1 Viruses Affecting Tropical and Subtropical Crops: Biology, Diversity, Management

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1.1 Introduction

Viruses are the most abundant biological entities throughout marine and terrestrial ecosystems. They interact with all life forms, including archaea, bacteria and eukaryotic organisms and are present in natural or agricultural ecosystems, essentially wherever life forms can be found (Roossinck, 2010). The concept of a virus challenges the way we define life, especially since the recent discoveries of viruses that possess ribosomal genes. These discoveries include the surprisingly large viruses of the *Mimiviridae* (Claverie and Abergel, 2012; Yutin *et al.*, 2013), the Pandoraviruses that lack phylogenetic affinity with any known virus families (Philippe *et al.*, 2013) and Pithovirus sibericum that was recovered from Siberian permafrost after being entombed for more than 30,000 years (Legendre *et al.*, 2014). Apparently they co-occurred and even predated cellular forms on our planet, yet arguably they have no certain place in our current view of the tree of life (Brüssow, 2009; Koonin and Dolja, 2013; Thiel *et al.*, 2013).

Besides their potential role in evolution, viruses have facilitated the understanding of various basic concepts and phenomena in biology (Pumplin and Voinnet, 2013; Scholthof, 2014). However, they have also long been considered as disease-causing entities and are regarded as major causes of considerable losses in food crop production. Pathogenic viruses imperil food security by decimating crop harvests as well as reducing the quality of produce, thereby lowering profitability. This is particularly so in the tropics and subtropics where there are ideal conditions throughout the year for the perpetuation of the pathogens along with their vectors. Viruses account for almost half of the emerging infectious plant diseases (Anderson *et al.*, 2004). Moreover, technologies of DNA and RNA deep sequencing (Wu *et al.*, 2010; Adams *et al.*, 2012; Grimsley *et al.*, 2012; Zhuo *et al.*, 2013; Barba *et al.*, 2014; Kehoe *et al.*, 2014), as well as genomics and metagenomics (Adams *et al.*, 2009; Kristensen *et al.*, 2010; Roossinck *et al.*, 2010; Rosario *et al.*, 2012), have allowed for the discovery of new species of plant viruses – some of which have been isolated from symptomless plants (Roossinck, 2005, 2011; Kreuze *et al.*, 2009; Wylie *et al.*, 2013; Saqib *et al.*, 2014). Recent investigations suggest that some viruses actually confer a

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 Virus Diseases of Tropical and Subtropical Crops (eds P. Tennant and G. Fermin)

range of ecological benefits upon their host plants (Mölken and Stuefer, 2011; Roossinck, 2011, 2012; Prendeville *et al*., 2012; MacDiarmid *et al*., 2013), for example, traits such as tolerance to drought (Xu *et al*., 2008; Palukaitis *et al*., 2013) and cold (Meyer, 2013; Roossinck, 2013). Studies of viruses associated with non-crop plants have only just begun, but findings so far indicate that overall very little is known about viruses infecting plants (Wren *et al*., 2006). It is becoming increasingly evident that the view of viruses as mere pathogens is outdated. These entities possess the potential for facilitating a variety of interactions among macroscopic life. Therefore, a lot of work is needed in terms of research dealing with the diversity, evolution and ecology of viruses to truly comprehend their rich contribution to all human endeavours, including agriculture and food security. This introductory section focuses on some of the topics that are of current interest and relevance to tropical and subtropical regions where a number of plant diseases that threaten food security are caused by viruses.

1.2 Biology: Structure, Taxonomy and Diversity

Of the ca. 2000 viruses listed in the 2013 report of the International Committee for the Taxonomy of Viruses, less than 50%, or ca. 1300, are plant viruses. Viruses, which by definition contain either a RNA or DNA genome surrounded by a protective, virus-coded protein coat (CP) are viewed as mobile genetic elements, and characterized by a long co-evolution with their host. Many plant viruses have a relatively small genome; one of the smallest among plant viruses is a nanovirus with a genome of about 1 kb while the closterovirus genome can be up to 20 kb. Despite this apparent simplicity, nearly every possible method of encoding information in nucleic acid is exploited by viruses, and their biochemistry and mechanisms of replication are more varied than those found in the bacterial, plant and animal kingdoms (MacNaughton and Lai, 2006; Koonin, 2009).

