Chapter 3
Emerging Plant Viruses: a Diversity of Mechanisms and Opportunities

Maria R. Rojas(✉) and Robert L. Gilbertson

3.1 Introduction 28
3.2 What are Some Plant Viruses that Presently are Considered as Emergent? 29
3.3 What Factor(s) Lead to the Emergence of a Plant Virus? 30
3.3.1 Long-Distance Movement 30
3.3.2 Emergence of Insect Vectors Precedes and Mediates Emergence of New Viruses from Pools of Viral Genetic Diversity in Reservoir Hosts 33
3.4 Reassortment and Recombination: Effective Mechanisms of Variability for DNA Viruses 37
3.4.1 Recombination 38
3.4.2 Reemergence of Cassava Mosaic Disease in Africa: a Role for Reassortment and Recombination 39
3.5 Tripartite Begomovirus Complexes: A Way for Bipartite Begomoviruses To Fight Host Defense Responses? 39
3.6 Acquisition of Novel Viruslike Entities: Monopartite Begomoviruses and their Satellite DNAs 40
3.7 Emergence of Diseases Caused by Novel Viruslike Agents 42
3.8 Multiple Mechanisms often Underlie the Emergence of Plant Viruses 42
3.8.1 Long-Distance Movement and an Emergent Vector: *Cucurbit yellow stunting disorder virus* 42
3.8.2 Mutation, Recombination and Long-Distance Movement: *Plum pox virus* 43
3.9 Bringing Them All Together: Tomato Yellow Leaf Curl/Leaf Curl Disease in West Africa 43
3.10 Emergence of a New Virus is not always Catastrophic: Failure of New Viral Diseases to Emerge Following a Major Change in the Whitefly Vector Population 44
3.10.1 A History of Whitefly-Transmitted Viruses in the Imperial Valley of California 44
3.10.2 Emergence of CuLCrV in the Imperial Valley 46
3.11 Conclusions 47
References 48

Abstract Although emerging plant viruses receive much less publicity than their animal- or human-infecting cousins, they pose a serious threat to worldwide

Maria R. Rojas
Department of Plant Pathology, University of California, Davis, CA, USA
mjrojas@ucdavis.edu

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agricultural production. These viruses can be new (i.e., not previously known) or already known; however, they share the common characteristic of occupying and spreading within new niches. Factors driving the emergence of plant viruses include genetic variability in the virus, changes in agricultural practices, increases in the population and/or distribution of insect vectors and long-distance transport of plant materials. In recent years, individual as well as entire groups of viruses have emerged, and this has involved a variety of mechanism(s), depending on the virus and the environment. Here, we will discuss some of these viruses, and highlight the mechanisms that have mediated their emergence. Special emphasis is placed upon the whitefly-transmitted geminiviruses (begomoviruses) and the thrips-transmitted tosposviruses, which have emerged as major threats to crop production throughout the world. Other examples include the recent emergence of novel viruslike agents, the acquisition and role of satellite DNA or RNA molecules in emergence of plant viruses, and cases where emerging viruses have had only a transient impact. It seems clear that global movement of plant materials, expansion of agriculture and large-scale monoculture will continue to favor emergence of plant viruses. However, improved diagnostics should allow for rapid identification of emerging viruses and better understanding of viral biology. This information can be used in the development of effective management strategies, which will hopefully minimize impact on agricultural production.

3.1 Introduction

Emerging viruses pose a major threat to animal, human and plant health. Some of these viruses have already greatly impacted human health, whereas others have caused catastrophic losses to crop and animal production. There are many definitions of what constitutes an emerging virus. We consider an emerging virus to be one that has recently changed or appeared to occupy and spread within a new niche. Emerging viruses can be new, i.e., not previously known; however, they are often known viruses that have become more apparent owing to changes in the environment/ecosystem and/or generation of a new variant, thereby providing the virus with an opportunity to expand into new niches. In the case of plant viruses, changes in agricultural practices and long-distance transport of plant materials are key factors mediating virus emergence. Some emerging viruses gain considerable public recognition and attention because of actual or potential health or economic losses, e.g., development of animal or human diseases or economic losses due to disease epidemics in crop plants. In other cases, the emergence of a virus or group of viruses may not result in catastrophic disease or economic losses.

Viruses that have emerged as animal or human pathogens are usually much more highly publicized than are emergent viruses of plants. However, the rate of emergence of plant viruses does not seem to be any less than that for human and other animal viruses. Furthermore, some common mechanisms underlie the emergence of animal and plant viruses, irrespective of the nature of the host. For example, an increasingly important mechanism is inadvertent long-distance transport of viruses,

which is mediated by increased global movement of plants and people. Another common mechanism is the capacity of viruses to jump from reservoir hosts into new hosts; these viruses can cause serious diseases in their new hosts, in great part owing to the lack of adaptation that tends to moderate the toll that the virus imparts on the host. Examples of emergent human viruses thought to have come from animal reservoirs include *Human immunodeficiency virus* (HIV), thought to have emerged from a progenitor virus in chimpanzees (Gao et al. 1999); the novel coronavirus associated with severe acute respiratory syndrome (SARS), thought to have emerged from an animal reservoir (Guan et al. 2003); and the hemorrhagic viruses Ebola and Marburg (Leroy et al. 2005; Towner et al. 2006). Following the introduction into humans, emergence of all three of these viruses, especially HIV, also was facilitated by long-distance transport of humans (or monkeys in the case of Marburg virus). Human and other animal viruses also can be introduced via insect vectors, and the emergence of the West Nile virus in the USA is an example of an Old World virus that was introduced into the New World, with subsequent spread into bird, animal and human hosts mediated by indigenous mosquito vectors (Lanciotti et al. 1999).

In the case of plant viruses, the appearance of emergent viruses is usually mediated via an insect vector. However, with increasing global trade, the emergence of a virus in a new geographical region may be initiated by the introduction of infected plant materials (e.g., plants, propagative materials or seeds). Once introduced, the successful emergent virus expands into a new niche via activity of an existing insect vector or, less frequently, through spread by physical contact.

Finally, new forms of animal, human and plant viruses also emerge through common mechanisms of genetic variability, including mutation, reassortment and recombination. A classic example of this is the influenza virus, which can rapidly generate new strains with significantly altered virulence, via reassortment and recombination. Hence, the current worldwide apprehension that the "bird flu" strain of influenza (H5N1) will mutate and emerge as a virus with a greater capacity to be spread among the human population (Li et al. 2004).

