

Viruses nature, architecture, multiplication and transmission.

General Concepts

Structure and Function

Viruses are small obligate intracellular parasites, which by definition contain either a RNA or DNA genome surrounded by a protective, virus-coded protein coat. Viruses may be viewed as mobile genetic elements, most probably of cellular origin and characterized by a long co-evolution of virus and host. For propagation viruses depend on specialized host cells supplying the complex metabolic and biosynthetic machinery of eukaryotic or prokaryotic cells. A complete virus particle is called a virion. The main function of the virion is to deliver its DNA or RNA genome into the host cell so that the genome can be expressed (transcribed and translated) by the host cell. The viral genome, often with associated basic proteins, is packaged inside a symmetric protein capsid. The nucleic acid-associated protein, called nucleoprotein, together with the genome, forms the nucleocapsid. In enveloped viruses, the nucleocapsid is surrounded by a lipid bilayer derived from the modified host cell membrane and studded with an outer layer of virus envelope glycoproteins.

Morphology

Plant viruses come in different shapes and sizes. Nearly half of them are elongate (rigid rods or flexuous threads), and almost as many are spherical (isometric or polyhedral), with the remaining being cylindrical bacillus-like rods (Figs. 14-4 and 14-5). Some elongated viruses are rigid rods about 15 by 300 nanometers, but most appear as long, thin, flexible threads that are usually 10 to 13 nanometers wide and range in length from 480 to 2,000 nanometers. Rhabdoviruses are short, bacilluslike, cylindrical rods, approximately three to five times as long as they are wide (52–75 by 300–380nm). Most spherical viruses are actually polyhedral, ranging in diameter from about 17 nanometers (tobacco necrosis satellite virus) to 60 nanometers (wound tumor virus). Tomato spotted wilt virus is surrounded by a membrane and has a flexible, spherical shape about 100 nanometers in diameter. Many plant viruses have split genomes, i.e., they consist of two or more distinct nucleic acid strands encapsidated in different-sized particles made of the same protein subunits. Thus, some, like tobacco rattle virus, consist of two rods, a long one (195 by 25 nm) and a shorter one (43 by 25 nm), whereas others, like alfalfa mosaic virus, consist of four components of different sizes (Fig. 14-4E). Also, many isometric viruses have two or three different components of the same size but containing nucleic acid strands of different lengths.

In multicomponent viruses, all of the nucleic acid strand components must be present in the plant for the virus to multiply and perform in its usual manner. The surface of viruses consists of a definite number of protein subunits, which are arranged spirally in the elongated viruses and packed on the sides of the polyhedral particles of the spherical viruses (Fig. 14-5). In cross section, the elongated viruses appear as hollow tubes with the protein subunits forming the outer coat and the nucleic acid, also arranged spirally, embedded between the inner ends of two successive spirals of the protein subunits. In spherical viruses the visible shell consists of protein subunits, while the nucleic acid is inside the shell and is arranged in an as yet unknown manner. Rhabdoviruses, and a few spherical viruses, are provided with an outer

lipoprotein envelope or membrane. Inside the membrane is the nucleocapsid, consisting of nucleic acid and protein subunits.

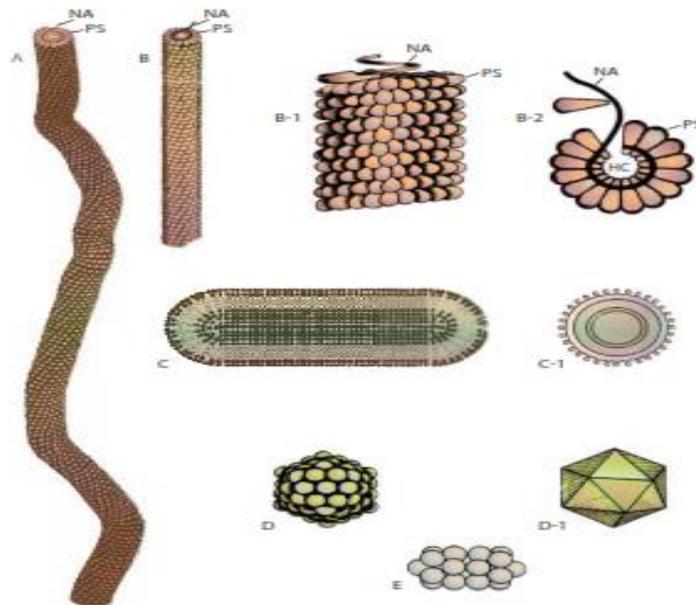


FIGURE 14-5 Relative shapes, sizes, and structures of some representative plant viruses. (A) Flexuous thread-like virus. (B) Rigid rod-shaped virus. (B-1) Side arrangement of protein subunits (PS) and nucleic acid (NA) in viruses A and B. (B-2) Cross-section view of the same viruses. HC, hollow core. (C) Short, bacillus-like virus. (C-1) Cross-section view of such a virus. (D) Isometric polyhedral virus. (D-1) Icosahedron representing the 20-sided symmetry of the protein subunits of the isometric virus. (E) Geminivirus consisting of twin particles.

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Composition and Structure

Each plant virus consists of at least a nucleic acid and a protein. Some viruses consist of more than one size of nucleic acid and proteins, and some of them contain enzymes or membrane lipids. The nucleic acid makes up 5 to 40% of the virus, protein making up the remaining 60 to 95%. The lower nucleic acid percentages are found in the elongated viruses, whereas the spherical viruses contain higher percentages of nucleic acid. The total mass of the nucleo-

protein of different virus particles varies from 4.6 to 73 million daltons. The weight of the nucleic acid alone, however, ranges only between 1 and 3 million ($1-3 \times 10^6$) daltons per

virus particle for most viruses, although some have up to 6×10^6 daltons and the 12 component wound tumor virus nucleic acid is approximately 16×10^6 daltons. All viral nucleic acid sizes are quite small when compared to 0.5×10^9 daltons for mollicutes and 1.5×10^9 daltons for bacteria.

Composition and Structure of Viral Protein

Viral proteins, like all proteins, consist of amino acids. The sequence of amino acids within a protein, which is dictated by the sequence of nucleotides in the genetic material, determines the nature and properties of the protein. The protein shells of plant viruses are composed of repeating subunits. The amino acid content and sequence for identical protein subunits of a given virus are constant but vary for different viruses and even for different strains of the same virus. Of course, the amino acid content and sequence are different for different proteins of the same virus particle and even more so for different viruses. The content and sequences of amino acids are known for the proteins of many viruses. For example, the protein subunit of tobacco mosaic virus (TMV) consists of 158 amino acids in a constant sequence and has a mass of 17,600 daltons (often written as 17.6 kDa, 17.6 kd, or 17.6 K). In TMV the protein subunits are arranged in a helix containing $16 \frac{1}{3}$ subunits per turn (or 49 subunits per three turns). The central hole of the virus particle down the axis has a diameter of 4 nanometers, whereas the maximum diameter of the particle is 18 nanometers. Each TMV particle consists of approximately 130 helix turns of protein subunits. The nucleic acid is packed tightly in a groove between the helices of protein subunits. In rhabdoviruses the helical nucleoproteins are enveloped in a membrane. In polyhedral plant viruses the protein subunits are packed tightly in arrangements that produce 20 (or some multiple thereof) facets and form a shell. Within this shell the nucleic acid is folded or otherwise organized.