According to Baltimore (Fig. 1.1), classification of viruses comprises seven independent classes, based on the nature of the nucleic acid making up the virus particle: double-stranded (ds) DNA, single stranded (ss) DNA, dsRNA, ss (+) RNA, ss (–) RNA, ssRNA (RT) or ssDNA (RT). The entities are further categorized by the Committee on Taxonomy of Viruses into five hierarchically arranged ranks: order, family, subfamily, genus and species. The polythetic species concept (van Regenmortel, 1989) as applied to the definition of the virus species recognizes viruses as a single species if they share a broad range of characteristics while making up a replicating lineage that occupies a specific ecological niche (Kingsbury, 1985; van Regenmortel, 2003). There are also proposals for the consideration of virus architecture in the higher-order classification scheme (Abrescia *et al*., 2009). Arguably, the defining feature of a virus is the CP, the structure of which is restricted by stereochemical rules (almost invariably icosahedral or helical) and genetic parsimony. Hurst (2011) introduced another proposal, namely the consideration of dividing life into two domains (i.e. the cellular domain and the viral domain), and thus the adoption of a fourth domain for viruses, along with entities such as viroids and satellites. It is opined that leaving viruses out of evolutionary, ecological, physiological or conceptual studies of living entities presents an incomplete understanding of life at any level. The proposed title of this domain is Akamara, which is of Greek derivation and translates to *without chamber* or *without void*; aptly referring to the absence of a cellular structure.

1.2.1 Virus evolution and the emergence of new diseases

Viruses are recognized as the fastest evolving plant pathogens. Genetic variation allows for the emergence and selection of new, fitter virus strains, as well as shapes the dynamics surrounding plant–virus and plant–vector interactions. Genetic changes are typically accomplished by mutations, the rate of which is greatest among RNA viruses because of non-proofreading activity of their replicases (i.e. RNA-dependent RNA polymerases). Recombination, either homologous or heterologous, is another source of virus variation. Recombination in potyviruses, for instance,

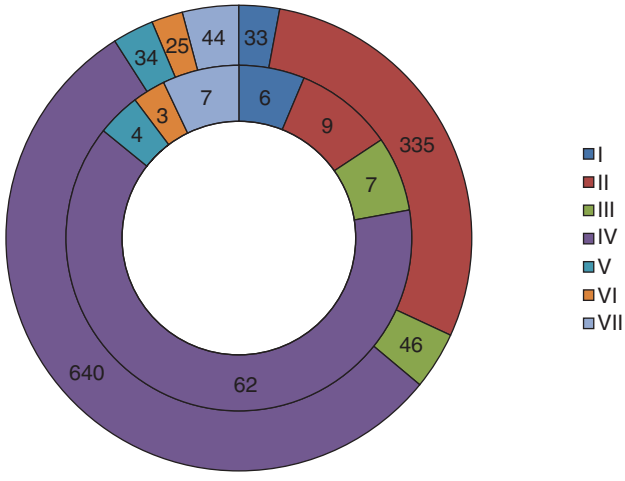

Fig. 1.1. The current plant virosphere (from the term viriosphere coined by Suttle in 2005) is comprised of (pathogenic) viruses belonging to all groups under Baltimore's classification. Group I (viruses with dsDNA genomes) include members of the family *Phycodnaviridae*; Group II (viruses with ssDNA genomes) those of the families *Geminiviridae* and *Nanoviridae*; Group III (viruses with dsRNA genomes) members of the families *Amalgaviridae*, *Endornaviridae*, *Partitiviridae* and *Reoviridae*; Group IV (viruses with (+) ssRNA genomes) that includes viruses from the families *Alphaflexiviridae*, *Betaflexiviridae*, *Benyviridae*, *Bromoviridae*, *Closteroviridae*, *Luteoviridae*, *Potyviridae*, *Secoviridae*, *Tombusviridae*, *Tymoviridae*, and *Virgaviridae*; Group V (viruses with (–) ssRNA genomes) with virus species of the families *Bunyaviridae*, *Rhabdoviridae* and *Ophioviridae*; Group VI (ssRNA-RT viruses with a DNA intermediate in their replication cycle) that consists of the plant virus families *Pseudoviridae* and *Metaviridae*; and finally, Group VII (dsRNA-RT viruses possessing an RNA intermediate in their replication cycle) with the members of the family *Caulimoviridae*. The inner circle provides the number of genera per group, while the outer circle includes the total number of species per group as of 2014. ds, double-stranded; RT, reverse transcriptase; ss, single-stranded.

has been shown to be especially frequent (Chare and Holmes, 2006). In other groups, like the family *Bunyaviridae*, reassortment of their genome segments seems to represent the underlying source of variation (Briese *et al.*, 2013).

