Molecular studies on Okra Yellow Veins Mosaic Virus (OYVMV) biotypes

CHAPTER 1

INTRODUCTION

1 Introduction:

Okra (Abelmoschus esculentus L) is commonly known as bhindi or lady’s finger belonging to family Malvaceae. It is an important fruit vegetable crop cultivated in various states of India. Several species of the genus Abelmoschus are grown in many parts of the world among them Abelmoschus esculentus is most commonly cultivated in Asia and has a great commercial demand due to its nutritional values.

The genus Abelmoschus was established by Medikus in 1787. However most authors followed deCandolle (1883) and treated it as a section of Hibiscus. It was Hochreutiner in 1924, who reinstated the genus Abelmoschus of Medikus, stating that calyx, corolla and stamens are fused together at the base and fall as one piece after anthesis whereas in case of Hibiscus these are distinct. Though the genus is of Asiatic origin, the origin of cultigen A. esculentus has been reported to be variable from India; Ethiopia, West Africa and Tropical Asia. However, Zeven and Zhukovasky, 1975 believed it to have originated from India. This view is strengthened from the Sanskrit words, Tindisha and Gandhmula found to designate Bhindi. Thus it is likely that the cultigen might have originated in Asia or it might originally have been present in Africa and India as a polyphyletic species. Again there is not much evidence available to show as to when and how cultivated Bhindi got introduced in India. There is no mention in ‘Ain-e-Akbari’ or any other archeological records. This shows that it does not have a long history of cultivation in this country. Probably it reached India by the end of 19th century.

1.1 Uses of okra:

Okra is cooked with meat for flavoring and because of high mucilaginous content, the fruits are ideal for both thickening and flavoring.
stews and soups. The fruits can also be boiled or fried and eaten as a vegetable.

Okra is cultivated for its immature fruits to be consumed as a fresh and canned food as well as for seed purpose. Fruits of okra contain a mucilaginous substance that thickens the soup and stews. Okra has a relatively good nutritional value and is a good complement in developing countries where there is often a great alimentary imbalance. It is a good source of vitamin A, B, C and is also rich in protein, carbohydrates, fats, minerals, iron and iodine. Fruit contains Moisture (89.6 percent), K (103 mg), Ca (90 mg), Mg (43 mg), P (56 mg), vitamin C (18 mg) in 100 g of fresh fruit. Metals such as iron and aluminium are found between 500 and 4000 ppm. The nitrogen percentage is 16 per cent dry weight; the amino acids Asp and Arg are each present at nearly 10 per cent.

1.2 Cytogenetics of okra

Very little work has been carried out on the chromosome complement of okra species. There are significant variations in the chromosome numbers and ploidy levels of different species in the genus *Abelmoschus*. The lowest number reported is 2n=56 for *A. angulosus*, whereas the highest chromosome number reported are close to 200 for *A. manihot var. caillei*. The most frequently observed somatic chromosome number, however, is 2n=130, although it is suggest that the numbers 2n=72, 108, 120, 132 and 144 are in regular series of polyploids with n=12. The existing taxonomical classifications at the speices level in the genus *Abelmoschus* are unsatisfactory. Detailed cytogenetical observations on Asian material of okra and related species are likely to provide more examples of the existence of amphidiploids in the genus.

1.3 Climate and soils for cultivation of okra:

A warm-weather plant prefers temperature between 22°C and 35°C. Okra is susceptible to frost and temperatures below 12°C. Okra can be grown on a wide range of soils, provided the internal soil drainage is
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good. Soils high in organic matter are preferred. The addition of lime or dolomite may be necessary during soil preparation to bring the pH to about 6.0 to 6.5.

1.4 Harvesting of Bhindi:

The success of okra growing depends on careful handling and packing of the product and rapid cooling of the fruits as soon as possible after harvest. The fruits should be ready for harvesting within 10 weeks of planting, and regular picking every 2 to 3 days is essential for maximum yields. Mature fruits left on the plant will reduce flowering and fruit set. The market demand is for young tender fruits about 7 to 10 cm long. Older, tough or stringy fruits are unsalable. Fruits showing spine growth on the fruit are discarded.

Yields of 0.3 to 0.5 kg/plant can be expected. The tender fruits are cut from the stalks and handled carefully; otherwise they may bruise and discolor. It is best to pick fruit into a waist bag to reduce skin damage to the fruit and avoid excessive bending over. It is advisable to wear rubber gloves when harvesting and handling okra fruits as the sap will irritate most skins.

