Review of literature

1. Sugarcane

1.1 Botany

Sugarcane (Saccharum spp. hybrid) is a tall perennial tropical or sub-tropical grass that belongs to the genus Saccharum of the tribe Andropogoneae in the family Gramineae. It tillers at the base to produce unbranched stems from 2-3 m or more tall and to around 5 cm in diameter with a special characteristic of containing high percentage of sugar (Fig.1). Generally sugarcane grows successfully under varied soil and climatic conditions between latitudes 35° N and 35° S with intermediate area round about 18° latitude being the most favoured climatologically. Sugarcane can be grown upto 5,000 feet elevation nearer to the equator and upto 2,000 feet further north or south (Tate and Ellis, 2004).

The genus Saccharum comprises six species Saccharum officinarum, S. spontaneum, S. robustum, S. edule, S. barberi and S. sinense (D'Hont et al., 1998). S. officinarum is known as the noble cane and has high sucrose content and thick stems. The remaining five species are referred to as wild canes and have low sucrose content and thin stems. Noble canes, despite having high sucrose content, are susceptible to stress and many diseases making them poor commercial varieties. In contrast, species such as S. spontaneum have important agronomic traits such as stress tolerance and disease resistance, but possess lower sucrose content and hence are also poor commercial sugarcanes. In order to obtain better commercial sugarcane varieties, extensive breeding programs have been undertaken. Currently most of the commercially cultivated sugarcane varieties are complex interspecific hybrids with complex polyploid that were developed through intensive selective breeding of species within the Saccharum genus (Cox et al., 2000). As sugarcane does not produce true-to-type seed, propagation is via stalks (setts) cut so that at least one bud is present per piece.
1.2 Economic importance

Sugarcane is one of the important commercial crops and is the principal source of raw material for sugar, accounting for about 78% of world's sugar production (Smith and Rott, 2003). It is not only food cum cash crop but also has the ability to promote industrialization of rural areas. Sugarcane produces valuable by-products and co-products. Currently sugarcane is considered as one of the best converter of solar energy into biomass and sugar. The biomass contains fibre, lignin, pentosans and pith which can be converted into value added products by application of suitable chemical, biochemical and microbial technologies. Sugarcane is a versatile crop that is a rich source of food (sucrose, jaggery and syrups), fibre (cellulose), fodder (green leaves and tops of cane plant, bagasse, molasses and to some extent press mud), fuel and chemicals (bagasse, molasses and alcohol). The sugar industry by-products are vast potential reserves for human and animal consumption as well as capable of providing energy as renewable source (Yadav and Solomon, 2006).
1.3 Production in the world

More than 100 countries produce sugarcane, primarily in the tropical and sub-tropical zones of the southern hemisphere. It is one of the important crops of the world and in the year 2007 sugarcane was cultivated over an area of 21.98 million hectares with a total production of 1,557.66 million tons (MT) of cane. During 2007, India is the second largest producer of sugarcane (355.52 MT) next only to Brazil (514.08 MT) which is the top producer. Other major sugarcane growing countries are China (105.65 MT), Thailand (64.37 MT), Pakistan (54.75 MT), Mexico (50.68 MT), Colombia (40.00 MT), Australia (36.00 MT), USA (27.75 MT) and the Philippines (25.30 MT) (FAOSTAT, 2008). The major sugarcane growing regions of the world are depicted in the figure 2.

![Map showing major sugarcane growing regions](image)

Fig. 2: Map showing major sugarcane growing regions of the world. Red dots (●) indicate the distribution of SStMV.

1.4 Production in India

India ranks as one of the top producers of sugarcane in the world. During 2007, sugarcane is cultivated in India in about 4.90 million hectares with a total cane production of 355.52 MT (FAOSTAT, 2008). It is the third largest crop in terms of value next to rice and wheat and the crop is grown extensively in Uttar Pradesh, Maharashtra, Karnataka, Tamil Nadu, Andhra Pradesh, Gujarat, Haryana, Uttarakhand, Punjab, Bihar and Madhya Pradesh states.
2. Sugarcane diseases

Despite continuous efforts to improve cultivation practices, sugarcane will always be subjected to a variety of stresses like salt, draught, temperature and pathogens. Pathogen stress is one that is of high concern in most agro-ecologies causing losses that are usually substantial and sometimes devastating. Sugarcane throughout the world is susceptible to a range of plant pathogens from fungi, bacteria and phytoplasmas to viruses (Tate and Ellis, 2004). These diseases can cause yield losses in commercial production, limit the regions in which cultivars can be grown or restrict the importation of sugarcane germplasm. The risk of disease outbreaks or importation of unknown pathogens are major constraints in sustained sugarcane production.

2.1 Fungal, bacterial and phytoplasmal diseases

Over 160 fungal pathogens have been reported on sugarcane. These fungi belong to several genera including *Fusarium*, *Gibberella*, *Peronosclerospora*, *Puccinia* and *Ustilago* (Tate and Ellis, 2004). The bacterial pathogens of sugarcane belong to the genera *Pectobacterium*, *Pseudomonas*, *Acidovorax*, *Xanthomonas*, *Herbaspirillum* or *Clavibactur* (Tate and Ellis, 2004). Phytoplasmas have also been shown to cause various diseases in sugarcane, namely grassy shoot, green grassy shoot, white leaf and yellow leaf syndrome (Tate and Ellis, 2004). (Table. 1).

2.2 Viral diseases

Among plant pathogens, viruses are perhaps the most important group. Sugarcane production is significantly constrained by several viral diseases around the world (Braithwaite, 2001; Smith and Rott, 2003). The number of characterized viruses described from natural infections of sugarcane is expanding. Various characteristics of taxonomically characterized viruses reported to naturally infect sugarcane are listed in table. 2.
**Table 1: Certain major diseases of sugarcane**
(Source: Smith and Rott, 2003; Tate and Ellis, 2004)

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Disease</th>
<th>Causal agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Red rot</td>
<td><em>Glomerella tucumanensis</em></td>
</tr>
<tr>
<td>2.</td>
<td>Pineapple disease</td>
<td><em>Ceratocystis paradoxa</em></td>
</tr>
<tr>
<td>3.</td>
<td>Orange rust</td>
<td><em>Puccinia bukhnii</em></td>
</tr>
<tr>
<td>4.</td>
<td>Common rust</td>
<td><em>Puccinia melanocephala</em></td>
</tr>
<tr>
<td>5.</td>
<td>Pokkah boeng</td>
<td><em>Gibberella fujikori</em></td>
</tr>
<tr>
<td>6.</td>
<td>Brown stripe</td>
<td><em>Bipolaris stenospila</em></td>
</tr>
<tr>
<td>7.</td>
<td>Eye spot</td>
<td><em>Bipolaris sacchari</em></td>
</tr>
<tr>
<td>8.</td>
<td>Yellow spot</td>
<td><em>Mycovellosiella koapkei</em></td>
</tr>
<tr>
<td>9.</td>
<td>Smut</td>
<td><em>Ustilago scitaminia</em></td>
</tr>
<tr>
<td>10.</td>
<td>Leaf scorch</td>
<td><em>Stagonospora sacchari</em></td>
</tr>
<tr>
<td>11.</td>
<td>Downy mildew</td>
<td><em>Peronosclerospora sacchari</em></td>
</tr>
<tr>
<td>12.</td>
<td>Wilt</td>
<td><em>Fusarium sacchari</em></td>
</tr>
</tbody>
</table>

**Fungal diseases**

**Bacterial diseases**

| 1.    | Bacterial mottle         | *Pectobacterium chrysanthemi*       |
| 2.    | Leaf scald               | *Xanthomonas albilineans*           |
| 3.    | Ratoon stunting disease  | *Clavibacter xyli subsp. xyli*      |
| 4.    | Red stripe               | *Acidovorax avenae subsp. avenae*   |
| 5.    | Gumming disease          | *Xanthomonas axonopodi*             |
| 6.    | Stinking rot             | *Pseudomonas desaiana*              |
| 7.    | Mottled stripe           | *Herbaspirillum rubrisubalbicans*   |
| 8.    | Bacterial sun spot       | *Pseudomonas sp.*                   |

**Phytoplasmal diseases**

| 1.    | White leaf               | *Phytoplasma*                       |
| 2.    | Grassy shoot             | *Phytoplasma*                       |
| 3.    | Green grassy shoot       | *Phytoplasma*                       |
| 4.    | Ramu stunt               | *Phytoplasma*                       |
Table 2: Certain characteristics of taxonomically characterized viruses naturally infecting sugarcane

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Virus (Acronym)</th>
<th>Genus (Family)</th>
<th>Distribution</th>
<th>Transmission</th>
<th>Morphology &amp; size (nm)</th>
<th>CP species &amp; M₀ (kDa)</th>
<th>Genomes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>Sugarcane streak mosaic virus (SSSMV)</td>
<td>— (Potyviridae)</td>
<td>Asia (India, Pakistan, Bangladesh, Sri Lanka, Thailand, and Vietnam)</td>
<td>Mechanical, sets</td>
<td>Flexuous filamentous 890x15</td>
<td>One 34.5</td>
<td>ssRNA, linear, Unipartite, 9.5-10</td>
</tr>
<tr>
<td>3.</td>
<td>Sorghum mosaic virus (SmV)</td>
<td>Potyvirus (Potyviridae)</td>
<td>Worldwide</td>
<td>Mechanical, aphids, sets</td>
<td>Flexuous filamentous 702-714x13</td>
<td>One 34-41</td>
<td>ssRNA, linear, Unipartite, 9.5</td>
</tr>
<tr>
<td>4.</td>
<td>Sugarcane streak virus (SSV)</td>
<td>Mastrevirus (Geminiviridae)</td>
<td>Worldwide</td>
<td>Leafhoppers, sets</td>
<td>Geminate 20x30</td>
<td>One 28</td>
<td>ssDNA, Circular, Unipartite, 2.76</td>
</tr>
<tr>
<td>5.</td>
<td>Fiji disease virus (FDV)</td>
<td>Fijivirus (Reoviridae)</td>
<td>Pacific Islands, Australia, Fiji; Papua New Guinea, Philippines, Thailand, Samoa, U.S.A.</td>
<td>Leafhoppers, sets</td>
<td>Isometric 71 or 54</td>
<td>Seven 36-150</td>
<td>dsRNA, linear 10 parts, 1.7-4</td>
</tr>
<tr>
<td>6.</td>
<td>Sugarcane bacilliform virus (SCBV)</td>
<td>Bachuvirus (Caulimoviridae)</td>
<td>Cuba, Morocco, USA.</td>
<td>Mealybugs, sets</td>
<td>Bacilliform 131x31</td>
<td>Two 27, 35</td>
<td>dsDNA, circular, 8 kbp</td>
</tr>
<tr>
<td>7.</td>
<td>Sugarcane mild mosaic virus (SCMMV)</td>
<td>Closterovirus (Closteroviridae)</td>
<td>Australia</td>
<td>Mealybugs, sets</td>
<td>Flexuous filamentous 1504-2200x10-18</td>
<td>--</td>
<td>ssRNA, linear, Unipartite</td>
</tr>
<tr>
<td>8.</td>
<td>Sugarcane yellow leaf virus (SclTYV)</td>
<td>Luteovirus (Luteoviridae)</td>
<td>Brazil, USA, Australia</td>
<td>Aphids, sets</td>
<td>Isometric 23-26</td>
<td>--</td>
<td>ssRNA, linear, Unipartite</td>
</tr>
<tr>
<td>9.</td>
<td>Peanut stunt virus (PCV)-sugarcane isolate</td>
<td>Furovirus</td>
<td>France, India, Australia</td>
<td>Mechanical, Fungus, sets</td>
<td>Rigid rods 190-245x21</td>
<td>--</td>
<td>ssRNA, Bipartite</td>
</tr>
</tbody>
</table>