Composition and Structure of Viral Nucleic Acid

The nucleic acid of most plant viruses consists of RNA, but a large number of viruses have been shown to contain DNA. Both RNA and DNA are long, chain-like molecules consisting of hundreds or, more often, thousands of units called nucleotides. Each nucleotide consists of a ring compound called the base attached to a five-carbon sugar [ribose (I) in RNA, deoxyribose (II) in DNA], which in turn is attached to phosphoric acid (Fig. 14-6). The sugar of one nucleotide reacts with the phosphate of another nucleotide, which is repeated many times, thus forming the RNA or DNA strand. In viral RNA, only one of four bases, adenine, guanine, cytosine, and uracil, can be attached to each ribose molecule. The first two, adenine and guanine, are purines and interact with the other two, uracil and cytosine, the pyrimidines. The chemical formulas of the bases and one of their possible relative positions in the RNA chain are shown in Fig. 14-6 (structure III). DNA is similar to RNA with two small, but very important differences: the oxygen of one sugar hydroxyl is missing and the base uracil is replaced by the base methyluracil, better known as thymine (IV). The size of both RNA and DNA is expressed either in daltons or as the number of bases [kilobases (kb) for single-stranded RNA and DNA or kilobase pairs (kbp) for double-stranded RNA and DNA], or as the number of nucleotides or nucleotide pairs. The sequence and the frequency of the bases on the RNA strand vary from one RNA to another, but they are fixed within a given RNA and determine its properties. Healthy cells of plants always contain doublestranded DNA and single-stranded RNA. Of the nearly 1,000 described plant viruses, most (about 800) contain

single-stranded RNA, but 50 contain double-stranded RNA, 40 contain double-stranded DNA, and about 110 contain single-stranded DNA.

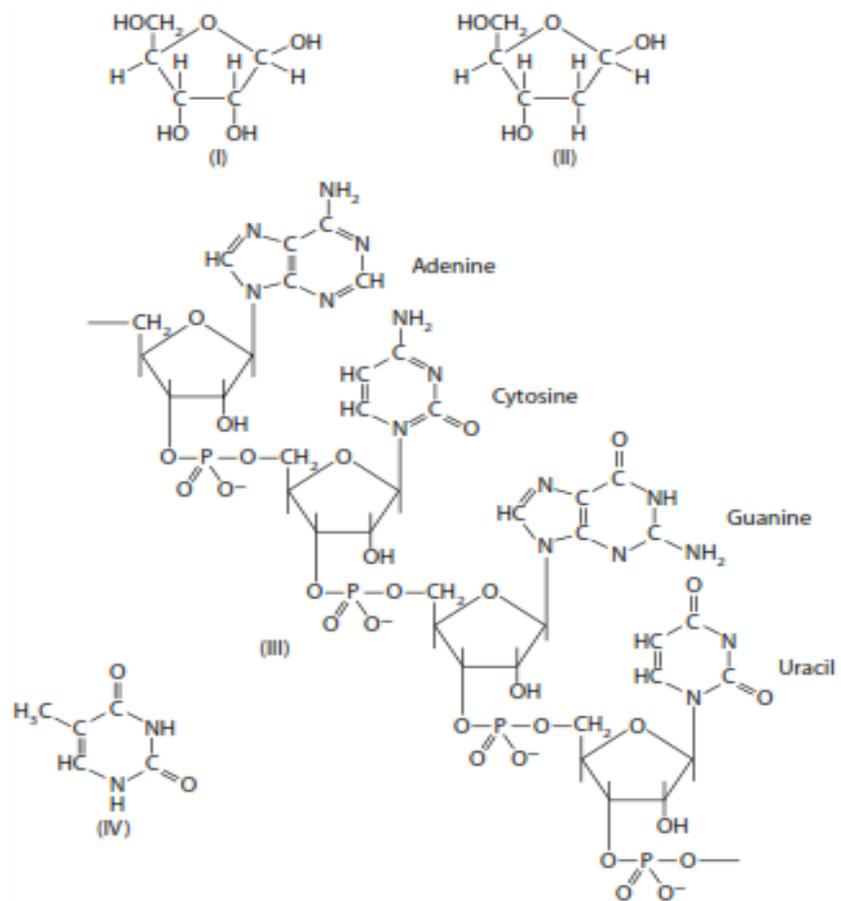


FIGURE 14-6 Chemical formulas of ribose (I), deoxyribose (II), ribonucleic acid or RNA (III), and thymine (IV).

NOMENCLATURE AND CLASSIFICATION OF PLANT VIRUSES

Many plant viruses are named after the most conspicuous symptom they cause on the first host in which they have been studied. Thus, a virus causing a mosaic on tobacco is called tobacco mosaic virus, whereas the disease itself is called tobacco mosaic; another virus causing spotted wilt symptoms on tomato is called tomato spotted wilt virus and the disease is called tomato spotted wilt, and so forth. Considering, however, the variability of symptoms caused by the same virus on the same host plant under different environmental conditions, by different strains of a virus on the same host, or by the same virus on different hosts, it becomes apparent that this system of nomenclature leaves much to be desired. All viruses belong to the kingdom Viruses. Within the kingdom, viruses are distinguished as RNA viruses and DNA viruses, depending on whether the nucleic acid of the virus is RNA or DNA. Viruses are further subdivided depending on whether they possess one or two strands of RNA or DNA of either positive or negative sense, either filamentous or isometric. Within each of these groups there may be viruses replicating via a polymerase enzyme (+RNA or DNA viruses) or via a reverse transcriptase (-RNA or DNA viruses). Most viruses consist of nucleic acid surrounded by coat protein, but some also have a membrane attached to them. Some viruses

have all their genome in one Particle (monopartite viruses), but the genome of other (multipartite) viruses is divided among two, three, or, rarely, four particles. Other characteristics in the classification of viruses include the symmetry of helix in the helical viruses, or number and arrangement of protein subunits in the isometric viruses, size of the virus, and, finally, any other physical, chemical, or biological properties. Figure 14-24 shows diagrammatically the various families and genera of plant viruses. The current nomenclature and classification scheme of plant viruses, along with the type species and the means of transmission of each virus genus, are as follows.

