% for the complete genomic sequence, indicating wide genetic diversity among them, and share 70-82% identity with *Sugarcane bacilliform Ireng Maleng virus* (SCBIMV) and *Sugarcane bacilliform Morocco Virus* (SCBMV), as well as 43-46% identity with *Banana streak virus* (BSV) and BSV-related SCBV species and this variation indicates the distinctness of Indian SCBV population. It is concluded that the symptoms associated with badnaviruses in sugarcane in India are caused by at least three new species, SCBBbV, SCBBoV and SCBBruV, besides SCBIMV and SCBMV represented by SCBV-BT and SCBV-Iscam, respectively.

Earlier based on serology and differential host studies presence strains of several *Sugarcane mosaic virus* (SCMV) and *Sorghum mosaic virus* (SCMV) were reported. However, this has caused much confusion in identifying the virus or virus strains and with application of molecular techniques, association of a new virus *Sugarcane streak mosaic virus* (SCSMV) was reported with sugarcane mosaic. Detailed studies using RT-PCR conducted at SBI established that sugarcane mosaic in India is caused by SCMV and SCSMV in combination or separately (Viswanathan *et al*., 2007). In addition to diagnosis of these viruses, the molecular studies also facilitated in identifying variations in virus genomes. Molecular characterization of several SCMV and SCSMV isolates established variation in

coat protein genome of the respective viruses for the first time in India. Occurrence of nine strains of SCSMV in India was established. SCSMV was characterized as a new genus "Susmovirus" in the family *Potyviridae* based on its distinct coat protein genome (Viswanathan et al., 2008a). Recently ICTV, has renamed the genus as *Poacevirus* based on host range of the species in the genus. Recently complete nucleotide sequence of an SCSMV isolate from India, SCSMV-IND was determined. It is a linear single stranded positive sense RNA genome of 9786 nucleotides in length (excluding the Poly A tail) and it comprises a large open-reading frame encoding polyprotein of 3131 amino acid residues (Parameswari *et al*., 2012).

Among the viral diseases yellow leaf disease (YLD) caused by *Sugarcane yellow leaf virus* (SCYLV) is the recently identified disease and it has caused more damage than any other viruses in India. The impact of the virus is much more than some of the major fungal diseases in certain locations in the country. The disease was first reported in 1999 in India based on serological assays (Viswanathan, 2002). Subsequently, molecular diagnostic assays were developed and association of the virus was established after sequencing the virus genome. Detailed molecular characterization established occurrence of three genotypes of the virus including the genotype SCYLV-IND in India. Recently we have sequenced complete genome of four isolates of SCYLV (5875 nt) Phylogenetic analyses established that all the isolates belong to the genotype SCYLV-IND and the genotype reported from China CHNI shared a very close relationship with our genotype and they showed a separate lineage, probably of Asian genotypes (Chinnaraja et al., 2012).

Overall, with the adoption of molecular biological tools, our virus etiology with the diseases and genomic variation in the viruses was established beyond doubt. The pathologist also more authentic in associating sugarcane viruses with the disease, since symptoms of many of viral diseases in sugarcane camouflage with nutrient disorders and in certain cases, the virus disease expression is stage specific or asymptomatic. Also knowledge on virus genome helped us in developing precise diagnostic tools to index different viruses in sugarcane. Earlier we developed a multiplex RT-PCR to detect all the major RNA viruses, SCMV, SCSMV and SCYLV in a single reaction. This assay facilitated detection of three viruses in different sugarcane varieties (Viswanathan et al., 2010). In addition, duplex RT-PCR assays were developed to detect any of the two viruses together in a reaction. The sensitive techniques also efficiently detected the suspected viruses in tissue culture seedlings. Meristem tip culture along with RT-PCR is recommended to eliminate SCYLV from sugarcane while developing YLD-free nurseries. This practice has helped many sugar industries to revive severely degenerated varieties and SBI plays a pivotal role in indexing for sugarcane viruses in the country. Additionally, to simplify virus diagnostics, recombinant antisera were raised against SCSMV-coat protein and DAC-ELISA and immuno capture RT-PCR were developed for the diagnosis of the virus. These techniques will have applications in routine diagnosis of the virus. Similarly production of recombinant antisera against other viruses is in progress. To conclude, application of molecular techniques has comprehensively identified and characterized sugarcane viruses in India for the first time and they are applied in quarantine and healthy seed nursery programmes. Sugar industry is to be sensitized on the impact of viral diseases in sugarcane to improve sugarcane productivity in the India and other developing countries.

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CHANGING SPECTRUM OF SUGARCANE DISEASES AND IMPERATIVE NEEDS FOR DISEASE MANAGEMENT

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With the advent of current century, an all time high production of sugarcane (299.3 million tonnes) was achieved in the year 2000 and further in 2007, about 355.5 million tonnes of cane was produced. The achievement was certainly an outcome of research and development efforts. Nevertheless, sugarcane productivity of 71.3 tonnes/ha achieved about 16 years ago in 1994-95, is yet to be achieved. Although numerous factors related to soil, water, nutrients, environment, etc., could be responsible for the productivity stagnation, the diseases of sugarcane are equally responsible. According to an estimate of FAO, sugarcane diseases can inflict losses in cane yield up to 19%. For decades diseases like red rot, smut, wilt, grassy shoot, ratoon stunting and mosaic have been occurring on different varieties in mild to severe form. All through these years, it has been noticed that the most important disease in a region became less important and the disease not so important or a minor one occupied most important rank. Such a change in disease scenario may have taken place due to the following reasons:

(i) Change in varietal spectrum

(ii) Introduction of disease in a new region by seed transmission or through air or water

(iii) Abberations in weather

(iv) Change in cropping system

Changing spectrum of diseases

Red rot: The epidemics were frequently encountered in the last century till sixties. Many ruling varieties like Co 213, Co 312 and Co 1148 were phased out of cultivation. Since last one decade, the