* Type, conformation, number of species and M₀ (kbp)  
Data source: Hema et al., 2001; Fauquet et al., 2005.
3. Molecular characterization of plant viruses

In attempts to control viral diseases, the primary step is to characterize the disease causal virus by studying its properties and to establish the identity of the virus. Several hundred virus diseases of plants have been described and apparent new ones continue to be reported. Often the causal agents are described as new viruses on totally inadequate grounds. When an unknown virus disease is being investigated, it has to be determined if the virus concerned is identifiable with any that are already known. In some instances may need to go to determination of particle morphology to determine to which group the virus belongs, followed by an appropriate serological test to identify the virus. If the virus under investigation is not easily assigned to any known taxon, the every property possible is to be investigated, to be sure that the virus is 'new', to discover its relationships and to publish an adequate description. The molecular level information on virions is helpful to develop molecular detection tools, to understand genome organization and expression strategies, to predict structural organization of polypeptides in the virions, to develop gene vectors and to exploit the virus genomes or sequences in producing transgenic plants (Hull, 2002).

3.1 Characterization of viral proteins

Most of the small geometric plant viruses with ssRNA as their genetic material have only one kind of protein subunit in the virus particle. In some viruses with (+) sense ssRNA genomes the basic capsid structure is made up of two coat protein species (e.g. comoviruses). In other, some of the coat protein subunits may be variants of the major coat protein; these are frequently associated with interaction with their vector (Hull, 2002). The number of polypeptides and molecular weight of the coat protein are very useful in assigning a virus to a taxonomic group. Amino acid composition and peptide profiling of coat protein are also important in distinguishing viruses. The properties of viral proteins, and in particular the amino acid sequences are of great importance in virus classification at all levels: for delineating strains, viruses and for indicating evolutionary relationships between families and groups of viruses. The amino acid sequence data of plant viral proteins can be used in concurrence with nucleotide sequence and serology to divide viruses of a group into distinct members. Coat protein amino acid sequence homologies
have been used to distinguish between distinct potyviruses and strains of these viruses and to estimate degrees of relationship within the group. Sometimes, phylogenetic relations based on one protein may not be similar to that on another protein. This is suggestive of recombination between viruses. In spite of the fact that coat proteins represent only a small fraction of the information in most viral genomes, they appear to be the most useful gene products for delineating many distinct viruses and virus groups (Shukla and Ward, 1989 a, b; McKern et al., 1992, 1993).

3.2 Characterization of viral nucleic acids

Most plant viruses have their genomes enclosed in either a tube shaped or an isometric shell made up of many small protein molecules. The majority of viral genomes are RNA with most of these being (+) sense RNA. Some viruses have (-) sense or dsRNA genomes while others have ssDNA or dsDNA. The genomes of, at least, one member of each virus genus has now been sequenced. Number of genome segments, their sizes, presence of 5'-linked protein, presence of 3'-poly (A) tracts, infectivity, melting profiles and nucleotide sequence are useful in precise identification of taxon of a virus.

The organization and strategy of viral genomes is now of prime importance for placing of viruses into families, and genera or for the establishment of a new family or genus. There is great interest in comparing the nucleotide and derived amino acid sequences of different viruses, from the point of view of virus evolution, virus classification and the functional roles of viral genes and controlling elements. The size of a viral nucleic acid is perhaps the most important property of the virus that can be expressed as a single number. The nucleotide sequences for many viral RNAs and DNAs have been determined. The ability to generate cDNA libraries covering the full extent of viral RNA genomes allowed DNA sequencing methods to be applied to determine the complete nucleotide sequence of such RNAs. These allow precise determination of molecular weight (Hull, 2002).

4. Potyviridae

The family Potyviridae constitutes the largest family of plant viruses responsible for a number of economically important plant diseases. This family
contains 198 definitive and possible members, accounting for more than one third of all viruses known to infect plant species around the world. Potyviruses are found throughout the world infecting more than 2000 plant species in more than 550 genera in 81 families of plants. As a family, potyviruses cause greater crop losses than all other plant viruses combined. Nearly 30% of all known plant viruses belong to this family. New members are being discovered and added to the list of potyviruses more frequently than for any other virus group (Ward et al., 1992; ICTV VIII report).

4.1 General properties (ICTV VIII report)

Potyvirus particles are flexuous filamentous rods with no envelope and are 11-15 nm in diameter, with a helical pitch of about 3.4 nm. Particle lengths of members of some of the six genera differ. The genome is a single molecule of linear, positive sense, single stranded RNA. However, in bymoviruses it is bipartite. Based on their genome organization and expression strategy, potyviruses have been included in the super group of picorna-like viruses (Urcuqui-Inchima et al., 2001).

All the members of the family Potyviridae form cytoplasmic cylindrical inclusion (CI) bodies during infection. The CI is an array of a viral protein which possesses ATPase and helicase activities. The viruses also encode NIa and NIb proteins which form pinwheel inclusions, but inclusion bodies composed of these proteins are not formed in all instances. NIa and NIb are also found in the cytoplasm. Amorphous inclusion bodies are also evident in the cytoplasm during certain potyvirus infections and represent aggregations of the protein HC-Pro. The inclusion bodies resulting from infection are characteristic of individual viruses and groups of viruses. Based on inclusions potyviruses have been assigned to one or other of four subdivisions and are useful in their identification (Edwardson et al., 1993).

Some of the members of the family Potyviridae have a narrow host range; most members infect an intermediate number of plants, and a few members infect species up to 30 families. Transmission to most hosts is readily accomplished by mechanical inoculation. Many viruses are widely distributed. Distribution may be aided by seed transmission in some cases.
4.2 **Current classification** (ICTV VIII report)

Currently, six genera are recognized in the family Potyviridae: Bymovirus, Ipomovirus, Macluravirus, Potyvirus, Rymovirus and Tritimovirus. Overview of Potyviridae classification is shown in the table 3.

**Table 3: Overview of Potyviridae classification**

<table>
<thead>
<tr>
<th>Genus</th>
<th>Type species</th>
<th>Number of species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bymovirus</td>
<td>Barley yellow mosaic virus (BaYMV)</td>
<td>6</td>
</tr>
<tr>
<td>Ipomovirus</td>
<td>Sweet potato mild mottle virus (SPMMV)</td>
<td>4</td>
</tr>
<tr>
<td>Macluravirus</td>
<td>Maclura mosaic virus (MacMV)</td>
<td>6</td>
</tr>
<tr>
<td>Potyvirus</td>
<td>Potato virus Y (PVY)</td>
<td>129</td>
</tr>
<tr>
<td>Rymovirus</td>
<td>Ryegrass mosaic virus (RGMV)</td>
<td>3</td>
</tr>
<tr>
<td>Tritimovirus</td>
<td>Wheat streak mosaic virus (WSMV)</td>
<td>3</td>
</tr>
<tr>
<td>Unassigned</td>
<td>Sugarcane streak mosaic virus (SSStMV)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Spartina mottle virus (SpMV)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tomato mild mottle virus (TMMV)</td>
<td></td>
</tr>
</tbody>
</table>

The data is based on ICTV Taxonomy of Viruses 2008.

4.2.1 **Genus Bymovirus**: (Type species: Barley yellow mosaic virus)

The virions are of 13 nm width and model lengths, 250-300 and 500-600 nm. The genome is divided between two RNA species. The longer particle contains RNA of 7.5-8.0 kb in size and the shorter particle has RNA of 3.5-4.0 kb in size. The capsid is made up of multiple copies of a single protein species of 28-33 kDa. Members of this genus are transmitted by the plasmodiophora fungus Polymyxa graminis.

4.2.2 **Genus Ipomovirus**: (Type species: Sweet potato mild mottle virus)

Virus particles are 800-950 nm long containing a genome of 10.8 kb encapsidated in multiple copies of a single protein species of about 38 kDa. The natural vector of ipomoviruses is whitefly Bemisia tabaci and transmitted in non-persistent manner.
4.2.3 Genus *Macluravirus*: (Type species: *Maclura mosaic virus*)

Macluravirus particles are 650-675 nm long and contain an RNA of about 8 kb encapsidated in multiple copies of a single protein species of 33-34 kDa. They are transmitted by aphids in a non-persistent manner.

4.2.4 Genus *Potyvirus*: (Type species: *Potato virus Y*)

Virus particles are 680-900 nm long and 11-13 nm in diameter encapsidating a genome of about 9.7 kb with multiple copies of a single protein species of 30-47 kDa. Potyviruses are transmitted by aphids in a non-persistent manner using a helper component. Some of the members of this genus are also seed transmitted.

4.2.5 Genus *Rymovirus*: (Type species: *Rye grass mosaic virus*)

Particles of this genus are 690-720 nm long and 11-15 nm diameter that contain an RNA genome of 9-10 kb with the capsid comprising many copies of a single protein species of about 29 kDa. They are transmitted by eriophyid mites.

