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Sources of Genetic Variation in Plant Virus Populations

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Plant viruses are the cause of important diseases of crop plants; a vast majority of plant viruses have single-stranded RNA genome and variation is an intrinsic property of living entities. Various mechanisms that lead to variation in virus population are mutation, addition, deletion, inversion of nucleic acid base sequences, recombination, suppression, re-assortment and mixed infection. Most reported work had a phylogenetic or taxonomic goal, and attempts to quantify the variability of virus populations. Thus evolutionary biology of plant virus populations is an exciting area of research, both intellectually challenging and relevant to everyday life.

Key words, Variation, Virus evolution, Population structure.

Plant viruses are the cause of important diseases of crop plants, which result in diminished production and may even compromise food supply in large areas. A vast majority of plant viruses have single-stranded RNA genomes, and since the late 1970s work with RNA viruses that infect bacteria and mammals has shown the large potential for variation of RNA genomes[1].

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Variation is an intrinsic property of living entities. An individual that differ genetically from their parents are generated by mutation due to addition, deletion, inversion of nucleic acid base sequences, recombination, suppression, re-assortment and mixed infection lead to evolution of different strains and new viruses. The distribution of genetic variants in the population of an organism may change with time, in the process called evolution.

Virus Evolution

The constant change of a viral population in face of selective pressures is the definition of virus evolution. The new science (or art) of virus molecular systematics is, however, shedding a great deal of light on the distant relationships of, and in some cases on the presumed origins of many important groups of viruses.

Geminiviridae, are a diverse group of viruses - with different genera having different numbers of genes and genome components - that presumably have a common origin. If one takes into account geographical diversity, and genetic divergence of vectors and of plant hosts.

Sources of genetic variation

Mutation

In TMV, more than 400 strains were reported due to mutation[2]. The rate of spontaneous mutation is a key evolutionary parameter, yet accurate estimations are available only for a handful of organisms. One of the most widely accepted differences in mutation rates is the one between RNA viruses and dsDNA organisms, the former mutating orders of magnitude more frequently than the latter. Such elevated mutation rates, though not necessarily evolved in response to natural selection, can explain why RNA virus show rapid evolution at the molecular level, although other factors such as replication speed, transmission mode or genomic architecture have to be considered as well[3-5].

High genetic stability has frequently been reported for plant RNA viruses, although substitution rates within the range of animal RNA viruses have also been reported[6-10]. This peculiar behavior might be due to stronger selective constraints acting on plant viruses, weaker immune mediated positive selection, the existence of strong bottlenecks during cell-to-cell movement and systemic colonization of distal tissues Another, more obvious, possibility is that plant viruses show reduced rates of spontaneous mutation[11-13].

Spontaneous Virus Mutants

Spontaneous mutations occur in the absence of known mutagens. There are 16 spontaneous mutants reported in TMV among them six had one Amino acid exchange in the coat protein, one had two amino acids exchange in the coat protein and one had three amino acids exchange in the coat protein.

Induced Mutations

Induced Mutations results from treatment with known mutagen. Chemical mutagens can be divided into two types. Those may be *in vitro or in vivo* mutagens.

The high efficiency of nitrous acid as an in vitro mutagen for TMV. (Gierer and mundry, 1958). By incorporating 5-fluorouracil, it replaces the uracil residues in the RNA and can leads to changes uracil to cytosine and adenine to guanine because it is analogue of uracil.

Evolution by Mutations

RNA viruses are replicated with less fidelity than DNA viruses, thus there are more mutations occurring in RNA viruses. This is due to the absence of fidelity of the RNA polymerase, which doesn't have a proof reading function as in DNA polymerase). One mis-incorporation in 10^{-4} or 10^{-5} nucleotides replicated for RNA viruses. However, this is 300-fold less for DNA viruses (Table 1).

Table 1. Intra-population nucleotide diversities in some plant viruses

Virus	Diversity value	Estimated from
PMMV	0.018	Rnase T1 fragment analysis
TMGMV		
Spain	0.022	Rnase T1 fragment analysis
Spain	0.020	Sequence analysis
Australia	0.022	Sequence analysis
World	0.057	Sequence analysis
WSMV	0.031	RFLP analysis
CMV-satRNA	0.064	RPA analysis
BCTV		
CHF	0.026	RFLP analysis
Worland	0.021	RFLP analysis
CLCuV		
CP	0.019	RFLP analysis
AC1	0.024	RFLP analysis

a) From[14]

b) PMMV = Pepper mild mottle virus, TMGMV = Tobacco mild green mosiac virus, WSMV = wheat streak mosaic virus, CMV-satRNA = satellite RNA of cucumber mosiac virus, BCTV = Beet curly top virus, CLCuV = Cotton leaf curl virus.

