Molecular approaches towards analyzing the viruses infecting maize (*Zea mays* L.)

Kamal Sharma[1,2,3] and Raj Shekhar Misra[1]*

[1]International Institute of Tropical Agriculture, PMB 5320, Ibadan, Nigeria.
[2]Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala -695017, India.
[3]International Institute of Tropical Agriculture, PMB 5320, Ibadan, Nigeria.

Abstract

Information on virus diseases of maize still remains scanty in several maize growing countries. Therefore it is hoped that this description will stimulate more research, which will lead to better understanding of viruses infecting maize in Africa. Plant viruses are a major yield-reducing factor for field and horticultural crops. The losses caused by plant viruses are greater in the tropics and subtropics, which provide ideal conditions for the perpetuation of both the viruses and their insect vectors. Management of viral diseases is more difficult than that of diseases caused by other pathogens as viral diseases have a complex disease cycle, efficient vector transmission and no effective viricide is available. Traditionally, integration of various approaches like the avoidance of sources of infection, control of vectors, cultural practices and use of resistant host plants have been employed for the management of viral diseases of plants. All these approaches are important, but most practical approach is the understanding of seed transmission, symptom development, cell-to-cell movement and virus multiplication and accurate diagnosis of viruses. This update aims to continue on this course while simultaneously introducing additional levels of complexity in the form of microbes that infect plants. Rather than serving as a standard literature review, the objective is to provide a broad conceptual introduction to the field of molecular plant-microbe interactions, virus multiplication, transmission and virus diagnosis and various immunodiagnostic and molecular diagnostic methods such as enzymes linked immunosorbent assay (ELISA), immunosorbent electron microscopy (ISEM), polymerase chain reaction (PCR), nucleic acid hybridization, dot immunoblotting assay (DTBIA) found suitable for diagnosis of viruses infecting maize. These techniques do not only provide information for epidemiological purposes, but also help to develop disease free stock of maize. Therefore, these various techniques with symptoms and history are of immense value to diagnose maize viruses and are the cornerstone of the management of maize viruses. This information will be useful to researchers in understanding of maize viruses. Information on symptomatology, transmission, geographical distribution and properties of viruses is summarized here based on literature review.

Keywords: Maize, diagnosis, ELISA, transmission, virus.

INTRODUCTION

Maize (*Zea mays* L.) is one of the major cereal crops; it ranks third in production following wheat and rice with an average of 784,786,580 tons produced annually by 10 countries (FAO, 2007) (Table 1). It is the world's most widely grown crop in almost all tropical areas of the world including tropical highlands over 3000 m in altitude, to temperate areas as far north as the 65th latitude. Maize is one of a few important grasses that humanity has cultivated for centuries to provide food and a considerable number of industrial products (Galinat, 1977). Maize has often been described as "the grain that civilized the New World." Maize, or corn as it is called, has a multitude of uses and ranks third among the world's cereal crops in terms of total production. Also, because of its worldwide distribution and lower prices relative to other cereals, maize has a wider range of uses than any other cereal. Within the developing world, there are a number of

Table 1. Top ten maize producers in 2007.

Country	Production (Tonnes)	Area harvested	Yield Hg/ Ha
United States	332,092,180	35022300	94823
China	151,970,000	28074000	54131
Brazil	51,589,721	13827500	37309
Mexico	22,500,000	7800000	28846
Argentina	21,755,364	2838072	76655
India	16,780,000	7770000	21595
France	13,107,000	1481000	88501
Indonesia	12,381,561	3450650	35881
Canada	10,554,500	1361100	77543
Italy	9,891,362	1081680	91444

Source: Food and Agricultural Organization of United Nations: Economic And Social Department: The Statistical Division. http://faostat.fao.org/site/567/ 17.2.09

countries where maize is a major staple food and the per capita human consumption reaches high levels. Maize is a major staple food for most of the indigenous, rural populations in Africa (CIMMYT, 1990). Africans consume nearly one-fourth of the total feed (livestock) maize produced in Africa, and for many countries, the per capita consumption of maize may be as high as 100 kg per year (CIMMYT, 1990) Major maize producing countries in Africa are presented in Table 3. A number of diseases affect the productivity of maize (Bacterial, fungal, viral, virus like, nematode and parasitic).

Because of existence of different ecological conditions between the temperate areas and the tropics, the insect vectors and their disease agents also are different under these different conditions. To date, at least 21 viral plant pathogens affecting maize in the hot and humid tropics have been reported (Table 2). Most of the viruses and molecules referred to as etiological agents of diseases on maize have been found in several parts of Africa. Viruses found infecting maize on the African continent include: Maize streak virus (*geminvirus*), maize mottle/chlorotic stunt virus, maize eyespot virus, guinea grass mosaic virus (*potyvirus*), cynodon chlorotic streak virus (*rhabdoviridae*), maize yellow stripe virus (*tenuivirus*), brome mosaic virus (*bromoviridae* and *bromovirus*), barley stripe mosaic virus (*hordeivirus*), and barley yellow dwarf virus (*luteovirus)* (Thottappilly et al., 1993).

Plant viruses are intracellular pathogens that perform genome replication and encapsidation within the infected cells. In order to use the resources of the host cells efficiently for their genome replication, plant viruses have to interact with the host cells, manipulate host cell pathways, and, ultimately, transform the host cells into "viral factories." The magnitude of physiological and phenotypic changes in the host during viral infection suggests the involvement of a large number of host genes (Golem and Culver, 2003; Whitham et al., 2006). Thus, the intimate interaction between a plant virus and its host is complicated by the systemic nature of infection

and global alterations in host gene expression (Maule et al., 2002; Whitham and Wang, 2004).

All these pathogens represent potential threat to maize productivity. In the event of epidemiological surges, the ability to rapidly and precisely diagnose and identify the causal agents will be necessary to design control measurements.

Genome structure of viruses

The majority of plant viruses have a plus stranded (+)RNA genome compatible with the protein translation apparatus of the host. The infection cycle of these viruses includes entry into the cell, disassembly of the virus capsids, translation of the viral RNA, genome replication and transcription, encapsidation, and cell-to-cell movement. The central event is the genome replication of (+)RNA viruses, which consists of a two-step process: First, the minus strand replication intermediates are produced, which are then used to direct synthesis of excess amounts of (+)RNA progeny by the unique viral replicases (such as RNA dependent RNA polymerase (RdRp), the key enzymes in viral replication. Replication is an asymmetric process leading to a 20- to 100-fold excess of the new (+)RNA progeny over minus-strand RNA. All known plant (+)RNA viruses assemble their own replicase complexes (RC), likely containing both viral-and host-coded proteins (Buck, 1996; Ahlquist, 2002; Ahlquist et al., 2003; Nagy and Pogany, 2006). The assembled viral RCs are associated with cellular membranes, such as the endoplasmatic reticulum and the membranes associated with cell organelles like mitochondria, vacuole, golgi complex, chloroplast, and peroxisome, which serve as sites of viral replication (Laliberte and Sanfacon, 2010).

One intriguing aspect of (+)RNA viruses is that their RNAs must participate in several competing processes, all of which are required for successful viral infections.

Table 2. Maize viruses and virus like diseases.

Diseases	Virus	Virus genus/group	Vectors	Seed transmission	Geographical distribution	Reference
Maize bushy stunt	Mycoplasma like organism (MLO)		Corn leafhopper, *D. maidis*	Unknown		Legrnad and Power,
Maize chlorotic dwarf	Maize chlorotic dwarf virus (MCDV)	IV: (+)sense RNA Viruses (Waikavirus)	Arthropods (*G. nigrifrons*, *Graminella sonora* and *Exitianus exitiosus*)	No	United States of America	ICTVdB, 2006
Maize chlorotic mottle	Maize chlorotic mottle virus (MCMV)	IV: (+)sense RNA Viruses (Machlomovirus)	Arthropods (*Cicadulina mbila*, *C. zeae*, *C. storeyi* and *C. triangula*)	No	Nigeria, Rwanda, Sao Tome and Principe, Tanzania, Togo, Zambia, and Zimbabwe	Rossel and Thottappilly
Maize dwarf mosaic	Maize dwarf mosaic virus (MDMV) strains A, D, E and F	IV: (+)sense RNA (Potyvirus)	Arthropods, insects	Yes	China, South Africa, and the United States of America	ICTVdB, 2006
Maize eyespot virus			Virus transmitted by mechanical inoculation	No	Cote d'Ivoire	Brunt et al., 1996
Maize line	Maize line virus (MLV)	-	Transmitted by an insect; *P. maidis*; Delphacidae	No	Kenya and Tanzania	Kulkarni, 1973
Maize mosaic (corn leaf stripe, enanismo rayado)	Maize mosaic virus (MMV)	V: (-) sense RNA Viruses (Nucleorhabdovirus)	Arthropods, by insects *P. maidis*	No	Australia, Colombia, Costa Rica, Fiji, India, Mauritius, Mexico, Peru, Spain, Tanzania, and the United States of America (and the Caribbean Islands).	ICTVdB, 2006
Maize Iranian mosaic			Transmitted by a vector; an insect; *Unkanodes tanasijevici, Laodelphax striatellus, P. maidis*; Delphacidae	No	Iran	Brunt et al., 1996
Maize rayado fino (fine striping disease)	Maize rayado fino virus (MRFV)	-	Transmitted by a vector; an insect; *D. maidis*; Cicadellidae	No	Argentina, Brazil, Colombia, Costa Rica, Mexico, Peru, Venezuela, and the USA (in the south).	Brunt et al., 1996
Maize mottle/chlorotic stunt virus			Transmitted by a vector; an insect; *C. mbila, C. zeae, C. storeyi* and *C. triangula*; Cicadellidae	No	Nigeria, Rwanda, Sao Tome and Principe, Tanzania, Togo, Zambia, and Zimbabwe	Brunt et al., 1996

Table 2. Contd.

Maize streak dwarf			Transmitted by a vector; an insect; *Laodelphax striatellus* (both adults and nymphs); Delphacidae	No	China	Brunt et al., 1996
Maize streak monogeminivirus			Transmitted by a vector; an insect; *C. mbila, C. arachidis, C. bipunctella, C. triangula, C. bimaculata, C. similis, C. latens, C. ghaurii, C. parazeae*; Cicadellidae	No	African region; India, Madagascar, Reunion, and Yemen	Brunt et al., 1996
Maize rough dwarf (nanismo ruvido)	Maize rough dwarf virus (MRDV)	III: dsRNA Viruses	Virus is transmitted by a vector (*Delphacodes propinqua, Dicranotropis hamata, L. striatellus, Javasella pellucida, Sogatella vibix*). Virus is transmitted by mechanical inoculation	No	Argentina, Czechoslovakia (former), France, Israel, Italy, Norway, Spain, Sweden, and Yugoslavia	ICTVdB Management, 2006
Maize yellow stripe			Transmitted by a vector; an insect; *C. chinai*; Cicadellidae	No	Egypt	Brunt et al., 1996
Maize streak	Maize streak virus (MSV)	Group II (ssDNA) (*Mastrevirus*)	African leafhopper, *C. mbila* Naudé	Unknown	Sub-Saharan Africa	ICTVdB Management, 2006
Maize stripe (maize chlorotic stripe, maize hoja blanca)	Maize stripe virus	V: (-) sense RNA Viruses (Tenuivirus)	Virus is transmitted by arthropods, by insects *P. maidis*.	No	Australia, Botswana, Guadeloupe, India, Kenya, Mauritius, Nigeria, Peru, the Philippines, Reunion, Sao Tome and Principe, the United States of America, and Venezuela	ICTVdB Management, 2006
Maize white line mosaic	Maize white line mosaic virus (MWLMV)	Virus unclassified	Insect	No	France, Italy, and the United States of America	ICTVdB Management, 2006

These highly regulated, coordinated, and compartmentalized processes include translation of viral RNA, replication, transcription to produce subgenomic RNA for some viruses, encapsidation, and cell-to-cell movement. Each process can be further divided into distinct steps based on recent detailed analyses of a single replication cycle of (+)RNA viruses. During genome replication, the steps include the following: (1) The recruitment/selection of the viral (+)RNA template for replication, including a requirement for switching of the genomic RNA from translation to replication; (2) Targeting of viral replication proteins to the site of replication; (3) Preassembly of the viral replicase components;

Table 3. Top twenty maize producers country in Africa'.

