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Review Article

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Begomovirus disease of pumpkin crop in India and its management strategies possibility: a review

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ABSTRACT

Pumpkin is an important commercial crop grown worldwide in tropical and subtropical regions. The whitefly-transmitted Pumpkin yellow vein mosaic disease seriously threatens pumpkin cultivation worldwide. The advent of transgenic technology in the 1980s revolutionized the possibilities for introducing virus resistance into agriculturally important plant species. It offered a powerful tool to enhance crop protection and provided a pathway to potentially unlimited sources of resistance against viral diseases. The ongoing research and development in this field continue to explore and refine conventional and non-conventional approaches for effective virus management in agriculture crops. This review focuses on developing transgenic resistance against begomoviruses and discusses possible management strategies to address these challenges.

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INTRODUCTION

Pumpkin (*Cucurbita pepo, C. Maxima & C. Moshchata*) is an important vegetable crop cultivated in almost all parts of India. In terms of area and production, pumpkin is India's second most important cultivated vegetable crop in the *Cucurbitaceae* family. In India, the cultivated area is about 1.10 lakh ha, production is 23.12 million tonnes, and 21.01 tonnes/ha productivity during 2021-22 (agricoop.nic.in). Many biotic and abiotic stresses have an impact on pumpkin production. Begomoviruses are the largest genera in the family *Geminiviridae*. Most geminiviruses contain a bipartite genome, consisting of DNA-A and DNA-B. Begomoviruses have a circular, single-stranded deoxyribonucleic acid (ssDNA) genome and are transmitted in nature by the whitefly (*Bemisia tabaci*), which causes significant yield losses in economically important crop plants worldwide (Varma & Malathi, 2003; Stanley *et al.*, 2005). Begomoviruses generally have bipartite genomes (DNA-A and DNA-B) and infect dicotyledonous plants. Based on their genome characteristics and phylogenetic relationships, begomoviruses have been divided broadly into Old World (OW) viruses (eastern hemisphere, Europe, Africa, Asia, and Australia) and the New World (NW) viruses (western hemisphere, the Americas) (Rybicki, 1994; Nawaz-ul-Rehman & Fauquet, 2009). Monopartite begomoviruses (have DNA-A genome only) are predominantly found in the Old World and are often associated with satellite DNAs (alpha- and betasatellite), which may or may not contribute to pathogenicity (Briddon *et al.*, 2008). The first begomovirus satellite DNA, referred to as defective DNA-β, was found with the *Tomato leaf curl virus* from Australia (Dry *et al.*, 1997). Many monopartite begomovirus and DNA-β complexes have since been identified in a wide variety of plant species growing throughout the Old World (Briddon *et al.*, 2003), DNA-β components have no significant homology with their helper begomoviruses, on which they are dependent for their replication, encapsidation, and movement within and between plants (Saunders *et al.*, 2000). Some nanovirus-like DNA components known as alphasatellites (DNA-1) have also been reported with many begomovirus disease complexes. Unlike DNA-β, the nanovirus-like component is not essential for the disease (Xie *et al.*, 2010). Cucurbits are an important group of vegetables cultivated extensively in India. Pumpkin is being cultivated as a vegetable. Depending on the species, virtually all parts of the plant can be used for food including leaves, shoots, roots, flowers, seeds, and immature fruits (Jacks *et al.*, 1972). Among the diseases, those caused by viruses are difficult to control. They can be destructive and their severity depends frequently on the complex relationships of pathogens, hosts, and/or vectors. Virus diseases caused by infection of more than 32 viruses had major impacts on the production and quality of various cucurbitaceous crops such as cucumber, watermelon, pumpkins, zucchini, squash, and melon. Virus diseases are a

major problem in cucurbits including pumpkin cultivation areas all over the world. Among viral diseases of cucurbits, yellow vein mosaic caused by whitefly-transmitted geminivirus resulted in major economic losses in India (Capoor & Ahmad, 1975). The relationship between the virus and the vector in the transmission of the disease has been widely discussed by Jayashree *et al.* (1999). Various methods have been tried to control Pumpkin Yellow Vein Mosaic Disease (PYVMD) with differing levels of success. These methods include conventional, non-conventional methods and using healthy transplants, employing chemical and physical control measures against the whitefly vector, and breeding for resistance to PYVMD. However, controlling the disease by targeting the whitefly vector population has proven impractical due to high costs and low efficiency. Excessive pesticide use has also led to the development of resistance in whiteflies against many insecticides (Horowitz *et al.*, 2005). Breeding and implementing resistant cultivars is considered the practical and long-lasting approach for managing PYVMD (Lapidot & Friedmann, 2002). A resistant host plant restricts the multiplication of virus particles, thereby inhibiting the development of disease symptoms. PYVMD resistance breeding programs involve various steps such as developing inoculation protocols, screening and validating sources of resistance, gene discovery, genetic mapping, transferring resistance genes to cultivated pumpkin varieties, and evaluating the introgressed lines in the field.