4.2.6 Genus *Tritimovirus*: (Type species: *Wheat streak mosaic virus*)

Tritimovirus particles are 690-700 nm long and contain an RNA of about 8.5-9.6 kb in size. The capsid comprises multiple copies of a protein of about 32 kDa. They are transmitted by eriophyid mites possibly in a persistent manner.

*Unassigned members*: Sugarcane streak mosaic virus (SSStMV), Spartina mottle virus (SpMV) and Tomato mild mottle virus (TMMV).

4.2.7 Species demarcating criteria in the genera:

- Genome sequence relatedness.
  - CP amino acid sequence identity less than about 80 %,
  - Nucleotide sequence identity of less than 85 % over whole genome,
  - Different polyprotein cleavage sites.
- Natural host range
  - Host range may be related to species but usually not helpful in identifying species. May delineate strains.
• Pathogenicity and cytopathology.
  • Different inclusion body morphology,
  • Lack of cross-protection,
  • Seed transmissibility or lack there of,
  • Some aspects of host reaction may be useful.

• Mode of transmission.
  • Different primary vectors (not of use in identification to virus species).

• Antigenic properties.

• Serological differences.

4.3 Genome organization (Urcuqui-Inchima et al., 2001; ICTV, 2008)

Members of the family Potyviridae have a genome of single stranded, positive sense RNA. The members of five genera (Potyvirus, Ipomovirus, Macluravirus, Rymovirus and Tritimovirus) and unassigned members have a monopartite genome that contain a single RNA molecule. Where as the members of the genus Bymovirus have a bipartite genome that contain two RNA molecules.

The genome of monopartite potyviruses is about 10 kb in length characterized by a covalently linked 5'-terminal viral protein (VPg). An obvious characteristic of potyviruses is the presence of a long untranslated region (UTR) at the 5' end of the genome, which ranges between 75 and 225 nucleotides in length. The 5'-UTR is rich in adenine residues and has only a few guanine residues. The 3'-UTR of different potyviruses have been described as heterogeneous in size (143-593 nts), sequence and predicted secondary structure. A common feature of all of them is the presence of AU rich segments and the fact that each sequence can fold into stable secondary structures. The poly (A) tails of the potyviral RNAs have been found to be very heterogeneous in length. Potyviruses lack a cap structure at the 5' end of the genomic RNA and may perform a so-called cap-independent leaky scanning mechanism of translation. The genome organization of potyviruses is illustrated schematically in figure. 3.
The potyviral genomic RNA contains a single long open reading frame (ORF) that encodes a single large polyprotein precursor which is subsequently cleaved by virus-encoded proteolytic enzymes to yield functional protein products. The order of these products from the N-terminus to the C-terminus of the polyprotein is: first protein (P1 protease), the helper component / protease (HC-Pro) protein, third protein (P3), 6K peptide (6K1), cylindrical inclusion (CI) protein (movement protein with RNA helicase activity), second 6K peptide (6K2), the nuclear inclusion a protein (NIA, consisting of a VPg and a protease), nuclear inclusion b protein (NIB, the RNA dependent RNA polymerase) and the coat protein (CP). Only two of these, VPg and CP are detectable in virus particles.

4.4 Polyprotein processing

The genomes of plant viruses in the family Potyviridae contain a single long ORF and encode large polyproteins that are cleaved by virus-encoded proteases to yield 10 functional mature protein products of different sizes. Potyviruses encode three proteases, the N-terminal 35 kDa P1 protein, the N-terminal part of the 52 kDa HC-Pro and the 27 kDa C-terminal part of the NIA protein. Each of these proteases cuts at sites with a distinctive sequence pattern. Processing of the potyvirus polyprotein is shown in the figure 4.
Fig. 3: Diagrammatic representation of typical genome organization of monopartite potyviruses (VIII ICTV report).
Fig. 4: Schematic representation of the processing of potyvirus polyprotein (Reichmann et al., 1992)
4.4.1 PI protease:

The C-terminus of PI is a S30 serine protease that cleaves PI from the PI-HC-Pro product. The catalytic triad His-(X_{7,11})-Asp-(X_{30,36})-Ser with GLy-X-Ser-Gly around the active site serine is strictly conserved. The protein cleaves itself from the polyprotein i.e. at the PI/HC-Pro junction (Adams et al., 2005b).

4.4.2 HC-Pro protease:

The C-terminal region of the potyvirus HC-Pro is a C6 cysteine protease. This protease has cysteine and histidine residues at its active site and resembles papain-like cysteine proteases (Oh and Carrington, 1989). In the cleavage site, positions -4, -2, -1 and +1 of the G:G cleavage site are crucial while -5, -3 and +2 were not (Carrington and Herndon, 1992).

4.4.3 Nla protease:

They have cysteine residue in the active site and are classified as C4 cysteine proteases. These are related to the 3C proteases of picornaviruses. This is the major protease of potyviruses and has a two-domain structure. Nla is autocatalytically released from the polyprotein and then catalyses the cleavage of the various junctions to release P3, 6K1, CI, 6K2, Nlb and CP. The cleavage sites have been identified for several viruses and the primary sites are considered to be QS, QG and QA with the motif V-X-X-Q/(A,S,G or V) thought to be conserved in all potyviruses (Adams et al., 2005b).

4.5 Functions of potyviral proteins

The proteolytic cleavage of potyviral polyprotein yields ten functional proteins viz. PI, HC-Pro, P3, 6K1, CI, 6K2, VPg, Nla, Nlb and CP. Major functions of the potyviral proteins are depicted in figure 5.

4.5.1 P1 protein:

\textit{P1 is a proteinase:} The P1 protein is a serine-type proteinase which catalyses autoproteolytic cleavage at a Tyr-Ser or Phe-Ser dipeptide between itself and the helper component-proteinase, HC-Pro (Yang et al., 1998). The C-terminal 147 aa residues of the P1 protein constitute the complete functional proteinase; the N-terminal 157 aa residues are dispensable for proteinase activity (Verchot et al., 1992).
**PI interacts with RNA:** In addition to its proteolytic activity, the PI protein has been shown to exhibit nonspecific RNA-binding activity (Soumounou and Laliberte, 1994). The RNA-binding properties of PI are similar to those described for known movement proteins of plant viruses (Osman et al., 1992, 1993).

**PI in cell-to-cell transport:** It has been suggested that PI could also be involved in cell-to-cell transport of virus in plants (Dougherty and Semler, 1993). However, it has been demonstrated by mutational and complementation analysis that deletion of the entire PI coding sequence had only minor effects on cell-to-cell and long distance transport but considerably reduced genome amplification of TEV mutants, suggesting that the function of PI is related to virus replication (Verchot and Carrington, 1995a, b). The role of the N-terminal part of PI in the life-cycle of potyvirus remained unclear.

### 4.5.2 Helper component protease (HC-Pro):

Potyviral helper component-protease (HC-Pro) is a multifunctional protein involved in aphid transmission, polyprotein processing, long-distance movement, genome amplification and symptom expression.

**HC-Pro in vector transmission:** The active form of HC-Pro is a homodimer and the N-terminal region is required for efficient genome amplification and aphid transmission (Guo et al., 1999).

**HC-Pro has protease activity:** The C-terminal domain of HC-Pro has cysteine-type protease activity that autocatalytically cleaves between HC-Pro and P3 protein at its C-terminus. In TEV the cleavage site between HC-Pro and P3 is G763-G764 (Carrington and Herndon, 1992). The HC-Pro/P3 dipeptide cleavage site is conserved in all monopartite members of the family Potyviridae (Adams et al., 2005b).

**HC-Pro in movement:** It is involved in systemic as well as in cell-to-cell movement of virions. The central region is required for long-distance movement in plants (Wang et al., 1998).
Fig. 5: Major functions of potyviral proteins (Data source - Urcuqui-Inchima et al, 2001).
**HC-Pro as gene silencing suppressor:** The helper component-proteinase (HC-Pro) of potyviruses acts as a suppressor of gene silencing in virus infected plants. The FRNK box in the central region of HC-Pro is highly conserved and is associated with the suppression of gene silencing in *Zucchini yellow mosaic virus* (ZYMV) infected plants (Shibolet *et al.*, 2007).

4.5.3 P3 protein:

The complete P3 protein function(s) still remain unclear. Immunogold labelling with the antiserum against the nonstructural P3 protein of TEV indicated that it is involved in early stages of viral replication (Langenberg and Zhang, 1997).

4.5.4 6K1 and 6K2 proteins:

The two small proteins 6K1 and 6K2 (~6 kDa proteins) are co-existing with NIa and NIb in nuclear inclusion, suggesting that they may play a role in RNA replication or function as NIa (Restreppo-Hartwig and Carrington, 1994).

4.5.5 Cylindrical inclusion protein (CI):

**CI is a major component of replication complex:** The CI protein contains a nucleotide binding motif typical of RNA helicases of the superfamily SF2 of RNA viruses (Lain *et al.*, 1990). This motif consists of seven conserved sequences of VGSGKST from N- to C-terminus. The CI protein motifs possess nucleotide binding and helicase activity (Gorbalenya *et al.*, 1988). Experimental data evidenced that the helicase activity of CI protein plays a role in virus RNA replication inside the infected cell.

**CI in cell-to-cell movement:** A study in PSbMV-infected pea cotyledons showed the cylindrical inclusion (CI) protein to exist in transient functional states, and the presence of the CI was linked with an apparent transient reduction in callose in the vicinity of the plasmodesmata in aiding virions to adjacent cell (Roberts *et al.*, 1998). Later, CI protein was analysed genetically using alanine-scanning mutagenesis and the subsequent results provided genetic evidence for a direct role of CI protein in potyvirus intercellular movement, and for distinct roles of the CI protein in genome replication and cell-to-cell movement (Carrington *et al.*, 1998).
4.5.6 Viral protein genome-linked (VPg):

The VPg is the N-terminal part of NIa that is covalently attached to the 5' end of viral RNA via its tyrosine residue (Murphy et al., 1991).

VPg in genome replication: It may serve as the primer for viral RNA synthesis (Shahabuddin et al., 1988), may also be involved in cleavage of the replicative form of RNA (Tobin et al., 1989). In addition, the VPg is the moiety responsible for the stimulation of polymerase activity (Fellers et al., 1998b).