Once variation is introduced, selection pressures that range from the action of host resistance genes to host shifts and environmental changes, or other mechanisms of genetic drift, contribute to changes in the genetic makeup of the virus population. Complementation between viruses in mixed infections can also lead to the maintenance of viruses with deleterious mutations, and hence increase the availability of variants that selection can act upon. Finally, current thinking suggests that genome organization, particularly in viruses showing 'overprinting', that is, gene overlapping, also plays a role. Gene overlapping, which allows for genome compression, can increase the deleterious effect of mutations in viruses as more than one gene is affected resulting in reduced evolutionary rates and adaptive capacity (Chirico *et al.*, 2010; Sabath *et al.*, 2012).

1.2.2 Wild or non-crop plants as reservoirs and targets of 'new' causal agents of disease

Many viruses and their respective vectors are associated with non-crop reservoirs that potentially act as bridges between crop plants. Conversely, crop viruses have the capacity to infect non-crop plants with similar probability (Vincent *et al.*, 2014). In either scenario, the simplicity of plant virus genomes allows for quick adaptation of viruses to new hosts, and generalist viruses tend to exhibit greater potential to cause more damage than

specialist viruses. While the scenario of increased virus invasion of native species is worrying and raises concern for the survival of endangered species, equally worrying is the effective jumping of viruses between native and crop species. Recent findings suggest that native plant communities are likely to contain potentially damaging viral pathogens (Kehoe *et al.*, 2014). Increased frequency of these reports is expected as new contact between native plants and introduced crops or weeds continues because of mans' activities and climate change.

1.2.3 Virus–virus interactions

Co-infection is another factor involved in shaping the genetic structure and diversity of plant viruses resulting in variations in symptom expression, infectivity, accumulation and/or vector transmissibility. Co-infections naturally occur due to the geographic overlap of distinct pathogenic types and appear to be the rule rather than the exception. The outcome of the mixed infection depends mainly on the plant species, virus strains, the order of infection and initial amount of inoculum. Antagonistic interactions between closely related viruses can lead to cross-protection and mutual exclusion. However, infections with different viruses in the same host can result in the appearance of more severe symptom expression than either single infection alone (viral synergism). Co-infection with *Clover yellow vein virus* (*Potyviridae*) and *White clover mosaic virus* (*Alphaflexiviridae*), for example, causes more severe disease development in pea (*Pisum sativum*), probably due to some unknown action of the potyvirus P3N-PIPO protein (Hisa *et al.*, 2014). Co-infection opens the possibility for inter-specific recombination or reassortment, and thus the generation of new viral species. Presumably virus–virus interactions are not only formidable forces that shape virus evolution, but also sources of emerging diseases in cases where viruses (including helper viruses or pseudotype viruses) do not share the same geographical distribution, but enter into contact because of germplasm movement, the introduction of vectors, habitat disturbance, etc. or a combination thereof (Da Palma *et al.*, 2010).

1.2.4 Plant–virus interactions

As alluded to earlier, bottleneck events limit genetic variation in virus populations. Various barriers in plants impose severe bottlenecks on populations of invading viruses. One such barrier is the host genetic restriction of virus colonization *in planta* and the disruption of long-distance movement (for reviews, see Waigmann and Heinlein, 2007; Kubinak and Potts, 2013). Another barrier is achieved via the reduction in the number of initial infection events to which a plant or plant population is exposed as well as concurrent interactions with alternate host reservoirs (Acosta-Leal *et al.*, 2011). Transmission events, both horizontal and vertical, also represent events that may impose a bottleneck. Work with *Cucumber mosaic virus* illustrates the complex interplay between the mode of transmission and host-parasite co-evolution in determining virulence evolution (Pagán *et al.* 2014). *Cucumber mosaic virus* is an ss (+) RNA virus that has the broadest host range described for a plant virus. It infects more than 1200 species in more than 100 plant families and is transmitted in a non-persistent manner by more than 80 species of aphids (Hemiptera: Aphididae) and through seed. Under experimental conditions, vertical passaging led to an adaptation to vertical transmission and a concomitant decrease in virus accumulation and virulence. This was attributed to reciprocal host adaptation. On the contrary, horizontal passaging was shown to have no effect on either virus accumulation or virulence.

1.3 Plant Virus–Vector Interactions

Virus entry into plant cells is only possible through the disruption of the cuticle and plant cell wall either by mechanical processes (wind, rain, hail or human- or herbivore-induced wounds) or by vectors. The latter

include a number of sap-sucking species of arthropods, for example, which deliver virus particles directly into the cell cytoplasm (and the vascular system) leading to the rapid dissemination of the virus through the whole plant. Although most viruses are naturally transmitted by vectors, only few plant–virus systems are well studied and characterized (Bragard *et al.*, 2013). The degree to which virus replication determines the rate of transmission and virulence (Froissart *et al.*, 2010), the effect of environmental impacts such as climate change on virus–vector interactions, among others, are mostly unexplored.