This provides an updated review of the occurrence of PYVMD, pathogen variability, and the development of resistant cultivars. It also highlights the future prospects of PYVMD resistance breeding in pumpkins.

BEGOMOVIRUSES DISEASE OCCURRENCES ON PUMPKIN CROPS IN INDIA

Pumpkin is a member of the *Cucurbitaceae* family, which includes roughly 130 species that may be found both in the wild and in cultivation worldwide. It is a source of many phytochemicals known to have positive effects on health. The genus *Cucurbita* contains roughly 20 species of pumpkins (Kulczyński & Gramza-Michałowska, 2019). The most widely grown pumpkin species include *Cucurbita maxima*, *C. pepo* L., and *C. moschata Duchesne ex Poir* in India. All plant parts are edible, however, the seeds and pulp are particularly crucial for the preparation and nutrient content of meals (Yadav *et al.*, 2010; Kwiri *et al.*, 2014).

Begomovirus affects pumpkin crop production of which, the *Pumpkin yellow vein mosaic* (PYVM) disease of pumpkin (*Cucurbita moschata*) was first known to occur in central-western India (Varma, 1995). Two different begomoviruses, *Tomato leaf curl virus* (Singh *et al.*, 2001), and *Squash leaf curl China virus* (SLCCNV) in pumpkin (Muniyappa *et al.*, 2003) have been identified as serious viral pathogens of pumpkins in India. There are a few reports on natural infection of begomoviruses: ToLCNDV on the pumpkin (AM286434: unpublished), SLCCV on the pumpkin (AY184487 (Muniyappa *et al.*, 2003); DQ026296 (Singh, 2005), which indicated that begomoviruses have emerged as a major constraint to the cultivation of these crops in India. Similar or other types of symptoms of begomovirus diseases have also been reported from time to time by various researchers. A yellow mosaic in pumpkin (*Cucurbita maxima*) was observed in and around Lucknow (Singh *et al.*, 2001). The yellow vein mosaic disease symptoms occurred frequently in pumpkins in southern India and diseased plants showed vein yellowing and chlorotic patches. Infected plants are caused by the *Pumpkin yellow vein mosaic virus* (Muniyappa *et al.*, 2003). Pumpkin yellow vein mosaic disease (PYVMD) is a major constraint for the cultivation of pumpkins in India (Jayashree *et al.*, 1999; Muniyappa *et al.*, 2003). The infection of two begomoviruses, *Squash leaf curl China virus* and *Tomato leaf curl New Delhi virus* caused yellow vein mosaic disease and significant damage to pumpkin production throughout South India in 2004 (Maruthi *et al.*, 2007b). The biological and molecular properties of the Squash leaf curl China virus from Varanasi, India (SLCCNV: [IN: Var:Pum]) were characterized. SLCCNV-IN [IN: Var:Pum] could be transmitted by grafting and through whitefly transmission (Singh *et al.*, 2009). *Squash leaf curl China virus* caused yellow vein mosaic disease of pumpkin (*Cucurbita maxima* L.) in fields at Lucknow (northern India) in 2006-2007. Naturally, infected pumpkin plants exhibited severe yellow vein mosaic and distortions on leaves. The infected plants had poor flowering and bear deformed and reduced-size fruits when compared with the healthy ones (Singh *et al.*, 2008). This is the first report of natural infection of *Tomato leaf curl Palampur virus* (ToLCPalV) in pumpkin and the association of *Pepper leaf curl betasatellite* (PepLCB) with yellow vein mosaic disease of pumpkin in India (Namrata *et al.*, 2010). The *Squash leaf curl China virus* (SLCCNV) and *Tomato leaf curl New Delhi virus* (ToLCNDV) are species of Begomovirus (whitefly-vector Geminiviridae) and cause serious damage to the pumpkin crops of the genus Cucurbita (pumpkin) in the areas of South and Southeast Asia, across Asia, the Middle East, and the Mediterranean, respectively (Dhillon *et al.*, 2021).

SYMPTOMS OF BEGOMOVIRUS IN PUMPKIN

These include seven species of begomovirus that cause significant harm to pumpkins in India, including the following: *Pumpkin yellow vein mosaic virus* (Varma, 1955; Sohrab *et al.*, 2006), *Tomato leaf curl virus* (Singh *et al.*, 2001), *Squash leaf curl China virus* (Muniyappa *et al.*, 2003; Maruthi *et al.*, 2007b; Singh *et al.*, 2008, 2009; Namrata *et al.*, 2012; Tiwari *et al.*, 2012; Baldodiya *et al.*, 2019; Kushvaha *et al.*, 2023b), *Tomato leaf curl New Delhi virus* (Maruthi *et al.*, 2007a; Phaneendra *et al.*, 2012; Kushvaha *et al.*, 2023a), *Tomato leaf curl Palampur virus* (Tiwari *et al.*, 2010, 2012), and *Mungbean yellow mosaic India virus* (Pandey & Verma, 2017). Symptoms of disease-infected pumpkin plants included mild mosaic, chlorotic patches, mosaic, vein banding, puckering of leaves, stunting, vein yellowing, crumpling of the leaves, and fruit deformation in pumpkin plants (Figure 1 & Table 1).