VPg in systemic movement: A naturally occurring variation of several amino acids in the VPg of TVMV enabled the virus to overcome va gene resistance in tobacco (Nicolas et al., 1997), suggesting that changes in configuration of VPg may affect the interaction between VPg and appropriate host components regarding systemic virus movement.

VPg in virulence: The mechanism of Pisum sativum pathotype-specific resistance to PSbMV was investigated and the coding region determinant of PSbMV virulence was defined (Keller et al., 1998). Challenging of P-1-resistant peas with infectious full length P-1/P-4 recombinant clones of PSbMV revealed that 21 kDa VPg of the P4 pathotype determines its virulence.

VPg interacts with plant translation initiation factors: VPg binds to the eukaryotic translation initiation factor eIF(iso)4E and this interaction has been shown to be important for virus infection. VPg is a novel eIF4E-binding protein required for virus infectivity and inhibits host protein synthesis at a very early stage of the initiation complex formation through the inhibition of cap attachment to the initiation factor eIF4E. In the case of TuMV, VPg-Pro interaction with eIF(iso)4E is involved in perturbing normal cellular functions. (Beauchemin et al., 2007).

4.5.7 Small nuclear inclusion protein (NIa):

NIa, together with NIb, forms nuclear inclusion bodies in the cells of most potyvirus-infected plants. NIa consists of two proteins, the N-terminal VPg and a C-terminal trypsin-like serine protease. VPg is cleaved from the C-terminal 27K proteinase domain at a suboptimal cleavage site.
The protease cleaves most of the proteins from the precursor protein, such as, the CI-NIa and Nla-NIb junctions and catalyses the production of CI, NIb and CP by cleaving the P3-Cl, and NIb-CP junctions (Carrington and Dougherty, 1988). Additional cleavages, to release VPg and 6K1 and 6K2 products also occur (Garcia et al., 1992). Amino acid alignment of potyvirus Nla with other virus and cellular trypsin-like serine proteinase suggested a catalytic triad of histidine, aspartate and cysteine with the histidine residue in the substrate binding pocket. The RNA binding activities of Nla protease domain (NlaPro) and full-length Nla protein were similar. Later the role of Nla both in protein cleavage and in cell to cell movement was evidenced by Nla gene transfer into plants (Maiti et al., 1993; Fellers et al., 1998a).

4.5.8 Large nuclear inclusion protein (NIb):

NIb is an RNA-dependent RNA polymerase (RdRp): It is responsible for replication of virus plus and minus RNA molecules (Deiman et al., 1997). The most prominent sequence feature of the NIb is a glycine-aspartate-aspartate (GDD) motif characteristic for almost all RNA viruses of bacteria, animals, and plants (Koonin, 1991). The NIb protein interacts with the VPg domain of the Nla protein and this interaction requires a functional RNA attachment site. This interaction may be important for the initiation of viral RNA synthesis in infected cells. The polymerase activity of the NIb protein can be stimulated by VPg and Nla proteins. The CP interacts with the NIb in a manner that is sensitive to changes in the highly conserved GDD motif. Besides the functions of RNA-dependent RNA polymerase, the NIb protein of TEV possesses other functions, including nuclear translocation activities (Li and Carrington, 1993). NIb is directed to replication complexes through an interaction with the Pro-domain of Nla. RdRp is required for viral genome replication. RdRp, probably together with other components of both virus and cellular sources, forms a replicase-complex to fulfil this process of viral genome replication.

4.5.9 Coat protein (CP):

CP is the major gene product in virions and accounts for 90-95 % of the mass of most virus particles. The structure and functions of CP are extensively characterised, and a large number of CP sequences of potyviruses have been obtained (Adams et al., 2005a).
**CP in vector transmission:** The highly variable N-terminal region displayed on the virion surface is involved in virus specific functions or host/vector-virus interactions. Sequence data as well as site directed mutagenesis experiments confirmed that the conserved DAG motif located at the N-terminus of CP, together with HC-Pro, is responsible for aphid transmission (Atreya et al., 1990; 1992).

**CP in cell-to-cell and systemic movement:** CP, together with CI, is also responsible for cell to cell or long distance movement of viruses (Carrington et al., 1998).

**CP in encapsidation of viral RNA:** The highly conserved central and C-terminal regions of CP are involved in encapsidation of viral RNA. Evidence was found that assembly of a potyvirus begins near the 5'-terminus of the viral RNA (Wu and Shaw, 1998).

**CP regulates viral RNA synthesis:** CP is involved in the replication of potyviruses and the interaction between CP and RNA may be either sequence-specific or nonspecific. In case of PVA, studies revealed that CP binds RNA in a sequence-nonspecific manner. (Merits et al., 1998)

### 4.6 Genome sequence data in Potyviridae classification

Before the advent of sequence data, species and strains were distinguished with difficulty using criteria such as host range, symptomatology and serology. However, the availability of CP, and then full genome sequences helped to clarify the Potyviridae taxonomy and led to the establishment of criteria that can be used in particular to distinguish closely-related virus species from those that are strains of the same species.

Coat protein amino acid sequence comparisons have been used as a criterion for potyvirus classification. Three patterns of CP amino acid identities can be found: different potyvirus species have identities that range from 55 to 75 %; closely related species have identities of 74 to 88 %, and isolates of the same species have identities ranging from 90 to 99 % (Shukla et al., 1994). Beside the CP amino acid sequence, the nucleotide sequence of the 3'-UTR can also be used as a marker for genetic relatedness. Isolates of the same species show a high degree of nucleotide identity (83-99 %), whereas the identities between different species are rather low (39-53 %) (Turpen, 1989; Bousalem et al., 2000). Thus, the CP and the 3'-UTR sequences have in many cases been very informative in determination of the taxonomic position of a virus isolate.
Since these comparisons were based on the CP which is encoded by less than 10% of the viral genome, the validity of this pairwise analysis is questionable. Nevertheless, pairwise comparison of full genome sequences indicated that the taxonomic discrimination inferred from the comparison of the complete genomes appeared to support the previously described from the comparison of the partial sequences. The potyviral genome sequence data have been examined to define the optimal sequence criteria for genus and species discrimination for each gene separately and for the whole ORF and also to determine how well the comparisons based on individual genes reflect those provided by the entire ORF (Adams et al., 2005a). The range of nucleotide and amino acid identities amongst the individual genes of all the fully sequenced ORFs of members of the family Potyviridae are given in the table. 4.

5. Detection of plant viruses

Unlike other plant pathogens, there are no direct methods available yet to control viruses and, consequently, the current measures rely on indirect tactics to manage the viral diseases. Hence, methods for detection and identification of viruses, both in plants and vectors, play a critical role in virus disease management. Correct identification of the virus or viruses infecting a particular crop is essential for effective control measures to be applied. Disease symptoms alone may be misleading. Availability of diagnostic methods provide greater flexibility, increased sensitivity, and specificity for rapid diagnosis of virus diseases in disease surveys, epidemiological studies, plant quarantine and seed certification, and breeding programs. Many methods have been developed for the detection and identification of plant viruses. A single diagnostic test or assay may provide adequate information on the identity of a virus, but a combination of methods is generally needed for unequivocal diagnosis (Martin, 1998; Hull, 2002).
Table 4: The range of nucleotide and amino acid identities in pairwise comparisons amongst the individual genes of all the fully sequenced ORFs of members of the family Potyviridae (Adams et al., 2005a)

<table>
<thead>
<tr>
<th>Between genera</th>
<th>% nt identity</th>
<th>% aa identity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Different genus</td>
<td>Same genus</td>
</tr>
<tr>
<td>P1</td>
<td>34.7–47.6</td>
<td>34.6+</td>
</tr>
<tr>
<td>HC-Pro</td>
<td>35.1–46.5</td>
<td>47.1+</td>
</tr>
<tr>
<td>P3</td>
<td>33.4–44.7</td>
<td>36.9+</td>
</tr>
<tr>
<td>CI</td>
<td>38.4–55.4</td>
<td>49.4+</td>
</tr>
<tr>
<td>VPg</td>
<td>33.2–55.7</td>
<td>42.3+</td>
</tr>
<tr>
<td>N1a-Pro</td>
<td>33.6–52.8</td>
<td>45.2+</td>
</tr>
<tr>
<td>N1b</td>
<td>42.2–59.4</td>
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</tr>
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<td>CP</td>
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<tr>
<td>Polypeptide</td>
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</tr>
<tr>
<td>5′-UTR</td>
<td>33.8–62.8</td>
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</tr>
<tr>
<td>3′-UTR</td>
<td>31.6–51.8</td>
<td>30.9+</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Between species</th>
<th>% nt identity</th>
<th>% aa identity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Different species</td>
<td>Same species</td>
</tr>
<tr>
<td>P1</td>
<td>34.6–68.9</td>
<td>41.4+</td>
</tr>
<tr>
<td>HC-Pro</td>
<td>35.1–75.7</td>
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<tr>
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<td>33.4–79.6</td>
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<tr>
<td>CI</td>
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<tr>
<td>VPg</td>
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</tr>
<tr>
<td>N1a-Pro</td>
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<td>76.6+</td>
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<tr>
<td>CP</td>
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</tr>
<tr>
<td>Polypeptide</td>
<td>38.6–74.7</td>
<td>77.1+</td>
</tr>
<tr>
<td>5′-UTR</td>
<td>32.0–74.2</td>
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</tr>
<tr>
<td>3′-UTR</td>
<td>30.9–84.0</td>
<td>71.9+</td>
</tr>
</tbody>
</table>
5.1 Biological

Since their inception, biological assays are still in widespread use, because they are simple, require minimal knowledge of the virus. Virus detection and identification techniques originated with mechanical transmission of the viruses to susceptible indicator plants and characteristic symptoms produced by these plants allow both the detection and identification of many viruses. Although host-range may not be a precise guide for virus identification, it is still used in many laboratories as an important assay in virus diagnosis. The reliability of host-range tests for diagnosis can be increased with hands-on experience and by using a suitable range of plant species. However, some isolates, despite multiplying in the host, can induce no symptoms, thus escaping detection and the major limitations are the long time (weeks to months) required to obtain results, and the impossibility of large-scale use (Hull, 2002).