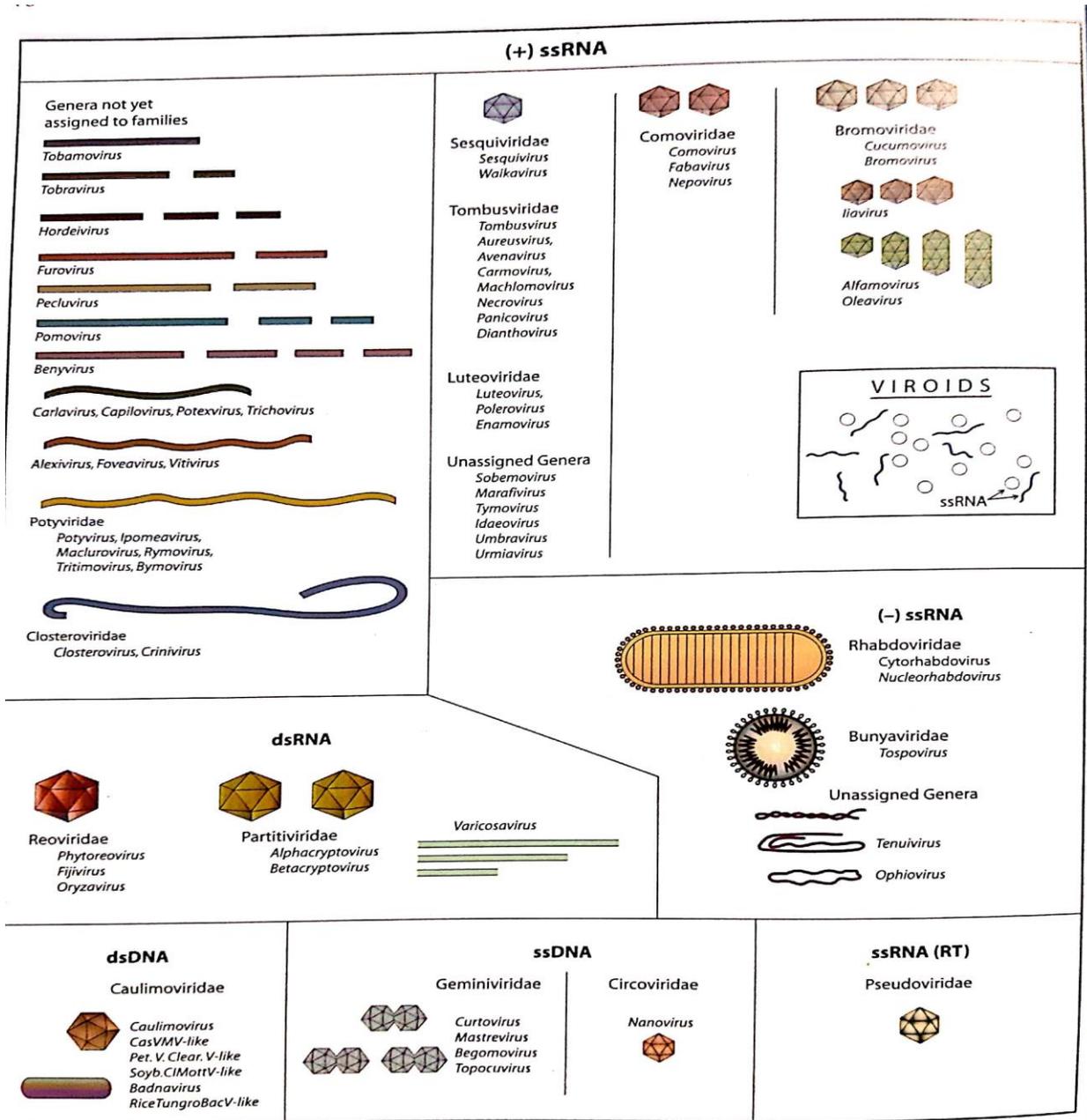


FIGURE 14-24 Schematic diagram of families and genera of viruses and of viroids that infect plants.



Kingdom: Viruses

Virus genera not yet assigned into families
 RNA viruses
 Single-stranded positive RNA [(+) ssRNA]

Rod-shaped particles	Family	Genus	Type species	Remarks
1 ssRNA	—	<i>Tobamovirus</i>	<i>Tobacco mosaic virus</i>	Contact transmission
2 ssRNAs	—	<i>Tobravirus</i>	<i>Tobacco rattle virus</i>	Nematode transmission
3 ssRNAs	—	<i>Hordeivirus</i>	<i>Barley stripe mosaic virus</i>	Seed transmission
2 ssRNAs	—	<i>Furovirus</i>	<i>Soilborne wheat mosaic virus</i>	Fungal transmission
3 ssRNAs	—	<i>Pecluvirus</i>	<i>Peanut clump virus</i>	Fungal and seed transmission
4 ssRNAs	—	<i>Pomovirus</i>	<i>Potato mop-top virus</i>	Fungal transmission, dicots
	—	<i>Benyvirus</i>	<i>Beet necrotic yellow vein virus</i>	Fungal transmission

Filamentous particles

1 ssRNA	—	<i>Allexivirus</i>	<i>Shallot virus X</i>	Eriophyid mite transmission
	—	<i>Carlavirus</i>	<i>Carnation latent virus</i>	No vector
	—	<i>Foveavirus</i>	<i>Apple stem pitting virus</i>	By contact only
	—	<i>Potexvirus</i>	<i>Potato virus X</i>	No vector. Some seed transmission
	—	<i>Capillivirus</i>	<i>Apple stem grooving virus</i>	No vector. Some seed transmission
	—	<i>Trichovirus</i>	<i>Apple chlorotic leafspot virus</i>	No vector. Some seed transmission
	—	<i>Vitivirus</i>	<i>Grapevine virus A</i>	Mealybugs, scale insects, aphids

Isometric particles

1 ssRNA	—	<i>Sobemovirus</i>	<i>Southern bean mosaic virus</i>	Seedborne, beetles, myrids
	—	<i>Marafivirus</i>	<i>Maize rayado fino virus</i>	In Gramineae, leafhoppers
	—	<i>Umbravirus</i>	<i>Carrot mottle virus</i>	Do not code coat proteins
	—	<i>Tymovirus</i>	<i>Turnip yellow mosaic virus</i>	Aphids w/ helper virus
	—			By beetles
2 ssRNAs	—	<i>Idaeovirus</i>	<i>Raspberry bushy dwarf virus</i>	By pollen and seed

Bacilliform particles

3 ssRNAs	—	<i>Ourmiavirus</i>	<i>Ourmia melon virus</i>	No vectors known
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Virus families

Filamentous viruses

1 ssRNA	Potyviridae	<i>Potyvirus</i>	<i>Potato virus Y</i>	Aphids, w/ helper virus
	Potyviridae	<i>Ipomovirus</i>	<i>Sweet pot mild mottle</i>	Whitefly <i>Bemisia tabaci</i>
	Potyviridae	<i>Macluravirus</i>	<i>Maclura mosaic virus</i>	Aphids
	Potyviridae	<i>Rymovirus</i>	<i>Ryegrass mosaic virus</i>	Eriophyid mites
	Potyviridae	<i>Tritimovirus</i>	<i>Wheat streak mosaic virus</i>	Eriophyid mites
	Potyviridae	<i>Bymovirus</i>	<i>Barley yellow mosaic virus</i>	Gramineae, fungal transmission

1 or 2 ssRNA				
	Closteroviridae	<i>Closterovirus</i>	<i>Beet yellows virus</i>	Aphids, mealybugs, or whiteflies
	Closteroviridae	<i>Crimivirus</i>	<i>Lettuce inf. yellows virus</i>	Whiteflies
Isometric viruses				
1 ss(+)RNA				
	Sequiviridae	<i>Sequivirus</i>	<i>Parsnip yellow fleck virus</i>	Aphids
	Sequiviridae	<i>Waikavirus</i>	<i>Rice tungro spherical virus</i>	Leafhoppers or aphids
	Tombusviridae	<i>Tombusvirus</i>	<i>Tomato bushy stunt virus</i>	Soilborne, but vector unknown
	Tombusviridae	<i>Aureusvirus</i>	<i>Pothos latent virus</i>	Soilborne
	Tombusviridae	<i>Arenavirus</i>	<i>Oat chlorotic stunt virus</i>	Soilborne
	Tombusviridae	<i>Carmovirus</i>	<i>Carnation mottle virus</i>	—
	Tombusviridae	<i>Dianthovirus</i>	<i>Carnation ring spot virus</i>	Soilborne, unknown
	Tombusviridae	<i>Machlomovirus</i>	<i>Maize chlorotic mottle virus</i>	Seed, beetles, thrips
	Tombusviridae	<i>Necrovirus</i>	<i>Tobacco necrosis virus</i>	Fungal transmission
	Tombusviridae	<i>Panicovirus</i>	<i>Panicum mosaic virus</i>	Gramineae, mechanical
	Luteoviridae	<i>Luteovirus</i>	<i>Barley yellow dwarf virus</i>	Gramineae, aphids
	Luteoviridae	<i>Polerovirus</i>	<i>Potato leafroll virus</i>	Monocot or dicot plants
	Luteoviridae	<i>Enamovirus</i>	<i>Pea enation mosaic virus</i>	Mechanically, aphids
2 ss(+)RNAs				
	Comoviridae	<i>Comovirus</i>	<i>Coupea mosaic virus</i>	Chrysomelid beetles
	Comoviridae	<i>Fabavirus</i>	<i>Broad bean wilt virus</i>	Aphids
	Comoviridae	<i>Nepovirus</i>	<i>Tobacco ring spot virus</i>	Nematodes
3 ss(+)RNAs				
	Bromoviridae	<i>Bromovirus</i>	<i>Brome mosaic virus</i>	Beetles, mechanically
	Bromoviridae	<i>Cucumovirus</i>	<i>Cucumber mosaic virus</i>	Aphids
	Bromoviridae	<i>Alfavirus</i>	<i>Alfalfa mosaic virus</i>	Aphids
	Bromoviridae	<i>Illavirus</i>	<i>Tobacco streak virus</i>	Pollen, seed
	Bromoviridae	<i>Oleavirus</i>	<i>Olive latent virus 2</i>	No vector known
dsRNA				
	Reoviridae	<i>Phytoreovirus</i>	<i>Wound tumor virus</i>	Leafhoppers
	Reoviridae	<i>Fijivirus</i>	<i>Fiji disease virus</i>	Gramineae, planthoppers
	Reoviridae	<i>Oryzavirus</i>	<i>Rice ragged stunt virus</i>	Planthoppers
	Partitiviridae	<i>Alphacryptovirus</i>	<i>White clover crypto. virus 1</i>	Nonenveloped, latent
	Partitiviridae	<i>Betacryptovirus</i>	<i>White clover crypto. virus 2</i>	Same, seed
	Partitiviridae	<i>Varicosavirus</i>	<i>Lettuce big-vein virus</i>	Fungal transmission
(-) ssRNA				
Bacilliform particles				
	Rhabdoviridae	<i>Cytorhabdovirus</i>	<i>Lettuce necrosis yellows virus</i>	Leafhoppers, planthoppers, aphids
	Phaboviridae	<i>Nucleorhabdovirus</i>	<i>Potato yellow dwarf virus</i>	Same
Membranous circular particles				
	Bunyaviridae	<i>Tospovirus</i>	<i>Tomato spotted wilt virus</i>	Thrips
Thin flexuous multipartite viruses				
	—	<i>Tenuivirus</i>	<i>Rice stripe virus</i>	Gramineae, planthoppers
	—	<i>Ophiovirus</i>	<i>Citrus psorosis virus</i>	No vector known
dsDNA				
Isometric				
	Caulimoviridae	<i>Caulimovirus</i>	<i>Cauliflower mosaic virus</i>	Aphids
	Caulimoviridae	<i>Soybean chlorotic mottle virus-like</i>		Aphids
	Caulimoviridae	<i>Cassava vein mosaic virus-like</i>		Aphids
		<i>Petunia vein clearing virus-like</i>		Aphids