GENOME ORGANIZATION OF BEGOMOVIRUS

Pumpkin-infecting ssDNA viruses are allied to the family: Geminiviridae (genus begomoviruses) is an important pathogen

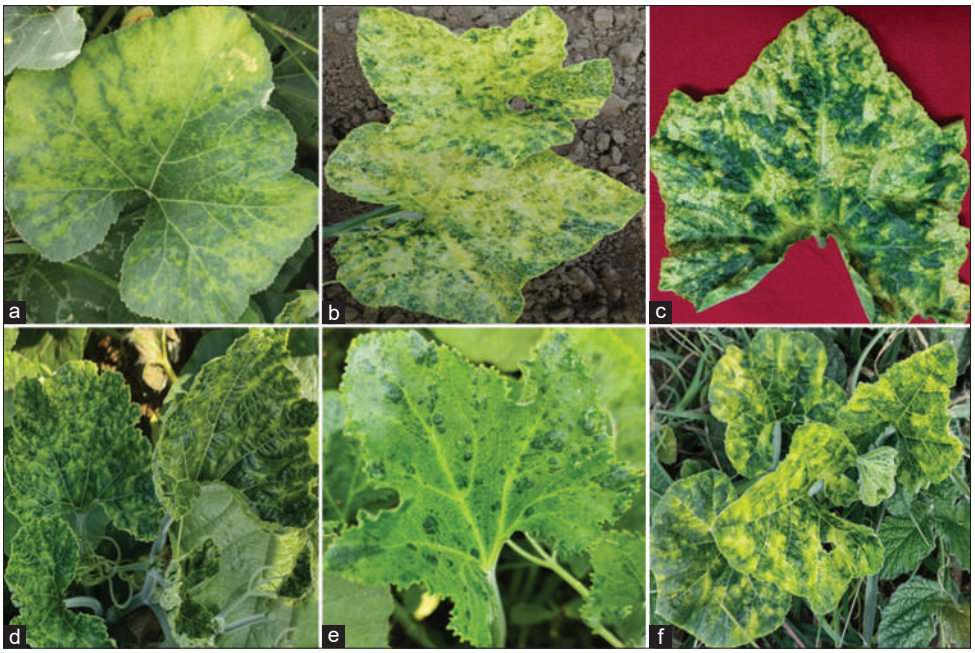

Figure 1: Symptoms of begomoviruses in pumpkin plant: a) Leaf yellowing, b) Yellow vein mosaic, c) Yellow spot with mosaic, d) Severe mosaic and leaf curling, e) Leaf narrowing and f) Plant stunted

Table 1: Detailed studies on begomoviruses such as Pumpkin yellow vein mosaic virus (PYVMV), *Tomato leaf curl virus* (TLCV), *Squash leaf curl China virus* (SLCCV), *Tomato leaf curl New Delhi virus* (ToLCNDV), *Tomato leaf curl Palampur virus* (ToLCPV), *Pumpkin yellow mosaic virus* (PYMV), and *Mungbean yellow mosaic India virus* (MYMIV) reported on pumpkin crops in India