In general, plant viruses are hosted by many plant species, but are transmitted by very few specific vectors (Power and Flecker, 2003). Diverse members of the phyla Arthropoda (vastly represented by insects of the order Hemiptera) and Nematoda, as well as zoosporic species belonging to the kingdoms Fungi and Stramenopiles and some protists *sensu lato* (including plasmodiophorids) are known to transmit plant viruses. A puzzling case of mosquitoes harbouring tymoviruses expands the repertoire of insects serving as plant virus vectors (Wang *et al.*, 2012). Aphids are, however, among the most studied of the insect vectors (Powell *et al.*, 2006) – they easily feed on plants using their piercing–sucking mouthparts and become viruliferous after brief probing on an infected plant. Since in many, if not all cases, viruses are transmitted as intact virions, the CP represents the first and most important virus protein that interacts with the vector and determines the specificity of virus transmission. Depending on the virus group, other proteins play a role in the first steps of contact between the virus and its vector, like the helper component-proteinase (HC-Pro) of potyviruses. After making contact with the aphid's stylet, virions are retained for a period thereafter and then released by salivation. In the case of circulative viruses, it has been postulated that insect cell receptors mediate the internalization of the circulating virions. In other cases, where propagation also occurs, interactions are more complex and necessitate the intervention of host-specific proteins to guarantee virus replication. In some insects, plant viruses can be transmitted via sexual reproduction. The whitefly, *Bemisia tabaci* B biotype, for example, transmits *Tomato yellow leaf curl virus* (*Geminiviridae*) between males and females.

Broadly speaking, non-circulative viruses only interact with the mouthparts of their vectors; acquisition occurs in minutes, inoculation periods are in the range of seconds to minutes and there are equally short retention periods. On the contrary, in the circulative and propagative modes of transmission, interaction between the virus and the vector involves the haemocoel and replication of the virus within the vector. In both cases, however, the acquisition time ranges from minutes to hours, and once viruliferous, virus transmission to other plants occurs after a few days and up to weeks. In the circulative non-propagative mode of transmission, the vector remains viruliferous for hours to weeks, while the vector remains viruliferous during its lifespan in the propagative mode of transmission. In the latter case, the virus can be inherited by the progeny of the viruliferous vector. Irrespective, the mode of transmission possibly affects the evolution of virus virulence, as well as the virus' ability to colonize and exploit vectors in order to facilitate their own transmission (Froissart *et al.*, 2010; Gray *et al.*, 2014).

Thrips-transmitted viruses belong to four genera, *Tospovirus, Ilarvirus*, *Carmovirus* and *Sobemovirus.* Transmission in the latter three genera is characterized by movement of infected pollen and entry of the viruses through wounds generated during feeding. Tospoviruses, on the other hand, are persistently and propagatively transmitted. A distinguishing feature is the acquisition of the viruses only by larvae of the thrips species. The virus passes from the larvae to the adult during pupation (Wijkamp *et al.*, 1995; Whitefield *et al.*, 2015). Virus replication occurs in both larval stages and adults. Much effort has been directed to understanding the intricate mechanisms that underlie the circulation of the viruses through the developing animal.

Transmission by mites is semi-persistent and in some cases circulative. Both processes of acquisition and transmission involve the

virus CP. Whiteflies, however, feed on phloem cells and if virus-infected, facilitate a persistent or semi-persistent relationship with the virus. CP also plays a fundamental role in retention during transmission. In the case of circulative begomoviruses, virus particles on their way from the haemolymph to the salivary glands interact with a GroEL homologue produced by an endosymbiont in the insect (reviewed by Kliot and Ghanim, 2013). Presumably, the interaction protects against proteolysis. Hoppers can persistently transmit different species of plant viruses belonging to a wide range of families (mostly to monocots) in a circulative and propagative manner.

Some 30 species of nematodes are known to transmit at least 14 species of viruses. These viruses were initially classified in the genera *Nepovirus* and *Tobravirus*; however, reclassification to other genera was performed when transmission by aphids or mites, not nematodes, was demonstrated (Bragard *et al.*, 2013). Plant virus-transmitting nematodes feed mostly near or at the root tip using a spear-shaped structure at the anterior part of the body, and this allows the animal to puncture the plant cell and extract cell contents – including virions if the plant is infected. Virions are retained on the surface of the spear and in the area surrounding the oesophageal cavity via the CP or some other virus-encoded protein.