The fruits are graded into various sizes and then packaged in a 9 liter fiberboard container. Okra should be cooled and sent to the market as soon as possible after harvest. During transport the fruits should be held between 7°C and 10°C and 90 to 95% humidity, to prevent wilting. Okra fruits are susceptible to chilling injury at lower temperatures. Containers are labelled with the word 'okra' along with the name and address of the grower. After harvest, the crop can be ratooned by cutting the plants back to about 15 to 20 cm height and re-fertilized.

1.5 Pests and diseases

A number of fungi, bacteria, viruses, mycoplasma, nematodes and insect pests attack this crop. The total loss of vegetable on this account
has been estimated up to 20-30%, but if the pathogens are allowed to develop, this loss may increase up to 80-90%.

1.6 Insects and pests

There are many insect pests which may attack okra, but among those most likely to be troublesome are whitefly, heliothis, rough bollworm, looper caterpillars and green vegetable bugs. Aphids and mites may also occur on okra crops.

Okra appears to be one of whitefly's favored hosts. The whitefly (*Bemisia tabaci* Genn) is a best carrier of okra yellow vein mosaic virus and also transmits this virus into the Bhindi crop. Although there are chemicals which will assist with management of this pest, they should only be used under strictly supervised conditions otherwise the insect will quickly develops resistance to them.

Insect management in okra is very difficult as only a small number of insecticides are registered. Some of these have only limited effectiveness against some pests for which they are registered. Monitoring the crop regularly for pests is essential.

1.7 Fungal Diseases

*Verticillium* wilt is the most common disease affecting okra. The most conspicuous symptom is a yellowing of the older leaves, which often develop a burnt appearance, particularly around the margins, followed by wilting of the plant. The only control measures recommended are crop rotation and the destruction of diseased plants.

Powdery mildew can become a major leaf problem in drier tropical regions. It may result in heavy leaf shed. No chemicals are registered for control of powdery mildew on okra.

*Ascochyta* leaf spot and *Cercospora* leaf spot have been recorded on okra. No definite control measures have been suggested, but spraying with a registered fungicide may be helpful if either of the leaf spots becomes destructive.
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Root-knot nematodes (*Meloidogyne* species) cause severe galling on okra roots. Infested soil should be treated with a registered nematicide before planting. Crop rotations are an important management tool in preventing a build-up of nematodes or other soil-borne organisms.

1.8 Viral Diseases

In economic terms, viruses are only of importance if it is likely that they will spread to crops during their commercial lifetime, which of course varies greatly between very short extremes in horticultural production and very long extremes in forestry. Plant viruses face special problems initiating an infection. The outer surfaces of plants are composed of protective layers of waxes and pectin, but more significantly, each cell is surrounded by a thick wall of cellulose overlying the cytoplasmic membrane (Fig. 1.1).

To date, no plant virus is known to use a specific cellular receptor of the type that animal and bacterial viruses use to attach to cells. Rather, plant viruses rely on a mechanical breach of the integrity of a cell wall to directly introduce a virus particle into a cell. This is achieved either by the vector associated with transmission of the virus or simply by mechanical damage to cells. After replication in an initial cell, the lack of receptors poses special problems for plant viruses in recruiting new cells to the infection.

![Figure 1.1 Plant cell showing organization cell walls](image-url)

Figure 1.1 Plant cell showing organization cell walls
1.9 Transmission of Plant Viruses

There are a number of routes by which plant viruses may be transmitted:

- **Seeds**: These may transmit virus infection either due to external contamination of the seed with virus particles, or due to infection of the living tissues of the embryo. Transmission by this route leads to early outbreaks of disease in new crops which are usually initially focal in distribution, but may subsequently be transmitted to the remainder of the crop by other mechanisms (below).

- **Vegetative propagation/grafting**: These are cheap and easy techniques of plant propagation but provide the ideal opportunity for viruses to spread to new plants.

- **Vectors**: Many different groups of living organisms can act as vectors and spread viruses from one plant to another:
  - Bacteria (e.g. *Agrobacterium tumefaciens* - the Ti plasmid of this organism has been used experimentally to transmit virus genomes into plants).
  - Fungi.
  - Nematodes.
  - Arthropods: Insects - aphids, leafhoppers, planthoppers, beetles, thrips, etc.
  - Arachnids – mites.