Acc. No.	Virus identified	Genome	Symptoms	Locations	References
Complete DNA-A and DNA-B					
AY184487	SLCCNV	DNA-A: 2738	Yellow vein	Southern India	Muniyappa *et al.*, 2003
AM286434	ToLCNDV	DNA-A: 2739	Yellow vein mosaic	New Delhi	Maruthi *et al.*, 2007a
AM286433	ToLCNDV	DNA-A: 2738	Leaf curl	North India	Maruthi *et al.*, 2007b
AM286435	ToLCNDV	DNA-B: 2694	Yellow vein mosaic	New Delhi	Maruthi *et al.*, 2007b
EU573715	SLCCNV	DNA-A: 2738	Yellow mosaic	Lucknow	Singh *et al.*, 2008
FJ859881	SLCCNV	DNA-B: 2704	Severe yellow mosaic	Varanasi	Singh *et al.*, 2009
DQ026296	SLCCNV	DNA-A: 2758	Yellow Vein mosaic	Eastern U.P.	Singh *et al.*, 2009
FJ931537	ToLCPalV	DNA-A: 2756	Yellow vein mosaic	Varanasi	Namrata *et al.*, 2010
JN129254	ToLCNDV	DNA-A:2740	Pumpkin leaf curl	IARI, New Delhi	Phaneendra *et al.*, 2012
Partial DNA-A					
EU366164	ToLCPalV	DNA-A: 771	Yellow mosaic	Gorakhpur	Tiwari *et al.*, 2012
AY396151	SLCCNV	DNA-A: 771	Yellow mosaic		
GQ225736	SLCCNV	DNA-A: 771	Yellow vein mosaic	Faizabad	
GQ225735	SLCCNV	DNA-A: 771	Yellow vein mosaic		
GQ225734	SLCCNV	DNA-A: 771	Yellow vein mosaic		
GQ225733	SLCCNV	DNA-A: 771	Yellow vein mosaic		
GQ225732	SLCCNV	DNA-A: 771	Yellow vein mosaic	Varanasi	Namrata *et al.*, 2012
GQ225737	ToLCNDV	DNA-A: 771	Yellow vein mosaic		
GQ225738	ToLCPalV	DNA-A: 771	Yellow vein mosaic		
EU366164	ToLCPalV	DNA-A: 771	Mild mosaic	Gorakhpur	
MF737341	MYMIV	DNA-A: 506	Mosaic and crumpling	Madhya Pradesh	Pandey & Verma, 2017
KX087160	SLCCNV	DNA-A: 1221	Pumpkin leaf curl disease	Assam	Baldodiya *et al.*, 2019
OQ320768	ToLCNDV	DNA-A: 771	Leaf curling		
OQ320770	ToLCNDV	DNA-A: 771	Severe Mosaic		
OQ320774	ToLCNDV	DNA-A: 771	Mild Mosaic	Bhopal, Madhya Pradesh	
OQ116977	ToLCNDV	DNA-A: 771	Leaf curling with severe mosaic		Kushvaha *et al.*, 2023a
OQ116978	ToLCNDV	DNA-A: 771	Mosaic		
OQ427110	SLCCNV	DNA-A: 771	Leaf yellowing		
OQ320771	SLCCNV	DNA-A: 771	Yellow vein mosaic		
OQ320773	SLCCNV	DNA-A: 771	Yellow spot		
OQ320772	SLCCNV	DNA-A: 771	Yellow spot with leaf curling	Bhopal, Madhya Pradesh	Kushvaha *et al.*, 2023b
OQ320775	SLCCNV	DNA-A: 771	Mild mosaic		

infecting generally dicotyledonous plants. It contains either DNA-A or DNA-B genomic components and is spread by whiteflies (*Bemisia tabaci*). DNA-A has six ORF regions, which encode proteins for replication, encapsidation, and movement. In the complementary sense, open reading frames AC1, AC2, AC3, and AC4 (four) can be found. The AC1 encodes for a Rep (replication-associated protein) and AC2 for a TrAP (transcriptional activator protein), whereas the protein encoded by AC3 is the Ren (replication enhancer protein), while the protein encoded by AC4 functions as a suppressor of RNA silencing. The other two ORFs (AV1 and AV2) are found in the virion sense, where AV1 codes for coat protein and AV2 for a protein of unclear function. Similarly, the DNA-B component also has two ORFs, BC1 in the complementary and BV1 in the virion sense. Mostly monopartite ssDNA viruses reported from Asia, Europe, Middle East, and Australia are associated with satellite molecules known as betasatellite (previously recognized as DNA-B) (Figure 2).

MANAGEMENT STRATEGY OF BEGOMOVIRUSES DISEASE

According to Valkonen (1998), any type of chemical treatment in the crop field can't prevent viral diseases. On the other hand, it might be controlled using techniques for preventing viral infection in crops. Conventional and Non-conventional management techniques have been described and suggested over the years (Figure 3).

The Conventional Method of Virus Management

Pest control

Begomoviruses are spread from one plant to another plant via insect vectors. Therefore, diseases can be effectively controlled by reducing the number of whitefly vectors through the use of appropriate/proper pesticides. The whitefly, a dreadful

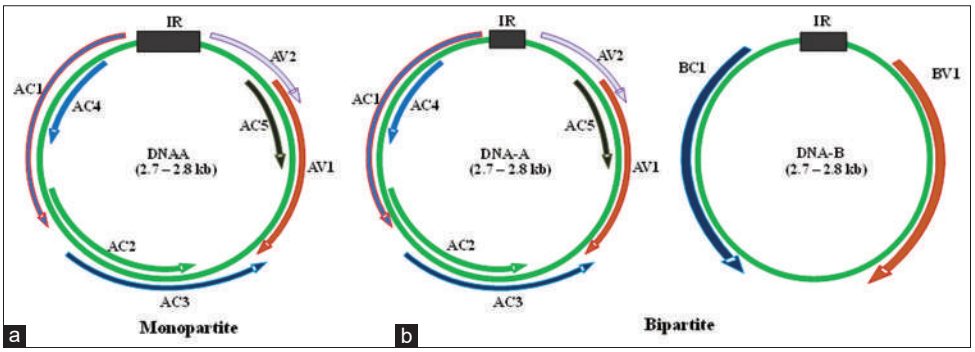

Figure 2: Begomovirus Genome: a) Monopartite begomovirus contains only DNA-A and b) Bipartite: DNA-A segment found along with DNA-B