Finally, a limited number of soil-borne zoosporic endoparasites belonging to the plasmodiophorids (Rhizaria: Cercozoa) and chytrid fungal (Fungi: Chitridiomycota) groups are known to transmit several plant viruses belonging to the families *Potyviridae* and *Virgaviridae* and the genus *Benyvirus*, as well as the families *Ophioviridae* and *Tombusviridae* and the genera, *Potexvirus* and *Varicosavirus*, respectively (Bragard *et al.*, 2013). These viruses are acquired externally (e.g. the chytrid *Olpidium* sp.) or internally within infected plant tissue and carried by resting spores and zoospores. Glycoprotein receptors seem to play a role in attachment of the virions in a CP-dependent manner. Mechanisms of delivery to plant cells and the involvement of other virus and cell factors are not clear.

Plant resistance mechanisms against vectors by antixenosis (modification of vector behaviour in terms of feeding preferences) or antibiosis (increased mortality or reduced fitness or reproductive capacity of the vector) have been reported (Gómez *et al.*, 2009). In both cases pre-existing physical barriers, metabolites or deterrents act to prevent transmission from the vector to the plant. Additionally, resistance to aphids, nematodes or whiteflies exists at different functional and morphological levels (Montero-Astúa *et al.*, 2014; Sundaraj *et al.*, 2014). Viruses can also affect plant hosts in a manner that favours vector attraction or behaviour, and hence, transmission (Palukaitis *et al.*, 2013). As mentioned earlier, the CP plays an integral role in virus–vector interactions and transmission (see Urcuqui-Inchima *et al.*, 2001; Ni and Cheng Kao, 2013). CPs not only give structure to the virions (encapsidating and protecting the virus genome), but also facilitate interactions with receptors, chaperones and other factors of the vector and the virus itself during acquisition, movement, replication and transmission. Additionally, it has recently been shown that the structure of *Potato virus A* (*Potyviridae*) virions is characterized by the presence of a significant fraction of disordered segments in its intravirus CP subunits (Ksenofontov *et al.*, 2013). It is posited that since intrinsically disordered segments of proteins enlarge the range of their specifically recognized partners, such 'promiscuity' might explain in part the spectacular efficiency of this protein in all interactions it establishes with plant (and vector) factors. This finding gives support to prior observations that vector transmission of plant viruses requires conformational changes of virions (Kakani *et al.*, 2004). Nonetheless, CP interactions alone do not explain virus transmission in all cases. For many viruses, if not all, the presence of virus inclusions or aggregates of different sizes in infected cells have been demonstrated. These aggregates apparently participate in virus transmission by the controlled release and uptake of virions. They seem to be essential for the successful transmission of *Cauliflower mosaic virus* by its aphid vector (Moshe and Gorovits, 2012; Bak *et al.*, 2013). Moreover, the

generation of more ordered, complex virus-derived structures within the vector itself facilitates, for example, the intercellular spread of *Rice dwarf virus* through leafhopper cells and transmission of the virus by this insect (Chen *et al.*, 2012). Although not a direct consequence of the interaction between a virus and its insect vector, some insects induce the production of a volatile alcohol (methanol) in plants on feeding. As a consequence, methanol sensitizes the plant and allows for virus entry and spread within the plant and between plants by insect vectors (Komarova *et al.*, 2014).

Many challenges lie ahead in terms of our understanding of virus–vector interactions and the ways we can use this knowledge to design control strategies relevant for multi-host plant viruses. For example, expansion of investigations into the role of ubiquitination-related enzymes linked to viral infection to a system wide analysis involving virus vectors could provide insights into how these mechanisms can be exploited for the development of new antiviral strategies (Alcaide-Loridan and Jupin, 2012). A complete understanding of the mechanisms and factors surrounding phloem transport of plant viruses (Hipper *et al.*, 2013) could also facilitate manipulation or avoidance of vector feeding and thus control virus transmission. Recently, it was demonstrated that the expression of viral glycoproteins in transgenic plants interfered with virus acquisition and effectively blocked virus transmission by insect vectors (Montero-Astúa *et al.*, 2014).

1.4 Diagnosis and Crop Protection Technologies

Because effective management of virus diseases requires an integrated approach aimed at preventing or delaying infection, timely and accurate diagnosis of virus infections is of paramount importance. There is the added challenge of discrimination of unrelated strains and the reliable detection and characterization of related strains. International attempts to develop and standardize diagnostic protocols for plant viruses, are coordinated by the European and Mediterranean Plant Protection Organization and by the International Plant Protection Convention.