Suppression

Suppression is the inhibition of a mutant phenotype by a second suppressor mutation. Genetic suppression results in an apparently wild-type phenotype from a virus which is still genetically mutant - a pseudorevertant.

Reversion

Mutant viruses can therefore appear to revert to their original phenotype by three pathways: Back mutation of the original mutation to give a wild-type genotype/phenotype (true reversion), compensatory mutation may occur in the same gene as the original mutation correcting it.

Recombination in viruses

Plant RNA virus recombination was first demonstrated for Brome Mosaic Virus (BMV)[15]. Mutation within helicase resulted in an increase in frequency of recombination and distribution of cross over sites.[16]

Forms of recombination in viruses

Homologous recombination: It occurs between base sequences that are same or very similar at cross over point. Non homologous recombination: it occurs at a site where there is micro homology or no obvious homology. Recombination is the physical interaction of virus genomes during super infection resulting in gene combinations not present in either parent. There are three mechanisms by which the recombination can occur, depending on the organization of the virus genome (Fig. 1).

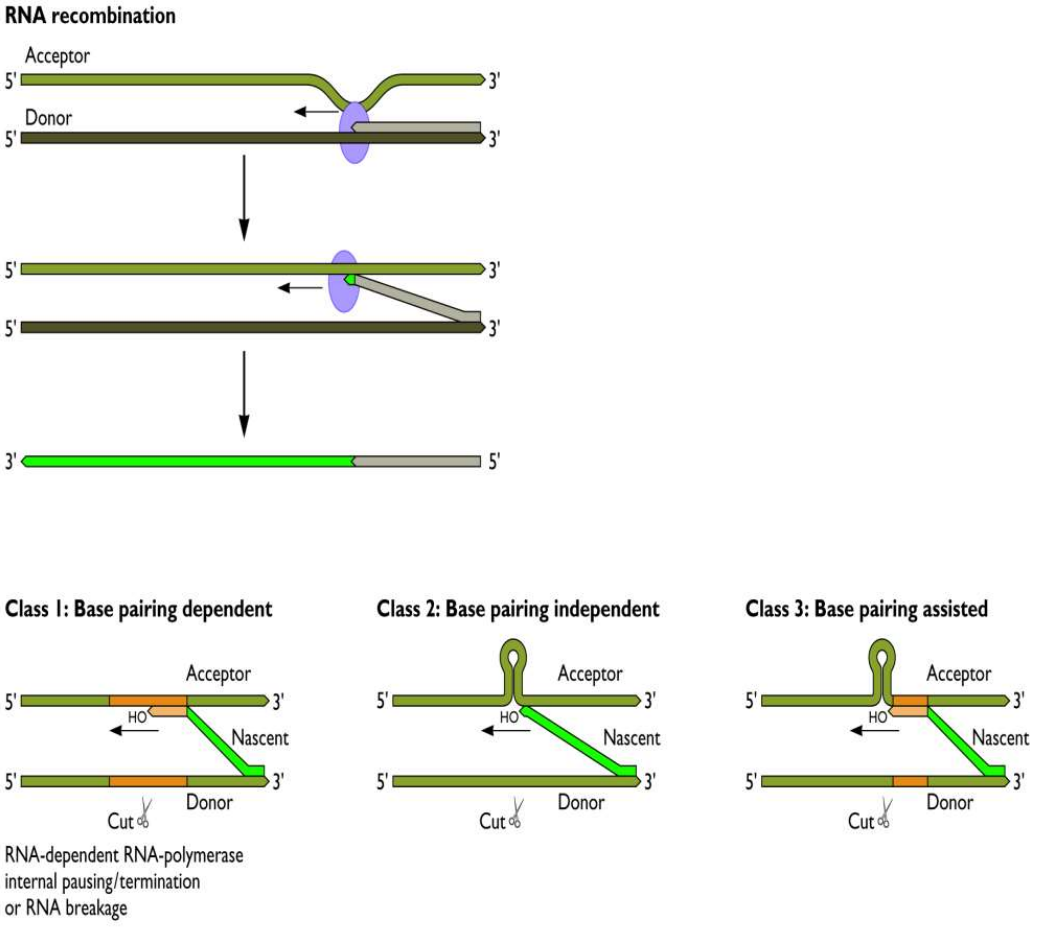

Fig. 1. Mechanisms of recombination of viral RNAs

Intramolecular recombination by strand breakage & re-ligation

This process occurs in all DNA viruses & RNA viruses which replicate via a DNA intermediate. Believed to be mediated by cellular enzymes, since no virus mutants with specific recombination defects have been isolated.

Intramolecular recombination by 'copy-choice' process occurs in RNA viruses (it has been known in picornaviruses since the 1960s, In intramolecular recombination, the probability that breakage-reunion or strand-switching will occur between two markers (resulting in recombination) is proportional to the physical distance between them.