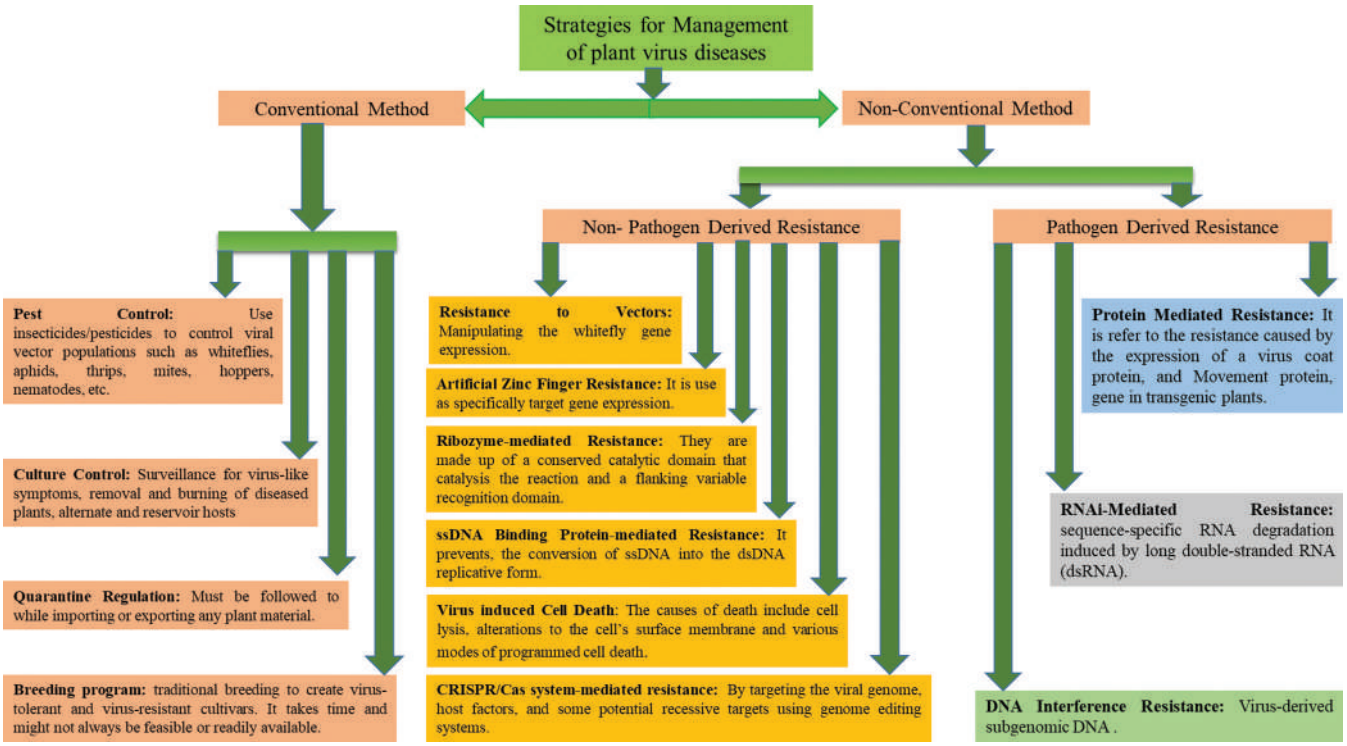

Figure 3: Strategies for Management of begomovirus diseases through conventional and non-conventional methods

sap-sucking insect pest, is known for seriously harming crops and reducing yields through direct feeding by both nymphs and adults as well as by the spread of viruses and diseases (Abubakar *et al.*, 2022). It can be achieved by a combination of physical and mechanical approaches, indigenous technical knowledge, biological control, plant-based products, the spray of synthetic pesticides, biotechnological strategies, and IPM strategies (Padhi & Misra, 1987; Liu & Meister, 2001; Quesada-Moraga *et al.*, 2006; Razze *et al.*, 2016; Ibrahim *et al.*, 2017; Isman, 2017; Perring *et al.*, 2018; Shejulpatil *et al.*, 2019; Elango *et al.*, 2020; Abubakar *et al.*, 2022). This entails managing the pest that serves as a means of spreading begomoviruses from diseased to healthy plants. Insecticides/pesticides have been employed as a direct attack on vectors since the 1930s by killing the insects that serve as vectors. The development of integrated disease management systems that involve the spraying of oils, viricides, insecticides, and pesticides has been found to be effective in minimizing yield loss by many workers due to a variety of viral infections. Malathion (50% E.C.) was effective to reduce the populations of whitefly if sprayed at 21 days of intervals (Raj *et al.*, 2012).