5.2 Physical assays

The physical properties of a virus (e.g., thermal inactivation point, dilution end point, and longevity in vitro), taken to be a measure of infectivity of the virus in sap extracts, were previously used to identify plant viruses. However, these properties are unreliable and no longer recommended for virus diagnosis (Francki, 1980). Electron microscopy (EM) provides very useful information on the morphology of the virus particles. Many plant viruses induce distinctive intracellular inclusions, or develop large crystalline accumulations of virus particles, and their detection by light microscopy or EM can provide a simple, rapid, and relatively inexpensive method to confirm viral infection (Edwardson et al., 1993). Because of the uniqueness of inclusions produced as a result of infection by some viruses, unknown viruses can sometimes be identified to the genus level based on inclusion bodies observed using selective stains. Plant virus inclusion technology, however, requires extensive hands-on experience and is seldom used by the novice for routine viral disease identification.

5.3 Viral protein based assays

Serological or immunological assays are based on viral coat protein and include precipitation/agglutination tests, immunosorbent electron microscopy (ISEM), enzyme-linked immunosorbent assay (ELISA), and immunoblotting.

28
5.3.1 Immunoprecipitin tests:

Precipitin tests (either in liquid medium or in agar/agarose) rely on the formation of a visible precipitate when adequate quantities of virus and specific antibodies are in contact with each other. Precipitin and microprecipitin tests are routinely used by some investigators, but agglutination and double diffusion tests are more commonly used. The double diffusion method can be used to distinguish related, but distinct, strains of a virus or even different but serologically related viruses. However, disadvantages of this method include lack of sensitivity in detecting viruses that occur in low concentration (e.g., luteoviruses and most viruses of woody hosts), the need to dissociate filamentous or rod-shaped viruses to allow them to diffuse through the gel matrix, and the need for large quantities of antibodies (van Regenmortel, 1982).

5.3.2 Immunosorbent electron microscopy (ISEM):

ISEM combines the specificity of serological assays with the visualization capabilities of the EM. Virus particles are selectively trapped on antibody-coated grids with little contaminating host-plant material. Hence, the technique is more sensitive for detecting viruses than the leaf dip method. In addition to diagnosis, ISEM can also be used to estimate the degree of serological relationship between viruses. Although ISEM is a sensitive technique, it has the same drawbacks as EM. Nonetheless, it is ideal for confirmatory tests using small numbers of samples, if the EM facility and specific antisera are available (Milne, 1993).

5.3.3 Enzyme immunoassays:

Applying enzyme-linked immunosorbent assays (ELISAs) to viruses revolutionized diagnosis, making the analysis of large number of samples feasible, simpler, and with both low cost and high sensitivity (Sward and Eagling, 1995). Although polyclonal antibodies frequently presented problems of specificity, the availability of specific monoclonal and recombinant antibodies solved this problem (Kohler and Milstein, 1975; Terrada et al., 2000). They offer a number of advantages for routine diagnostic purposes including a detection limit in the range of 1–10 ng virus/mL, easy handling of a large number of samples, specificity for differentiation of serotypes and assessment of the assay result by eye. Although it
has met the need of routine diagnosis for the detection of most plant viruses in plant material, the poor detection limit is its limitation. Sometimes it is not suitable for detecting very low virus concentrations (Martin, 1998). Dot immunoblotting assay (DIBA) can be used to detect viruses in both plants and vectors. The technique is similar to ELISA except that the plant extracts are spotted on to a membrane rather than using a microtitre plate as the solid support matrix. Unlike in ELISA, where a soluble substrate is used for colour development, a precipitating (chromogenic) substrate is used for virus detection in the DIBA. Hydrolysis of chromogenic substrates results in a visible coloured precipitate at the reaction site on the membrane. Chemiluminescent substrates, which emit light upon hydrolysis, can also be used and the light signal detected with X-ray film (Leong et al., 1986). An optimized DIBA is as sensitive as ELISA, simple, relatively inexpensive, and the results can be scored visually. Tissue immunoblotting assay (TIBA) is a variation of DIBA in which a freshly-cut edge of a leaf blade, stem, leaf, tuber, root or an insect is blotted on the membrane, followed by detection with labelled antibodies as described above (Navot et al., 1989; Hsu and Lawson 1991; Polston et al., 1991). This method is also simple, does not require elaborate sample preparation or extraction, and provides information on the distribution of viruses in plant tissues (Lin et al., 1990). The disadvantages of DIBA and TIBA are possible interference of sap components with the subsequent diagnostic reactions. Sometimes the colour of the sap will prevent weak positive reactions from being observed and the results cannot be readily quantified. Nevertheless, their sensitivity, the relatively short time required to assay large numbers of samples, the need for minimum laboratory facilities for the assay, the ability to store blotted membranes for extended periods, and low costs favour TIBA and DIBA as useful diagnostic techniques. The other advantage is that the samples can be blotted onto the membranes right in the field and such membranes can be carried or shipped by mail for further processing at a central location either within the country or in a different country.

5.4 Viral genome based assays

Currently, nucleic acid-based methods are sensitive, specific and allow genetic relationships to be determined. These include nucleic acid hybridization assays, polymerase chain reactions (PCRs) and microarrays (Webster et al., 2004).
5.4.1 Nucleic acid hybridization:

In nucleic acid hybridization assays, a single-stranded complementary nucleic acid (either DNA or RNA), which has been labelled with a reporter molecule is used as a probe to form a hybrid with the target nucleic acid. The double-stranded probe-target hybrid molecules are then detected by several methods, depending on the reporter molecule used.

The dot- or spot-blot hybridization assay is a commonly used technique in plant virus diagnostics (Webster et al., 2004). Complementary DNA (cDNA) clones, specific to any region of the viral genome, are commonly used as a probe to detect virus in plant extracts. To produce cDNA clones, the viral RNA is usually converted to double-stranded DNA and cloned into suitable vectors (Sambrook and Russel, 2001). Radioactive isotopes like $^{32}$P are used for labelling nucleic acid probes and the signal detected by autoradiography. Radioisotopes have a short half-life (causing supply problems), can be hazardous to health if improperly handled, and require extensive and costly procedures to meet safety regulations. In recent years, these problems have been overcome by using nonradioactive labelling and detection methods using either biotin/streptavidin (Langer et al., 1981) or digoxigenin (DIG) / antiDIG systems (Holtke et al., 1995). There are certain disadvantages of the biotin / streptavidin system, such as the presence of endogenous biotin in the samples and the tendency of streptavidin to stick non-specifically to solid phases like nylon membranes, resulting in severe background problems. Therefore, the DIG/antiDIG system has been widely employed for detection of several viruses. In this system the membranes are exposed, subsequent to hybridization, to antiDIG antibodies coupled to an enzyme (alkaline phosphatase or horseradish peroxidase), and the signal is generated by adding a suitable substrate that results in either a precipitated product (chromogenic detection) or chemiluminescence (chemiluminescent detection) which is detected by autoradiography.

5.4.2 Microarrays:

In the microarray detection, the nucleic acid sample to be examined is typically fluorescently labelled in an enzymic reaction and this fluorescent nucleic acid is hybridized to the array. The fluorescence allows the hybridization events on the solid support to be identified, and the identity of the genome fragment is deduced.
from the position on the array. Nucleic acids can be bound to the solid support in extremely small spots, allowing the many different genes to be investigated simultaneously in a highly parallel fashion. The virus detection arrays reported to date have been developed for fairly specific areas, targeting small numbers of viruses as model systems for assay development. For example, the methods published used different approaches for the detection and identification of a range of potato viruses and cucurbit-infecting tobamoviruses. (Boonham et al., 2007).

5.4.3 Polymerase chain reaction (PCR):

In plant virology, the most frequently used molecular technique has been, first, the polymerase chain reaction (PCR). Compared to traditional methods, PCR offers several advantages, because, viruses do not need to be cultured before their detection, it affords high sensitivity at least theoretically, enabling a few target molecules to be detected in a complex mixture, and it is also rapid and versatile. In fact, the different variants of PCR, have increased the accuracy of detection and diagnosis, and opened new insights into our knowledge of the ecology and population dynamics of many plant viral pathogens, providing a valuable tool for basic and applied studies in plant pathology (Webster et al., 2004).

This procedure is applicable directly to DNA plant viruses (caulimo, gemini, and badnaviruses); however, for diagnosis of plant viruses with RNA genomes, the RNA target has to be converted to a complementary DNA (cDNA) copy by reverse-transcription before PCR is begun. The cDNA provides a suitable DNA target for subsequent amplification. During the initial cycles of PCR, a complementary strand of DNA will be synthesized from the cDNA template, and thereafter the reaction will proceed as for double-stranded DNA described above. This process of amplification is called reverse transcription-polymerase chain reaction (RT-PCR). PCR can be used in conjunction with restriction fragment length polymorphism (RFLP) or sequencing of the amplified DNA to study the variability of viruses at the molecular level (Almond et al., 1992; Tenllado et al., 1994; Candresse et al., 1995).

Based on the nucleotide sequence information of several different viruses, specific oligonucleotide primers can be designed and used in PCR to detect and differentiate viruses at the family, genus, or strain level (Robertson et al., 1991; Omunyin et al., 1996), or for simultaneous detection of unrelated viruses in a sample
by using a mixture of virus-specific primer pairs (Multiplex PCR; Hauser et al., 2000; Nassuth et al., 2000; Webster et al., 2004). Multiplex PCR is very useful in plant pathology because different viruses frequently infect a single crop or host (Lopez et al., 2006). There are several examples of simultaneous detection of viruses (Olmos et al., 2007).

A technique that combines the technical advantages of PCR with the practical advantages of ELISA, called immunocapture (IC)-RT-PCR, was developed for the detection of several different plant RNA viruses (Wetzel et al., 1992; Nolasco et al., 1993). In this assay, the virus particles are first concentrated by trapping onto a solid surface (either microcentrifuge tube or ELISA plate) using virus specific antibodies. The trapped virus particles are disrupted and the released viral nucleic acid amplified by RT-PCR. This results in greater sensitivity, and problems encountered with RNA extraction are minimized and inhibitors of RT-PCR washed away prior to amplification. Thus IC-RT-PCR is a very useful alternative for RT-PCR in virus detection from plant material and insect vectors (Webster et al., 2004).