3.2 What are Some Plant Viruses that Presently are Considered as Emergent?

Emergent plant viruses can be placed into two broad categories: entire groups of viruses (e.g., genera or families) or individual viruses. Some examples of plant virus groups that are emergent, on a global level, include (1) the whitefly-transmitted begomoviruses (genus *Begomovirus*, family *Geminiviridae*), (2) the thrips-transmitted tospoviruses (genus *Tospovirus*, family *Bunyaviridae*) and (3) the criniviruses (genus *Crinivirus*, family *Closteroviridae*). Some individual viruses that have emerged relatively recently include the potexvirus, *Pepino mosaic virus* (PepMV), an emergent tomato virus; and the sobemovirus, *Rice yellow mottle virus*. In addition, there also can be outbreaks of "new" viruses, such as those causing

necrosis-associated diseases of tomato in Mexico, Spain and Guatemala (Verbeek et al. 2007). Another example is the emergence of a novel whitefly-transmitted potyvirus infecting cucurbits in Florida (Adkins et al. 2007).

3.3 What Factor(s) Lead to the Emergence of a Plant Virus?

There can be many factors that facilitate the emergence of a plant virus. These include genetic mechanisms such as random mutations, recombination, reassortment; long-distance movement to new agroecosystems; changes in vector population dynamics; and acquisition of novel viruslike entities. Quite often, the emergence of a plant virus involves more than one of these mechanisms (Table 3.1). In the rest of this chapter, we will consider these mechanisms and provide examples of viruses that have used these mechanisms in their emergence.

3.3.1 Long-Distance Movement

3.3.1.1 *Pepino mosaic virus*: Emergence of a Virus via Seed Dissemination

A new viral disease of tomato appeared in greenhouse-produced tomatoes in the Netherlands in 1999. The symptoms of this disease were variable, but included various degrees of mosaic (including a bright yellow mosaic) in leaves, distorted leaf growth and mottling in fruits. In some cases, plants would senesce prematurely and take on a grayish appearance (referred to as "thistletop"), whereas in other cases (conditions favorable for plant growth) plants would be symptomless. The causal agent was identified as PepMV, a member of the genus *Potexvirus*, plus-sense single-stranded RNA viruses with flexous rod-shaped virions. PepMV was not a new virus; it was first identified in Peru in 1974 infecting pepino (*Solanum muricatum*), a local solanaceous plant with a sweet-tasting fruit. Shortly after its identification in the Netherlands, the disease began to appear throughout Europe (e.g., France, Italy, Spain and the UK). In 2001, it was reported from the USA, where it has been increasing in incidence ever since. The disease is not particularly devastating to tomato production, causing losses of approximately 5–15%, much of which is due to reduced fruit quality. However, the virus is rapidly spread via mechanical means (i.e., touch, pollination, grafting and pruning) and it is extremely persistent owing to the stability of the virions. Thus, PepMV clearly fits the definition of an emergent virus.

One of the big questions was how did PepMV move from South America to Europe. Analysis of the nature of the initial outbreaks of the disease revealed an association with certain seed lots. Subsequent studies provided evidence of the association of the virus with seed, although at low levels and probably as an external contaminant. However, given how rapidly the virus can spread, plant-to-plant,

Table 3.1 Examples of emergent viruses/virus groups and mechanisms associated with their emergence

	Mechanism of emergence					
Virus/virus group	Long-distance movement	Changes in insect vector	Appearance of new species	Reassortment/ recombination	Acquisition of novel agents	Novel virus
Whitefly-transmitted begomoviruses	X	X	X	X	X	
Criniviruses	X	X	X			
Tospoviruses	X	X	X			
Pepino mosaic virus (potexvirus)	X			X		
Plum pox virus (potyvirus)	X			X		
Tomato torrado virus						X

it would not require high rates of seed contamination for the virus to become established in a tomato production system. Another factor that probably played a role in the emergence of PepMV is the practice of producing hybrid tomato seeds in countries such as Peru, which have favorable weather conditions and low labor costs. Thus, it is possible that PepMV was carried into Europe on hybrid tomato seed produced in Peru. If this were the case, then it would be expected that isolates of the virus from Peru, Europe and North America would be closely related. Indeed, studies of the population genetics of PepMV revealed high levels of nucleotide sequence similarity among tomato-infecting isolates from Peru, Europe and North America; consistent with emergence of a single genetic type (Verhoeven et al. 2003; Pagan et al. 2006). On the other hand, strains of the virus differing in genetic and biological properties (e.g., capacity to induce symptoms in tomato) also have been identified in Europe and elsewhere. There is evidence that these strains also may have originated from Peru, suggesting multiple introduction events into Europe. Finally, mixed infections of PepMV strains were detected in Spain, and recombinant isolates were detected (Pagan et al. 2006). Management of PepMV will require use of pathogen-free seed (facilitated by development of effective seed treatments or seed assays) and strict sanitation in tomato greenhouses; development of PepMV-resistant tomato varieties should be a long-term goal.

3.3.1.2 *Tomato yellow leaf curl virus*: Introduction of an Old World Virus into the New World

Tomato yellow leaf curl disease (TYLCD) was first described in the Middle East around 1940 (Cohen and Antignus 1994). In 1991, an isolate of the causal begomovirus, *Tomato yellow leaf curl virus* (TYLCV), from Israel was characterized and shown to possess a monopartite genome (Navot et al. 1991). Evidence that this single DNA component comprised the viral genome came from the development of TYLCD following introduction of the viral DNA into tomato plants via agroinoculation, a method where the plant pathogenic bacterium *Agrobacterium tumefaciens* is used to deliver the viral DNA instead of the whitefly vector (*Bemisia tabaci*). DNA sequence analysis revealed that the genome organization of TYLCV was similar to that of the DNA-A component of the bipartite begomoviruses, but with an extra open reading frame (ORF) on the virion-sense DNA strand (V1 ORF). With use of the cloned viral DNA as a probe and the sequence to generate TYLCV-specific PCR primers, TYLCV was found distributed throughout the Middle East (e.g., Israel, Egypt, Jordan, Lebanon and Cyprus). Now, over 15 years since the characterization of this TYLCV isolate (TYLCV-IL, now considered as the "type" isolate), this virus has spread to the New World, where it has emerged as a major constraint on tomato production. Here, we examine how this has happened.

TYLCV-IL was introduced into the Dominican Republic in the early 1990s (Salati et al. 2002). The identification of TYLCV in this Caribbean island country was the first report of the emergence of an Old World monopartite begomovirus in the New World. TYLCV, like all geminiviruses, is not seed-transmitted, and the

whitefly vector is not capable of transcontinental flight. Thus, it was not surprising that anecdotal reports indicated that tomato transplants had been imported into the Dominican Republic from Israel in the early 1990s, owing to destruction of local transplants by heavy rains. Unfortunately, this inadvertent introduction was all TYLCV needed to gain a foothold in the Western Hemisphere. The Dominican Republic was well suited for the establishment of TYLCV: hot dry weather, overlapping crops of susceptible tomato varieties and large populations of the whitefly vector.