Recombination in RNA viruses

It was first demonstrated on BMV[15]. Bromovirus contains three divided +ve strand RNA genomes. RNA1 and 2 participates in viral replication and transcription, and RNA 3 necessary for cell to cell movement and production of coat protein. Formation of heteroduplex between two RNA could induce non homologous recombination and it must be more than 30nt. Recombination mechanism is favored by template switching by viral replicase and to induce the replicase to jump from one to another. Mutations in replicase would affect the frequency of recombination in the recombination sites. Mutations within the helicase resulted in an increase in frequency of recombination and distribution of crossover sites. Mutation in replicase decreased the frequency of recombination[16].

Recombination in DNA viruses

In theory, a cell's nuclear membrane guards its contents by barring access to potential foes. In reality, pathogens employ a diverse bag of tricks to circumvent this barrier. The murine leukemia virus (a retrovirus), for example, waits until the nuclear membrane degrades during cell division. Other retroviruses, like HIV and so-called pararetroviruses, enlist protein escorts that help them slip through undetected. Pararetroviruses include both animal viruses, such as hepatitis B, and plant viruses, such as the cauliflower mosaic virus (CaMV). Once inside the nucleus, the double-stranded DNA genome of the CaMV is transcribed into an RNA transcript (called 35S RNA), thanks to the activity of the 35S promoter. This CaMV promoter is widely used to drive transgenic expression in plants.

Replication proceeds through reverse transcription as a viral enzyme reverse transcribes the 35S RNA into genomic DNA that is then packaged into viral particles. During replication, genetic material can pass between different viral genomes when two viral particles infect the same host cell. These exchanges can create novel viruses. But with little data on viral recombination rates in multicellular organisms, it's unclear how these recombinant viral genomes are influencing host infection. In a new study, the cauliflower mosaic viral infection in one of its natural hosts, the turnip plant (*Brassica rapa*), to measure the frequency of viral recombination. Recombination was evident in over half of the recovered viral genomes, suggesting that recombination is routine for this plant virus. And since recombination events are linked to both expanded viral infection and increased virulence, understanding the rate of recombination could help shed light on mechanisms underlying the evolution and pathology of a virus—insight that could prove critical for developing methods to inhibit or contain an infection[17].

Reassortment

In viruses with segmented genomes, the genome segments can be randomly shuffled during super infection. Progeny viruses receive (at least) one of each of the genome segments, but probably not from a single parent, e.g. influenza virus has eight genome segments, therefore in a mixed infection, there could be 28 = 256 possible progeny viruses. In reassortment, the frequency of recombination between two markers is either very high (indicating that the markers are on two different genome segments) or comparatively low (which means that they are on the same segment). Reassortment of genome segments occurs between genotypes within a tospovirus species. Reassortment may have altered properties e. g. responses to plant resistance, thrips transmission properties.

Mixed virus assembly

Mixed infection between RNA of one strain of virus and the coat protein of another strain of virus can be seen. Protoplasts were inoculated with TMV together with ToMV. Some of the individual rods were coated with a mixture

of two coat proteins[18]. Assembly between the RNA of one strain of a virus and the coat protein of another strain of virus. Between RNA and protein from unrelated viruses[19]. Mixed particles are called pseudotypes absent in enveloped plant viruses but present in Rhabdoviridae.

Phenotypic mixing

Protein coat consist of a mixture of proteins from the two viruses. Protoplast was inoculated with TMV together with ToMV some of the individual rods were coated with a mixture of the two coat proteins[18]. Tobacco leaves doubly infected with TMV+PVX, PVY+PVX, no assembly of one the viral RNA in coat protein of another could be detected). It may be due to closely related strains of virus replicated in same region of the cell. Different viruses may be assembled from components accumulated in separate site in the same cell so such separation may not always be complete. Efficient and specific virus assembly favored by localization of RNA and protein sub units in compartments within the cell.

CONCLUSION

The heterogeneous nature of plant virus population has been evident since the early 1920s and 1930s. This evidence was mostly overlooked until the past two decades when techniques became available that allowed the rapid typing of viral genomes and the spread, from animal virology, of the quasispecies concept of virus populations. The new awareness of the genetic variability of plant viruses has resulted in the appearance in the past 15 years of numerous analyses of virus variation through the molecular characterization of isolates, mainly by the determination of nucleotide sequence. Most reported work had a phylogenetic or taxonomic goal, and attempts to quantify the variability of virus populations, or to characterize their genetic structure, are comparatively scarce. Attention has been given by a few plant virologists to the evolution of virus populations and showed that the evolutionary biology of plant virus populations is an exciting area of research, both intellectually challenging and relevant to everyday life.

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