WORKING WITH AND MANAGING PLANT VIRUSES

(+)ssDNA

Caulimoviridae	<i>Badnavirus</i>	<i>Commelina yellow mottle virus</i>	Mealybugs
Caulimoviridae	<i>Rice tungro bacilliform virus-like</i>		Leafhoppers
Geminiviridae	<i>Mastrevirus</i>	<i>Maize streak virus</i>	Gramineae, leafhoppers
Geminiviridae	<i>Curtovirus</i>	<i>Beet curly top virus</i>	Dicot, leafhoppers
Geminiviridae	<i>Begomovirus</i>	<i>Bean golden mosaic virus</i>	2 DNAs, whiteflies
Geminiviridae	<i>Topocovirus</i>	<i>Tom. pseudocurly top virus</i>	Treehopper
Circoviridae	<i>Nanovirus</i>	<i>Subteranean clover stunt virus</i>	6 DNAs

ssRNA (RT) Pseudoviridae: retrotransposons

What are Bacteriophages?

Bacteriophages are obligate intracellular parasite on bacteria that uses bacterial machinery system for its own multiplication and development. These are commonly referred as “phage”. Bacteriophages were jointly discovered by Frederick Twort (1915) in England and by Felix d’Herelle (1917) at the Pasteur Institute in France. “Bacteriophage” term was coined by Felix d’Herelle. Some of the examples of bacteriophages are, Spherical phages such as ϕ X174 (ssDNA), Filamentous phages such as M13(ssDNA), T-even phages such as T2, T4 and T6 that infect E.coli, Temperate phages such as λ and μ .

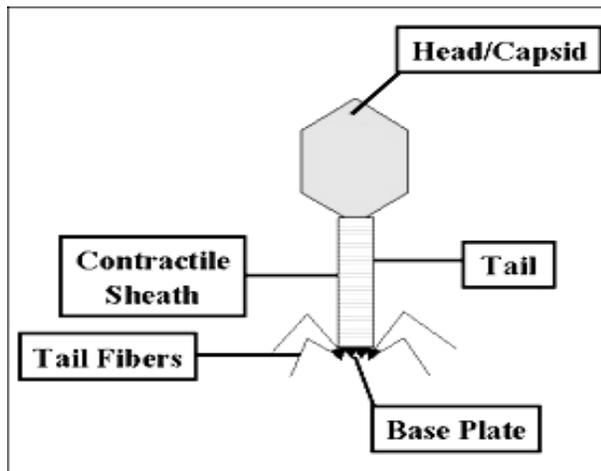


Fig. 8. Basic structure of Bacteriophages

Composition:

All bacteriophages contain nucleic acid as genetic material and protein. Depending upon the phage, the genetic material may be either DNA or RNA. Certain unusual modified bases are present in the genetic material of phages which protect the phage genetic material from nucleases during infection. Protein surrounds the genetic materials and protects to the surrounding environment.

Structure:

The basic structural features of T4 bacteriophages are illustrated in Figure 2. It is approximately 200 nm long and 80-100 nm wide. Size of other phages is of 20 – 200nm in length. All bacteriophages contain head and tail part. Head part is also termed as capsid which is composed of one or different types of proteins. Genetic materials are present inside and protected by capsid. Tails are attached to the capsid in most of the phages. These are hollow tube like structure through which viruses inject their genetic material inside the host during infection. Tail part is more complex structure in phages. In T4 phage, tail part is surrounded by a contractile sheath and basal plate like structure present at the end of tail from which certain tail fibres are attached. Tail fibres help in attachment of phages to bacteria and contractile sheath helps in contraction during infection. Some of the phages do not contain tail fibres at the end. Certain other structures are involved in these phages for binding to the bacterium during infection.

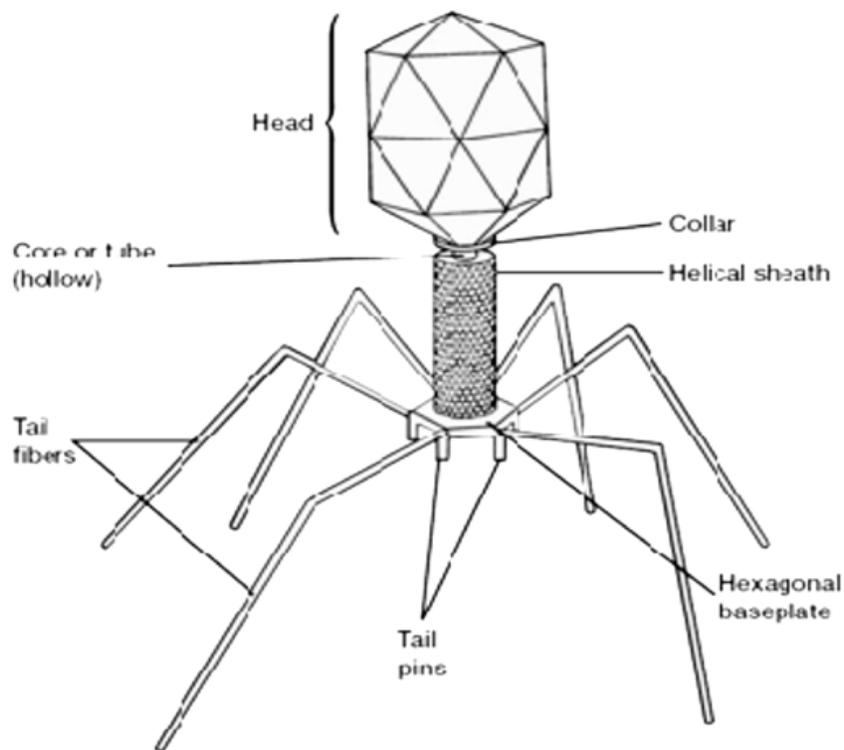


Fig. 9. Structure of T4 Bacteriophage

Multiplication/Infection of Host Cells

The first step in the infections is binding of phage to bacterium which is mediated by tail fibres are some other structures on those phages that lack tail fibres. Binding of phage tail fibre to bacterium is through adsorption process and it is reversible. There are specific receptors are present on bacterial cell surface through which phages bind on it by its tail fibre. These receptors are proteins, lipopolysaccharides, pili and lipoproteins of bacterium. Some phages lacking basal plate and tail fibre bind tightly to bacterial cell surface and it is reversible.