Traditionally, the detection of virus infections has relied on biological testing or indexing. Indexing is based on the detection of the virus pathogen and associated symptoms following grafting on an appropriate indicator plant. The technique is still widely used as part of the certification programs against certain pathogens (e.g. *Citrus tristeza virus*, and *tomato spotted wilt*, *impatiens necrotic spot* and *watermelon silver mottle* tospoviruses) (EPPO, 2014). Nonetheless, it is necessary that visual inspection for symptoms is accompanied with other confirmatory tests to ensure accurate diagnosis. Among the various diagnostic techniques, immuno-based methods are routinely used for virus detection (Hull, 2002), specifically some form of antibody-based enzyme immunoassay utilizing polyclonal antibodies that have been generated against purified viral CP (van Regenmortel, 1982) or viral proteins expressed as recombinant fusion proteins in instances where the virus is intrinsically poorly immunogenic or is difficult to purify from host tissues (Raikhy *et al.*, 2007; Lee and Chang, 2008; Gulati-Sakhuja *et al.*, 2009; Rani *et al.*, 2010; Rana *et al.*, 2011; Khatabi *et al.*, 2012; Mandal *et al.*, 2012). More recently, tests employing a cocktail of polyclonal antibodies also derived from fusion constructs of viral gene sequences of two or three different viruses are being developed (Kapoor *et al.*, 2014). This approach will facilitate the detection of mixed virus infections which are usually observed in the field.

Other diagnostic tests include the PCR, RT-PCR and hybridization-based techniques (Gilbertson *et al.*, 1991). These tests have proven rapid, sensitive and reasonably inexpensive to conduct. Degenerate primers are used typically in PCR (Rojas *et al.*, 1993; Wyatt and Brown, 1996). Degenerate primers have facilitated the identification of, for example, most geminiviruses, but mixed infections and the presence of satellite DNA, which are commonly found in association with monopartite begomoviruses in South-East Asia (Dry *et al.*, 1997; Mansoor *et al.*, 2003), interfere with the identification of viruses present in

samples. Often combinations of ELISA and PCR technologies are employed in an attempt to improve sensitivity and to avoid problems with inhibitors. Advances in real-time quantitative PCR technology have enabled large-scale detection of many plant RNA and DNA viruses. Recent developments in multiplex real-time PCR show promise for future identification, genotyping and quantitation of viral targets in a single, rapid reaction (Fageria *et al.*, 2013). But for now, microarray technologies provide the option of multi-pathogen detection (Hammond *et al.*, 2015). Labelled nucleic acids isolated from samples are hybridized to a large number of diagnostic probes spotted on a platform. The array is subsequently scanned to produce a file of fluorescence intensities for the probes (Nam *et al.*, 2014). An amplification step prior to hybridization is often included to increase the sensitivity for low titre viruses.

A new cadre of techniques are emerging, which unlike the traditional methods, do not require an *a priori* prediction of the viruses likely to be present in the sample. They include for example, rolling-circle amplification coupled with restriction fragment length polymorphism and next-generation sequencing of small RNAs isolated from infected plants. Although these approaches are powerful and flexible, they may not prove suitable for routine diagnostic procedures, and are more likely to facilitate the identification of novel or unknown viruses (Schubert *et al.*, 2007; Kreuze *et al.*, 2009; Hagen *et al.*, 2012). Before long, the field of nanotechnology is likely to bring on board new diagnostic tools. Electrochemical DNA biosensors, for example, provide a novel technique for the recognition of target DNA by hybridization (Malecka *et al.*, 2014). Essentially, target DNA is captured in a recognition layer. The probe–target complex then triggers a signal for electronic display and analysis. Potential advantages of these devices include rapid detection, portability and adaptability.

Efforts to identify and implement control strategies against virus diseases vary with the crop and the region. Typically, dissemination within and between regions is often addressed through quarantine controls in addition to other government interventions that restrict the movement of plant materials within the region. As regards to on farm practices, these range from the interference of vector-mediated virus transmission, the implementation of biological and cultural management practices and the development of host–plant resistance. Prevalent among farmers, however, is the policy of 'living with the disease'. There is willingness on their part to change to varieties that offer more tolerance or are resistant, and until they become available, to continue with the existing varieties and harvest as much as possible or increase the area under production to achieve the production required. But tolerant and/or resistant varieties are not always available or they are not readily combined with other desirable horticultural attributes. Alternate approaches to the development of host resistance have emerged that utilize molecular techniques either in the form of linked molecular markers to speed up and simplify the selection of resistance genes or pathogen-derived or transgenic resistance.