Country	Production (Tonnes)	Area harvested	Yield Hg/Ha
Nigeria	7800000	4700000	16595
South Africa	7338738	2551800	28759
Egypt	7045000	868000	81163
Ethiopia	4000000	1468000	27248
Malawi	3444700	1688500	20400
Tanzania	3400000	3000000	11333
Kenya	3240000	1600000	20250
Mozambique	1579400	1505400	10491
Zambia	1366158	872800	15652
Uganda	1262000	844000	14952
DR Congo	1155000	1480000	7804
Ghana	1100000	750000	14666
Zimbabwe	952600	1445800	6588
Cameroon	923000	480000	19229
Benin	900000	700000	12857
Angola	570000	1115000	5112
Togo	500000	380000	13157
Chad	200000	200000	10000
Rwanda	90000	110000	8181
Sudan	60000	80000	7500

Source: Food and Agricultural Organization of United Nations: Economic And Social Department: The Statistical Division. http://faostat.fao.org/site/567/ 17.2.09.

(4) Activation/final assembly of the viral RC containing the (+)RNA template on intracellular membranous surfaces; (5) Synthesis of the viral RNA progeny by the RC, including minus- and plus-strand synthesis; (6) Release of the viral (+)RNA progeny from the RC to the cytosole, and (7) Disassembly of the viral RC (Nagy and Pogany, 2006).

Geminiviruses are DNA viruses which are known to infect plants and have a small genome which encode only a few proteins. The genome encodes for both structural and non-structural proteins. Geminiviruses have circular single-stranded DNA. Therefore, their DNA replication cycle relies largely on the use of cellular DNA replication proteins. The genome is either in two segments or not segmented at all. The non-segmented genome is 2500 to 3000 nucleotides long, and the segmented genome is 4800 to 5600 nucleotides long. The strategy used by geminiviruses to replicate their single-stranded DNA (ssDNA) genome consists of a first stage of conversion of ssDNA into double-stranded DNA (dsDNA) intermediates and, then, the use of dsDNA as a template to amplify viral dsDNA and to produce mature ssDNA genomes by a rolling-circle replication mechanism (Gutierrez, 1999).

The characteristic twinned or "geminate" particles, which consist of two joined, incomplete $T = 1$ icosahedra, are unique among viruses. Structure of a geminivirus particle, the Nigerian strain of Maize streak virus (MSV-N), was determined. The particle, of dimensions 220 ×

380 Å, has an overall 52-point-group symmetry, in which each half particle "head" consists of the coat protein (CP) arranged with quasi-icosahedral symmetry (Zhang et al., 2001). Whereas the structure of the maize streak virus genome (Kenyan isolate, MSV-K), as determined from the sequence of clones obtained from DNA isolated from virus particles, is composed of one major DNA component of about 2.6 kb. MSV virion DNA was partially double-stranded, composed of a full-length virion (V) strand and a short (70 to 80 b) primer (P) strand. The primer strand has a fixed 5-end capped with alkaline labile material, presumably 1 to 2 ribonucleotides. The MSV genome has two major coding regions oriented on opposite strands and flanked by two small intergenic regions. The coding region on the P strand is composed of two major open reading frames (ORFs), arranged in tandem and in the same reading frames (Howell, 1984). The genomes of plant (+)RNA viruses code for 4 to 10 proteins, including 1 to 4 proteins involved in viral RNA replication. The replication proteins include one RNA-dependent RNA polymerase (RdRp) protein and auxiliary proteins with helicase, RNA capping or other functions. Host factors contribute to these functions, provide additional functions, and likely participate in each step of the (+)RNA virus replication. Based on recent genome-wide studies (Kushner et al,, 2003, Jiang et al,, 2006), the emerging picture is that the identified host factors are mostly highly conserved genes, suggesting that (+)RNA viruses might selectively target conserved host functions

as opposed to species factors. This strategy can help viruses to have a broader host range and to expand infections to new host species.

Host factors play crucial roles in plant (+)RNA virus replication and infection. Host factors affect host-virus interactions, virus pathology, virus evolution, and they are also key determinants of host range of a given virus (Nagy and Pogany, 2006).

Maize stripe virus (MStV) is a member of newly recognized *tenuivirus* group (Family: Tospoviridae). This group exhibits several unique properties different from other characterized RNA plant viruses (Gingery, 1988). Thin, filamentous, sometime circular infectious nucleoprotein particles have been associated with *tenuivirus* infected plants (Chen et al., 1993; Tsai and Falk, 1993). The nucleoprotein particles are composed of a ca. 35,000 Mr nucleocapsid protein and 4 to 5 species of RNA (Toriyama, 1982; Chen et al., 1993; Tsai and Falk, 1993). When analyzed by denaturing gel electrophoresis, the 5 RNAs have molecular weights of 0.52, 0.78, 0.81, 1.18 Kd and Mr 3.01×10^6. The 16,000 Mr (16 K) protein which has been referred to as the nuclear coat protein (NCP) is found abundantly in MStV-infected plants (Falk et al., 1987) and it can readily be found in sap from infected plants as crystals by phase-contrast light microscopy and the crystals react with antiserum to MStV NCP in immunofluorescence microscopy (Bradfute and Tsai, 1990). Recent studies showed that fibrous intracellar inclusions can be readily found in paradermal sections of the leaf sheath of MStV infected maize (Overman et al., 1992). The nucleotide sequence of MStV NCP gene has also been determined. The purified Maize mosaic virus (MMV, Nucleorhabdovirus) virions contained a single-stranded RNA of Mr 4.2×10^6 (Falk and Tsai, 1983). MMV virions contain three major structural proteins of Mr 75,000, 54,000, and 30,000 as analyzed by SDS-PAGE. Various sizes of MMV virions have been reported. Dimensions of 255×90 nm for negatively-stained partially-purified preparations, and 242×48 nm for particles in thin sections of MMV-infected tissues have been reported for the Venezuelan isolate (Lastra, 1977); 224×68 nm and 234 to 325×63 nm have been reported for purified virions and those in MMV-infected cells, respectively, for the Florida isolate (Bradfute and Tsai, 1983; Falk and Tsai, 1983); and dimensions of 204×67 nm for bullet-shaped particles, 245×80 nm for bacilliform particles have been reported for the Hawaiian isolate of MMV (McDaniel et al., 1985). Both the perinuclear accumulation of virus particles in the infected cells (Bradfute and Tsai, 1983) and the presence of particle in the cytoplasm of epidermal, mesophyll, and vascular parenchyma cells and phloem and xylem elements of infected plants have been reported (Lastra, 1977; McDaniel et al., 1985). The granular masses were found to surround the nuclei of the epidermal strips of MMV infected leaves and roots using light microscopy (Nault et al., 1984). The viral genome of maize dwarf

mosaic virus (MDMV) was 9515 nt and contained an open reading frame encoding 3042 amino acids, flanked by 3 - and 5 -UTRs of 139 and 250 nucleotides, respectively. The RNA of maize dwarf mosaic virus (Potyvirus) strain A (MDMV-A) was characterized and compared with that of strain B (MDMV-B) . Glyoxal-treated MDMV-A RNA has a Mr of 3.32×10^6 measured in agarose gels, compared with that of MDMV-B which is 3.41×10^6 under the same conditions. MDMV-A RNA has a Tm of 50.7°C and a hyperchromicity (increase in absorbance during unfolding of a higher structure) of 23.3%, which are higher than those reported for MDMV-B RNA (Berger et al., 1989) . MDMV-Bg was more conserved in the coding region (52.9%) than in the UTRs (45.8%) when compared to the 15 other potyviruses (Kong and Steinbiss, 1998) . In Maize chlorotic mottle virus (MCMV), sub-genomic RNA is present in infected cells; encoding the coat protein. The genome expression is based on RNA production which can be analyzed by the dsRNA patterns found in the infected tissues. Usually, there are 2 virus specified dsRNA species found in infected cells. Size of largest virus specified dsRNA 4.4 kb and 2nd largest 1.1 kb. The genome has four ORFs encoding proteins of 32 and 50 kDa (possibly the polymerase), 9 and 25.1 kDa (coat protein) (Lommel et al., 1991). The complete nucleotide sequence of the single-stranded RNA genome of maize rayado fino virus (MRFV), the type member of the genus *Marafivirus (*Family: Tymoviridae), is 6305 nts in length and contains two putative open reading frames (ORFs). The largest ORF (nt 97 to 6180) encodes a polyprotein of 224 kDa with sequence similarities at its N-terminus to the replication-associated proteins of other viruses with positive-strand RNA genomes and to the papain-like protease domain found in tymoviruses (Hammond and Ramirez, 2001). The C-terminus of the 224-kDa ORF also encodes the MRFV capsid protein. A smaller, overlapping ORF (nt 302 to 1561) encodes a putative protein of 43 kDa with unknown function but with limited sequence similarities to putative movement proteins of tymoviruses (Hammond and Ramirez, 2001). Morphologically, Maize chlorotic dwarf virus (MCDV) virions are 30 nm diameter icosahedrons with a buoyant density of 1.507 g/ml (Gingery, 1976). The genome consists of a single-stranded RNA molecule with an estimated molecular mass of 9.4 kDa (Gingery et al., 1981). These properties of MCDV resemble those of rice tungro spherical virus (RTSV) within the family of Sequiviridae (Shen et al., 1993). These two viruses and anthriscus yellow virus have been placed in the genus *Waikavirus* (Mayo et al., 1995). Maize necrotic streak virus (MNeSV) has 32 nm isometric particles that encapsidate a single stranded RNA genome *ca.* 4.3 kb in size, and is a tentative member of the genus *Tombusvirus* . Maize necrotic streak virus has isometric particles 32 nm in diameter, with a poorly resolved surface structure, that encapsidate a single-stranded

positive-sense RNA genome of *ca.* 4.3 kb (Louie et al., 2000). Viral RNA has a genome structure and organization that are similar to those of tombusviruses. However, MNeSV is classified as a tentative species in the genus *Tombusvirus* because it is not transmissible by leaf-rub inoculation, has a low rate of vectorless transmission through the soil, and has a small coat protein (CP) that is more similar in size (29 kDa), structure, and sequence to those of necroviruses and sobemoviruses than to those of tombusviruses (de Stradis et al., 2005). The molecular masses of maize fine streak virus (MFSV) the proteins by SDS-PAGE of purified virions were reported 82±2, 50±3 and 32± 2 KDa with mean of ± SD, n = 9. A ribonuclease A-sensitive nucleic acid of more than 10 kb was isolated from purified MFSV. No other RNA species observed in nucleic acid preparations. The open reading frame (ORF) encoded by the MFSV G3A cDNA homologous to the N terminus of *rhabdoviral* L proteins. An ORF encoded by the 5' 1.4 kb of the MFSVG6A cDNA had similarity with the nucleocapsid (N) protein of rhabdoviruses (Redinbaugh et al., 2002). MSV has twin quasi-icosahedral particles (18 × 30 nm) with a coat protein of Mr 26000. The genome is composed of a single 2.7 kb single-stranded circular DNA (Mullineaux et al., 1984).