After binding of phage to bacterium, there is contraction in tail by contractile sheath and phages inject their genetic material through hollow tube like tail. Some phages also contain certain enzymes that digest the bacterial cell surface. Phages that don't contain tail fibre and contractile sheath, uses different mechanism for inject its own genetic material inside the host. Only genetic material of phages enters inside the bacterium and the remainder of phages (ex. capsid) remain outside of bacterium.

Life Cycle:

There are two different types of life cycle present in phages: (i) Lytic cycle and (ii) Lysogenic cycle. Lytic (virulent) cycle kill the host cell that they infect, while lysogenic (temperate) cycle establishes a persistent infection without killing the host cell.

(A.) Lytic Cycle:

These are also known as virulent cycle because phages multiply inside the host and lyse the cell at the end of its life cycle. After attachment of tail fibre to host, genetic materials are injected inside the host. The time period between the entry of genetic material inside the host and release of mature phage after end of life cycle is termed as eclipse period. Synthesis of phage components and its packaging into mature phages takes place in this period. After infection, the genetic material of phages uses host biosynthetic machinery for replication, transcription and translation. Structural proteins of phages (capsid, tail etc.) are also synthesized inside the host using host biosynthetic machinery. After synthesis, genetic materials are packed inside the capsid and tail is attached on it. This process is called as maturation of phages. In lytic phage, phages also synthesized lysis protein. Bacterial cells are lysed due to accumulation of phage lysis protein and mature phages are released into the medium. Around 10-1000 phages are released from the bacterial cell. The average yield of phages per infected bacterial cell is known as burst size.

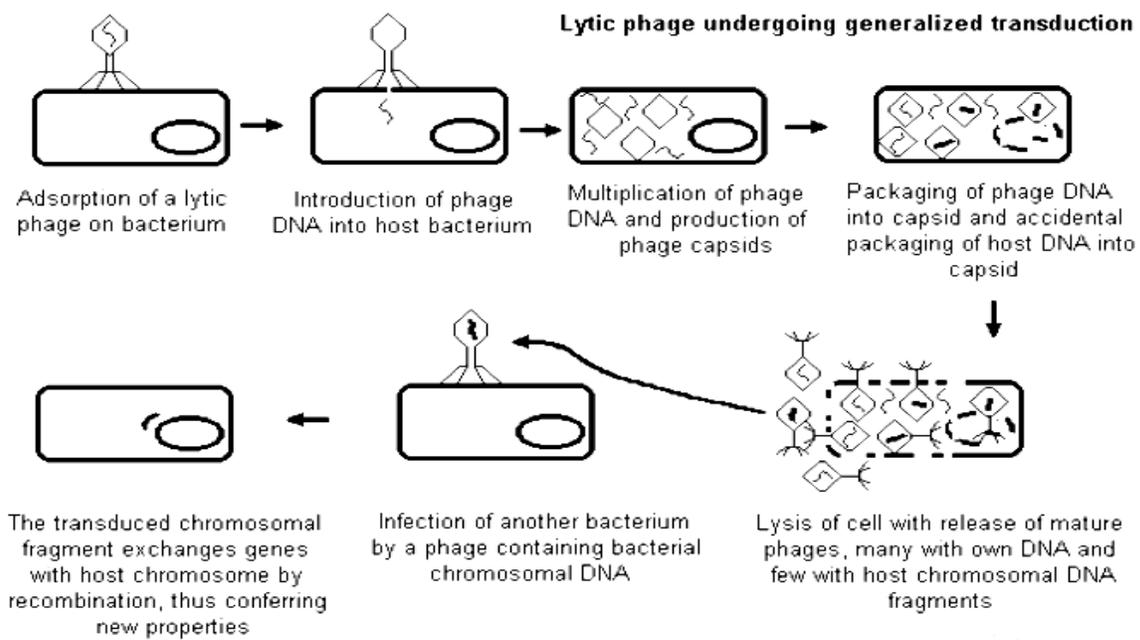


Fig. 10. Lytic cycle of bacteriophage infection

(B.) Lysogenic cycle:

These are also known as temperate phage because phages multiply via the lytic cycle or may enter a dormant state inside the cell. After entry of genetic material into the host cell, the phage DNA integrates into the host chromosome and starts replication along with it and passed to the daughter cells of host. Integration of phage DNA to host chromosome is termed as prophage and bacteria is termed as lysogenic bacteria. Due to integration of phage DNA to host chromosome, extra genes carried by phage get expressed in the host cell and it may change the properties of bacterial cell. This process is termed as lysogenisc or phage conversion.

Due to exposure to UV rays, ionizing radiations, mutagenic chemicals etc, DNA of phage is released from host chromosome and separated phage DNA initiates lytic cycle resulting in the lysis of cell and release of phages into the medium.

Significance of bacteriophages:

- New characteristics are acquired using lysogenic conversion
- Insertional mutation can be induced in bacterial chromosome by random insertion of genes or nucleotides.
- Latent infection in mammalian cells by retroviruses can be studied using Lambda phage as model system.
- In genetic engineering, phages are used extensively where they serve as cloning vectors.
- Phages are used to maintain libraries of genes and monoclonal antibodies
- Natural removal of bacteria from water bodies can be done using bacteriophages

Ultrastructure of TMV

DESIGN AND ASSEMBLY TMV is the classical example of a rod-shaped virus. Its rod shape results from its basic design, namely a regular helical array of identical protein subunits, in which framework is embedded a single molecule of RNA wound as a helix (figure 1b). It is, of course, the RNA that carries the genetic information, i.e. the capacity to instruct the host cell to make many copies of the virus. This general picture was already complete by 1958, when Rosalind Franklin died, and it seemed easy to comprehend how a structure of this type might be built out of identical subunits: the subunits might assemble themselves by repeated identical interactions, like adding steps in a spiral staircase, enclosing the RNA as a corkscrew-like thread as the rod extends. In other words, the assembly might be likened to growth at a screw dislocation in a crystal. We now know that this simple picture of assembly is wrong in all its essentials. The isolated protein can aggregate in different polymorphic forms, and Caspar (1963) suggested that these might play the role of intermediates in the assembly of the helical aggregate. The virus, in fact, assembles in a much more complex way, for what, with hindsight, we can see to be good physical and biological reasons. The story of how my colleagues and I came to suspect that the simple scheme was deficient and how the path of assembly was found has been told elsewhere (Klug 1979). I can only summarize the results here. When the RNA and the coat protein of the virus are taken apart, the protein molecules alone under physiological conditions aggregate, not into a long helix, but into a 20S aggregate. We identified this as a 'disk' (figure 2) — a two-layer cylindrical structure, each layer consisting of a ring of 17 molecules, compared with the $16\frac{1}{3}$ molecules present in each turn of the assembled helix. The disk can be crystallized, but because of the large molecular mass of the disk (600 000 Da) the determination of its exact structure by X-ray methods posed formidable technical and analytical problems.

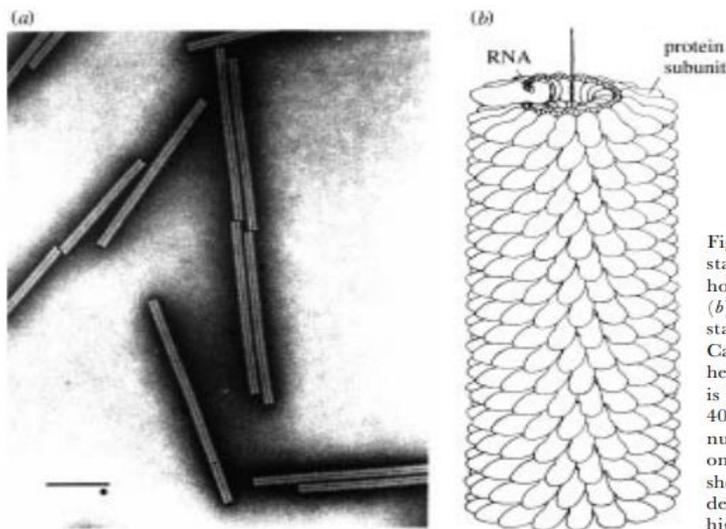


Figure 1. (a) Electron micrograph of negatively stained TMV particles (J. T. Finch). Note the hole down the middle. Scale bar, 100 nm. (b) Diagram summarizing the results of the first stage of structure analysis of TMV (Klug & Caspar 1960). The protein subunits form a tight helical array with $16\frac{1}{3}$ units per turn, and the RNA is packed between the turns at a radius of about 40 Å from the helix axis. There are three nucleotides per protein subunit. Only about one-sixth of the length of a complete particle is shown. The detailed X-ray structure was first determined by Stubbs *et al.* (1977), then carried to higher resolution by Namba & Stubbs (1986) and Namba *et al.* (1989).