- **Mechanical**: Mechanical transmission of viruses is the most widely used method for experimental infection of plants and is usually achieved by rubbing virus-containing preparations onto the leaves, which in most plant species are susceptible to particular infection. However, this is also an important natural method of transmission. Virus particles may contaminate soil for long periods and may be transmitted to the leaves of new host plants through wind-blown dust or rain-splashed mud.
Transmission of plant viruses by insects: This is of particular agricultural importance. Extensive areas of monoculture and the inappropriate use of pesticides which kill natural predators can result in massive population booms of insects such as aphids. Plant viruses rely on a mechanical breach of the integrity of a cell wall to directly introduce into a cell. This is achieved either by the vector associated with transmission of the virus or simply by mechanical damage to cells. Transfer by insect vectors is a particularly efficient means of virus transmission. In some instances, viruses are transmitted mechanically from one plant to the next by the vector and the insect is merely a means of distribution or through insect flying with wind ways may be carried to long distances (sometimes hundreds of miles). Insects which bite or suck plant tissues are, of course, the ideal means of transmitting viruses to new hosts. This is known as non-propagative transmission. However, in other cases the virus may also infect and multiply in the tissues of the insect (propagative transmission) as well as those of host plants. In these cases, the vector serves as a means not only of distributing the virus, but also of amplifying the infection. Begomoviruses (Geminiviridae) are transmitted by whiteflies. These viruses cause a great deal of crop damage in plants such as tomatoes, beans, squash, cassava, okra and cotton and their spread may be directly linked to the inadvertent world-wide dissemination of the "B" or silverleaf biotype of the whitefly Bemisia tabaci. This vector is an indiscriminate feeder, encouraging rapid and efficient spread of viruses from indigenous plant species to neighboring crops.
1.10 Multipartite Plant Viruses

I. Segmented virus genomes are those which are divided into two or more physically separate molecules of nucleic acid, all of which are then packaged into a single virus particle.

II. Although multipartite genomes are also segmented, each genome segment is packaged into a separate virus particle.

<table>
<thead>
<tr>
<th>Family:</th>
<th>Segments</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Begomovirus</em> (Geminiviridae) (single-stranded DNA)</td>
<td>Bipartite / Monopartite</td>
</tr>
<tr>
<td><em>Comovirus</em> (single-stranded RNA)</td>
<td>Bipartite</td>
</tr>
<tr>
<td><em>Furovirus</em> (single-stranded RNA)</td>
<td>Bipartite</td>
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<tr>
<td><em>Tobravirus</em> (single-stranded RNA)</td>
<td>Bipartite</td>
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<tr>
<td><em>Partitiviridae</em> (double-stranded RNA)</td>
<td>Bipartite</td>
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<tr>
<td><em>Bromoviridae</em> (single-stranded RNA)</td>
<td>Tripartite</td>
</tr>
<tr>
<td>* Hordeivirus* (single-stranded RNA)</td>
<td>Tripartite</td>
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1.11 Pathogenesis of Plant Virus Infections

Initially, most plant viruses multiply at the site of infection, giving rise to localized symptoms such as necrotic spots on the leaves. Subsequently, the virus may be distributed to all parts of the plant either by direct cell to cell spread or by the vascular system, resulting in a systemic infection involving the whole plant. However, the problem these viruses face in re-infection and recruitment of new cells is the same as they face initially how to cross the barrier of the plant cell wall. Plant cell walls necessarily contain channels called plasmodesmata which allow plant cells to communicate with each other and to pass metabolites between them. However, these channels are too small to allow the passage of virus particles or genomic nucleic acids. Many plant viruses have evolved specialized movement proteins which modify the plasmodesmata. One of the best known examples of this is the 30kd protein of tobacco mosaic virus (TMV). This protein is expressed from a sub-genomic mRNA and its function is to modify plasmodesmata causing genomic RNA coated with protein to be transported from the infected cell to neighbouring cells. Other viruses, such as cowpea mosaic virus (CPMV...
Molecular studies on Okra Yellow Veins Mosaic Virus (OYVMV) biotypes - Comovirus family) have a similar strategy but employ a different molecular mechanism. In CPMV, the 58/48kd proteins form tubular structures allowing the passage of intact virus particles to pass from one cell to another. Typically, virus infections of plants might result in effects such as growth retardation, distortion, mosaic patterning on the leaves, yellowing, wilting, etc.