It did not take long for the virus to spread throughout the northern and southern processing tomato regions of the Dominican Republic, threatening to destroy an industry that had made the island self-sufficient for this commodity (Salati et al. 2002). Furthermore, the virus spread to Cuba, Jamaica, Puerto Rico and other islands in the Caribbean. In 1999, TYLCV was first reported in the USA from Florida, where it was discovered in tomato plants sold at retail stores. The inoculum source was hypothesized to be viruliferous whiteflies blown into Florida via high winds (Polston et al. 1999). Subsequently, TYLCV was reported from Georgia, Louisiana and even from North Carolina. More recently, TYLCV has emerged in northern Mexico (the states of Sinaloa and Tamulipas), where it caused major losses to fresh and processing tomato production in the state of Sinaloa during the 2005–2006 growing season (Brown and Idris 2006). The virus was subsequently identified in Texas and Arizona at the end of the 2006 growing season and, in March 2007 it was first identified in California (Rojas et al. 2007). TYLCV was also identified in Guatelmala in 2006.

DNA sequence analyses have confirmed that the TYLCV isolates throughout the New World are isolates of TYLCV-IL (i.e., total sequences more than 95% identical). Thus, since the initial introduction into the New World in the early 1990s, TYLCV has emerged, in a relatively short period of time, as a serious constraint on tomato production throughout the Caribbean, northern Mexico and the southern USA. While the factors mediating the rapid spread of this damaging pathogen in the Western Hemisphere are not completely understood, it is likely that it reflects a combination of migratory movements of the whitefly vector, together with movement of infected tomato transplants and/or other plants that are infected with TYLCV or harbor viruliferous whiteflies. The emergence of TYLCV represents an excellent example of the potential dangers associated with global movement of plant materials.

3.3.2 Emergence of Insect Vectors Precedes and Mediates Emergence of New Viruses from Pools of Viral Genetic Diversity in Reservoir Hosts

The *Begomovirus* and *Tospovirus* genera have emerged through a remarkable proliferation of new viral species over the past 10–20 years (Varma and Malathi 2003; Fauquet and Stanley 2003; Rojas et al. 2005; Whitfield et al. 2005; Prins and

Goldbach 1998). In both cases this was preceded by increases in the distribution and population of a polyphagous insect vector, often via the same mechanisms that facilitate long-distance dispersal of viruses. In the case of the begomoviruses, the vector is the sweet potato whitefly, *B. tabaci* (Brown et al. 1995); whereas for the tospoviruses, it is the western flower thrips (WFT), *Frankliniella occidentalis* (Prins and Goldbach 1998; Whitfield et al. 2005). Another factor that has contributed to the emergence of these viruses is the apparent availability of a pool of progenitor viral genetic information present in reservoir hosts (e.g., weed or native plants). Finally, the interaction of these vector–virus combinations is mediated by increasing land conversion and intensification of agricultural production, e.g., through the use of new high-yielding varieties, improvements in irrigation and irrigation technology (especially drip irrigation), and increased use of pesticides and fertilizers (Matson et al. 1997). Indeed, it is often at the interface of land conversion/agricultural intensification where emergence of new viruses commonly occurs.

3.3.2.1 Emergence of Thrips and Tospoviruses

One of the definitions of an emerging group of viruses is the relatively rapid emergence of new virus species in diverse geographic locations. RNA viruses of the genus *Tospovirus* are the only plant-infecting viruses in the family *Bunyaviridae*. The type member *Tomato spotted wilt virus* (TSWV) has been known since 1919, but it is only recently that new tospovirus species have proliferated (Whitfield et al. 2005). Thus, the tospoviruses qualify as a group of emergent viruses on the basis of the fact that new species are being described from a range of host plants in a diversity of geographical locations (Prins and Goldbach 1998; Whitfield et al. 2005). This proliferation has been correlated with tremendous increases in thrips populations, especially the polyphagous WFT, which has been mediated by agricultural intensification (Prins and Goldbach 1998). In the case of thrips, overuse or reliance on a few insecticides has also led to the generation of insecticide-resistant populations. Together with the difficultly of applying insecticides to flowers and other places where thrips feed and reproduce, this has limited the effectiveness of vector control for management of these tospovirus diseases. Most of the new tospovirus species have originated in tropical regions of Asia, perhaps suggesting a “hot spot” of viral genetic diversity in reservoir hosts. However, two species are emerging as significant economic threats in temperate North America: *Impatiens necrotic spot virus* (INSV) and *Iris yellow spot virus* (IYSV).

INSV was first discovered as a virus affecting ornamental plants in greenhouses. It has become widely distributed in the USA, presumably via the movement of infected ornamental plants and/or in viruliferous thrips carried on ornamentals (Daughtrey et al. 1997). Thus, INSV has emerged as an important virus in the ornamental plant industry. More recently, INSV has been reported causing diseases in a number of crop plants. For example, INSV was identified as the cause of tomato spotted wilt-like symptoms in peppers in Georgia and

lettuce in California (Naidu et al. 2005; Koike, Kuo and Gilbertson, unpublished data). It remains to be seen whether INSV will become an emergent virus of vegetable crops. The availability of rapid detection methods such as immunostrips for TSWV and INSV will greatly facilitate identification and monitoring of these tospoviruses.

Another emergent tospovirus is IYSV, which infects onions and other members of the onion family (Gent et al. 2006). It fits the description of an emergent virus because it initially had a limited distribution but, more recently, it has been reported from a wide range of geographic locations. Although the nature of the emergence of IYSV is not clear, the recent plethora of reports of the virus may relate to improved diagnosis, including better recognition of the symptoms (i.e., the chlorotic diamond-shaped lesions on leaves and stems) and availability of serological detection tools (Gent et al. 2006). The potential for IYSV to cause losses to onion seed production, through the lodging of seed stalks at points with virus lesions, makes this an important emerging virus in the onion industry.

Finally, the finding of limited genetic diversity among isolates of INSV and IYSV from different hosts and geographic locations seems to support an initial emergence event followed by effective long-distance dispersal. This dispersal has undoubtedly been favored by the fact that the key hosts of INSV and IYSV are vegetatively propagated, thereby avoiding the limitation imposed by the lack of efficient tospovirus seed transmission.

3.3.2.2 *Bemisia tabaci*: an Insect Vector that has Mediated the Emergence of Begomoviruses and Criniviruses

There is perhaps no greater culprit in the emergence of new plant viruses than the sweet potato whitefly, *B. tabaci* biotype B (=silverleaf whitefly, *Bemisia argentifolii*). Thought to have originated in the Middle East/Asia, this insect has emerged as a major pest of vegetable production worldwide (Brown et al. 1995). Though a significant pest in its own right, it has mediated the emergence of the begomoviruses and the criniviruses over the past 20 years.