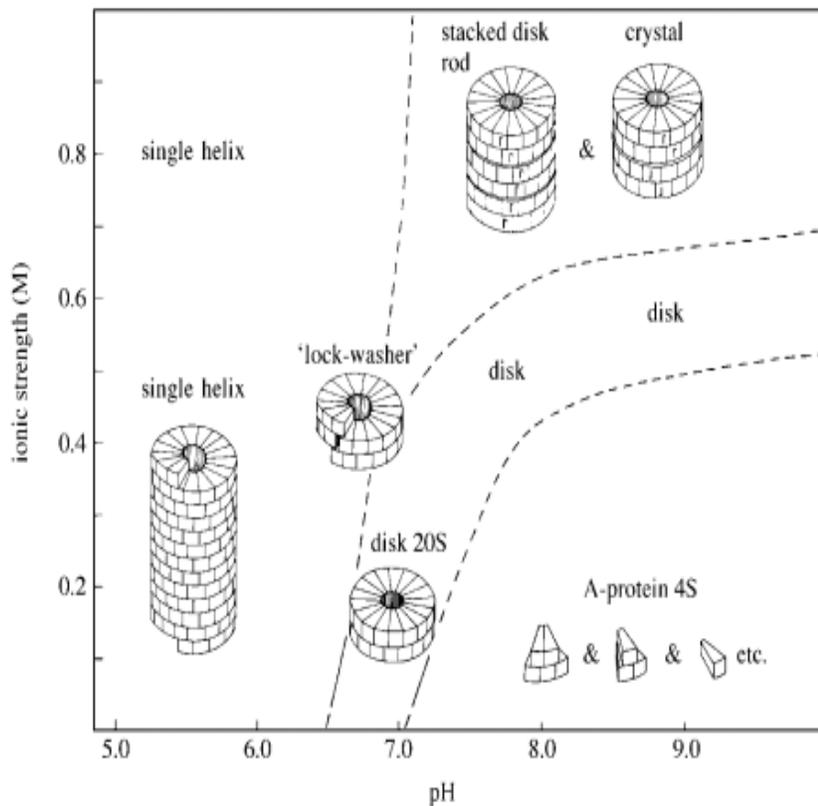


Figure 2. Diagram showing the ranges over which particular species of TMV protein participate significantly in the equilibrium (after Durham *et al.* 1971). This is not a conventional phase diagram: a boundary is drawn where a larger species becomes detectable and does not imply that the smaller species disappears sharply. The 'lock-washer' indicated on the boundary between the 20S disk and the helix is not well defined and represents a metastable transitory state observed when not enough time is allowed for the transition (fig. 1 in Butler, this issue). The boundaries are approximately correct for a protein concentration of 5 mg ml^{-1} at 20°C , but not all species that have been observed are shown. The predominant species at pH 7.0 and $I=0.1$ is the '20S' aggregate (more accurately *ca.* 19.5S), which we identify as a two-layer polar disk. As the pH falls below pH 7, the sedimentation constant increases to values greater than 20S, characteristic of short helical aggregates (Butler *et al.* 1992). The stacked-disk structure is formed by prolonged incubation at alkaline pH, and represents an irreversible half-proteolysed aggregate (Durham 1972; Unwin & Klug 1974).

These were overcome and after a dozen years it was possible to construct an atomic model (Figure 3) showing the detailed structure of the protein subunit and how it interacts with its neighbours (Bloomer *et al.* 1978). This study was pursued to the end, not merely for the sake of knowing the structure of a protein subunit, but because we had shown earlier that the protein disk plays a crucial role in the assembly of the virus from its RNA and protein (Butler & Klug 1971). The disk combines with a specific initiation tract on the single-stranded viral RNA, which is located about 1000 bases from the 3' end (Zimmern & Butler 1977; Zimmern & Wilson 1976), and then dislocates to begin helix growth. Foreign RNAs that do not have this tract are rejected. When the sequence of bases of this initiation tract, or 'origin of assembly' as it is now called, was determined (Zimmern 1977), it was seen that it could be folded so that the initial binding site is exposed at the apex of a stem-loop structure. Now the X-ray studies had shown that the two layers of the protein disk are so arranged as to leave a gap between them, thus in effect extending the central hole of the

disk, rather like a pair of 'jaws', as if waiting to 'bite' the RNA (Figure 3). So we were able to develop the picture of initiation, or nucleation as a physicist would say, of assembly shown in Figure 4 (Butler et al. 1976). The RNA hairpin loop inserts through the central hole of the disk, and the stem of the loop opens up and binds in the 'jaws' formed by the two layers of protein. The disk then dislocates into a helical structure, entrapping the RNA, after which elongation proceeds by the incorporation of further disks, pulling up more RNA through the central hole. There was some controversy surrounding this picture of growth after the initial stages, but there was no doubt about the role of the 20S aggregate (the disk) in initiating assembly, and also in further growth.

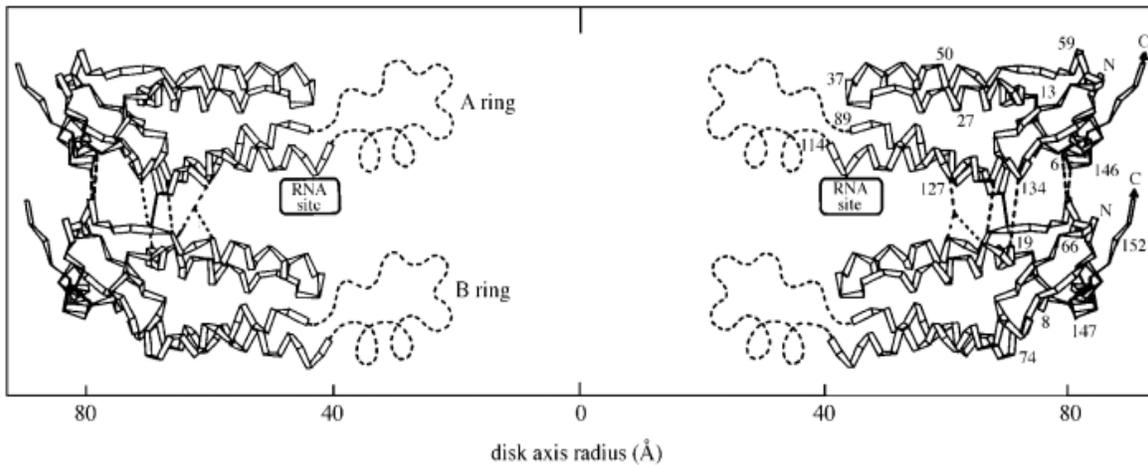


Figure 3. Section through a two-layer disk along its axis, reconstructed from the results of X-ray analysis to a resolution of 2.8 Å (Bloomer *et al.* 1978). The ribbons show the path of the polypeptide chain of the protein subunits. Subunits of the two rings can be seen touching over a small area toward the outside of the disk (where the side-chain contacts that occur between the rings have been drawn in as dashed and solid lines) but opening up into the 'jaws' toward the centre. The dashed lines at low radius indicate schematically the mobile portion of the subunits in the disk, extending in from near the RNA binding sites (between the rings *ca.* 40 Å radius as shown) to the edge of the central hole, which has a radius of 20 Å.