1.12 Impact of Geminiviruses

Geminiviruses belong to the family Geminiviridae and have circular single stranded (ss) DNA genome which is responsible for major crop losses worldwide. Geminiviruses have been divided into four genera Mastoviruses, Begomoviruses, Curtoviruses and Topoviruses, based on genome organization, insect vector and host ranges (Fig. 1.2). The names of these genera are derived from that of the type of member of each genus which are Maize streak virus (MSV), Bean golden mosaic virus (BGMV), Beet curly top virus (BCTV) and Tomato pseudo curly top virus (TPCTV). The family Geminiviridae is the second largest among the plant viruses with 133 officially recognized species. The genus Begomovirus of this family is the largest having 117 species transmitted by whitefly Bemisia tabaci and infect dicot plants.

1.13 Begomoviruses

Begomoviruses are small plant viruses with single-stranded circular DNA genomes that are encapsidated in twinned quasi-icosahedral particles.

Their genomes can be mono or bipartite. They cause significant and often total yield losses of important food and industrial crops in tropical and subtropical regions of the Western and Eastern Hemispheres. High incidences of begomoviruses are associated with high populations of whiteflies and serious losses in several crops in the Americas and the Caribbean Basin. This reflects the economic importance and enormous
diversity of geminiviruses resulting from widespread distribution and host adaptation.

1.14 Virus impact on okra

One of the major problems with this crop is infection and yield loss due to the fast growing, widely spread okra (Bhindi) yellow vein mosaic virus, a begomovirus. The okra yellow vein mosaic virus (OYVMV) disease is characterized by a homogenous interwoven network of yellow vein enclosing islands of green tissue within its leaf. In extreme cases, infected leaves become yellowish or creamy color. If plants are infected within 20 days after germination, their growth is retarded, few leaves and fruits are formed and the loss may be 94%. The extent of damage declines with delay in infection of the plants. Plants infected 50 to 65 days after germination suffer a loss of 49% and 84% to respectively.

The vector transmitting the okra yellow vein mosaic virus is Bemisia tabaci Genn. Several attempts have been made to manage the whitefly. But the recombinations occurring within the genome of geminiviruses created difficulties to develop the resistance or effective tolerance against the okra yellow vein mosaic virus.

1.15 Genome organization of begomoviruses

Begomovirus genomes have either one (monopartite) or two (bipartite) DNA components ranging from 2.5 to 2.8 kb in size. The bipartite genome has DNA-A and β-DNA, both showing common region (CR) of ~200 nucleotides within the intergenic region. These viruses replicate in the host cell nucleus via a double-stranded (ds) DNA intermediate, termed replicative form (RF). The RF is used as a template for transcription as well as replication. Both strands code for viral proteins.

DNA-A component encodes the proteins required for viral DNA replication while the β-DNA encoded two proteins that are essential for systemic movement and symptom expression. Recently certain monopartite Begomoviruses indicating Agerantum yellow vein mosaic
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virus (AYVMV), Cotton leaf curl virus (CLCuV), some Begomoviruses infecting tomato (TYLCV) and tobacco (TLCV) in China and Okra (Bhindi) yellow vein mosaic virus (OYVMV) in India have been found to require a satellite molecule called β (beta) for introduction of disease symptoms in the same host plants.

**DNA-A:** The DNA-A of bipartite begomoviruses and monopartite begomoviruses have very similar genome organization and encodes 5-6 overlapping open reading frames (ORFs). The virion-sense strand (V) of DNA A encodes the coat protein (CP, AV1/V1) that encapsidates the viral ssDNA. The DNA-A of Old World begomoviruses encodes an additional ORF AV2/V2 that has been implicated in virus movement. The DNA A complementary-sense (C) strand encodes the replication-associated protein (Rep, AC1/C1), a transcriptional activator protein (TrAP, AC2/C2), and a replication enhancer protein (REP, AC3/C3). TrAP is involved in the control of both viral and host gene expression. Some DNA A of bipartite viruses and all monopartite viruses encode AC4/C4 that participates in cell-cycle control. The DNA-B encodes two ORFs, a virion-sense nuclear shuttle protein (NSP, BV1) and a complementary-sense movement protein (MP, BC1). The DNA A and DNA B share no homology except for a ~150nt to 250 nt common region which contains the origin of replication and regulatory regions for bi-directional transcription. This region which contains a conserved non coding sequence (TAATATTAC) is located in the stem loop structure. Monopartite geminiviruses also contain similar non-coding intergenic region.