A novel real-time quantitative PCR assay (TaqMan technology) was developed for the detection and quantification of plant viruses (Dietzgen et al., 1999; Mumford et al., 2000; Eun et al., 2000; Roberts et al., 2000). In addition to sensitivity and specificity, this technique has certain advantages over RT-PCR; it reduces the risk of cross-contamination, obviates post PCR manipulations, provides higher throughput, and enables quantification of virus load in a given sample.

The potential of PCR technology can be effectively exploited in epidemiological studies and in breeding programs for virus resistance, and especially in situations where detection is otherwise difficult with other techniques (Rush et al., 1994; Harrison et al., 1997; Candresse et al., 1998a).

The sensitivity levels of various detection techniques used for plant viruses were given in table. 5.
6. Detection of potyviruses

The development of effective control strategies against potyviruses is dependent on the availability of reliable methods of identification and detection. This has not been seemed possible for this group of plant viruses, because of its size, complexity and immense variation. In the past, characteristics used for distinguishing viruses and strains were host range, symptomatology, cross-protection, morphology of cytoplasmic inclusions and serology. But they have not provided a workable solution for the potyvirus group as a whole because of the large size and extensive biological and antigenic variation. With advancements in virology, it was found that coat protein amino acid and nucleotide sequence data are most useful criteria, as they can be used to distinguish viruses from strains and to establish evolutionary relationships between groups of distinct potyviruses (Adams et al., 2005a). There are several methods of detection of potyviruses based on the viral protein and genomes.
6.1 Serological techniques

The molecular basis of potyvirus serology is well established. Serology, which successfully differentiates viruses in other plant virus groups, has proven unsatisfactory when applied to potyviruses where the serological relationships between related strains and distinct members are complex and inconsistent (Barnet et al., 1987). The N- and C-termini of CP are located on the surface of virions. The N-terminus is the most variable and immunodominant in the CP. The epitopes contained in this region, therefore, generate virus-specific antibodies. The N- and C-termini of CP are easily degraded during virus purification and further storage. The conserved core region of CP enables production of antibodies which can be used to detect a broad range of potyviruses (Shukla et al., 1998; Shukla and Ward, 1989b). Therefore, the antibodies targeted to the N-terminus of the potyvirus coat protein should be virus specific where as those generated to the coat protein core should be group specific. Monoclonal antibodies were produced to a number of distinct potyviruses and have been found to display extreme diversity in their specificity (Jordan, 1992).

6.2 Nucleic acid based techniques

A more precise approach to discriminate potyviruses and related strains is to use detection methods based on viral nucleic acids.

6.2.1 Hybridization techniques:

Defined nucleic acid probes were made to specific regions of the genomic RNA, including the 3' untranslated region (UTR). The 3'-UTR of potyvirus genomic RNA has great value in the identification of potyviruses and can be used to distinguish viruses from strains. Frenkel et al. (1989) showed that the 3'-UTR nucleotide sequences of potyvirus strains are highly conserved (83-99 %) while distinct potyviruses had only 30-53 % nucleotide sequence similarity. Using this approach many of the potyviral strains were identified and classified. Nucleic acid hybridization involving the 3'-UTR of the potyviral genomes was used to support the proposal that Watermelon mosaic virus-2 (WMV-2) and Soybean mosaic virus-N are strains of the same virus (Yu et al., 1989). Using the same approach MDMV-B was confirmed to be a strain of SCMV (Frenkel et al., 1992). This cDNA
hybridization procedure also confirmed that BYMV was distinct from CYVV and also supported that Pea mosaic virus was distinct from BYMV (Barnett et al., 1987; Tracy et al., 1992).

6.2.2 Reverse transcriptase-Polymerase chain reaction (RT-PCR):

The compiled molecular data on the potyviruses showed that the CP and 3'-UTRs are found to be most useful regions in the taxonomic analysis. Use of degenerate primers derived from conserved regions of CP gene, RT-PCR, cloning and sequence analysis allowed to rapidly determine the taxonomic status of the potyviruses and their strains. Gibbs and Mackenzie (1997) identified a degenerate primer pair for amplifying part of 3'-terminii of all potyvirids by RT-PCR. Pappu et al. (1993) developed a group-specific PCR detection method for the RT-PCR amplification of part of CP and whole of the 3'-UTR of DsMV from crude leaf extracts. A universal PCR primer to detect members of the Potyviridae and its use to examine the taxonomic status of several members of the family were also reported (Chen et al., 2001).

7. Management of plant viral diseases

Management of virus diseases is much difficult as compared to diseases caused by other pathogens because virus diseases have complex disease cycles, efficient transmission modes and non-availability / lack of effective viricidal chemicals (Hull, 2002). No direct method for the control of virus diseases is yet available. Most of the procedures involve measures designed to reduce sources of infection inside and outside the crop, to limit spread by vectors and to minimize the effect of infection on yield. Generally, such measures offer no permanent solution to a virus disease problem. Integration of various approaches has been used for the management of virus diseases of plants. Such measures are reported to only minimize the impact of viruses on crops to variable degrees and environmental effects and consumer concern about pesticide residues in food are associated with chemical control of virus vectors.

Strategies of plant virus disease management are largely aimed at preventing virus infection by: (i) eradicating the source of infection to prevent the virus from reaching the crop, (ii) minimizing the spread of the disease by controlling its vector,
(iii) generating virus-free stocks of seed and vegetative propagules, and (iv) producing transgenic plants containing viral genes that confer resistance to the virus (Hull, 2002). A major objective of plant breeding programmes has been to develop cultivars resistant to viral pathogens. However, this approach also has limitations like durability of resistance, difficulty in transferring the genes across the species and long time required to develop resistant cultivars. The advantages of resistance are countered by the ability of virus populations to evolve and overcome the protection conferred by resistance factors and thus the resistance has often been short-lived. The main factor determining the durability of resistance is not the nature of the resistance genes but rather the evolutionary potential of the pathogen population. Garcia-Arenal et al. (2003) analysed the evolutionary forces of 29 plant virus species that have genetic and biological diversity and indicated that evolutionary potential may be an important determinant of durability of resistance against plant viruses.

7.1 Virus-free planting material in the management of plant viruses

Virus-free stocks of seed may provide a very effective means for control of a disease where a virus is transmitted through the seed. Such transmission may be an important source of infection because it introduces the virus into the crop at a very early stage, allowing infection to be spread to other plants while they are still young. To obtain effective control by the use of virus-free or low-virus seed, a certification scheme is necessary, with seed plants being grown in appropriate isolation.

For many vegetatively propagated plants, the main source of virus is infection in the plant itself. With such crops, one of the most successful control measures involves the development of virus-free clones, that is, clones free of the particular virus (Faccioli and Marani, 1998). First, a variety which is free of the virus must be found. When a particular variety is 100 % infected, procedures must be developed to free a plant or part of a plant from the virus. A variety of appropriate indexing methods are essential for identification of virus-free propagation material. Occasionally, individual plants of a variety, or plants in a particular location, may be found to be free of the virus. However, many vegetatively reproduced varieties appear to be virtually 100 % infected with a virus, and, with these one or more of the special treatments and methods have to be used to obtain a stock of virus-free material.
7.2 Tissue culture methods to generate virus-free plant material

Since the viruses are obligate parasites and are systemic in nature, thermotherapy, chemotherapy and tissue culture techniques are used to eliminate the viruses from the plant material.

7.2.1 Thermotherapy:

Heat treatment has been a most useful method for freeing plant material from viruses. Many viruses have been eliminated from at least one host plant by heat treatment. At present, there is no basis for predicting that tissue from a certain plant species can, or cannot, be freed of a particular virus. The mechanisms underlying the elimination of viruses are not yet understood (Mink et al., 1998). Many viruses replicate at a slower rate when the plants are grown at an elevated temperature. Plants are usually exposed to 36°C or higher within the limits of temperature tolerance. The period of exposure may range from several weeks to months. When the plants are thus kept, the length of the shoot tips which are usually virus-free increases, which can be excised and used for grafting. While the viruses are inactivated by high temperature, the viroids are inactivated by low temperature exposures.

7.2.2 Chemotherapy:

It is used to eliminate a number of RNA and DNA viruses. Many synthetic compounds like ribavirin (Virazole), 5-dihydro aza uracil (DHT), and diacetyl - dihydro aza uracil (DA-DHT) are used. They may either be sprayed on or incorporated into the in vitro culture medium (Walkey, 1978; Faccioli and Marani, 1998).

7.2.3 Meristem tip culture:

Culture of meristem tips has also proved to be an effective way of obtaining vegetatively propagated plants free from certain viruses. The smaller the excised tips at the time of removal, the better the chance that they will give rise to virus-free plants (Faccioli and Marani, 1998). A wide range of nutrient media has been used by different workers. The basic ingredients are an appropriate selection of mineral salts (macro- and micro-nutrients), sucrose, and one or more vitamins and / or growth regulators, sometimes in agar. Only a proportion of meristem tip cultures yield virus-free plants.
Plants found to be apparently free of the virus at an early stage of growth may develop infection after quite a long incubation, so that, it is very important that apparently virus-free plants obtained by meristem tip culture be tested over a period before release. Virus-free plants of several important tropical plants have been obtained using meristem tip culture (Faccioli and Marani, 1998; Grout, 1999). Meristem-tip culture has been used widely for the production of virus-free plant material in many species propagated mainly or exclusively by vegetative means (Kartha and Gamborg 1975; Brown et al., 1988; Ayabe and Sumi 2001).

In general, it is well known that virus levels in cultured cells can be very low and sometimes the virus may be lost (Wang and Hu, 1980). Somatic embryogenesis can efficiently eliminate several phloem-limited grapevine viruses (Goussard et al., 1991). Similar results were obtained in citrus (D’Onghia et al., 2001) and in sugarcane (Parmessur et al., 2002). It has been noted that there was no translocation of phloem-limited viruses from infected tissue to somatic embryos (Goussard et al., 1991; Parmessur et al., 2002), while somatic embryogenesis alone was not effective in eliminating Grapevine fanleaf virus (GFLV) (Goussard and Wiid, 1992; D’Onghia et al., 2001).

Virus testing of in vitro-derived planting material is vital for producing virus-free propagation material. Enzyme-linked immunosorbent assay (ELISA) is currently the main diagnostic tool for large-scale testing of fruit tree viruses. However, virus diagnosis in in vitro planting material requires a more reliable and sensitive method. Reverse transcriptase-polymerase chain reaction (RT-PCR) can detect minute amounts of target RNA and is suitable for detecting viruses in very small tissue samples with extremely low virus titre, as found in meristem-tip culture (Dovas et al., 2001).