Over the past 20 years, *B. tabaci* (biotype B as well as other biotypes) has mediated the emergence of well over 100 new begomovirus species. This has resulted in the whitefly-transmitted begomoviruses supplanting potyviruses as the group of plant viruses with the largest number of recognized viral species. In this chapter, we will provide examples of the mechanisms involved in the emergence of new begomovirus species (Fig. 3.1). For detailed reviews of this subject, the reader is referred to other reviews (Polston and Anderson 1997; Seal et al. 2006; Varma and Malathi 2003).

The worldwide emergence of begomovirus diseases has been facilitated, in part, by parallel evolution. Here, genetically distinct begomovirus species evolve in distinct geographical regions (e.g., continents) to cause the same or similar disease symptoms in a given crop plant (Fig. 3.1). This occurs as whiteflies introduce genetically diverse progenitor begomoviruses, existing in reservoir hosts in these geographically

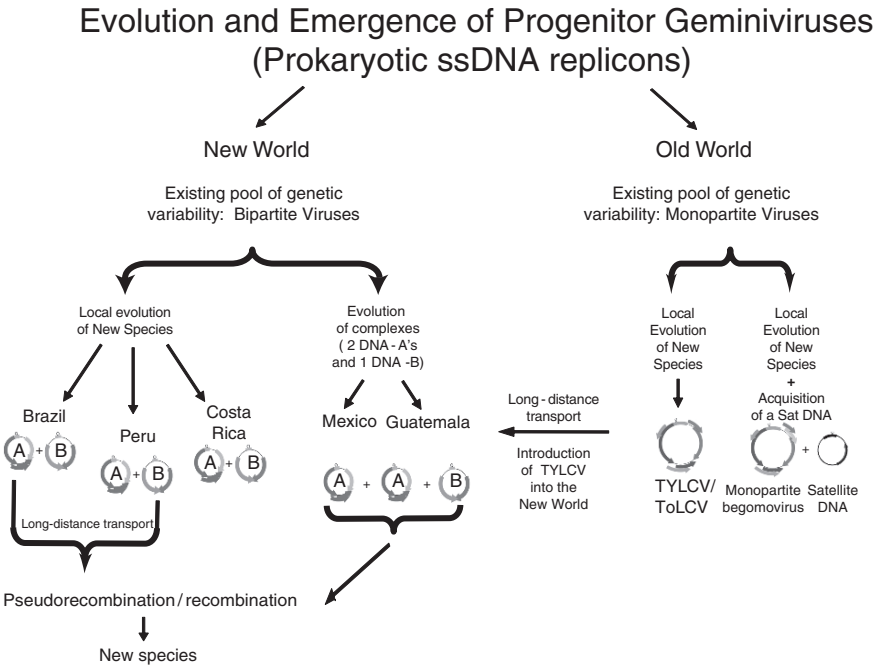

Fig. 3.1 Mechanisms of emergence of whitefly-transmitted begomoviruses (genus *Begomovirus*, family *Geminiviridae*). *TYLCV*, *Tomato yellow leaf curl virus*; *ToLCV*, *Tomato leaf curl virus*

distinct regions, into widely grown susceptible crops (e.g., common bean, cotton, cucurbits, peppers and tomato). This results in the simultaneous or parallel evolution of new crop-infecting begomovirus species in multiple locations. However, while the viruses are genetically diverse, the common mechanisms of begomovirus pathogenicity in these genetically uniform hosts results in the induction of similar disease symptoms.

A good example of this phenomenon is the disease bean golden mosaic. It has been established that two genetically distinct lineages of begomoviruses emerged in South America (Brazil) and Mesoamerica, each of which causes the disease bean golden mosaic (Gilbertson et al. 1993a). Evidence that viruses representing these two lineages are genetically distinct includes sequence divergence (approximately 80% identity), inability to generate infectious reassortants, and differences in sap transmissibility and germ plasm susceptibility. On the basis of these differences, viruses representing these lineages were named *Bean golden mosaic virus* (the initially described virus from Brazil) and *Bean golden yellow mosaic virus* (a cluster of related Mesoamerican viruses). This diversity can complicate breeding for disease resistance and deployment of resistant varieties (i.e., when a variety has resistance against only a subset of the viruses that cause the same or similar symptoms). A similar situation has since been described for begomoviruses associated with other diseases, including African cassava mosaic, cotton leaf curl and tomato leaf

curl (Varma and Malathi 2003). This situation has undoubtedly been facilitated by the emergence of the polyphagous *B. tabaci* biotype B, which feeds on a wide range of plants and, thus, would have a higher probability to acquire, mix and deliver a diversity of begomovirus components into potential new hosts.

3.4 Reassortment and Recombination: Effective Mechanisms of Variability for DNA Viruses

In terms of viral evolution, it is widely held that geminiviruses are at a disadvantage when compared with their RNA virus counterparts because of the lower frequency of mutations introduced during replication of the viral genome. However, it is now clear that geminiviruses have made up for this deficiency, if it exists, through reassortment and recombination (Padadim et al. 1999; Rojas et al. 2005). In the geminiviruses, recombination is facilitated by a type of rolling circle replication referred to as recombination-dependent replication, which favors recombination and generates a diversity of viral DNA forms (Preiss and Jeske 2003).

Initial evidence for recombination in bipartite begomoviruses came from studies in which deleterious mutations were repaired by replacement with wild-type DNA sequences from the same species (Roberts and Stanley 1994). It was further hypothesized that the bipartite genome might facilitate recombination between components, even among distinct viral species. However, initial studies showed a limited capacity for reassortment among components of begomoviral species, owing to the specificity of the replication-associated (Rep) protein for the cognate origin of replication [common region (CR) of the components of a bipartite begomovirus]. The formation of infectious reassortants between the cloned DNA components of closely related bipartite begomovirus species *Bean dwarf mosaic virus* (BDMV) and *Tomato mottle virus* (ToMoV) suggested that specificity of the Rep protein was less stringent than initially thought (Gilbertson et al. 1993b). Additional reports confirmed these findings, and revealed that reassortment was common among closely related begomoviruses; this even was reported to occur between distantly related bipartite begomoviruses (i.e., from different phylogenetic clades; Garrido-Ramirez et al. 2000).

Key insight into how these processes could mediate the emergence of new bipartite begomoviruses came from results showing that passage, through plants, of a less fit reassortant (ToMoV DNA-A/BDMV DNA-B) led to emergence of a more pathogenic virus through a recombination event that resulted in the exchange of the ToMoV DNA-A CR with that of the BDMV DNA-B component. This allowed the emergence of a fitter bipartite begomovirus, in large part due to the increased replication of the BDMV DNA-B (Hou and Gilbertson 1996). The question remained about the relative importance of these mechanisms in nature. Compelling evidence for a role of CR exchange in begomovirus evolution in nature came from a study of cassava-infecting begomoviruses in India. Here, a new bipartite begomovirus, *Sri Lankan cassava mosaic virus* (SLCMV), was identified that appeared to have emerged from a monopartite begomovirus (SLCMV) that acquired a DNA-B

component from another bipartite begomovirus (*Indian cassava mosaic virus*) by CR exchange (Saunders et al. 2002).