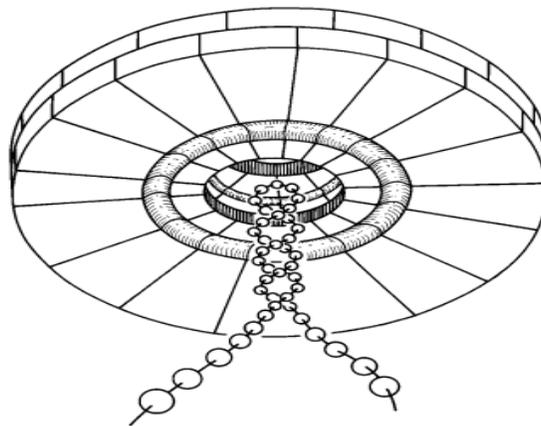


Figure 4. Nucleation of virus assembly is believed to occur by the insertion of a stem-loop hairpin structure of RNA into the central hole of the protein disk and between the two layers of subunits. This loop, formed by the nucleation region of the RNA origin of assembly, binds round the first turn, opening up the base-paired stem as it does so, and causes the disk to dislocate into a short helix. This 'closes the jaws', entrapping the RNA between the turns of protein subunits, and gives a start to the nucleoprotein helix (which can then elongate rapidly to some minimum stable size).

There has also been a controversy about the exact nature of the 20S aggregate, which we identified as a two-layer polar disk. The disk is thus an obligatory intermediate in the assembly of the virus which, simultaneously, fulfills both the physical requirement for nucleating the growth of the helical particle and the biological requirement for specific recognition of the viral RNA. A most intricate structural mechanism has been evolved to give the process an efficiency and purposefulness, whose basis we now understand. TMV is self-assembling, self-nucleating and self-checking. The general conclusion derived from the story of TMV assembly is that one must distinguish between the design of a structure and the construction process used to achieve it. In the TMV structure all 2130 coat protein subunits (except the few at the ends of the particle) make the same non-covalent contacts with each other, and this specific bonding pattern repeated many times leads to a symmetrical final structure. There is nothing in the design of the completed structure which gives a hint that different bonding patterns, and nonequivalent ones at that, are required during the process of assembly. The protein subunit under physiological conditions is designed not to form an endless helix, but a restricted two-layer variant of it—the disk—which is stable and which can be readily converted to the lock-washer, or helical, form. The disk therefore represents an intermediate subassembly by means of which the entropically difficult problem of nucleating helical growth is overcome. At the same time nucleation by the disk subassembly furnishes a mechanism for recognition of the homologous viral RNA (and rejection of foreign RNAs) by providing a long stretch of nucleotide binding sites for interaction with the special sequence of bases on the RNA. In this way specificity for the homologous viral RNA is achieved.

TRANSMISSION OF PLANT VIRUSES

Plant viruses are transmitted from plant to plant in a number of ways. Modes of transmission include vegetative propagation, mechanically through sap, through seed, pollen, dodder, and by specific insects, mites, nematodes, and fungi.

Transmission of Viruses by Vegetative Propagation

Whenever plants are propagated vegetatively by budding or grafting, by cuttings, or by the use of tubers, corms, bulbs, or rhizomes, any viruses present in the mother plant from which these organs are taken will almost always be transmitted to the progeny (Fig. 14-15). Considering that almost all fruit and many ornamental trees and shrubs, many field crops, such as potatoes, and most florist's crops are propagated vegetatively, this means of transmission of viruses is the most important for all these types of crop plants. In cases of propagation by budding, the presence of a virus in the bud or in the root stock may result in an appreciable reduction of successful bud unions with the rootstock and, therefore, in poor stands. The transmission of viruses may also occur through natural root grafts of adjacent plants, particularly trees (Fig. 14-15). For several tree viruses, natural root grafts are the only known means of tree-to-tree spread of the virus within established orchards.

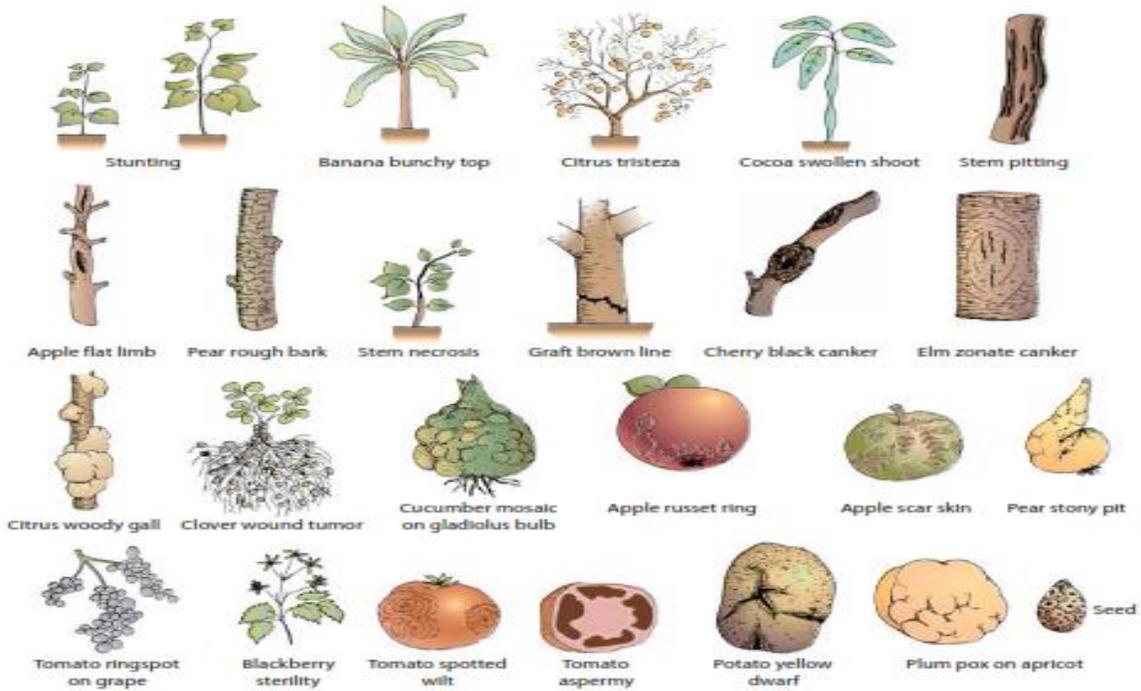


FIGURE 14-14 Types of systemic symptoms caused by viruses and viroids in plants.

Mechanical Transmission of Viruses through Sap

The mechanical transmission of plant viruses in nature by the direct transfer of sap through contact of one plant with another is uncommon and relatively unimportant. Such transmission may take place after a strong wind injures the leaves of adjacent diseased and healthy plants or when plants are wounded during cultural practices by tools, hands, or clothes, or by animals feeding on the plants, and the sap carrying virus is transferred to wounded plants (Figs. 14-16 and 14-17). Of the important plant viruses, *potato virus X*, *tobacco mosaic virus*, and *cucumber mosaic virus* are transmitted through sap in the field and may cause severe losses.

The greatest importance of mechanical transmission of plant viruses stems from its indispensability in studying the viruses that cause plant diseases. For mechanical transmission of a virus, young leaves and flower petals are ground to crush the cells and release the virus in the sap (Fig. 14-16). Often a buffer solution is added to stabilize the virus. The sap may be strained to remove tissue fragments and is then applied to the surface of leaves of young plants dusted previously with an abrasive such as Carborundum to aid in wounding of the cells. The sap is applied by rubbing the leaves gently with a cheesecloth, finger (Fig. 14-13A), glass spatula, or painter's brush dipped in the sap or by using a small sprayer. In successful inoculations, the virus enters the leaf cells through the wounds made by the abrasive or through broken leaf hairs and initiates new infections.