MOLECULAR ASPECTS OF MULTIPLICATION OF MAIZE VIRUSES

All viruses are intracellular molecular parasites on eukaryotic cells since they possess minimum of essential genetic information. Consequently, they have developed the capacity to utilize metabolic machinery of the host cell for production of their DNA and RNA during viral nucleic acid multiplication and viral proteins during translation process. The invading virus genome thus takes control of and subverts the normal cellular processes and usurps the natural cellular machinery as well as cellular factors for replication and transcription of their DNA and RNA genomes and synthesis of viral proteins.

Propagation is the fundamental aspect of biology of any organism. So it is in plant viruses also. Apart from its basic role in increasing population and perpetuating a virus species, it has great importance in genome recombination, in generating hybrid genomic molecules, in producing defective-interfering RNAs/DNAs, in patho-genesis, and in several other viral functions (Zaitlin and Palukaitis, 2000). It involves four chronologically overlapping fundamental steps: (1) Decapsidation resulting in unmasking of genomic RNA and making it available for various viral functions; (2) Translation during which viral genomic RNA serves as mRNA and produces structural and non-structural proteins coded by viral RNA; (3) Replication of viral RNA genome to yield progeny RNA molecules, and (4) Encapsidation leading to assembly of progeny RNA molecules with the cognate

capsid protein molecules to produce the progeny virus particles. Each of these stages is a complex phenomenon requiring specific conditions and certain essential proteins that perform vital functions during these stages (Salonen et al., 2005). For viral RNA replication, these essential proteins include polymerases, helicases, DNA- binding proteins, capping enzymes, elements needed for binding of host factors, and others (Ahlquist et al., 2003). Additionally, the cis - and transacting nucleotide sequence motifs and RNA secondary structures within 5'-termini of viral genomic RNAs are central to virus RNA replication. Such events, enzymes and factors are also functional in each of the other three stages. Phylogenetically, replication-associa-ted genes constitute the core elements of RNA virus genomes while other gene modules are considered as accessory elements. Very little was known about molecular aspects of plant viral RNA replication (Teycheney et al., 2000) and its initiation enzymatic studies on virus multiplication constitute the frontier area.

Most of the research focused on RNA viruses evolution, which are generally subject to relatively high rates of mutation due to their dependence on error- prone DNA dependent RNA polymerases. RNA viruses have been shown to evolve at rates between 10^{-3} to 10^{-5} substitutions per site per year (subs/site/year) (Malpica et al., 2002). However recently, DNA viruses evolution study has also gained equally importance, in contrast with the hypothesis that polymerase fidelity influences evolution rates double stranded DNA (dsDNA) papillomaviruses and polyomaviruses evolve at rates in the region of 10^{-9} subs/site/year (Drake, 1991; van der Walt et al., 2008).

The genome replication of most DNA viruses takes place in the cell's nucleus. If the cell has the appropriate receptor on its surface, these viruses enter the cell sometimes by direct fusion with the cell membrane or more usually by receptor-mediated endocytosis. Most DNA viruses are entirely dependent on the host cell's DNA and RNA synthesizing machinery, however, viruses with larger genomes may encode much of this machinery themselves.

Geminiviruses utilize three replication modes: Complementary-strand replication (CSR), rolling-circle replication (RCR) and recombination-dependent replication (RDR). Here RCR mechanism is briefly explained

Rolling-circle replication

A characteristic feature of RCR is the involvement of a replication initiator protein (Rep) with a nicking-closing activity similar to that found in topoisomerases. RCR occurs in three stages. In the first stage (SS RF synthesis), viral ssDNA (+ strand) enters the cell and is converted into a covalently closed dsDNA replicative form (RF) in a process involving host-directed, RNA-primed synthesis of a complementary (-) template for further

replication. The purpose of the second stage of RCR (RF RF synthesis) is to generate additional RF DNA (Saunders et al., 1991). This step is initiated by viral Rep protein, gene A protein (gpA) in the case of X174, whose function is to nick the plus strand at a specific sequence. Following phosphodiester bond cleavage, Rep protein covalently binds to the 5' terminus via a phosphotyrosine linkage. The 3' -OH terminus is used as a primer for the synthesis of nascent plus strand, which displaces the parental plus strand from the intact minus-strand template. Synthesis again is carried out by host replication proteins (DePamphilis, 1988) . Completion of the nascent plus strand regenerates the origin of replication, which again is nicked by Rep, this time acting as a terminase to release the displaced unit-length plus strand, which is simultaneously ligated to circular form by the closing activity. Rep is transferred to the newly created 5' terminus. Early in the replication cycle, the circularized ssDNA is used as template for synthesis of minus-strand DNA, resulting in the amplification of RF. The third stage of RCR (RF SS synthesis), which occurs late in the replication cycle, is responsible for the accumulation of viral genomes for encapsidation. This stage is similar to RF RF synthesis, except that priming is prevented and ssDNA is the predominant product (DePamphilis, 1993; Bisaro, 1994).

Multiplication/propagation of maize viruses

Propagation is well studied for several economic important viruses for ex. maize stripe virus (Nault and Gordon, 1988), maize chlorotic mottle virus (Lommel et al., 1991). Here it is briefly explained with another economically important virus maize dwarf mosaic virus (MDMV). The incidence of this virus disease is usually less than 5%, but levels as high as 65% been reported (Seifer and Hackerott, 1987). The virus is spread from plant to plant and field to field by several species of aphids. The most common carriers (vectors) are the greenbug and the corn leaf aphid. Initial infections may occur when over wintering aphids feed on infected weed hosts and then move into a field. Additionally, the virus can spread to great distances when virus-carrying aphids are moved by strong winds associated with weather fronts (Seifer and Hackerott, 1987). MDMV is also readily transmissible by aphids in a non-persistent manner which means that both virus acquisition and inoculation by aphids can occur in a few seconds. At least 25 species of aphids have been reported to be vectors of plant viruses (Knoke et al., 1983a). The transmission efficiency varies greatly depending upon aphid species, environmental conditions, virus strains and host plants. The virus can survive in perennial grasses or in the seed of annual or perennial grasses which represent important sources for both MDMV and the aphids that transmit it. The aphid species known to be efficient vectors of MDMV are:

The green bug, *Schizaphis graminum* (Rondani), the corn root aphid, *Aphis maidiradicis* Forbes, the cowpea aphid, *Aphis craccivora* Koch, the bean aphid, *Aphis fabae* Scopoli, the melon aphid, *Aphis gossypii* Glover, the boat gall aphid*, Hyalopterus atriplicis* (L.), the pea aphid, *Acyrthosiphon pisum* (Harris), the green peach aphid*, Myzus persicae* (Sulzer), the English grain aphid, *Macrosiphum avenae* (F.), the blue grass aphid, the corn leaf aphid, *Rhopalomyzus poae* (Gillette) and *Rhopalosiphum padi* (L.) (Knoke et al., 1983a). Maize mosaic virus (MMV) is solely transmitted by *Peregrinus maidis* in a persistent-propagative manner. The rate of MMV transmission by *P. maidis* by means of plant acquisition ranged from 5 to 42% (Lastra, 1977; Falk and Tsai, 1985). *P. maidis* was able to acquire MMV in less than 15 min and the patterns of transmission were often erratic (Falk and Tsai, 1985). The efficiency of MMV transmission by *P. maidis* could be increased from 20 to 43% by injection with either purified MMV or with sap from MMV-infected corn plants (Falk and Tsai, 1985).

INCIDENCE AND ECONOMIC IMPORTANCE OF VIRUSES INFECTING MAIZE

An economically devasting maize disease throughout the southeastern part of the United States, commonly known as maize chlorotic dwarf virus (MCDV) is caused by a complex of strains of MCDV. It is considered to be the second major corn virus disease in the USA (Knoke and Louie, 1981). Maize dwarf mosaic virus (MDMV) is one of the most important widely distributed virus diseases of corn (*Z.mays* L.) in the temperate regions of the world especially in U.S.A. and Hawaii. This disease has not been reported as a serious disease in the Tropics and Subtropics (Tsai and Brown, 1989). This disease caused severe yield losses in the early 1960's particularly in dent corn (Williams and Alexander, 1965). Yield losses as high as 40% have been attributed to MDMV wherever maize and sugarcane are cultivated, however, occur predominantly in the United States and Australia. Distribution of the diseases caused by other viruses generally reflects the geographic distribution of their host. However, the crop losses varied greatly depending on the susceptibility of the corn genotype, virus strains, plant age and environmental factors. MDMV was named by Williams and Alexander (1965) and is closely related to sugarcane mosaic virus (SCMV) which has at least 13 strains. The leafhopper-borne maize yellow stripe virus (MYSV) is a tentative member of the *tenuivirus* group. MYSV symptom-types include fine stripe, coarse stripe, and chlorotic stunt; these symptom-types usually appear on different leaves of the same plant (Ammar et al., 1990). Maize streak virus (MSV) remains an economically important disease of maize in much of Africa. Yield loss as high as 100% have been reported under favorable conditions for disease development (susceptible varieties