There are two categories of transgenic resistance in host plants that show significant promise for disease management. First is the use of plant-derived genetic resistance that was reviewed by Truniger *et al.* (2008) and Fraile and Garcia-Arenal (2010). The second exploits the post-transcriptional gene silencing machinery to generate small interfering RNAs (siRNAs) that target viral genomes or critical host factors for degradation. One favoured strategy for engineering resistance to plant viruses is the expression of hairpin (hp) RNA constructs composed of inversely repeated viral RNA sequences separated by an intron spacer. The hpRNAs are processed by Dicer into siRNAs and these can provide whole plant resistance to virus infection. This strategy has shown greater than 90% effectiveness in combating virus infection. For *Plum pox virus* resistance, several constructs consisting of overlapping portions of P1/HC-Pro, HC-Pro, and HC-Pro/P3 coding regions were generated and tested (Hily *et al.*, 2004; Di Nicola-Negri *et al.*, 2005, 2010; Kundu *et al.*, 2008; Ilardi and Nicola-Negri, 2011). The 5′ UTR/P1 fragment was found to be the most effective for broad-spectrum transgenic resistance. A related strategy was

used to create resistance to cucurbit-infecting potyviruses. Here an inverted repeat construct was prepared using a large fragment of the *Zucchini yellow mosaic virus* (ZYMV) HC-Pro gene which also showed substantial similarity with that of *Watermelon mosaic virus* (WMV) and *Papaya ringspot virus* serotype W (PRSV-W). Transgenic cucumber and melon lines inoculated with ZYMV or WMV failed to accumulate viral RNAs, while plants inoculated with PRSV-W exhibited significantly lower levels of virus than non-transformed plants (Leibman *et al.*, 2011). This is an exciting example of the engineering of small RNAs for resistance to related virus strains or even related species.

Another silencing approach that is proving to be effective is the silencing of host factors that are crucial for virus susceptibility. One of the most common factors used by members of the family *Potyviridae* is an isoform of the translation initiation factor 4E (eIF(iso)4E). Mutation in eIF4E family is a common component of recessive resistance against plant viruses. The mechanism of recessive resistance to potyviruses, especially mediated by eIF4E and eIF(iso)4E is explained in detail by Truniger and Aranda (2009). This type of resistance blocks virus multiplication in inoculated leaves. Examples of recessive eIF4E-mediated resistance to species members of the *Potyvirus* supergroup include: *mo1* (*Lettuce mosaic virus*) in lettuce, *lsp1* (*Tobacco etch virus*) in *Arabidopsis*, *cum1-1* (*Clover yellow vein virus*) in cucumber, *pvr2* (*Pepper veinal mottle virus*) in pepper and *sbm-1* (*Pea seed-borne mosaic virus*) in pea. Interestingly, eIF4E and IF(iso)4E resistance is the result of failed interactions with the potyvirus VPg. There is only one reported example of recessive eIF4E-mediated resistance to members of the *Bean common mosaic virus* (BCMV) supergroup and that is *bc-3* (BCMV) in bean (Naderpour *et al.*, 2010). Silencing eIF(iso)4E can confer resistance to ZYMV and *Moroccan watermelon mosaic virus*, which are both members of the BCMV supergroup (Rodríguez-Hernández *et al.*, 2012). This exciting advance in engineered resistance demonstrates that certain recessive resistance mechanisms provide broad-spectrum resistance to potyvirus infection that can extend to other members of the BCMV supergroup. Therefore, in crops where recessive resistance genes are not available for breeding elite cultivars, siRNA or hpRNA silencing can be used to provide protection against infection either by targeting the virus itself or a critical host factor (Truniger *et al.*, 2008).

1.5 Virus Diseases Threaten Food Security in Tropical and Subtropical Regions

Although accurate figures for crop losses due to virus infections are not readily available, it is widely accepted that among the plant pathogens, viruses are second only to fungal pathogens with respect to economic losses. Human actions are extensively implicated in virus disease outbreaks and epidemics, as is the appearance of new viruses that switched host species or new variants of classic viruses that acquired new virulence factors or different epidemiological patterns. While technological advances in, for example, diagnostic and agronomic practices have reduced the risk of epidemics in developed countries more so than developing countries, virus diseases remain a threat to global food security and have the potential to be widespread with subsequent economic, social and environmental impacts.