Cultural control

To eliminate a potential source of the begomovirus, perennial weeds should be eliminated from the vicinity of greenhouses, gardens, and fields, because weeds act as a reservoir of begomovirus during the non-cropping period. Cleaning and eradication of Begomovirus reservoirs from fields with diseased plant components were found to be a very successful strategy. Along with this, it was discovered that measures including early plantation, plant spacing, and the application of silver or white-colored mulches were useful in lowering the incidence of disease and achieving the highest crop production. For a very long time, weeds have been recognized as important sources of viral infections. Perennial weeds should be removed from the vicinity of greenhouses, gardens, and fields to get rid of any potential virus sources (Khan, 2006). In India, begomovirus infection has been found in several weeds, including *Ageratum conyzoides*, *Acalypha indica*, *Eclipta alba*, *Sida cordifolia*, *Jatropha gossypifolia*, *Malva parviflora*, *Croton bonplandianum*, *Malvesrtum coromandalianum*, *Coccinia grandis*, *Launaea procumbens*, *Physalis minima*, *Sonchus oleraceus*, *Nicotiana plumbaginifolia*, *and Parthenium hysterophorus* (Somvanshi *et al.*, 2009; Snehi *et al.*, 2018).

Quarantine regulation

Quarantine, also known as plant and seed health testing, is a crucial step in the disease management process. Obtaining disease-free vegetative propagated plants from nuclear stock after several stages of propagation under circumstances that ensure that stated health standards are met has been defined by the European and Mediterranean Plant Protection Organisation (EPPO) as a certification scheme. Since then, pathological research has been done, and there is currently more international collaboration in certification-related activities (Ji *et al.*, 2007). With this certification, both the nurseryman who sells plant material that has been vegetative propagated and the farmer who purchases the nurseryman's goods are protected. In order to ensure the quality of propagative material to trueness-to-type (genetic purity), certification systems have been devised to conform to official standards/regulations imposed by the national and international authorities. Assessing the hazards (from viruses and pests), choosing planting material that is supposedly clean, pre-detecting the virus, micro-propagation, and genetic fidelity testing are the main steps in the procedure. In the end, a certificate is only given to the plants that are produced in accordance with the scheme's guidelines.

Breeding program

The best method for preventing begomoviral diseases is to breed resistance to the virus or its vector. According to Valkonen (1998), virus-resistant varieties are preferable since they don't need additional inputs for virus-free planting materials or virus/vector management and boost production profitability by increasing yields in both a qualitative and quantitative way. Gene introgression from *Solanum peruvianum*, *Solanum chilense*, *Solanum pimpinellifolium*, and *Solanum habrochaites* is one of the most obvious examples of breeding-mediated resistance to begomovirus infection in tomato (Pelham *et al.*, 1970). The conventional methods for controlling virus diseases have limitations, primarily due to the reliance on single dominant genes that breeders typically use to develop resistant cultivars. However, this approach often fails in the field (Lecoq, 1998). Additionally, identifying resistance genes is challenging as the underlying mechanisms for resistance in many crop species remain largely unknown in most cases (Valkonen, 1998).

Non-Conventional Methods of Virus Management

Genetic transformation offers to resist pathogen infection by introducing selected or specific virus resistant genes directly into the host plant genome. There were two major methods that have been used against plant pathogenic viruses, including geminiviruses which results in proving transgenic resistance: Pathogen derived resistance (PDR) and Non-Pathogen derived resistance (NPDR).

Pathogen derived resistance

In this process, resistance is derived directly from the pathogen, including its nucleic acid sequence resulting in encoding functional or non-functional protein, providing resistance. PDR includes "mild strain interference" that means plants infected with less virulent strains often develop resistance against highly virulent strains of that particular virus (Alves-Junior *et al.*, 2009). But in some cases no cross-protection has been seen and are being either benign or synergistic (Owor *et al.*, 2004). Some evidence suggested that it might be useful for the protection of cassava against begomoviruses that cause cassava mosaic disease in Africa. In this method, host plants are transformed by closely related pathogens. It has been reported that PDR can be protein-mediated resistance which provides low but expansive resistance and nucleic acid-mediated resistance which usually protects against very high levels of inoculum and is highly specific.

PDR technique is widely based upon silencing of viral genes. It can be done in two different ways – a) TGS, transcriptional gene silencing and b) PTGS, post transcriptional gene silencing. TGS is mainly based upon DNA methylation or histone modifications which confer wrapping on the histone region of the gene which ultimately leads to beyond reach for other promoter proteins and enzymes which are responsible for mRNA transcription. Whereas in PTGS, degradation of mRNA is done by introducing double stranded RNA which results in silencing and repression of some specific proteins with the help of RNA interference. This includes important factors like RNA dependent RNA polymerase (RDR 2), Drosha and Dicer Like (DCL 3) and ARGONAUTE (AGO 4), RNA- induced silencing complex (RISC) and viral RNA as a template which gets processed and results in forming small interference RNA (siRNA) later on causing degradation of mRNA (Lindbo *et al.*, 1993).