3.4.1 Recombination

TYLCV is clearly a virus that is well adapted to its primary host, tomato. While it is able to infect other plants, including certain crop plants (e.g., peppers and common bean), ornamentals (e.g., lisianthus) and weeds, these infections are often symptomless and associated with low viral titers (Salati et al. 2002). The virus either lacks a factor needed for effective colonization of these non-tomato hosts, or the hosts possess some defense factor(s) or response(s) that prevents efficient infection. If selective pressures are placed on TYLCV that influence the capacity of the virus to infect tomato (e.g., the introduction of a resistant variety or an extensive tomato-free period), the virus may respond by genetic variation, leading to the emergence of a new variant with a broader host range.

Indeed, sequence analyses of TYLCV isolates from around the world have revealed evidence of extensive recombination (Fauquet and Stanley 2003). The first isolate of TYLCV to be fully characterized was the previously mentioned TYLCV-IL (Navot et al. 1991). A second isolate from Israel, TYLCV-Mld[IL], was subsequently described; it was a recombinant, having a genome comprising approximately 75% TYLCV-IL and approximately 25% of an unknown begomovirus. The recombinant portion of the genome included the intergenic region and the 5′ end of the C1 ORF, which encodes the Rep protein. Subsequently, epidemics of TYLCD in southern Italy in the late 1980s were shown to be caused by a distinct begomovirus species (less than 80% identical to TYLCV), and it was named *Tomato yellow leaf curl Sardinia virus* (TYLCSV). The emergence of TYLCSV likely reflected an independent parallel evolution event mediated by increases in whitefly populations in southern Italy.

In 1992, TYLCD appeared in southern Spain and, consistent with geographic proximity to Italy, the causal agent was a strain of TYLCSV (TYLCSV-ES). In 1997, TYLCV-Mld[IL] was detected in southern Spain and, in 1999, a recombinant TYLCV was identified that was composed equally of TYLCSV-ES and TYLCV-Mld[IL] sequences. This recombinant, named TYLCMalV, was hypothesized to have emerged because of a selective advantage over the parental viruses in terms of a wider host range, more efficient whitefly transmission and being more infectious in TYLCV-resistant tomato varieties (Monci et al. 2002). Evidence for the selective advantage of this recombinant came from results of a study of the population structure of begomoviruses associated with TYLCD in southern Spain from 1999 to 2003 indicating that TYLCMalV had become established and had spread throughout this important tomato production region (Garcia-Andres et al. 2007). In addition, mixed infections of TYLCV were common, and a new type of recombinant, between the type strain of TYLCV and TYLCSV-ES, was detected. This clearly demonstrates how recombination can

generate novel begomovirus forms that can allow for adaptation and expansion into new environments, and that can compete with existing viruses.

3.4.2 Reemergence of Cassava Mosaic Disease in Africa: a Role for Reassortment and Recombination

Further evidence that these mechanisms play a key role in emergence of new begomoviruses has come from analysis of begomoviruses associated with the reemergence of cassava mosaic disease (CMD) in Africa. This disease has long been known on the African continent, and causes significant economic losses to production of this staple crop. Multiple begomovirus species can cause CMD, and these likely arose via parallel evolution. On the basis of the biology of these viruses and the nature of cassava cultivation, management strategies were implemented, including use of virus-free propagative material, deployment of resistant varieties and roguing of infected plants. This approach seemed to keep the disease at manageable levels (Legg 1999).

However, in the 1980s the incidence and severity of CMD increased markedly in East Africa (Legg 1999; Legg and Fauquet 2004). This was associated with the emergence of highly virulent forms via reassortment and recombination. For example, recombination between *East African cassava mosaic virus* (EACMV) and *African cassava mosaic virus* (ACMV), in which capsid protein (CP) gene sequences of ACMV were exchanged with homologous sequences in EACMV, has given rise to a highly virulent recombinant (EACMV-UG2) that has been implicated in these disease outbreaks. In addition, reassortment between other recombinant EACMV components has led to the emergence of other highly virulent forms in other parts of southeast Africa (Pita et al. 2001). Together with increases in whitefly populations on cassava, these emerging viruses pose a major threat to cassava production on the African continent.

3.5 Tripartite Begomovirus Complexes: A Way for Bipartite Begomoviruses To Fight Host Defense Responses?

The bipartite begomovirus genome has evolved to allow for efficient replication and spread of a single-stranded DNA replicon in plants, in addition to providing opportunities for viral variability (Rojas et al. 2005). Thus, attempts to generate viable viruses having dramatically altered genome size, or with more than two components, have generally been unsuccessful. However, there is increasing evidence that complexes of more than two bipartita DNA components have emerged as primary causal agents of diseases such as chino del tomate in Mexico, leaf curl of tomato in Guatemala and African cassava mosaic in Uganda (Fig. 3.1). Because such complexes are typically not viable, there must be a selection pressure to generate and maintain these specific complexes.

Evidence for the existence of this type of synergistic interaction among bipartite begomovirus components came from the observation that the tripartite combination of *Pepper huasteco yellow vein virus* (PHYVV) DNA-A and DNA-B and *Pepper golden mosaic virus* (PepGMV) DNA-A induced strikingly more severe disease symptoms in *Nicotiana benthamiana*, tomato and pepper plants compared with symptoms induced by PHYVV alone (Sharp et al. 1999). The specificity of this interaction was demonstrated by the consistent detection of all three components in plants with the severe symptom phenotype, and the inability to form such a complex with the DNA-A component of another bipartite tomato-infecting begomovirus from Mexico. Furthermore, this complex was detected in the field in Mexico, consistent with its emergence and transmission by *B. tabaci* (Mendez-Lozano et al. 2003). While the mechanism underlying the synergism among these begomovirus components is not known, the CP was not involved because the synergism was not altered with a PepGMV DNA-A CP mutant.

Possible insight into the mechanism underlying these synergistic interactions comes from analyses of complexes associated with CMD in Uganda (Pita et al. 2001). Here, evidence has been provided that the product of the AC4 ORF can function as a suppressor of gene silencing, and that the relative effectiveness of the suppression varies among DNA-A components. Thus, the selection pressure to maintain more than one DNA-A component may well reflect the need to have a compliment of AC4 suppressors, thereby allowing for effective suppression of this powerful antiviral defense response (Vanitharani et al. 2005).