Plant protection plays an important role in minimizing the losses incurred by virus diseases and improving food security, that is, in satisfying the demand worldwide for both the quality and quantity of agricultural goods (Savary *et al.*, 2012). There are many possible intervention points in the crop–pathogen interaction, but decisions on which are to be prioritized will depend on a combination of feasibility and likely effects. Nonetheless, interventions require initial investment in capacity and resource building accompanied with cost estimates of adoption. Other costs will be incurred from investments in evaluation research and diagnostic programs (Oerke, 2006), as well as education programmes aimed at scientists and regulators on the diseases and prophylactic

approaches. Since the basic biology of some cultivated plants and their pathogens are still poorly understood, particularly in the developing world, the emergence of new diseases adds a complicating dimension to food production and availability. Thus the challenge that lies ahead in terms of food security involves increased investment in basic and applied research, particularly in the fields of plant, vector and virus gene expression and the identification of new viruses, as well as biodiversity, distribution, adaptation and ecology of the biotic protagonists (Wren *et al.*, 2006; Mehta *et al.*, 2008; Kundu *et al.*, 2013; MacDiarmid *et al.*, 2013). However, none of these objectives will be effectively attained if the use of technologies already developed are not maximized and accompanied with the generation and exploitation of new scientific discoveries (Schumann, 2003; Walthall *et al.*, 2012; UK Plant Science, 2014). Genomics along with the other '-omics' technologies facilitate the identification of genes affecting important traits and a greater understanding of how they function, which invariably will contribute to the transfer of genes to elite varieties via marker assisted breeding or transgenic approaches. The latter technology has spurred considerable public debate over recent years that is likely to continue in the broader context of other uses of biotechnology and their consequences for human societies. Issues such as cost, safety and benefit ought to be dispassionately evaluated (Thomson, 2002, 2008; Ronald, 2011). Finally, global partnerships must also be fostered if we are to honestly pursue the final goal of nutritious, cheap and widely available food for all. Prevention and remediation of the impact of plant diseases is high and a burden for countries less prepared. Nonetheless, it has been estimated that the benefits associated with prevention and protection programs for virus transmitted diseases far surpass the costs of the protection program (Cembali *et al.*, 2003, 2004). Additionally, disease control can mitigate effects of climate change in addition to contributing to sustainable crop production (Mahmuti *et al.*, 2009).

The chapters that follow provide up-to-date information on selected viruses of important crops, including their distribution, their biological and molecular characteristics, and the approaches that control the diseases they elicit and sustain productive agricultural systems. These entities were chosen based on their potential impact on food security. They differ considerably in host range, their longevity in the host and dissemination. Many of the viruses, as discussed in this book, belong to the family *Potyviridae* (Chapters 4, 7, 8, 9, 10, 11 and 16) and others of Group IV (*Bromoviridae*, Chapter 6; *Closteroviridae*, Chapters 14 and 17; and *Secoviridae*, Chapter 15). Viruses belonging to the most important family of plant viruses, at least in terms of the number of species, the family *Geminiviridae* (Group II), are covered in Chapters 3, 5 and 13. The impressively successful *Tomato spotted wilt virus* (family *Bunyaviridae*, Group V) is examined in Chapter 12, while other important viruses belonging to Groups II (*Nanoviridae*) and VII (*Caulimoviridae*) are reviewed in Chapters 2 and 15, respectively. The overall impact of the virus diseases on crop production is considered in the individual chapters. These crops (rice, wheat, maize, potato, cassava, soybean, yam, sweet potato, tomato, citrus, banana and plantain, and pineapple, among others) are regarded as important staples in tropical and subtropical areas worldwide. They are mainly consumed directly and are major contributors to human calories and proteins. They are also targets of a diverse array of viruses (Rybicki and Pietersen, 1999; Kumar *et al.*, 2013; Rybicki, 2015). Notable examples of virus pathogens that challenge food security in sub-Saharan Africa are the mosaic viruses of cassava. The tuberous roots of cassava are the major source of dietary starch in sub-Saharan Africa. The crop was presumably introduced to the western coast of Africa in about the sixteenth century by Portuguese traders as a safeguard against periods of famine that consistently plague the region (Alabi *et al.*, 2011). Today, cassava is considered the crop of the future not only because of its contribution to food security, but also because it represents a significant income earner for smallholders, and promises immense potential as a source of industrial

raw materials like glucose and starch. The crop is widely used in many countries of Africa, where unfortunately the prevalence of viral disease is high; however, these viruses are not known in South America, which is the centre of origin of cassava. Finally, although not a crop essential for food security (debatable as this statement might be), papaya, and its worst enemy, *Papaya ringspot virus* (*Potyviridae*), was included because it represents a case where the use and implementation of modern strategies of disease control cannot be defined as other than successful.

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