and favorable climatic conditions for leafhopper development). MSV is an economically significant pathogen in maize, cereals and sugar-cane throughout Africa (Damsteegt, 1983; Rose, 1978) and has also been isolated from grasses such as *Coix* spp., *Panicum* spp., *Paspalum* spp. and *Setaria* spp. in Africa (Storey and McClean, 1930; Rose, 1978). Maize stripe virus (MStV) was first described in 1936 in East Africa by Storey who recognized two types of symptoms, one with narrow yellow stripes on the leaves, the other with broad stripes (Storey, 1936). Kulkarni (1973) demonstrated that two symptoms of maize stripe were associated with two distinct pathogens and were transmitted persistently by the corn delphacid, *P. maidis* (Ashmead). Later Bock et al. (1974) proved that the narrow yellow stripe was caused by a *rhabdovirus*. To date, maize stripe has been reported from Venezuela, Florida the Philippines, Mauritius, Australia, Peru and Taiwan (Tsai and Falk, 1993). Maize mosaic virus (MMV) was first reported in 1914 in Hawaii (Kunkel, 1921). It is considered a serious disease in the tropics and subtropics, and has been speculated as a possible cause of the collapse of Mayan civilization (Brewbaker, 1980). MMV is also transmitted by *P. maidis* in a persistent manner. Maize mosaic has often been confused with maize stripe in the literature because of their similarity. MMV has been reported in Central and South America, Mexico, India, Mauritius, Reunion, Madagascar, and Tanzania (Tsai and Falk, 1993). Maize rayado fino virus (MRFV) was first reported in El Salvador in the 1960's (Ancalmo and Davis, 1961). Later, Gamez (1983) demonstrated a Costa Rican isolate of MRFV transmission by the corn leafhopper, *Dalbulus maidis* . This disease has also been found in Uruguay, Brazil, Colombia, Panama, Guatemala, Honduras, Nicaragua, Mexico, Peru, Venezuela, Ecuador and the U.S. (Nault et al., 1980; Toler et al., 1985). Yield losses in Central America may be up to 40 to 50% of early infected plants. Losses and incidences may reach 100% for newly introduced cultivars (Gamez, 1983). The isolation, culture and characterization of Maize fine streak virus (MFSV; *rhabdovirus*) obtained from leaf samples of Decatur County in Southwestern Georgia that exhibited chlorotic vein streaking. Based on symptoms incited by the virus, it has been previously named maize fine streak virus. The initial symptoms included chlorotic spots and short streaks on small veins that were unevenly distributed. Symptoms were fully developed with continuous streaks on intermediate and small veins, on leaves four to seven, between 3 and 4 weeks post-vascular puncture inoculation (VPI). The streaks enlarged as the plants matured, such that after approximately 6 weeks post-VPI the leaves appeared white with isolated green spots. Alternatively, some plants showed a partial recovery with only scattered chlorotic spots and streaks on later leaves. The virus has been found only in limited areas of Southwesteren Georgia in two fall-growing seasons, suggesting the virus is currently of limited agronomic importance (Redinbaugh et al., 2002).

MAIZE VIRUS TRANSMISSION AND VIRUS-VECTOR INTERACTIONS

Viruses are transmitted predominantly by several genera of aphids, leafhoppers for ex. *Cicadulina arachidis* but can also be transmitted mechanically and through seed. Yield losses due to viral disease may be extensive. 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 to long extremes from crop to crop. 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 (Hull, 1989).

Most plant viruses are absolutely dependent on a vector for plant-to-plant spread. Although a number of different types of organisms are vectors for different plant viruses, phloem-feeding hemipterans are the most common and transmit the great majority of plant viruses (Ng and Falk, 2006). The complex and specific interactions between hemipteran vectors and the viruses they transmit have been studied intensely, and two general strategies, the capsid and helper strategies, are recognized. Both strategies are found for plant viruses that are transmitted by aphids in a non persistent manner. Evidence suggests that these strategies are also found for viruses transmitted in a semipersistent manner. Recent applications of molecular and cell biology techniques have helped to elucidate the mechanisms underlying the vector transmission of several plant viruses (Harris et al., 1981). The apparent absence of sites for virus retention and accumulation in the non-vector, *D. maidis,* provides a plausible explanation for Maize chlorotic dwarf virus (MCDV) leafhopper transmission specificity. MCDV is restricted to the phloem of infected plants (Harris and Childress, 1983), and virions retained in the foregut would be bathed by fluids ingested from the phloem. Overall, the characteristics of semi-persistent, non-circulative virus transmission seem compatible with an internal vector retention site. The vector's loss of the ability to inoculate through moulting (non-transstadial) is a characteristic of non- circulative transmission; shedding of the intima during moulting would result in loss of MCDV and, hence, its transmission (Childress and Harris, 1989).

MStV is transmitted by *P. maidis* in a persistent-propagative manner. Nymphs of *P. maidis* transmitted

.

MStV with ca. twice the efficiency after a 24, 48, 68, 96 and 192 h acquisition access period (AAP) as did adults. Macropterous adults were slightly more efficient transmitters than brachypterous adults (Tsai and Falk, 1993). MRFV is transmitted by *D. maidis* in a persistent manner. A protracted incubation period in the vector is required. The rate of MRFV transmission by *D. maidis* was usually low ranging from 10 to 34% (Nault et al., 1980; Gamez, 1983). Nymphs were more efficient transmitters than the adults (Gamez, 1983). The average incubation period (IP) in *D. maidis* varied from 12.5 to 16 days. The average retention period in *D. maidis* ranged from 16.5 to 20.2. The infectivity of partially purified MRFV was demonstrated by vector injection and membrane feeding (Gamez, 1983). This author demonstrated that the transmission rate for *D. maidis* injected with partially purified MRFV was dosage dependent. Using ELISA tests, MRFV was shown to multiply in *D. maidis* in a time course study (Gingery et al., 1982; Rivera and Gamez, 1986). The Texas isolate of MRFV has also been experimentally transmitted by *D. elimatus, Stirellus bicolor*, and *Graminella nigrifrons* (Gamez, 1983). Symptoms caused by the severe strain of MCDV include severe stunting, leaf discoloration (reddening and yellowing), and leaf- tearing of maize (Bradfute et al., 1972; Gordon and Nault, 1977). A consistent, diagnostic symptom of MCDV-S infection is chlorosis of the tertiary leaf veins (vein banding) (Gordan and Nault, 1977; Pratt et al., 1994). Transmission is by the detocephaline leafhopper, *G. nigrifrons* (Forbes), in a semi-persistent manner (Gingery et al., 1981). Maize dwarf mosaic virus, a subgroup of the sugarcane mosaic virus (SCMV) complex of potyviruses (Gingery, 1981), has a wide host range in the Gramineae and is nonpersistently transmitted by more than 20 aphid species (Knoke and Louie, 1981). MDMV consists of several strains initially characterized by host range and later by serological differences (Hill et al., 1973; Hill and Benner, 1976). The maize stripe virus (MStV) is a member of the newly described *tenuivirus* group (Gingery, 1988). Maize streak virus (MSV) is transmitted by leafhoppers of the genus *Cicadulina* (Rose, 1978; Van Rensburg, 1981).).Maize fine streak virus (MFSV) is not transmitted by any of the insects tested under nonpersistent or semipersistent conditions. Because electron microscopy indicated the pathogen was a *rhabdovirus*, vector transmission under persistent conditions was tested with species of the Aphididae, Delphacidae, and Cicadellidae. Transmission by the known maize rhabdovirus vectors *P. maidis* (MMV) and *Endria inimical* (WAMSV) was unsuccessful. Only the leafhopper, *G. nigrifrons*, transmitted MFSV. G. nigrifrons also transmitted MFSV to barley (*Hordeum vulgare*), wheat (*Triticum aestivium*), oat (*Avena sativa*), gaint foxtail (*Seteria faberi*), and rye (*Secale cereale*), but did not transmit the virus to sorghum (Sorghum bicolor 'Atlas') or Johnsongrass (*Sorghum halpense*)

(Redinbaugh et al., 2002).

COMMONLY USED DIAGNOSTIC TOOLS IN MAIZE VIRUS DETECTION

PCR based tools play a vital role in diagnosis, detection and identification of viruses in plants. Traditional diagnosis of plant viruses requires bioassay, an indicator plant, determination of host range, symptomatology, virus particle morphology (size and shape), and vector relations. A single diagnostic test or assay may provide adequate information on the identity of a virus but a combination of methods is generally needed which are specific, sensitive and inexpensive (Naidu and Hughes, 2003). However, progress in molecular biology, biochemistry and immunology has led to the development of many new, accurate, rapid and less labour -intensive methods of virus detection. Technologies for the molecular detection of plant pathogens have already undergone two major breakthroughs well over the past three decades. The first was the advent of antibody based detection, in particular monoclonal antibodies and enzyme- linked immunosorbent assay (Kohler and Milstein, 1975; Clark and Adams, 1977). There are various immuno-diagnostic and molecular-diagnostic techniques presently available in the field of virology and these are divided into two: Protein based techniques which include precipitation/agglutination tests, enzymes linked immunosorbent assay (ELISA), Immunosorbent electron microscopy (ISEM), fluorescent antibody test, dot immunoblotting assay (DTBIA). Viral nucleic acid based techniques are dot blot hybridization/slot blot hybridization, polymerase chain reaction (PCR), nucleic acid hybridization with radio labelled and nonradio-labelled probes, DNA/RNA probes. Appropriate screening procedures have been conducted in order to certify any plant free of certain pathogen using ELISA, PCR, DNA probes. Occurrence of virus in maize in several African, Asian and American countries has been reported (Thottappilly et al., 1993). ELISA and other modified forms e.g. direct antigen coating enzymes linked immunosorbent assay (DAC-ELISA), double antibody sandwich ELISA (DAS-ELISA), antigen-coated plate (ACP-ELISA), plate trapped antigen (PTA-ELISA), triple antibody sandwich (TAS-ELISA).

Consequently, several laboratories have developed methods either based on DNA detection using the polymerase chain reaction (PCR) technique, or based on protein detection using ELISA (Anklam et al., 2002). However, the methods vary in their reliability, robustness and reproducibility; in combination with different levels of cost, complexity, and speed etc. Moreover, there is no one method that is applicable in all circumstances. A further consideration is the claim of very high sensitivity reached in the analysis even in absence of clearly proven detailed performance studies.

Serology was the first method adopted in the evolution of rapid plant pathogen detection and identification (Clark, 1981; Miller and Martin, 1988). This technique is based on the recognition of antigens with antibodies produced to them. In its initial application by plant virologists, serology had been used routinely to identify virus species and strains but was not amenable to high throughput assays. ELISA is based on a nearly decade earlier demonstration by Avrameas (1969) that glutaraldehyde cross-linked enzyme-antibody conjugates retained both the specificity of the IgG molecule and the catalytic properties of the enzyme. ELISA allows qualitative and quantitative analysis, high throughput, and high sensitivity and was adopted rapidly and widely (Miller and Martin, 1988).

Virions of viruses that are sap-transmissible to herbaceous hosts usually can be purified in milligram amounts and high purity for serial injection into rabbits or goats and recovery of polyclonal antibody from serum, or into chickens, for recovery from yolk. Examples are members of the genera *Nepovirus*, *Ilarvirus* , *Trichovirus* and *Vitivirus.* These preparations have been used for the production of polyclonal antibodies and also to inject into mice to produce monoclonal antibodies (Mabs), the production of which requires special facilities and protocols (Halk and DeBoer, 1985; Hu et al., 1990; Torrance, 1995; Schieber et al., 1997; Boscia et al., 2001). Mabs often have less avidity than polyclonal antibodies, but the high specificity of Mabs allows strain differentiation and eliminates the problem of cross-reaction with host material (Permar et al., 1990; Nemchinov et al., 1996). If strain specificity is not desired, a broad-spectrum reagent can be produced by combining Mabs generated from several cell lines. Where sequence information is available but purified virions have not been obtained, antibodies with reactivity to the virion may be raised against synthetic peptides (Robinson et al., 1997; Ling et al., 2000).