Many examples under PDR are proven to be successful which are driven by protein-mediated pathogenic RNA viruses to form transgenic plants but the mechanism of resistance is still unknown. According to Lindbo *et al.* (1993), CP of *Tobacco etch virus* was expressed in transgenic tobacco plants as a resistance mechanism, in which mRNA of the transgene in involved rather than a protein mediated mechanism. RNA interference also includes a crucial component called micro RNA (miRNAs), having a hairpin like structure. In the nucleus, it is mostly expressed by RNA polymerase II as primary miRNA. It is generally identified and processed by nuclear RNaseIII enzyme and its co-factor DiGeorge critical region (DGCR8) and then in the form of stem-loop miRNA export out of the nucleus via exportin5 nuclear transport receptor with co factor RanGTP (Kim, 2005). Then in cytoplasm, it is further cleaved by Dicer to form long double stranded miRNA which leads to inhibition of translation by blocking the site of mRNA because it has partial complementarity with miRNA so it readily pairs with one another (Leung & Sharp, 2007). As the cell cycle increases there will be consequential defencelessness against virus infections because TGS might become more drastic which results in the deactivation of transgene. However, PTGS associated mechanisms are more reliable and have less unfortunate phenotypes on the growth of transgenic plants.

Protein mediated resistance

From the studies, it was evident that sequences other than the CP gene could serve as pathogen-derived resistance genes, which led researchers to stray from a strategic connection to viral assembly-disassembly events or conventional "cross-protection" theories. This is due to it has bipartite begomovirus which has longer latent periods between inoculation and the appearance of symptoms and CP is not that important for infection. That's the main cause of failure for protein mediated resistance mechanism against bipartite begomovirus. CP expression could also be interfered by the insect transmission is still unknown. Some studies showed receptors in the digestive tract of vectors have specificity for CP expression in geminiviruses (Briddon, 1990). It has been reported that most of the protein mediated PDR against geminiviruses used replication associated protein (Rep) as an important virus replication factor. Strategies to restrict and hamper Rep results in effective resistance if interfered properly but overexpression may result in necrotic effects in plants.

RNA interference-mediated resistance

Lucioli *et al.* (2003) first investigated virus-specific siRNA in geminiviruses. When TYLCV infected a tomato plant, its RNA extracts showed siRNA have polarity towards both sense and anti-sense of its viral Rep gene. Synthetic siRNA was designed to demonstrate the confirmations of RNA silencing to target the coding region of ACMV *(African cassava mosaic virus*) Rep which inhibits mRNA and ultimately results in the reduction of virus replication in cultured cells. Some transgenic plants show synergistic interaction and cause critical disease due to dual infection of two different viruses (ACMV and EACMCV). This is because these plants show full-length expression of the Rep gene of ACMV in sense orientation against varying CMBs and express a crucial wide variety of resistance. RNAi mediated resistance has successfully targeted the non-coding region of geminiviruses in the form of intron-spliced hairpin structure which inhibits the expression of the CP gene sequences (Zrachya *et al.*, 2007).

DNA interference

Geminiviruses generally have very small sized circular single-stranded DNA genomes. They may contain virus-derived subgenomic DNA which is the resultant of error in replication due to deletion, duplication, inversion, rearrangement and may be the insertion of a non-viral DNA sequence. They are known as defective interfering (DI-DNA). It often produced by the plant if transmitted with geminiviruses and its accumulation causes delay in infection and symptoms. This mechanism subsequently interferes with the Rep-producing gene and reduces the amount of viral DNA and its expression. Stanley *et al.* (1990) studied ACMV DNA-B transmitted plants show ameliorated symptoms as compared to untransformed plants due to destructive and improper replication of genomic components (DNA-B 70% and DNA-A 20%) which episomally synthesizes full length virus genome. But when challenged with either BCTV or TGMV observed that it is virus specific with no phenotypic resistant characters. It was concluded that virus with small size genome are less effective to be resistance in the presence of DI-DNA and easily move from one cell to another. Manipulation like encoding Rep molecule or deletion of gene (βC1 gene) in alpha and beta satellite in viral genome show varying resistance effects in transgenic plants. Beta satellite is much more promising than the alpha (Briddon *et al.*, 2004; Saunders *et al.*, 2008). Because beta-satellite can interact with more than one begomoviruses (Tao & Zhou, 2004) but investigations show the proper delivery of resistance molecules in plants have not been reported yet.

Non-pathogen derived resistance

It refers to the resistance derived from resistance sequences other than the pathogen.

Resistance to vectors

In 2002, a functional genomics project was initiated, which generated several thousand expressed sequence tags (ESTs) of *B. tabaci*; the vector of Begomoviruses. This project provided the basic information to design experiments aimed at understanding and manipulating whitefly gene expression. Using sequences generated by this project Ghanim *et al.* (2007) provided evidence that the RNAi mechanism discovered in many organisms is also active in *B. tabaci*. By injecting long dsRNA molecules specifically directed against genes uniquely expressed in the midgut and salivary glands in the body cavity, the expression of the targeted mRNA was significantly inhibited. Gene expression levels in RNAi-silenced whiteflies were reduced up to 70% compared to whiteflies injected with buffer or with a green fluorescent protein-specific dsRNA. These findings open up the possibility of expressing dsRNA constructs in plants that target insect genes (Baum *et al.*, 2007). As well as providing a possible means of protecting plants from insects, by down-regulating genes essential for insect survival, the system could also potentially be used to interfere with insect transmission of viruses by silencing insect genes involved in the uptake and circulation of the virus in the insect.