3.6 Acquisition of Novel Viruslike Entities: Monopartite Begomoviruses and their Satellite DNAs

Old World monopartite begomoviruses can be associated with a DNA satellite or "extrachromosomal" DNA. In studies of the etiology of a number of Old World geminivirus diseases [e.g., Ageratum yellow vein in *Ageratum conyzoides* (Singapore), Bhendi yellow vein mosaic in okra (India), cotton leaf curl in cotton (Pakistan), and tomato and tobacco leaf curl in tomato and tobacco, respectively (China)], a single begomovirus DNA component was detected, suggesting the disease was caused by a monopartite begomovirus (as had been shown for TYLCV). However, when introduced back into the natural hosts, the cloned DNA component was infectious but induced symptoms that were much milder than those observed in plants in the field (Mansoor et al. 2003). This puzzling observation was explained following the identification of sub-virus-sized satellite DNAs (satDNAs) associated with these diseases. When these satDNAs were introduced into plants, together with their cognate monopartite begomovirus, the characteristic disease symptoms were induced. These satDNAs, also referred to as DNA-βs, are approximately 1.4 kb (about half the size of the begomovirus genome) and require the helper begomovirus for replication and movement. The satDNAs share no sequence similarity with the helper virus except a stem-loop structure (presumably the origin of replication),

and have a single ORF (βC1). The βC1 protein is a symptom determinant, as an intact *βC1* gene is required for satDNA to mediate symptom development, and expression of βC1 in transgenic plants results in a symptomatic phenotype (Saunders et al. 2004; Saeed et al. 2005).

What is emerging from studies of Old World begomovirus-associated diseases is that most of these diseases are complexes of a monopartite begomovirus and a satDNA (Fig. 3.1). This conclusion is further supported by the identification of tremendous diversity of DNA-β satDNAs (more than 130 sequences in the GenBank) from a range of geographic locations (e.g., Africa, China, India, Indonesia, and Pakistan; Briddon et al. 2003). This level of diversity is consistent with the idea that this "unholy alliance" was a relatively ancient event, which has facilitated the emergence of monopartite begomoviruses as important pathogens of a range of crop plants in the Old World (Rojas et al. 2005). It is not clear where the satDNA originated from, but it has been suggested that it came from another, yet to be characterized, type of single-stranded extrachromosomal DNA (Mansoor et al. 2003).

The acquisition of the satDNA may be analogous to the acquisition of the DNA-B component, which facilitated the emergence of the bipartite begomoviruses in the New World. However, while it is well established that the bipartite begomovirus DNA-B component encodes two proteins required for movement (Rojas et al. 2005), the function of the satDNA is less clear. One key function may be the suppression of host defenses, such as gene silencing. Consistent with this hypothesis, functional analyses of the *βC1* gene of some satDNAs have revealed that it is a suppressor of gene silencing (Cui et al. 2005; Kon et al. 2007). This may be an important function, as gene silencing has been shown to target begomoviruses (Bisaro 2006; Rojas et al. 2005; Vanitharani et al. 2005). However, it is also possible that the satDNA and the βC1 may be involved in other functions, such as movement.

An important characteristic of these satDNAs that differentiates them from the bipartite begomovirus DNA-B component is their capacity to be maintained (replicated, moved and encapsidated) by multiple monopartite begomoviruses (Mansoor et al. 2003). This promiscuity has revealed more flexibility in Rep-protein-mediated replication than had been previously thought. Moreover, the capacity to be replicated by multiple begomoviruses means that satDNAs can form complexes with multiple monopartite begomoviruses, thereby allowing these viruses to increase their incidence and/or host range. Thus, it is clear that there is a strong selective advantage for monopartite begomoviruses to "partner" with a satDNA, and that this has had a major impact on the emergence of the begomoviruses in the Old World.

Symptoms induced by RNA viruses also can be moderated by satellite RNAs (satRNAs; Simon et al. 2004), such as the satRNAs associated with *Cucumber mosaic virus* (CMV). Like the begomovirus satDNA (e.g., DNA-β), the CMV satRNAs require the helper virus (CMV) for replication, and have little sequence similarity with the helper virus. In contrast to the begomovirus satDNA, the CMV satRNA are linear single-stranded RNAs, apparently do not encode for any proteins, and usually attenuate symptoms induced by the helper virus (Simon et al. 2004). However, certain CMV satRNAs increase disease symptoms, inducing either necrosis or chlorosis. The induction of necrosis has been shown to be associated with the initiation of programmed cell

death mediated by the satRNA minus-strand (Simon et al. 2004). The origins of begomovirus satDNAs and CMV satRNAs remain a mystery; however, whereas the begomovirus satDNAs are commonly found in nature, the CMV satRNAs tend to be found in experimental systems. Thus, the begomovirus/satDNA combination is a synergistic interaction, whereas the CMV/satRNA interaction seems to reflect a situation where the satRNA is a parasite of the virus.

3.7 Emergence of Diseases Caused by Novel Viruslike Agents

Though many emerging plant virus diseases are associated with previously characterized viruses or viruslike agents, some are caused by novel agents. For example, necrosis-associated diseases of tomato in Mexico [*marchitez manchada* (Sinoloa spotted wilt)], Spain [*torrado* (burned or roasted) disease; Verbeek et al. 2007] and Guatemala [*mancha de chocolate* (chocolate spot)] have emerged and appear to be caused by a novel virus or viruses. The symptoms of these diseases appear similar to those induced by the tospovirus TSWV; however, tests for known tomato-infecting viruses have given negative results. The diseases are also graft- and sap-transmissible, consistent with a viral etiology. It was reported recently that the torrado disease is caused by a novel plant picorna-like virus, most closely related to single-stranded RNA viruses in the genera *Sequivirus*, *Sadwavirus* and *Cheravirus*. The name proposed for this virus is Tomato torrado virus (Verbeek et al. 2007). It is not clear whether the necrosis-associated diseases in Guatemala and Mexico are caused by Tomato torrado virus or some other virus. It is also not clear what the vector(s) of these agents is. If these diseases turn out to be caused by the same or closely related viruses, their recent emergence in New and Old World locations may suggest a long-distance transport mechanism, e.g., in association with seeds or propagative materials.