Enzyme-linked immunosorbent assay (ELISA)

Two classes of ELISA protocols are used for surveillance (Koenig and Paul, 1982). Direct methods such as double antibody sandwich (DAS)–ELISA involves enzyme attachment to the antibody probe (Bar- Joseph and Salomon, 1980; Koenig, 1978; Rochow and Carmichael, 1979; Uyemoto, 1980). In the indirect method [(DASI)-ELISA], the antibody probe remains unlabeled. Instead, the enzyme is attached to a second antibody or protein, a reactive specifically to the probe antibody (Koenig and Paul, 1982; Rowhani et al., 1985). DASI-ELISA is favored over DAS-ELISA for its greater sensitivity, broader reactivity and convenience. Only a single enzyme conjugate is needed for assays of different viruses, and usually a suitable conjugate is available commercially. However, many factors may influence the sensitivity and

reliability of ELISA assay, among these are quality of antibodies, preparation and storage of reagents, incubation time and temperature, selection of appropriate parts of sample and the use of suitable extraction buffer (McLaughlim et al., 1981). ELISA is an excellent technique for detection of seed borne viruses (Bashir and Hampton, 1996). Generally a sample is regarded as positive if the absorbance value exceeds the mean value of a negative control by 2 to 3 standard deviations (Naidu and Hughes, 2003). An optimized DTBIA is as sensitive as ELISA, simple, relatively inexpensive and the DTBIA result can be scored visually, but differs from ELISA as the plant extracts are spotted on to a membrane rather than using a microlitre plate as the solid support matrix. Abdullahi et al. (2001) evaluated the detection capacity of ELISA to prove its reliability using a reverse transcriptase PCR assay, thus, PCR confirmed ELISA.

Two virus isolates associated respectively with the maize stripe (MStV) and the maize chlorotic stripe (MCStV) syndromes in Mauritius, have been purified and characterized by using antisera. The most sensitive diagnosis was achieved using $F(ab')_2$ ELISA (Roca de Doyle et al., 2007). Antisera to the 32,000 Mr (32 K) capsid and 16,500 Mr (16 K) In immunological assay the antiserum against the noncapsid protein was found very useful for detecting MStV infections in plants caused by *P. maydis* by indirect ELISA (Falk et al., 1987; Tsai and Falk, 1993). In the DAS-ELISA with purified virus of Taiwan isolate as antigen, specific reactions to two maize streak virus (MStV-FL) antisera were used. The 19.8 kDa antiserum of MStV-FL gave very strong reactions with crude sap and the noncapsid protein of MStV-T by indirect ELISA (Chen et al., 1993). MYSV was detected, through symptomatology and insect transmission, through ELISA and dot-blot methods, for detecting MYSV in several host plants and weeds as well as in the vector leafhopper *C. chinai* (Mahmoud et al., 1996). Using DAC-ELISA, MYSV antiserum and dot-blot methods the virus was detected in clarified extracts of *C. chinai* leafhoppers at a dilution of 10^3. However, DAC-ELISA is more economic and less complicated than other ELISA methods and is thus more useful in field surveys. However, the dot-blot method may be more sensitive for detecting MYSV in single leafhoppers (Ammar et al., 1990). The ELISA was very useful in demonstrating the virus titer and effects of factors for MDMV (Jenson et al., 1985). The serological and molecular diagnostic tools (ELISA and Western blots) were very useful tools for determining the virus distribution in maize and other hosts. The serological relationships among maize-infecting rhabdovirus have been reported using western blot analysis (Redinbaugh et al., 2002). Assays of corn for MDMV-A and MDMV-B and of Johnsongrass for MDMV-A detection was performed by enzyme linked immunosorbent assay (EIA) and distribution of MDMV and MCDV was studied (Knoke et al., 1983b). The information on the serological relationships between coat

proteins of MSV isolates has been obtained using polyclonal antisera (Dekker et al., 1988). Comparison of different ELISA methods was performed for MSV to determine which method is most suitable for serotyping of MSV group, that is, DAS-ELISA, Direct ACP ELISA, Indirect ACP ELISA, Indirect DAS F(ab')2 ELISA and PAS-ELISA (Pinner and Markham, 1990). Among these indirect ELISA procedures proved to be the most useful methods for serotyping the MSV isolates (Pinner and Markham, 1990). There is also report of MStV detection by ELISA in *P. maidis* vector and virus was successfully detected from the midgut of vector (Nault and Gordon, 1988). The ELISA method also proved useful for early detection of maize rayado fino virus and epidemiological studies (Gordon et al., 1985). Maize viruses, that is, MSV, MStV, MDMV- A were detected by using serological tests in Zimbabwe (Bonga and Cole, 1997). ELISA technique proved to be very useful for maize virus detection, distribution and epidemiology study.

Polymerase chain reaction (PCR)

PCR was developed in the mid-1980s (Saiki et al., 1988) and was rapidly adopted to identify pathogens through their DNA genetic materials. PCR assays are extremely sensitive, reliable, fast, and highly versatile. An alternative approach to virion purification is immuno-capture (IC), referred to as IC-RT-PCR (Wetzel et al., 1991; Nolasco et al., 1993; Minafra and Hadidi, 1994; Nemechinov et al., 1995; Rowhani et al., 1995).

The PCR DNA amplification technique was used to detect and typing of maize streak virus isolates with degenerate oligonucleotide primers based on short sequence of genomic DNA. The amplification was specific and extremely sensitive. In addition, the techniques was compared with ELISA and reported that endpoint dilution used in PCR was 10^4- fold lower than that routinely obtained in ELISA with purified virus (Rybicki and Hughes, 1990). MSV strain A has been associated with maize streak virus in Africa and only known strain reported in Africa. In Cameroon, maize streak virus is most important disease. PCR based method (rolling circle amplification) was used to identify the new strain MSV-A and first time reported in Cameroon (Leke et al., 2009). PCR approach are not only used for the diagnostic in maize but also used in genetic mapping of lines against maize streak virus resistance (Pernet et al., 1999), to study the transient and transgenic expression of MSV replication-associated protein mutants and demonstrated that rep[1-219Rb-] transgene is effective against a range of MSV strains (Shepherd et al., 2007). The presence of transgenes in landraces in local maize varieties was determined and transgene elements were detected with highly sensitive PCR-based markers (Ortiz-García et al., 2005). PCR-RFLP applied for genetic analysis of MSV isolates in

Uganda. Sixty-two full-genome sequences were determined, 52 of which were detectably recombinant in which two recombinants contained predominantly MSV-A(1) -like sequences. Interestingly, it was demonstrated that its characteristics in MSV are quite different from those observed in related African cassava-infecting geminivirus species (Owor et al., 2007).

Multiplex-PCR has a distinct advantage in that it allows the concurrent identification of viruses in plants with mixed infections, all in a single PCR experiment (Routh et al., 1988; Saldarelli et al., 1998; Saade et al., 2000; Wetzel et al., 2002; Dovas and Katis, 2003). Multiplex PCR procedures were applied for simultaneously detecting multiple target sequences in genetically modified (GM) soybean, maize, and canola. Simultaneous amplification profiling (SAP), rather than target specific detection was used for the identification of four GM maize lines. For maize nonspecific amplification was utilized as a tool for specific and reliable identification of one line of GM maize. SAP proved simple and has the potential to identify both approved and non approved GM lines in maize (James et al., 1999).

In real time- PCR, a fluorescent-labeled oligonucleotide (e.g., TaqMan fluorescent probe) in the reaction mixture and a laser-excited fluorescence detection monitor are utilized to assess the quantity of PCR product at the end of each PCR cycle. The TaqMan probe set consists of a pair of oligonucleotide primers and a TaqMan probe designed to hybridize to a site between the two primer binding sites. This method eliminates the need for product detection by gel electrophoresis. It is quantitative and highly sensitive (Korimbocus et al., 2002; Mackay et al., 2002; Marbot et al., 2003). In a recent study, application of real-time TaqMan RT- PCR was demonstrated. Maize chlorotic mottle virus is transmitted through infected maize seeds. It becomes difficult to detect this virus in the seeds to prevent its introduction and infection. A real-time TaqMan RT-PCR procedure for efficient detection of MCMV was developed and its sensitivity was tested. The sensitivity of the method was 4 fg of total RNA or 25 copies of RNA transcripts, which was approximately ten-fold higher than conventional RT-PCR gel electrophoresis method (Zhang et al., 2010).

Recently, a novel real-time quantitative PCR assay was developed for the detection and quantification of plant viruses (Heid et al., 1996). Polymerase chain reaction (PCR) is a molecular biology method for enzymatically copying target nucleic acid sequence without using a living organism, in which repeated replication of a given sequence forms millions of copies within a few hours. PCR technique is a DNA based technology that permits a small sample of target nucleic acid to be copied multiple times for analysis (Mullis and Faloona, 1987).

Flinders technology associates (FTA)

FTA is a paper-based technology designed for the

collection and archiving of nucleic acids, either in their purified form or within pressed samples of fresh tissue. Proprietary chemicals impregnated into the paper act to lyze cellular material and fix and preserve DNA and RNA within the fibre matrix (Whatman, 2004). After a short drying period, pressed samples can be stored at room temperature for extended periods and processed when required. Nucleic acids are recovered by removing small punches from the pressed area and washing with simple reagents. RNA and smaller DNA molecules, such as plasmids and viral genomic components, are eluted by a simple extraction buffer and used as template for amplification by PCR. Genomic DNA remains attached to the paper matrix but are available for amplification by PCR when the paper punch is included in the PCR reaction mix (Ndunguru et al., 2005). Predicted PCR products were obtained in 100 and 80% of the cassava leaf samples collected from the greenhouse and field respectively; with the entire MSV-infected field grown maize plants sampled yielding viral sequences. The studies described here demonstrate that FTA offers a simple, sensitive and specific tool appropriate for the diagnosis and molecular characterization of plant viral pathogens isolated from plant tissues and transgene sequences integrated into the plant genome (Ndunguru et al., 2005).

Conclusions

The ultimate goal in microbial testing is the ability to accurately and sensitively detect pathogens in real-time or as quickly as possible. Nucleic acid diagnostics (NAD) offer many advantages over traditional microbiological and immunological methods for the detection of infections microorganisms. These include faster processing time as well as greater potential for intra-species identification and identification of antibiotic susceptibility and strain typing based upon unique sequences. The original techniques of PCR and gel electrophoresis are being superseded by real-time PCR while the development of integrated sample preparation and amplification devices with a simplified user interface will allow for true point-of-care disease detection and suitably tailored treatments. This chapter describes the principles of maize virus incidence, host-pathogen interaction, ELISA and nucleic acid diagnostics including an overview of the technology's history as well as the general properties of diagnostics target. Special emphasis is placed upon the detection of pathogens relevant to maize. Ongoing developments in molecular detection platforms including nucleic acid based provide potential for new test methods that will enable multi-parameter testing and at-line monitoring for viral contaminants.