7.3 Tissue culture methods to generate virus-free sugarcane

Traditionally sugarcane has been cultivated vegetatively and, as a result, viral infections constitute a very serious problem. Almost all commercial sugarcane varieties have been shown to be infected with a complex of viruses. Tissue culture provides a useful technique for eliminating viruses from infected plants and for producing virus-free sugarcane plants.
Tissue culture of sugarcane can be performed using either meristem tip culture or by somatic embryogenesis from callus (Lee, 1987). Meristem tip culture is a viable, rapid and reliable method of virus elimination in plants. Virus distribution is uneven throughout the infected plants and the meristems are either free from the virus or carry a very low titer (Bhojwani and Razdan, 1980; Wang and Hu, 1980). Long-term acceptability of virus-free seed cane production method depends on the generation of healthy sugarcane plants that do not differ significantly in phenotypic and yield characteristics from the elite clones originally selected and released as commercial cultivars. Meristem tip culture is suitable for production of seed-cane, as sugarcane derived by meristem culture did not differ significantly from the original germplasm for any measured yield trait.

Attempts were made to eliminate viruses from infected sugarcane tissues. For example, apical meristem tip culture eliminated chlorotic streak virus (Walkey, 1978) and SCMV (Hendre et al., 1975; Dean, 1982) from infected sugarcane. Successful elimination of SCMV and Fiji disease virus (FDV) was reported in sugarcane through apical meristem tip or axillary bud culture (Leu, 1972; Wagih et al., 1995). The elimination of Sugarcane yellow leaf virus (SCYLV) using tissue culture was also reported (Chatenet et al., 2001; Fitch et al., 2001; Parmessur et al., 2002).

8. Mosaic disease of sugarcane

The literature survey revealed that mosaic disease of sugarcane is the most widespread virus disease occurring in almost all sugarcane growing countries. Several potyviruses like Sugarcane mosaic virus (SCMV), Sugarcane streak mosaic virus (SStMV), Maize dwarf mosaic virus (MDMV) and Sorghum mosaic virus (SrMV) are involved in the mosaic disease of sugarcane (Smith and Rott, 2003). All over the world, SCMV, a definitive species in the genus Potyvirus, family Potyviridae is the causative agent of the mosaic disease. But in India and several other Asian countries, SStMV, a distinct species of probably a new unassigned genus in the family Potyviridae is the major cause of the disease (Hema et al., 2008). In almost all sugarcane growing states of India, SStMV is widespread than SCMV (Rao et al., 2004; Viswanathan et al., 2008).
8.1 Economic importance

The economic importance of mosaic disease varies widely depending in part on the geographic distribution of the virus, partly on the variety infected. The mosaic disease of sugarcane was first described in Java and Indonesia and in the early decades of 20th century it was widely spread to other regions of the world through vegetative material. This led to severe outbreaks of the disease in almost all sugarcane growing areas of the tropics and sub-tropics, including Argentina, Brazil, Egypt, Hawaii, Louisiana and Puerto Rico (Ingelbrecht, 1999; Hema et al., 2008). In some areas the devastating effects of mosaic disease caused severe economic losses to commercial sugar companies and their employees. The sugarcane mosaic epidemic in the 1920s caused a near collapse of the sugar industry in Louisiana (USA), Argentina and Brazil (Ingelbrecht, 1999). In the 1930s the mosaic disease brought the sugar industry to its knees in South Africa (Anon, 1980).

The mosaic disease caused severe economic losses to sugar industry and is responsible for the decline of important commercial clones in several countries. Estimated yield losses from the disease vary greatly, losses of 30-40 % were commonly reported and some times 60-80 %. The incidence of mosaic disease in commercial sugarcane fields appears to be 100 % in some varieties in almost all sugarcane growing states of India (Agnihothri, 1996; Singh et al., 2003; Hema et al., 2008). Under field conditions it is difficult to distinguish between healthy and diseased plants except for chlorotic symptoms on young leaves of diseased plants and hence farmers may not realize the impact of the disease. Even 10-15% yield loss due to disease is significant because of vast cultivation of the crop. The cane juice of diseased plants contains less of sugars and more of starch (Jain et al., 1998).

8.2 Symptomatology

The leaves of infected stalks develop a typical mosaic pattern of pale green to yellow elongated streaks or patches, interspersed with similar streaks of normal colour (Fig. 6, 7). The conspicuousness of the symptoms varies widely according to the strain of the virus and the sugarcane variety that is infected. The symptoms are usually most readily seen towards the base of young leaves, but in some highly susceptible varieties symptoms may occur on the leaf sheaths and even on the stalks. The characteristic symptoms of the disease are more prominent on top 1-3 leaves
and they masked as the leaves aged. The main effect of mosaic infection on crop growth is stunting of infected stalks. No visual abnormalities in diseased plant growth are observed. Further, the above symptoms are more conspicuous in well fertilized and irrigated crop than in poorly managed crop.

Fig. 6: Healthy (A) and SSbMV infected (B) sugarcane leaves.

Fig. 7: Healthy (A) and SSbMV infected (B) sorghum
8.3 Sugarcane mosaic virus (SCMV) (Koike and Gillaspie, 1989; ICTV, 2008)

8.3.1 Geographical distribution: The virus is probably distributed worldwide.

8.3.2 Morphology: Virions consist of a capsid. Virus capsid is not enveloped. Capsid/nucleocapsid is elongated with helical symmetry. The capsid is flexuous filamentous with a clear modal length of 730-755 nm and a width of 13 nm. Axial canal is indistinct. Basic helix is obscure.

8.3.3 Physicochemical and physical properties: Virions have a buoyant density in CsCl of 1.3327 g cm⁻³. There is one sedimenting component found in purified preparations. The sedimentation coefficient S20w is 148-176. The thermal inactivation point (TIP) is at 50-60°C. The longevity in vitro (LIV) is 2-4 days. Although the titer is dependent on the host, the decimal exponent (DEX) of the dilution end point is usually around 2-4.

8.3.4 Nucleic acid: The genome constitutes 5.5-6% of the virion by weight. The genome is monopartite. Only one particle size of linear, positive-sense, single-stranded RNA is recovered. The complete genome is 9500 nucleotides long.

8.3.5 Proteins: Proteins constitute about 94-94.5% of the particle weight. The viral genome encodes structural proteins and non-structural proteins. Virions consist of one structural protein with Mᵦ, 28.5 - 36.5 kDa.

8.3.6 Antigenic relationships: The virus is serologically related to the potyviruses infecting sugarcane, maize and sorghum: 1) Johnson grass mosaic virus—SCMV-JG and Maize dwarf mosaic virus strain O. 2) Maize dwarf mosaic virus—MDMV-A, MDMV-D, MDMV-E and MDMV-F. 3) Sugarcane mosaic virus—MDMV-B, SCMV-A, SCMV-B, SCMV-D, SCMV-E, SCMV-Sc, SCMV-Bc and SCMV-Sabi. 4) Sorghum mosaic virus—SCMV-H, SCMV-I and SCMV-M.

8.3.7 Transmission and vector relationships: Virus is transmitted by arthropods, by insects of the order Hemiptera, family Aphididae; Dactyus ambrosiae, Hysteroneura setariae, Rhopalosiphum maidis, Toxopecta graminum. Virus is transmitted in a non-persistent manner. Virus is also transmitted by mechanical inoculation; transmitted by grafting; transmitted by seeds; not transmitted by pollen. The virus is also transmitted vertically through infected planting material.
8.3.8 Maintenance and propagation hosts: Most commonly used maintenance and propagation host species are Zea mays (sweet corn). Assay hosts are Sorghum genotypes.

8.3.9 Cytopathology: Inclusions are present in infected cells. Inclusion bodies in the host cell are found in the cytoplasm. Cytoplasmic inclusions are pinwheels. Inclusions do not contain mature virions. Other cellular changes include alteration of chloroplasts organisation (i.e. small gran with few thylakoids and uniform distribution of intergrana (stromal) lamellae).


8.3.11 Detection methods: Different serological methods like ISEM, ELISA and immunoblot enzyme assays are available for the detection of SCMV. Both polyclonal and monoclonal antibodies are available. Nucleic acid probes for the detection of SCMV were developed based on the conserved central domain of the CP. RT-PCR test was also developed. RT-PCR based RFLPs were developed for differentiation of closely related strains of SCMV. (Viswanathan and Mohanraj, 2001; Braithwaite and Smith, 2001).

8.3.12 Genetic diversity: It was reported that three non-Australian isolates grouped together, with a further branch separating the two US isolates from the South African isolate. Similarly, the Australian isolates were grouped according to their provenance with the Nambour and Brisbane isolates and the Isis and Bundaberg isolates grouped together, respectively. The SC isolate is the most dissimilar of the Australian isolates, perhaps because it was propagated in maize. (Handley et al., 1998). SCMV strains SC from sugarcane and MDB from maize, whose overall sequence identity between coat proteins was only 79%, were still considered as strains of the same virus. The main reason for this was the 94% similarity between CP cores, the 88% sequence identity between their 3'-non coding region, and the fact that a PCR amplified probe corresponding to the 3'-non coding region of the SC
strain hybridized only with the SCMV strains including MDB but not strains of JGMV, MDMV and SrMV. When compared with SCMV strains A, B, D, E, and SC (all isolated from sugarcane), the similarities for SCMV-MDB ranged from only 83 to 86% in the CP. (Alegria et al., 2003).

8.4 Sugarcane streak mosaic virus (SStMV) (Hema et al., 2008)

8.4.1 Geographical distribution: SStMV has been reported from USA in sugarcane imported from Pakistan. It was also reported from India and several other Asian countries like Bangladesh, Sri Lanka, Indonesia, Thailand and Vietnam.

8.4.2 Morphology: The virions are non-enveloped flexuous filamentous particles with dimensions of ca. 890 x 15 nm (Fig. 8).

![Electron micrograph of SStMV showing flexuous filamentous particles](image)

Fig. 8: Electron micrograph of SStMV showing flexuous filamentous particles (Hema et al., 2001).