3.8 Multiple Mechanisms often Underlie the Emergence of Plant Viruses

3.8.1 Long-Distance Movement and an Emergent Vector: Cucurbit yellow stunting disorder virus

The criniviruses represent a group of viruses that have emerged over the past 10–20 years in association with the worldwide emergence of whiteflies (Wisler et al. 1998). Another factor that has contributed to the emergence of these viruses is the recognition of their association with yellowing-type symptoms that were previously attributed to nutrient deficiencies. *Cucurbit yellow stunting disorder virus* (CYSDV) is a crinivirus that induces a striking interveinal yellowing of various cucurbits (cucumbers and melons) and is transmitted by *B. tabaci* (Celix et al. 1996; Wisler

et al. 1998). The virus emerged as a serious constraint on cucurbit production in the Middle East and Mediterranean regions in the early 1990s, and this was associated with the displacement of the greenhouse whitefly (*Trialeurodes vaporariorum*) by *B. tabaci*. In 2000, CYSDV was reported in Texas and subsequently in Guatemala. In 2006, a significant outbreak of CYSDV occurred in Arizona, California and northern Mexico (Kuo et al. 2007). Thus, CYSDV represents another example of an Old World virus being introduced into the New World. Moreover, as criniviruses are not seed-transmitted and CYSDV infects annual host plants, the virus was likely introduced via infected plants or viruliferous whiteflies carried on plants (hosts or nonhosts of CYSDV). Analyses of genetic diversity among CYSDV strains from different regions, as well as from a single region over an 8-year period, revealed a relatively homogenous population (Marco and Aranda 2005). Thus, CYSDV probably has emerged via long-distance transport followed by local spread mediated by existing *B. tabaci* populations.

3.8.2 Mutation, Recombination and Long-Distance Movement: Plum pox virus

Sharka disease, caused by the potyvirus *Plum pox virus* (PPV), is one of the most damaging diseases of *Prunus* spp. (e.g., peach, apricot, nectarine, plum and sweet and sour cherry). Prior to the 1990s, it was a disease exclusively found in the Old World (e.g., Europe and Asia); however, in 1994 it was detected in Chile and in 2000 it was detected in Canada and the USA (Candresse and Cambra 2006). Despite extensive quarantine efforts, this long-distance movement likely occurred through movement of infected propagative materials, with subsequent spread via aphids. Genetic analyses of PPV isolates, from a diversity of hosts and locations, have revealed extensive genetic variability, with at least six subgroups (strains or serotypes) recognized. Some of this diversity can undoubtedly be attributed to random mutation, mediated by the lack of proofreading activity of the viral replicase; however, at least one of these subgroups, PPV-Rec, emerged via recombination events between isolates of other subgroups (e.g., PPV-D and PPV-M; James and Glasa 2006). Extensive efforts are under way to eradicate this emergent virus from Canada and the USA as well as to develop resistant varieties of *Prunus* spp.

3.9 Bringing Them All Together: Tomato Yellow Leaf Curl/Leaf Curl Disease in West Africa

Tomato yellow leaf curl disease (TYLCD) and tomato leaf curl disease (ToLCD) have emerged as major constraints on tomato production in West Africa, including countries such as Mali, Benin, Burkina Faso and Senegal. The symptoms in infected plants include stunted and distorted growth, and varying degrees of leaf

curl and crumple, chlorosis and purpling. Recent investigation of these diseases has revealed a complex etiology, involving emergence of multiple new begomovirus species, a recombinant virus and a satDNA. Thus, three new monopartite begomovirus species have been associated with this epidemic: *Tomato leaf curl Mali virus* (ToLCMLV), *Tomato yellow leaf curl Mali virus* (TYLCMLV) and *Tomato yellow leaf crumple virus* (ToYLCrV). Analysis of the complete sequence of an infectious TYLCMLV clone revealed it was a recombinant virus, with the genome comprising approximately 80% TYLCV-IL, with approximately 20% from an uncharacterized begomovirus. The recombinant region of the genome included the intergenic region (up to the nicking site in the origin of replication) and the 5′ end of the C1 ORF, which encodes the Rep protein. In addition, a novel approximately 1.4 kb satDNA was cloned from TYLCMLV-infected plants and, when coinoculated with TYLCMLV, it increased symptom severity in tomato, common bean and *N. glutinosa*. With use of PCR and virus-specific primers, it was established that tomatoes with severe stunting and distortion symptoms in West Africa were associated with mixed begomovirus infection. Thus, the TYLCD/ToLCD epidemic in West Africa is caused by a complex of locally emergent begomoviruses, a satDNA and perhaps a progenitor virus (TYLCV) that was previously introduced into West Africa. Management of this disease complex will be challenging and will likely require an integrated approach (Rojas et al. 2005).

3.10 Emergence of a New Virus is not always Catastrophic: Failure of New Viral Diseases to Emerge Following a Major Change in the Whitefly Vector Population

3.10.1 A History of Whitefly-Transmitted Viruses in the Imperial Valley of California

While the emergence of whitefly-transmitted begomoviruses has led to the appearance of diseases that have caused significant economic losses to a wide range of crop plants throughout the world, this is not always the case. The Imperial Valley of California is an irrigated desert agricultural production area in southern California where a variety of vegetable and forage crops are grown, including alfalfa, carrots, cotton, melons and onions. This area historically has sustained significant populations of sweet potato whiteflies (*B. tabaci* biotype A). Two species of whitefly-transmitted bipartite begomoviruses, *Squash leaf curl virus* (SLCV) and *Cotton leaf crumple virus* (CLCrV) have also been described from this area; however, these viruses (or the diseases they cause) have been known for decades (since the early 1980s for SLCV and since the 1940s for cotton leaf crumple disease) and have not been a major economic problem. Thus, these viruses would not be classified as emergent.

However, in the early 1980s, a new whitefly-transmitted virus emerged that caused a severe yellows disease of lettuce and cucurbits. This virus was identified as closterovirus-like and, subsequently, it was shown to be a novel bipartite closterovirus. The virus was named *Lettuce infectious yellows virus* (LIYV), and it is the type member of the genus *Crinivirus* in the family *Closteroviridae* (Wisler et al. 1998). This emergent virus caused significant economic losses to lettuce and melon production in the Imperial Valley, and it was feared that LIYV could be a limiting factor for production of these crops.

In the early 1990s, this situation in the Imperial Valley changed with the introduction of the B biotype of *B. tabaci* (silverleaf whitefly). Massive outbreaks of whiteflies followed the introduction of this exotic insect (Perring et al. 1991; Toscano et al. 1998), and the B biotype quickly displaced the indigenous A biotype. These outbreaks resulted in crop losses owing to the sheer magnitude of the whitefly populations and their feeding-induced physiological abnormalities (e.g., irregular ripening of tomato and silverleaf of squash). Another big concern was that the insects would increase the incidence/severity of existing viruses and/or facilitate the emergence of new virus diseases. Unexpectedly, the incidence of LIYV decreased dramatically, and the infectious yellows disease essentially disappeared from the Imperial Valley. Whitefly transmission experiments, conducted with *B. tabaci* biotypes A and B provided the explanation: *B. tabaci* biotype B was an inefficient vector of LIYV. Thus, here is a rather unusual situation where introduction of an exotic insect has led to the reduction of an economically important emergent virus. Indeed, it appears that LIYV may well have become extinct in the Imperial Valley!