Mostly disease problems are first noticed in the field and depending on the scope and urgency, this will immediately be followed by applied efforts to contain, control, or eradicate the pathogen. If the problem is sufficiently relevant from an economic perspective and interesting from a fundamental viewpoint, research on molecular diagnostic most likely will be performed to find the actual causal agent. This can be the natural host or an alternative suitable plant species. Aided by genomics and functional genomics data, genes will be identified and/or manipulated to eliminate the pathogen, or protect the plant from either the infection or the symptoms.

Accurate identification and early detection of the viral diseases is the cornerstones of the management of maize cultivar. Maize viruses are difficult to identify using morphological criteria, which can be time consuming and challenging and requires extensive knowledge in taxonomy. Molecular and immunological detection such as ELISA and other modified forms, precipitation/agglutination, fluorescent antibody, PCR, nucleic acid hybridization are best suitable techniques to detect the various viruses, which include, MMV, MCMV, MSV, MStV, MMV and MDMV infecting maize. Until now, ELISA and other modified forms have been extensively used, because these are quick. However PCR has been widely used with the varying degree of modification for detection of viral genomes in infected plant in the last two decades. The disadvantage of PCR is that it requires sophisticated equipment like thermocycler which is expensive, where as ELISA/DAC-ELISA can be used for diagnoses even in field conditions and are very cost effective. Viruses and virus strains cannot be distinguished on the basic of common sources of resistance. These various immunological and molecular diagnostic tests with symptoms and history are of immense value to diagnose maize viruses, thus, these diagnostic techniques can become a routine in plant pathology research. This review will substantially accelerate to understand host-pathogen interaction, multiplication and their diagnostic assay. This should, in turn, lead to development of very effective and durable control measures against harmful pathogens.

Abbreviations: MStV, maize stripe virus; **MMV,** Maize mosaic virus; **MDMV,** maize dwarf mosaic virus; **MCMV,** maize chlorotic mottle virus; **MRFV,** maize rayado fino virus; **MCDV,** Maize chlorotic dwarf virus; **MNeSV,** maize necrotic streak virus; **MFSV,** maize fine streak virus; **SCMVN,** sugarcane mosaic virus; **MBSM,** maize bushy stunt mycoplasma; **MYSV,** maize yellow stripe virus; **MSV,** maize streak virus; **CSS,** corn stunt spiroplasma; **RSV,** rice stripe virus; **MCStV,** maize chlorotic stripe; **WASMV,** wheat american striate mosaic virus; **SSMV,** Sorghum stunt mosaic virus; **RBSDV,** rice black-streaked dwarf virus; **RTSV,** rice tungro spherical virus; **ISEM,** Immunosorbent electron microscopy; **DIBA,** dot immunoblotting assay; **DAC-ELISA,** direct antigen coating enzymes linked immunosorbent assay; **DAS-ELISA,** double antibody sandwich ELISA; **ACP-ELISA,** antigen-coated plate; **PTA- ELISA,** plate trapped antigen; **TAS-ELISA,** triple antibody sandwich; **DTBIA,** direct

tissue blot immunoassay; **NAD,** nucleic acid diagnostics; **IC-RT-PCR,** immuno-capture RT-PCR; **NCP,** nuclear coat protein; **UTR,** untranslated region; **ORFs,** open reading frames; **CP,** coat protein; nts, nucleotides; **IAP,** inoculation access period; **AAP,** acquisition access period; **Mabs,** monoclonal antibodies; **VPI,** vascular puncture inoculation ; **IP,** incubation period.

REFERENCES

Abdullahi I, Ikotun T, Winter S, Thottappilly G, Atiri A (2001). Investigation on seed transmission of cucumber mosaic virus in cowpea. Afr. Crop Sci. J., 9(4): 677-684.

Ahlquist P (2002). RNA-dependent RNA polymerases, viruses, and RNA silencing. Science, 296: 1270-1273.

Ahlquist P, Noueiry AO, Lee WM, Kushner DB, Dye BT (2003). Host factors in positivestrand RNA virus genome replication. J. Virol., 77: 8181-8186.

Ammar ED, Gingery RE, Gordon DT, Aboul-Ata AE (1990). Tubular helical structures and fine filaments associated with the leafhopper-borne maize yellow stripe virus. Phytopathology, 80: 303-309.

Ancalmo O, Davis WC (1961). Achaparramiento (corn stunt). Plant Dis., Rep., 45: 281.

Anklam E, Gadani F, Heinze P, Pijnenburg H, Eede GVD (2002). Analytical methods for detection and determination of genetically modified organisms in agricultural crops and plant-derived food products. Eur. Food Res. Technol., 214: 3-26.

Avrameas S (1969). Coupling of enzymes to proteins with glutaraldehyde. Immunochemistry, 6: 43-52.

Bar-Joseph M, Salomon R (1980). Heterologous reactivity of tobacco mosaic virus strains in enzyme-linked immunosorbent assays. J. Gen. Virol., 47: 509-512.

Bashir M, Hampton RO (1996). Detection and identification of seedborne viruses from cowpea (*Vigna unquiculata* (L.) walp) germplasm. Plant Pathol., 45: 54-58.

Berger PH, Luciano CS, Thornbury DW, Benner HI, Hill JH, Zeyen RJ (1989). Properties and *in vitro* translation of maize dwarf mosaic virus RNA. J. Gen. Virol., 70: 1845-1851.

Bisaro DM (1994). Recombination in geminiviruses: Mechanisms for maintaining genome size and generating genomic diversity. In homologous recombination and gene silencing in plants (ed. J. Paszkowski), Kluwer, Dordrecht, pp. 39-60.

Bock KR, Guthrie EJ, Woods RD (1974). Purification of maize streak virus and its relation-ship to viruses associated with streak diseases of sugarcane and *Panicum maximum*. Ann. Appl. Biol., 77: 289-296.

Bonga J, Cole DL (1997). Identification of viruses infecting maize in Zimbabwe. Afr. Plant Protect., 3(1): 1-9.

Boscia D, Digiaro M, Safi M, Garau R, Zhou Z (2001). Production of monoclonal antibodies to grapevine virus D and contribution to the study of its aetiological role in grapevine diseases. Vitis, 40: 69-74.

Bradfute OE, Tsai JH (1983). Identification of maize mosaic virus in Florida. Plant Dis., 67: 1339-1342.

Bradfute OE, Tsai JH (1990). Rapid identification of maize stripe virus. Phytopathology, 80: 715-719.

Bradfute OE, Gingery RE, Gordon DT, Nault LR (1972). Tissue ultrastructure, sedimentation and leafhopper transmission of a virus associated with a maize dwarfing disease (Abstract). J. Cell Biol., 55: 25a.

Brewbaker JL (1980). Diseases of maize in the wet lowland tropics and the collapse of the Maya civilization. Econ. Bot., 33: 101-118.

Buck KW (1996). Comparison of the replication of positive-stranded RNA viruses of plants and animals. Adv. Virus Res., 47: 159-251.

Chen CC, Tsai JH, Chiu RJ, Chen MJ (1993). Purification, characterizaiton, and serological analysis of maize stripe virus in Taiwan. Plant Dis., 77: 367-372.

Childress SA, Harris KF (1989). Localization of virus-like particles in the foreguts of viruliferous *Gramineila nigrifrons* leafhoppers carrying the semi -persistent maize chlorotic dwarf virus. J. Gen. Virol., 70: 247-251.

CIMMYT (1990). 1989/90 CIMMYT World Maize: Facts and trends: Realizing the potential of maize in sub-Sahara Africa. Mexico, DF, Mexico.

Clark MF, Adams AN (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol., 34: 475-483.

Clark MF (1981). Immunosorbent assays in plant pathology. Ann. Rev. Phytopathol., 19: 83-106.

Damsteegt VO (1983). Maize streak virus: I. Host range and vulnerability of maize germplasm. Plant Dis., 67: 734-737.

de Stradis A, Redinbaugh MG, Abt JJ, Martelli GP (2005). Ultra structure of maize necrotic streak virus infections. J. Plant Pathol., 87(3): 213-221.

Dekker EL, Pinner MS, Markham PG, Van-Regenmortel MHV (1988). Characterization of maize streak virus isolates from different plant species by polyclonal and monoclonal antibodies. J. Gen. Virol., 69: 983-990.

DePamphilis ML (1988). Transcriptional elements as components of eukaryotic origins of replication. Cell, 52: 635-638.

DePamphilis ML (1993). Eukaryotic DNA replication: Anatomy of an origin. Annu. Rev. Biochem., 62: 29-63.

Dovas CI, Katis NI (2003). A spot-nested RT-PCR method for the simultaneous detection of members of the *Vitivirus* and *Foveavirus* genera in grapevine. J. Virol. Meth., 170: 99-106.

Drake JW (1991). A constant rate of spontaneous mutation in DNA-based microbes. 1991. Proc. Natl. Acad. Sci. USA, 88: 7160-7164.

Falk BW, Tsai JH (1983). Physicochemical characterization of maize mosaic virus. Phytopathology, 73: 1536-1539.

Falk BW, Tsai JH (1985). Serological detection and evidence for multiplication of maize mosaic virus in the planthopper, *Peregrinus maidis*. Phytopathology, 75: 852-855.

Falk BW, Tsai JH, Lommell SA (1987). Differences in levels of detection for maize stripe virus capsid and major noncapsid proteins in plant and insect hosts. J. Gen. Virol., 68: 1801-1811.

FAO (2007). http://faostat.fao.org/site/567/default.aspx.

Galinat WC (1977). The origin of corn. In: Corn and Corn Improvement. (eds: G.F. Sprague). American Society of Agronomy. Madison, Wisconsin, USA, pp. 1-47.

Gamez R (1983). Maize rayado fino disease: The virus-host-vector interaction in neotropical environments. In: Proc. Int'l. Maize Virus Dis. Colloq. and Workshop, 2-6 August 1982. (eds: D.T. pp. 62-68.

Gordon JK, Knoke LR, Nault RMR (YYYY). The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, pp. 266.

Gingery R (1988). The rice stripe virus group. In: The Plant Viruses 4: The Filamentous Plant Viruses. (eds: R.G. Milne). Plenum, New York, pp. 297-329.

Gingery RE (1976). Properties of maize chlorotic dwarf virus and its ribonucleic acid. Virology, 73: 311-318.

Gingery RE (1981). Chemical and physical properties of maize viruses pp. 38-39. In: viruses and virus-like diseases of maize in the United States. (Eds: D.T. Gordon, J.K. Knoke and G.E. Scott). Wooster: Ohio Agricultural. Research Development Center.

Gingery RE, Gordon DT, Nault LR (1982). Purification and properties of an isolate of maize rayado fino virus from the United States. Phytopathology, 72: 1313-1318.

Gingery RE, Gordon DT, Nault LR, Bradfute OE (1981). Maize chlorotic dwarf virus pp.19-32. In: (eds: E.Kurstak). Plant Virus Infection and Comparative Diagnosis Elsevier/North Holland Biochemical Press, Amsterdam.

Golem S, Culver JN (2003). Tobacco mosaic virus induced alterations in the gene expression profile of *Arabidopsis thaliana*. Mol. Plant-Microbe Interact., 16: 681-688.