8.4.3 Physicochemical and physical properties: The virus has DEP of $10^{-5}$-$10^{-6}$, TIP of 55-60°C and LIV of 1-2 days at room temperature and 8-9 days at 4°C.

8.4.4 Nucleic acid: The virus contains one species of linear ss RNA with size of ~10 kb as its genome. SStMV genomic RNA has $A_{max}$ and $A_{max}$ at 258.2 and 234 nm and $A_{260/230}$ ratio is 1.98. The genome is partially sequenced from 3' end (4,445 nts, encoding 6K2, VPg-Nla, Nfb, CP and 3'-UTR) and deposited in GenBank (Accession number Y17738).
8.4.5 Proteins: The viral coat protein contains one major polypeptide with $M_\text{r}$ of ~40 kDa. The $M_\text{r}$ of CP deduced from CP gene nucleotide sequence is 34 kDa. The glycosylation of CP explains discrepancy between $M_\text{r}$ values determined by the two approaches.

8.4.6 Antigenic relationships: The virus strongly reacted with homologous and heterologous antisera of a potyvirus isolated from sorghum around Parbhani, Maharashtra state, sugarcane virus isolate from Uttar Pradesh and weakly with *Narcissus* *latent* *virus* from UK. The virus failed to react with antisera of potyviruses like SCMV strains, A, B, D, E, H, I, MDMV-A, black eye cowpea mosaic, peanut mottle, peanut stripe, tobacco etch, pea seed-borne mosaic, soybean mosaic, peanut green mosaic and PVY and potyvirus group specific antisera.

8.4.7 Transmission and vector relationships: Aphids (*Aphis craccivora* and *Rhopalosiphum maidis*) failed to transmit the virus. DAG / DAD triplet present in the coat protein of aphid transmitted potyviruses is absent in the CP of SStMV.

8.4.8 Maintenance and propagation hosts: *Sorghum bicolor* cv Rio.

8.4.9 Cytopathology: Thin sections of virus infected sugarcane and sorghum leaves showed cytoplasmic laminated aggregates and pinwheels, respectively.

8.4.10 Detection methods: Bioassay on *S. bicolor* cv Rio, conventional polyclonal antiserum based DAS- and DAC- ELISAs and $^{32}$P and DIG-labeled nucleic acid probe based slot-blot hybridization tests were developed for the detection of SStMV. Dot-blot immunobinding assay (DBIA), DB-PCR and IC-RT-PCR were also developed.

8.4.11 Genetic diversity: The 3'-terminal partial genome sequence comparisons and phylogenetic relationships revealed that this distinct virus was most similar to the *Wheat streak mosaic virus* (WSMV) and *Brome streak mosaic virus* (BStMV), members of the genus *Tritimovirus*.

The comparison of N-terminal and core region of the SStMV-CP revealed a wide range (89.9–100 %) of amino acid sequence similarity. However, SStMV isolates like KA, TA, TN, CB86071, CB94003 and CBKMS2095 greatly varied. The SStMV population structure greatly varied in the samples (e.g., CoC671)
collected from Tamil Nadu and Maharashtra. Two isolates from Co86032 sampled at different fields of the same place were relatively heterogenous. (Viswanathan et al., 2008)

The comparison of characteristics of SCMV and SSTMV are shown in Table. 6.

Table. 6: Comparison of characteristics of SCMV and SSTMV

<table>
<thead>
<tr>
<th>Property</th>
<th>SCMV</th>
<th>SSTMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution</td>
<td>World-wide</td>
<td>Asia (India, Pakistan, Bangladesh, Sri Lanka, Thailand, Vietnam)</td>
</tr>
<tr>
<td>Transmission</td>
<td>Setts, sap, aphids</td>
<td>Setts, sap</td>
</tr>
<tr>
<td></td>
<td>DAG triplet present in the coat protein</td>
<td>Aphids failed to transmit.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DAG triplet absent in the coat protein</td>
</tr>
<tr>
<td>Host range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural</td>
<td><em>Saccharum</em> spp., <em>Sorghum</em> spp., <em>Panicum</em> spp., <em>Eleusine</em> spp.,</td>
<td>Sugarcane, sorghum</td>
</tr>
<tr>
<td></td>
<td><em>Setaria</em> spp., <em>Zea mays</em></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td><em>Saccharum</em> sp., <em>Sorghum</em> sp., <em>Zea mays</em></td>
<td><em>Sorghum</em> species</td>
</tr>
<tr>
<td>Inclusions</td>
<td>Pinwheel inclusions with laminated and / or cylindrical arms</td>
<td>Cytoplasmic laminated aggregates (in naturally infected sugarcane).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pinwheel inclusions (in experimentally infected sorghum)</td>
</tr>
<tr>
<td>Virions</td>
<td>Non-enveloped, flexuous filamentous particles (730-755 x 13 nm)</td>
<td>Non-enveloped, flexuous filamentous particles (890 x 15 nm)</td>
</tr>
<tr>
<td>Coat protein</td>
<td>One major polypeptide with M, 28.5 - 36.5 kDa</td>
<td>One major polypeptide with M, 40 kDa (SDS-PAGE) and 34 kDa (from sequence of CP gene). Glycosylated, unrelated to several SCMV strains</td>
</tr>
<tr>
<td>Antigenic</td>
<td>Cross react with <em>Maize dwarf mosaic</em>, <em>European maize mosaic</em>,</td>
<td>Failed to react with antisera of potyviruses like SCMV strains A, B,</td>
</tr>
<tr>
<td>relationships</td>
<td><em>Sorghum red stripe</em> and <em>Abaca mosaic viruses</em>.</td>
<td>D, E, H, I, MDMV-A, <em>Black eye cowpea mosaic</em>, <em>Pea seed-borne mosaic</em>, <em>Soybean mosaic</em>, <em>Pea green mosaic</em> and <em>Potato virus Y</em> and potyvirus group specific antisera.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weakly reacted with <em>Narcissus latent virus</em>.</td>
</tr>
</tbody>
</table>

Contd.
### Genome

<table>
<thead>
<tr>
<th>Genome relationships</th>
<th>Complete sequence for several strains available (e.g.: for strain A GenBank accession number: AJ278405)</th>
<th>Partial sequence is available (e.g.: GenBank accession numbers: U75456, AY193784, Y17738, AM749393, AY189681, AY193783, EU883391, EF079667, EU650179, EF079666, DQ421788, DQ866750, DQ866749, DQ866748)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 % similarity at amino acid level</td>
<td>24 % similarity at amino acid level</td>
<td></td>
</tr>
<tr>
<td>Taxonomic position</td>
<td>Distinct species in the genus <em>Potyvirus</em>, family <em>Potyviridae</em>.</td>
<td>Distinct from the species of recognized six genera of <em>Potyviridae</em>. Suggested to create a new genus with this virus as type species in the family <em>Potyviridae</em>.</td>
</tr>
<tr>
<td>Diagnostics</td>
<td>ELISA, immunosorbent electron microscopy, electro-blot immunoassay, RT-PCR, RFLP supplemented RT-PCR</td>
<td>Bioassay on <em>Sorghum bicolor</em> cv Rio, conventional and recombinant coat protein polyclonal antibodies based DAS- and DAC-ELISAs and $^{32}$P and DIG-labeled nucleic acid probes (495 bp) based slot-blot hybridization tests, DBLA, DB-PCR and IC-RT-PCR</td>
</tr>
</tbody>
</table>

### 8.5 Management

Worldwide, the mosaic disease of sugarcane was reported to be caused by important potyviruses viz. *Sugarcane mosaic virus* (SCMV), *Sugarcane streak mosaic virus* (SSStMV) and *Sorghum mosaic virus* (SrMV) (Smith and Rott, 2003). Information available on epidemiology of potyviruses infecting sugarcane shows how these viruses are perpetuating in time and space in commercial sugarcane fields. Various practices, such as avoidance of sources of infection, cultural control, genetical resistance and use of biotechnological approaches have been suggested for the management of potyviruses (Koike and Gillaspie, 1989; Jain *et al.*, 1998). Management of potyviruses infecting sugarcane is also possible through practices, but it appears to be complicated as these viruses are perpetuating mainly through propagation material (setts), long duration and ratooning practice of the crop.

Infected material, alternate hosts and vectors are the primary source of infection and their avoidance provide an ideal strategy for management. Since sugarcane is a vegetatively propagated crop and potyviruses are transmitted through
seed-cane, use of virus-free planting material is imperative for raising healthy crop as well as ratoons (Jain et al., 1998). Meristem tip culture alone or in combination with thermotherapy has been found to be useful in generating SCMV-free planting material. Over 90% success was obtained in Co740 variety, when apical meristem tips of 0.05-1.5 mm long from mosaic disease affected sugarcane plants were used for tissue culture (Hendre et al., 1975).

A number of cultivated and wild grasses serve as natural reservoirs of SCMV where as sorghum is the only known alternate host for SStMV. These alternate hosts are important in SCMV spread to sugarcane as they are planted in areas adjacent to sugarcane. Eradication of these hosts at frequent intervals during the cropping season yields good results. Roguing is not worth to follow in the young sugarcane crop as the percent infection is very high (nearly 100%).

A number of aphid species are involved in the field spread of SCMV. The virus is more readily transmitted to or from alternate hosts to or from sugarcane. Though insecticidal control of vectors is commonly practiced for the management of viral diseases, it has found to be not useful in SCMV management (Jain et al., 1998). No aphid vector has been reported to be involved in the transmission of SStMV. Aphids (Aphis craccivora and Myzus persicae) failed to transmit SStMV from sorghum to sorghum under experimental conditions (Hema et al., 2008). Sithanantham et al. (1972) have reported mite (Aceria sacchari) transmission of streak virus disease of sugarcane in South India. The role of mites, if any, in the spread of the streak mosaic disease in India is to be established to control the vector transmission of SStMV.

Use of resistant sugarcane varieties is the pragmatic and environmentally sound approach to manage SCMV. Though resistance to SCMV essentially comes from wild canes, their genetic basis of resistance is not known. A number of clones have been identified as tolerant / resistant to SCMV in many countries. But continuous evolution and emergence of resistant breaking virus strains is a limitation.

Attempts have been made to develop transgenic sugarcane using CP gene of SCMV as transgene (Jain et al., 1998). Similar attempts may be extended to exploit the CP gene of SStMV and other potyviruses.