The B biotype is a known vector of begomoviruses, although it is generally a less efficient vector than the A biotype. However, the apparent reduced vectoring efficiency is compensated for by the biotype B feeding on a wider range of host plants and having a higher rate of reproduction. Thus, it was feared that the high populations of biotype B whiteflies would lead to the emergence of new begomoviruses. This could occur by introducing variants of indigenous weed-infecting begomoviruses into crop plants or mixtures of begomoviral components, resulting in the evolution of a new emergent crop-infecting virus via reassortment and recombination. This latter scenario has been proposed to explain the evolution of CLCrV (Idris and Brown 2004; Seo et al. 2006). However, this scenario has yet to unfold in the Imperial Valley, almost 20 years since the introduction of biotype B. Thus, the incidence of SLCV and CLCrV did not change significantly, nor did new crop-infecting begomoviruses emerge to threaten agricultural production. The fact that SLCV and CLCrV still appear, to some extent, every year, taken together with the subsequent introduction of CYSDV and TYLCV, indicates that the failure to observe the emergence of new begomoviruses was not due to an inability of the B biotype whiteflies to transmit viruses. More likely this reflects a limited genetic pool of indigenous viruses in this desert region (as compared with a tropical agroecosystem), or perhaps a lack of extensive cultivation of highly susceptible begomovirus hosts (e.g., tomato, pepper and common bean). Thus, the Imperial Valley agroecosystem

appeared to lack a component necessary for the rapid and widespread emergence of new begomoviruses.

3.10.2 Emergence of CuLCrV in the Imperial Valley

In the fall of 1998, when watermelon volunteers at the edge of the agricultural production area of the Imperial Valley showed symptoms of geminivirus infection, it was thought to be an outbreak of SLCV. However, while tests confirmed begomovirus infection in these plants, sequence analysis of the PCR-amplified fragments of the virus genome revealed only approximately 85% sequence identity with SLCV, the most closely related previously characterized begomovirus. Subsequent cloning and sequencing of the DNA-A and DNA-B components of this begomovirus established that it was a new species, and it was named *Cucurbit leaf crumple virus* (CuLCrV; Guzman et al. 2000). Phylogenetic analyses confirmed that CuLCrV was a distinct begomovirus species in the SLCV cluster of New World bipartite begomoviruses. The close relationship of CuLCrV and SLCV was further demonstrated by the finding that infectious reassortants could be generated between the cloned DNA components of these viruses (Brown et al. 2002). Thus, while the source of CuLCrV remains unknown, it is thought to have evolved from a progenitor begomovirus infecting an indigenous host plant that is distributed outside the cultivated lands of the Imperial Valley. A similar progenitor virus may have given rise to SLCV over 20 years earlier. This hypothesis could be tested by surveying weeds and other indigenous hosts (symptomatic and symptomless) for the presence of begomovirus infection by PCR with degenerate begomovirus primers (Rojas et al. 2005).

In the years following its identification, CuLCrV emerged as the cause of leaf crumpling, curling and chlorosis symptoms in cantaloupe, watermelon and squash in the Imperial and Coachella Valleys of California, southern Arizona and northern Mexico. Therefore, CuLCrV represents the first emergent begomovirus that has appeared in the desert southwest following the outbreaks of whitefly biotype B. In the case of squash, the virus caused in the Coachella valley economic losses. However, observation of CuLCr disease development in cantaloupe and watermelon revealed that, while severe symptoms initially developed in infected plants, symptoms eventually became markedly attenuated as the plants continued to grow. Eventually, plants showed few or no symptoms and fields with affected plants provided acceptable yields. This “recovery from infection” occurred in cantaloupe and watermelon, but not in squash or pumpkin. It also was consistently reproduced in the laboratory. Analysis of recovered tissue revealed a considerable reduction of viral titer compared with nonrecovered symptomatic tissues, high rates of methylation of viral DNA and the presence of small CuLCrV-derived RNAs. Furthermore, reinoculation of recovered tissue with CuLCrV failed to result in symptom development, whereas inoculation of recovered tissues with CMV led to the return of severe symptoms and increased CuLCrV titers. This latter result suggests that the

2b silencing suppressor of CMV may have interfered with the host defense mechanism that attenuated symptoms of CuLCrV. Taken together, these results indicate that cantaloupe and watermelon stage a vigorous defense response against CuLCrV infection involving virus-induced gene silencing. Thus, though CuLCrV is a new emergent virus, the major economic hosts of the virus in the Imperial Valley mount a vigorous defense response, limiting the economic importance of the virus.

3.11 Conclusions

Over the past 10–20 years, groups of plant viruses as well as individual viruses have emerged as significant constraints on crop production worldwide. This emergence can involve a range of mechanisms (Table 3.1), depending on the virus(es) involved and the environment. Existing viruses can be moved long distances via human activities, allowing for subsequent establishment and spread in compatible agroecosystems. Irrespective of whether the virus has a DNA or an RNA genome, mechanisms of variability such as mutation, reassortment and recombination allow for the generation of new forms of existing viruses that have the potential to emerge as important pathogens. This is facilitated by new selection pressures placed on the viral population, such as those associated with modifications of existing agroecosystems. However, the rate at which such variants are generated and their economic impact is a function of multiple factors (efficiency of vector transmission, the existing pool of viral genetic variability in a region, nature of the host, aspects of the agroecosystem, etc.). Novel viruses or viruslike entities can also emerge, and these often appear at interfaces of agricultural and undeveloped lands. In the case of plant viruses, emergence of variants of known viruses or novel agents is greatly facilitated by increases or emergence of insect vector populations. Entire groups of viruses (e.g., the begomoviruses, criniviruses and tospoviruses) have emerged following the worldwide emergence of their insect vectors. Moreover, given the continued global movement of plant materials and seeds, expansion of agriculture into new areas, and the propensity of large-scale commercial agriculture to favor development of large populations of insect pests, it is highly likely that new plant viruses will continue to emerge.

The challenge in dealing with emergence of new viruses is significant, but it has been lessened by improvements in technology and increased understanding of viral genetic diversity, ecology and genetics. Thus, with new detection technologies (PCR and sequencing and microarray-based technologies), potentially new emergent viruses can be identified sooner. However, identification of novel viruses can still be very challenging, and often requires a combination of new technology and innovative approaches. Once an emerging plant virus has been identified, effective detection tools need to be developed and applied to answer questions about the biology of the virus. This information can then be used to develop effective management strategies. Ideally, multiple approaches will lead to development of an

integrated pest management (IPM) approach. Such an approach has led to the effective management of the emergent TYLCV in the Dominican Republic, and tomato production has actually increased since the introduction of the virus (Salati et al. 2002). Similar management approaches are now being brought to bear on begomovirus diseases of tomato in West Africa and Central America. Thus, it is hoped that the use of IPM strategies, tailor-made for emergent viruses based on understanding of the biology of the virus, will minimize the impact of these viruses on world food production.

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