Gordon DT, Nault LR (1977). Involvement of maize chlorotic dwarf virus and other agents in stunting diseases of *Zea mays* in the United States. Phytopatholgy, 67: 27-36.

Gordon DT, Nault LR, Gordon NH, Heady SH (1985). Serological detection of corn stunt spiroplasma and maize rayado fino virus in field-collected *Dalbulus* spp. from Mexico. Plant Dis., 69: 108-111.

Gutierrez C (1999). Geminivirus DNA replication. Cell. Mol. Life Sci., 56(3-4): 313-329.

Halk EL, DeBoer SH (1985). Monoclonal antibodies in plant-disease

research. Ann. Rev. Phytopathol., 23: 321-330.

Hammond RW, Ramirez P (2001). Molecular characterization of the genome of *maize rayado fino virus*, the type member of the genus *Marafivirus*. Virology, 282(2): 338-347.

Harris KF, Childress SA (1983). Cytology of maize chlorotic dwarf virus infection in corn. Int. J. Trop. Plant Dis., 1: 135-140.

Harris KF, Treur B, Tsai J, Toler R (1981). Observations of leaf hopper ingestion-egestion behavior: its likely role in the transmission of noncirculative viruses and other plant pathogens. J. Econ. Entomol., 74: 446-453.

Heid CA, Stevens J, Livak KJ, Williams PM (1996). Real time quantitative PCR. Genome Res., 10: 986-994.

Hill JH, Benner HI (1976). Properties of potyvirus RNAs: turnip mosaic, tobacco etch, and maize dwarf mosaic viruses. Virology, 75: 419-432.

Hill JH, Ford RE, Benner HI (1973). Purification and partial characterization of maize dwarf mosaic virus strain B (sugarcane mosaic virus). J. Gen. Virol., 20: 327-339.

Howell SH (1984). Physical structure and genetic organisation of the genome of maize streak virus (Kenyan isolate). Nucleic Acids Res., 12(19): 7359-7375.

Hu JS, Gonsalves D, Boscia D, Namba S (1990). Use of monoclonal antibodies to characterize grapevine leafroll associated closteroviruses. Phytopathology, 80: 920-925.

Hull R (1989). The Movement of Viruses in Plants. Ann. Rev. Phytopathol., 27: 213-240.

ICTVdB Management (2006). 00.000.4.00.008. Maize white line mosaic virus. In: ICTVdB - The Universal Virus Database, version 4. Büchen-Osmond, C. (Ed), Columbia University, New York, USA.

ICTVdB Management (2006). 00.057.0.01.039. Maize dwarf mosaic virus. In: ICTVdB - The Universal Virus Database, version 4. Büchen-Osmond, C. (Ed), Columbia University, New York, USA.

ICTVdB Management (2006). 00.060.0.07.003. Maize rough dwarf virus. In: ICTVdB - The Universal Virus Database, version 4. Büchen-Osmond, C. (Ed), Columbia University, New York, USA.

ICTVdB Management (2006). 00.065.0.02.003. Maize chlorotic dwarf virus. In: ICTVdB - The Universal Virus Database, version 4. Büchen-Osmond, C. (Ed), Columbia University, New York, USA.

ICTVdB Management (2006). 00.069.0.01.002. Maize stripe virus. In: ICTVdB - The Universal Virus Database, version 4. Büchen-Osmond, C. (Ed), Columbia University, New York, USA.

ICTVdB Management (2006). 01.062.0.05.004. Maize mosaic virus. In: ICTVdB - The Universal Virus Database, version 4. Büchen-Osmond, C. (Ed), Columbia University, New York, USA.

James D, Jelkmann W, Upton C (1999). Specific detection of cherry mottle leaf virus using digoxygenin-labeled cDNA probes and RT-PCR. Plant Dis., 83: 235-239.

Jenson SG, Palomar MK, Ball EM, Samson R (1985). Factors influencing virus titer in maize dwarf mosaic virus-infected sorghum. Phytopathology, 75: 1132-1136.

Jiang Y, Serviene E, Gal J, Panavas T, Nagy PD (2006). Identification of essential host factors affecting tombusvirusRNAreplication based on the yeastTet promoters Hughes Collection. J. Virol., 80: 7394-404.

Knoke JK, Louie R (1981). Epiphytology of maize virus diseases. In: Gordon DT, Knoke JK, Scott GE (eds): Virus and virus-like diseases of maize in the United States. Southern Coop. Ser. Bull., 247: 92-102.

Knoke JK, Anderson RJ, Louie R, Modelen LV, Findley WR (1983a). Insect vectors of maize dwarf mosaic virus and maize chlorotic dwarf virus. pp. 130-138. In: (eds: Gordon DT, Knoke JK, Nault LR, Ritter RM). Proc. Int'l. Maize Virus Disease Calloq. and Workshop. 1982. Ohio Agriculture Research and Development Center, Wooster.

Knoke JK, Louie R, Madden LV, Gordon DT (1983b). Spread of maize dwarf mosaic virus from Johnsongrass to corn. Plant Dis., 67: 367-370.

Koenig R (1978). ELISA in the study of homologous and heterologous reactions of plant viruses. J. Gen. Virol., 40: 309-318.

Koenig R, Paul HI (1982). Variants of ELISA in plant virus diagnosis. J. Virol. Methods, 5: 113-125.

Kohler G, Milsten C (1975). Continuous culture of fused cells secreting antibody of predefined specificity. Nature, 256: 495-497.

Kong P, Steinbiss HH (1998). Complete nucleotide sequence and analysis of the putative polyprotein of maize dwarf mosaic virus

genomic RNA (Bulgarian isolate). Arch. Virol., 143(9): 1791-1799.

Korimbocus J, Coates D, Barker I, Boonham N (2002). Improved detection of Sugarcane yellowleaf virus using a real-time fluorescent (TaqMan) RT-PCR assay. J. Virol. Methods, 103: 109-120.

Kulkarni HY (1973). Comparison and characterization of maize stripe and maize line viruses. Ann. Appl. Biol., 75: 205-216.

Kunkel LO (1921). A possible causative agent for the mosaic disease of corn. Hawaii. Sugar Plantation Association Experimental Station. Bull. Bot. Ser., 3: 44-58.

Kushner DB, Lindenbach BD, Grdzelishvili VZ, Noueiry AO, Paul SM, Ahlquist P (2003). Systematic, genome-wide identification of host genes affecting replication of a positivestrand RNA virus. Proc. Natl. Acad. Sci. USA, 100: 15764-15769.

Laliberte JF, Sanfacon H (2010). Cellular Remodeling During Plant Virus Infection. Ann. Rev. Phytopathol., 48: 69-91.

Lastra RJ (1977). Maize mosaic and other maize virus and virus -like diseases in Venezuela. In: Proc. Int'l. Maize Virus Dis. Colloq. and Workshop, 16-19 Aug., 1976. (eds: Williams LE, Gordon DT and Nault LR). Ohio Agriculture Research Developmental Center, Wooster, pp. 30-39.

Leke WN, Njualem DK, Nchinda VP, Ngoko Z, Zok S, Ngeve JM, Brown JK, Kvarnheden A (2009). Molecular identification of Maize streak virus reveals the first evidence for a subtype A1 isolate infecting maize in Cameroon. Dis. Rep., 19: 36.

Ling K, Zhu HY, Jiang Z, Gonsalves D (2000). Effective application of DASELISA for detection of grapevine leafroll associated closterovirus -3 using a polyclonal antiserum developed from recombinant coat protein. Eur. J. Plant Pathol., 106: 301-309.

Lommel SA, Kendall TL, Siu NF, Nutter RC (1991). Characterization of maize chlorotic mottle virus. Phytopathology, 81: 819-823.

Louie R, Redinbaugh MG, Gordon DT, Abt JJ, Anderson RJ (2000). Maize necrotic streak virus, a new maize virus with similarity to species of the family Tombusviridae. Plant Dis., 84: 1133-1139.

Mackay IM, Arden KE, Nitsche A (2002). Survey and summary, real-time PCR in virology. Nucl. Acid Res., 30: 1292-1305.

Mahmoud A, Hotjvenel JC, Abol-Ela SE, Sewify GH, Ammar ED (1996). Detection of maize yellow stripe tenui-like virus by ELISA and dot-blot tests in host plants and leafhopper vector in Egypt. Phytopathol. Mediterranea, 35: 19-23.

Malpica JM, Fraile A, Moreno I, Obies CI, Drake JW, Garcia-Arenal F (2002). The rate and character of spontaneous mutation in an RNA virus. Genetics, 162: 1505-1511.

Marbot S, Salmon M, Vendrame M, Huwaert A, Kummert J (2003). Development of real-time RT-PCR assay for detection of *Prunus necrotic* ringspot virus in fruit trees. Plant Dis., 87: 1344-1348.

Maule A, Leh V, Lederer C (2002). The dialogue between viruses and hosts in compatible interactions. Curr. Opinion Plant Biol., 5: 279-284.

Mayo MA, Murant AF, Turnbull-Ross AD, Reavy B, Hamilton RI, Gingery RE (1995). Family-Sequiviridae In: (eds: Murphy F.A., Fauquet C.M., Bishop D.H.L., Ghabrial S.A., Jarvis A.W., Martelli G.P., Mayo M.A., Summers M.D): Virus Taxonomy. 6th Rep. Int. Committee on Taxonomy of Viruses. Springer-Verlag, Vienna and New York, pp. 337-340.

McDaniel LL, Ammar ED, Gordon DT (1985). Assembly, morphology, and accumulation of a Hawaiian isolate of maize mosaic virus in maize. Phytopathology, 75: 1167-1172.

McLaughlin MR, Barnett OW, Burrows PM, Bavm RH (1981). Improved ELISA conditions for detection of plant viruses. J. Virol. Methods. 3: 13-25.

Miller SA, Martin RR (1988). Molecular diagnosis of plant diseases. Ann. Rev. Phytopathol., 26: 409-432.

Minafra A, Hadidi A (1994). Sensitive detection of grapevine virus A, B, or leafroll associated III from viruliferous mealybugs and infected tissue by cDNA amplification. J. Virol. Methods, 47: 175-188.

Mullineaux PM, Donson J, Morris-Krsinich BAM, Boulton MI, Davies JW (1984). The nucleotide sequence of maize streak virus DNA. EMBO J., 3: 3063-3068.

Mullis KB, Faloona FA (1987). Specific synthesis of DNA in vitro via a polymerase catalyzed chain reaction. Methods Enzymol., 155: 335-350.

Nagy PD, Pogany J (2006). Yeast as a model host to dissect functions

.

of viral and host factors in tombusvirus replication. Virology, 344: 211-20.

Naidu RA, Hughes JDA (2003). Methods for the detection of plant viral diseases in plant virology in sub-Saharan Africa, Proceedings of plant virology, IITA, Ibadan, Nigeria. Eds. Hughes JDA, Odu B, pp. 233-260.

Nault LR, Gordon DT (1988). Multiplication of maize stripe virus in *Peregrinus maidis.* Phytopathology, 78: 991-995.