Artificial zinc finger resistance

Inhibiting the binding of viral Rep to the site of viral replication is a promising strategy for preventing virus replication (Sera, 2017). AZFNs (Artificial zinc finger nuclease) were constructed based on those that had the highest DNA-binding affinities. AZFPs (artificial zinc finger proteins) have specifically targeted gene expression (Negi *et al.*, 2008). Subsequently, the AZF technology was employed to address Tomato Yellow Leaf Curl Virus (TYLCV) by designing AZFPs (Antiviral Zinc Finger Proteins) that could inhibit the binding activity of TYLCV replication (Takenaka *et al.*, 2007). The key advantage of AZFPs lies in their high affinity and specificity, enabling effective resistance even at low expression levels (Ilyas *et al.*, 2010).

Ribozyme-mediated resistance

Small nucleolytic RNAs called ribonucleic acid enzymes (ribozymes) have been used to suppress gene expression. These AZFPs function as molecular scissors, catalytically cleaving target RNAs. They are composed of a conserved catalytic domain, which facilitates the catalytic activity, and a flanking variable recognition domain that is responsible for specifically identifying and binding to the target sequence. The fundamental advantage of ribozyme is that it may target any RNA by engineering the proper alterations in the recognition domain. The trans-cleaving capacity of a hammerhead ribozyme targeted to the Rep mRNA of the Mungbean yellow mosaic India virus was studied by Chilakamarthi *et al.* (2007). Under in-vitro circumstances, the ribozyme demonstrated approximately 33% cleavage activity on synthetic Rep transcript in 1 hour.

ssDNA binding protein-mediated resistance

Gene 5 protein is an ssDNA binding protein derived from E. coli phage M13 that strongly cooperates with DNA binding without obvious sequence specificity. While the single-stranded (progeny) viral DNA is being synthesized, it blocks the transformation of ssDNA into the replicative form of dsDNA (Salstrom & Pratt, 1971). The G5 protein was examined by Padidam *et al.* (1999) to see if it might stop the buildup of ToLCNDV ssDNA. They proposed that the expression of G5 in transgenic plants may offer a cutting-edge method of managing geminiviruses and that such resistance might be effective against all geminiviruses. Despite these encouraging outcomes, this technique for supplying resistance to geminiviruses in plants has not yet been researched (Ilyas *et al.*, 2010).

Virus-induced cell death

Natural plant toxins contain Ribosome-inactivating proteins (RIPs) for its general defence from different pathogens (Narayanan *et al.*, 2005). With the help of genetic engineering, a potent RIP isolated from *Dianthus caryophyllus* against ACMV to provide transgenic resistance in *N. benthamiana* (Hong *et al.*, 1996), and is done by ACMV virion-sense promoter. Bipartite begomovirus such as ACMV virion-sense promoter can control and ensure expression of toxin only to virus-infected cells specific location.

CRISPR (clustered regularly interspaced short palindromic repeats)/Cas system mediated resistance

The recent advent of the CRISPR/Cas system has introduced new techniques for developing resistance to geminiviruses. It has the potential as a specific editing tool and has shown to be effective against several different geminiviruses when expressed in plants (Zaidi *et al.*, 2016). The CRISPR/Cas systems, which are derived from bacteria and archaea, work as a crucial adaptive immunity against foreign nucleic acids. As our understanding of CRISPR/Cas systems has advanced fast, they have been transformed into practical tools for editing exogenous and endogenous DNA or RNA sequences in different organisms. Only a Cas effector protein and an easily modified guide RNA (gRNA) are present in a simplified CRISPR system. Tashkandi *et al.*, (2018) targeted the TYLCV (Tomato yellow leaf curl virus) genome with Cas9 sgRNA at the sequences encoding the coat protein (CP) or replicase (Rep) resulting in efficient virus interference, as evidenced by the low accumulation of the TYLCV DNA genome in the transgenic tomato and *Nicotiana benthamiana* plants. While CRISPR/Cas9 is the best tool for building geminivirus resistance in sensitive plants, it is not without flaws (Beam & Ascencio-Ibáñez 2020).

CONCLUSION

Begomovirus poses significant threats to pumpkin crops in India. It has a bipartite genome, consisting of DNA-A and DNA-B. Begomoviruses have a circular, single-stranded deoxyribonucleic acid (ssDNA) genome. These viruses are transmitted by whiteflies (*Bemisia tabaci*) and cause severe yield losses by inducing symptoms such as leaf curl, vein clearing, yellowing, and stunted growth. Managing begomovirus in pumpkin crops requires a multifaceted approach. Many conventional

and non-conventional management strategies are used to protect pumpkin crops. Continuous research and adaptation of strategies based on local conditions and virus dynamics are essential to effectively combat this threat and sustain pumpkin production in India.

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