**β-DNA:** Satellites are viruses with nucleic acids that depend on a helper virus for replication but lack nucleotide sequence homology to the helper virus. Satellite viruses code for their own coat protein, whereas RNA satellites are packaged in coat protein encoded by the helper virus. Many plant RNA viruses have RNA satellites associated with them. The majority of satellites interfere with the replication of helper virus resulting in
attenuated symptoms. Some satellites exacerbate disease symptoms induced by helper virus or produce a novel symptom which is usually not associated with helper virus infection. The first viral satellite DNA was found to be associated with *Tomato leaf curl virus* (ToLCV) from Australia. This 682 nt DNA depends on ToLCV for its replication and encapsidation, but its replication can also be supported by other geminiviruses. In contrast, DNA β satellites associated with some begomovirus infections are required for the induction of disease symptoms in some host plants. Like ToLCV sat-DNA, DNA β satellites require a helper virus for replication and encapsidation. The DNA β satellite associated with *Eupatorium yellow-vein virus* (EupYVV) has been linked to disease symptoms described about 1250 years ago. The ToLCV satellite DNA that lacks any ORF has been suggested to be a remnant of a DNA β that was at one time associated with this monopartite begomovirus. DNA β satellites have a conserved genome organization consisting of a single complementary-sense ORF (βC1), an adenine rich region, and a satellite-conserved region (SCR). The SCR contains a stem loop sequence TAA/GTATTAC which is similar to sequence in the origin of replication of geminiviruses.

Besides full-length molecules, naturally occurring DNA β deletion mutants have been reported. These deletion mutants did not induce DNA β associated symptoms in cotton or ageratum.
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Figure 1.2: Genome organization of geminiviruses. ORFs are designated as either being encoded on the virion (V) or complementary (C) strand. Gene functions are shown where these are known. The position of the stem-loop containing the conserved nonanucleotide (TAATATTAC) is indicated. CP/V1, coat protein; MP/V2, movement protein Rep/C1, replication associated protein; TrAP/C2, transcriptional activator protein; REP/C3, replication enhancer protein; pathogenicity determinant/C4; MP/BC1, movement protein; NSP/BV1, nuclear shuttle protein; LIR, large intergenic region; SIR, small intergenic region; IR, intergenic region; CR, common region.
The DNA β satellites contribute to the production of symptoms and enhanced levels of helper virus accumulation in certain hosts. Hence, it has been proposed that DNA β might affect the replication of helper virus by either facilitating their spread in host plants, or by suppressing the host gene silencing. DNA-β’s encode a protein, βC1 that is a determinant of pathogenicity and suppressor of gene silencing. DNA-A components are satellite-like molecules associated with many begomovirus DNA-β disease complexes. Sequence comparison of DNA A components shows that they have a conserved genome organization containing a single virion-sense Rep ORF, an A-rich region, and a hairpin structure. DNA 1 components are capable of self replication but require helper virus for encapsidation and movement within the host plant. DNA 1 components have no effect on symptom expression.

Molecular characterization of Indian biotypes of okra yellow vein mosaic virus (OYVMV), a begomovirus has not been carried out. As the review highlights this virus migrated from Pakistan to north India; and then from north to central and from there to south in India.

It is also reported that during migration, this virus gets itself organized for acclimatization in the new climatic zone by making necessary changes within its genome. These changes in the genome of OYVMV seen in different biotypes can be identified only after sequencing the genome. The molecular aspects of viruses are studied not only to satisfy curiosity, but also because such information is essential for the knowledge-based design of strategies for controlling viruses. There have been many such studies reported for animal viruses seeking evidence of virus origins, mutation rates, selection and fitness, the nature and biological significance of variation, and the mechanisms of reassortment and recombination, but there are fewer such studies on plant viruses.

The present work on molecular studies of okra yellow vein mosaic virus biotypes was therefore carried out for assessment of variability in their genomes. The specific objectives of the present work were;
i. to isolate both DNA A and β DNA genomes of different biotypes of OYVMV from the virus infected leaves of okra collected from north, central and south zones of India;

ii. to clone the isolated genomes of different biotypes and validate their sequence;

iii. to analyze the sequence similarity within the genomes of OYVMV and identify the conserved regions within the genomes and the variation in any of the genes;

iv. to identify the recombinational hot spots within the genome sequences of all biotypes.