Nault LR, Gingery RE, Gordon DT (1980). Leafhopper transmission and host range of maize rayado fino virus. Phytopathology, 70: 709-712.

Nault LR, Madden LV, Styer WE, Triplehorn BW, Shambaugh GF, Heady SE (1984). Pathogenicity of corn stunt spiroplasma and maize bushy stunt mycoplasma to its vector *Dalbulus longulus*. Phytopathology, 74: 977-979.

Ndunguru J, Taylor NJ, Yadav J, Aly H, Legg JP, Aveling T, Thompson G, Fauquet CM (2005). Application of FTA technology for sampling, recovery and molecular characterization of viral pathogens and virus-derived transgenes from plant tissues. Virol. J., 2: 45 doi:10.1186/1743-422X-2-45.

Nemchinov L, Hadidi A, Maiss E, Cambra M, Candresse T (1996). Sour cherry strain of plum pox potyvirus (PPV): molecular and serological evidence for a newsubgroup of PPV strains. Phytopathology, 86: 1215-1221.

Nemechinov L, Hadidi A, Candresse T, Foster JA, Verdervskaya TD (1995). Sensitive detection of Apple chlorotic leafspot virus from infected apple or peach tissue using RT-PCR, IC-RT-PCR, or multiplex IC-RT-PCR. Acta Hortic., 386: 51-62.

Ng JCK, Falk BW (2006). Virus-vector interactions mediating nonpersistent and semipersistent transmission of plant viruses. Ann. Rev. Phytopathol., 44: 183-212.

Nolasco G, de Blas C, Torres V, Ponz F (1993). A method combining immunocapture and PCR amplification in a microtiter plate for the detection of plant viruses and subviral pathogens. J. Virol. Methods, 45: 201-218.

Ortiz-García S, Ezcurra E, Schoel B, Acevedo F, Soberón J, Snow AA, Schaal BA (2005). Absence of Detectable Transgenes in Local Landraces of Maize in Oaxaca, Mexico (2003-2004). Proc. Natl. Acad. Sci. USA, 102(35): 12338-12343.

Overman MA, Ko NJ, Tsai JH (1992). Identification of viruses and mycoplasmas in maize by light microscopy. Plant Dis., 76: 318-322.

Owor BE, Martin DP, Shepherd DN, Edema R, Monjane AL, Rybicki EP, Thomson JA, Varsani A (2007). Genetic analysis of maize streak virus isolates from Uganda reveals widespread distribution of a recombinant variant. J. Gen. Virol., 88(3): 154-165.

Permar TA, Garnsey SM, Gumpf DJ, Lee RF (1990). A monoclonal antibody that discriminates strains of *Citrus tristeza* virus. Phytopathology, 80: 224-228.

Pernet A, Hoisington D, Franco J, Isnard M, Jewell D, Jiang C, Marchand JL, Reynaud B, Glaszmann JC, de Leon DG (1999). Genetic mapping of maize streak virus resistance from the Mascarene source.I. Resistnace in line D211 and stability against different virus clones. Theor. Appl. Genet., 99: 524-539.

Pinner MS, Markham PG (1990). Serotyping and strain identification of maize streak virus isolates. J. Gen. Virol., 71: 1635-1640.

Pratt RC, Anderson RJ, Louie R, McMullen MD, Knoke JK (1994). Maize responses to a severe isolate of maize chlorotic dwarf virus. Crop Sci., 34: 635-641.

Redinbaugh MG, Seifers DL, Meulia T, Abt JJ, Anderson RJ, Styer WE, Ackerman J, Salomon R, Houghton W, Creamer R, Gordon DT, Hogenhout SA (2002). Maize fine streak virus: a new leafhopper-transmitted rhabdovirus. Phytopathology, 92: 1167-1174.

Rivera C, Gamez R (1986). Mustiplication of maize rayado fino virus in the leafhopper vector *Dalbulus maidis*. Intervirology, 25: 76-83.

Robinson E, Galiakparov N, Radian S, Sela I, Tanne E (1997). Serological detection of grapevine virusA using antiserum to a non structural protein, the putative movement protein. Phytopathology, 87: 1041-1045.

Roca de Doyle MM, Autrey LJC, Jones P (2007). Purification, characterization and serological properties of two virus isolates associated with the maize stripe disease in Mauritius. Plant Pathol., 41(3): 325-334.

Rochow WF, Carmichael LE (1979). Specificity among barley yellow

dwarf viruses in enzyme-linked immunosorbent assays. Virology, 95: 415-420.

Rose OJW (1978). Epidemiology of maize streak virus. Ann. Rev. Entomol., 23: 259-282.

Routh G, Zhang YP, Saldarelli P, Rowhani A (1988). Use of degenerate primers for partial sequencing and RTPCR-based assays of grapevine leafroll associated viruses 4 and 5. Phytopathology, 88: 1238-1243.

Rowhani A, Maningas MA, Lile LS, Daubert SD, Golino DA (1995). Development of a detection system for viruses of woody plants based on PCR analysis of immobilized virions. Phytopathology, 85: 347-352.

Rowhani A, Mircetich SM, Shepherd RJ, Cucuzza JD (1985). Serological detection of cherry leafroll virus in English walnut trees. Phytopathology, 75: 48-52.

Rybicki EP, Hughes FL (1990). Detection and typing of maize streak virus and other distantly related geminiviruses of grasses by polymerase chain reaction amplification of a conserved viral sequence. J. Gen. Virol., 71: 2519-2526.

Saade M, Aparicio F, Sanchez-Navarro JA, Herranz MC, Myrta A (2000). Simultaneous detection of the three ilarviruses affecting stone fruit trees by nonisotopic molecular hybridization and multiplex reverse-transcription polymerase chain reaction. Phytopathology, 90: 1330-1336.

Saiki RK, Gelfank GH, Staffel S, Scharf SJ, Higuchi R (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science, 239: 487-491.

Saldarelli P, Rowhani A, Routh G, Minafra A, Digiaro M (1998). Use of degenerate primers in a RT-PCR assay for the identification and analysis of some filamentous viruses, with special reference to clostero- and vitiviruses of the grapevine. Eur. J. Plant Pathol., 104: 945-950.

Salonen A, Ahola T, Kaariainen L (2005). Viral RNA replication in association with cellular membranes. Curr. Topics Microbiol. Immunol., 285: 139-173.

Saunders K, Lucy A, Stanley J (1991). DNA forms of the geminivirus African cassava mosaic virus consistent with a rolling circle mechanism of replication. Nucl. Acids Res., 19: 2325-2330.

Schieber O, Seddas A, Belin C, Walter B (1997). Monoclonal antibodies for detection, serological characterization and immunopurification of grapevine fleck virus. Eur. J. Plant Pathol., 103: 767-774.

Seifer DL, Hackerott HL (1987). Estimates of yield loss and virus titre in sorghum hybrids infected with maize dwarf mosaic virus strain B. Agric. Ecosyst. Environ., 19: 81-86.

Shen P, Kaniewska M, Smith C, Beachy RN (1993). Nucleotide sequence and genomic organization of rice tungro spherical virus. Virology, 193: 621-630.

Shepherd DN, Mangwende T, Martin DP, Bezuidenhout M, Thomson JA, Rybicki P (2007). Inhibition of maize streak virus (MSV) replication by transient and transgenic expression of MSV replication-associated protein mutants. J. Gen. Virol., 88: 325-336.

Storey HH (1936). Virus diseases of East African plants. IV. A Survey of the viruses attacking the Gramineae. E. Afr. Agric. J., 1: 333-337.

Storey HH, McClean APD (1930). The transmission of streak disease between maize, sugarcane and wild grasses. Ann. Appl. Biol., 17: 691-719.

Teycheney PY, Aaziz R, Dinant S, Salanki K, Tourneur C, Balazs E, Jacquemond M, Tepfer M (2000). Synthesis of (-)-strand RNA from the 3' untranslated region of plant virus genomes expressed in transgenic plants upon infection with related viruses. J. Gen. Virol. 81: 1121-1126.

Thottappilly G, Bosque-Perez NA, Rossel HW (1993). Viruses and virus diseases of maize in tropical Africa. Plant Pathol., 42: 494-509.

Toler RW, Skinner G, Bockholt AJ, Harris KF (1985). Reactions of maize (*Zea mays*) accessions to maize rayado fino virus. Plant Dis., 68: 56-57.

Torrance L (1995). Use of monoclonal antibodies in plant pathology. Eur. J. Plant Pathol., 101: 351-63.

Tsai JH, Brown LG (1989). Maize dwarf mosaic virus. Division of Plant Industry. Fla. Dept. Agric. & Consumer Serv. Plant Path. Circular, No. 320.

Tsai JH, Falk BW (1993). Viruses and mycoplasmal agents affecting

maize in the Tropics. In: (eds: R.J. Chiu and Y.Yeh) Proc. Symp. Plant Virus Virus-like Dis., pp. 43-48.

Uyemoto JK (1980). Detection of maize chlorotic mottle virus serotypes by enzyme-linked immunosorbent assay. Phytopathology, 70: 290-292.

van der Walt E, Martin DP, Varsani A, Polston JE, Rybicki EP (2008). Experimental observations of rapid Maize streak virus evolution reveal a strand-specific nucleotide substitution bias. Virol. J., 5: 104.

Van-Rensburg GDJ (1981). Effect of plant age at the time of infection with maize streak virus on yield of maize. Phytophylactica, 13: 197-198.

Wetzel T, Candresse T, Ravelonandro M, Dunez J (1991). A polymerase chain reaction assay adopted to plum pox virus detection. J. Virol. Methods, 33: 355-365.

Wetzel T, Jardak R, Meunier L, Ghorbel A, Reustle GM (2002). Simultaneous RT/PCR detection and differentiation of arabis mosaic and grapevine fanleaf nepoviruses in grapevine with a single pair of primers. J. Virol. Methods, 101: 63-69.

Whatman (2004). Application of FTA-based technology for sample collection, transport, purification and storage of PCR-ready plant DNA. [http://www.whatman.co.uk/repository/documents/s3/usFtaPlantDna.pdf].

Whitham SA, Wang Y (2004). Roles for host factors in plant viral pathogenicity. Curr. Opinion Plant Biol., 7: 365-371.

Whitham SA, Yang C, Goodin MM (2006). Global impact: Elucidating plant responses to viral infection. Mol. Plant-Microb. Interact., 19: 1207-1215.

Williams LE, Alexander LJ (1965). Maize dwarf mosaic: A new corn disease. Phytopathology, 55: 802-804.

Zaitlin M, Palukaitis P (2000). Advances in understanding plant viruses and virus diseases. Ann. Rev. Phytopathol., 38: 117-143.

Zhang W, Olson NH, Baker TS, Faulkner L, Agbandje-McKenna M, Boulton MI, Davies JW, McKenna R (2001). Structure of the Maize Streak Virus Geminate Particle. Virology, 279(2): 471-477.

Zhang Y, Zhao W, Li M, Chen H, Zhu S, Fan Z (2010). Real-time TaqMan RT-PCR for detection of maize chlorotic mottle virus in maize seeds. J. Virol. Methods, doi:10.1016/j.jviromet.2010.11.002.