In local-lesion hosts, symptoms usually appear within three to seven days or more, and the number of local lesions is proportional to the concentration of the virus in the sap. In systemically infected hosts, symptoms usually take 10 to 14 days or more to develop. Sometimes the same plants may first develop local lesions and then systemic symptoms. In the mechanical transmission of viruses, the taxonomic relationship of the donor and receiving (indicator) plants is unimportant, as virus from one kind of plant, whether herbaceous or a tree, may be transmitted to dozens of unrelated herbaceous plants (vegetables, flowers, or weeds). Several viruses, however, especially of woody plants, are difficult or, so far, impossible to transmit through sap.

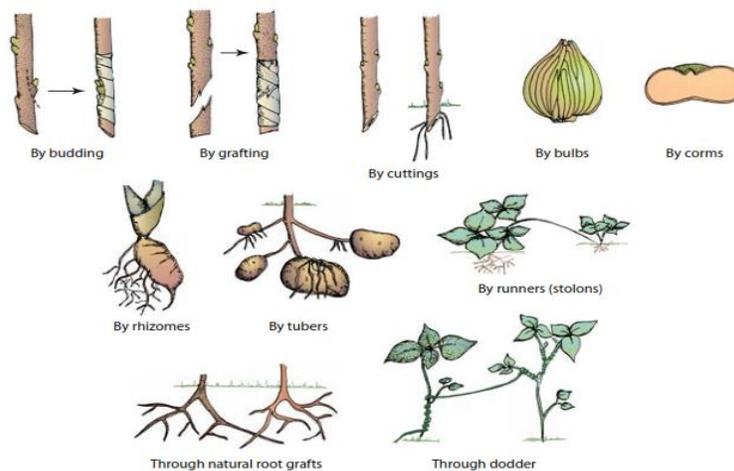


FIGURE 14-15 Transmission of viruses, mollicutes, and other pathogens through vegetative propagation, natural root grafts, and dodder.

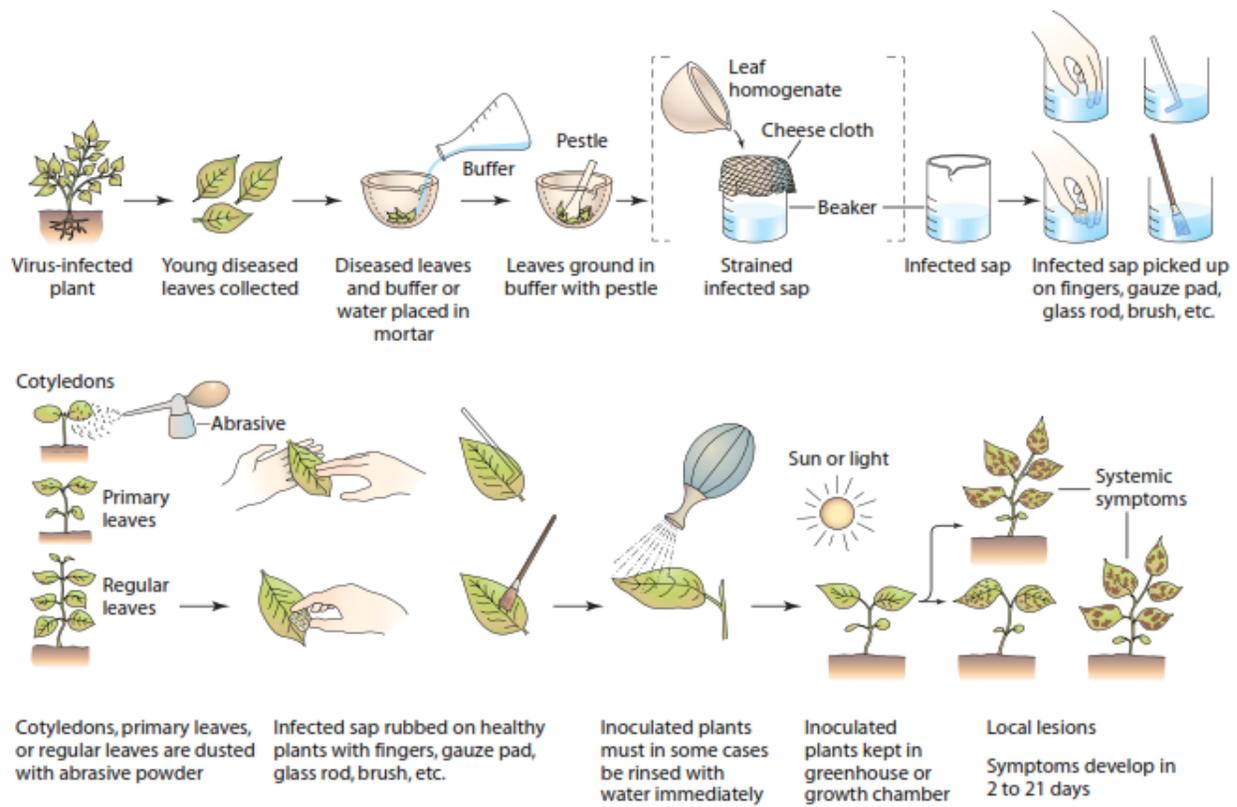


FIGURE 14-16 Typical steps in mechanical or sap transmission of plant viruses.

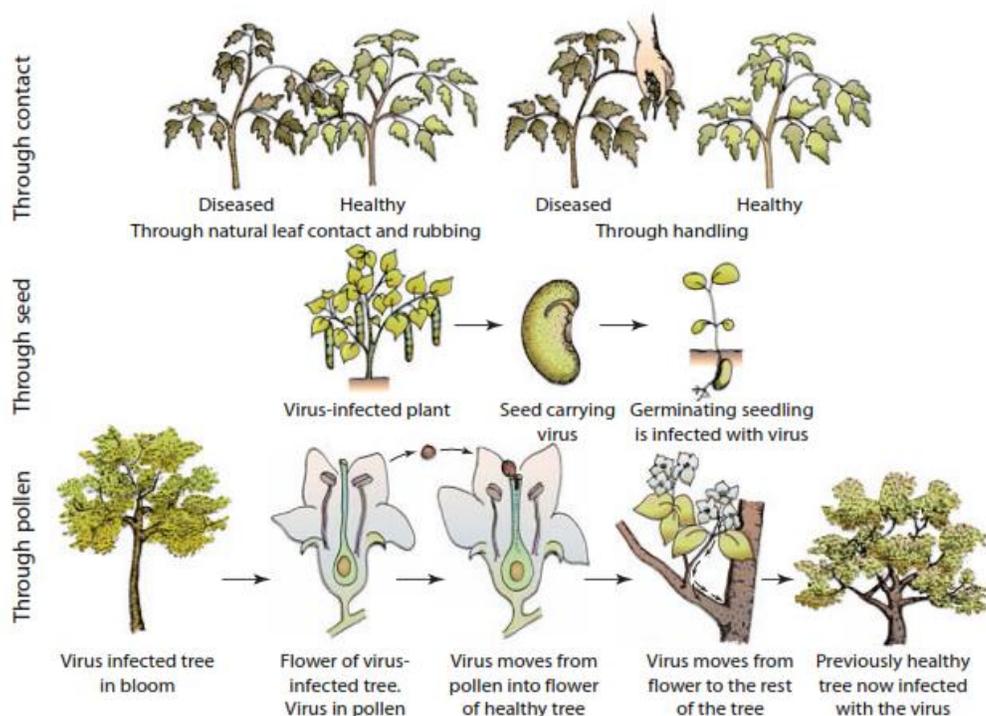


FIGURE 14-17 Plant virus transmission through direct contact of plants, handling, seed, and pollen.

Seed Transmission

More than 100 viruses are transmitted by seed to a smaller or greater extent. As a rule, only a small portion (1–30%) of seeds derived from virus-infected plants of only some hosts of the virus transmit the virus (Fig. 14-17). In some virus–host combinations, however, half or most of the seeds carry the virus, and in a few others 100% of the seeds carry the virus. The frequency of transmission varies with the host–virus combination and with the stage of growth of the mother plant when it becomes infected with the virus. In most seed-transmitted viruses, the virus seems to come primarily from the ovule of infected plants, but several cases are known in which the virus in the seed seems to be just as often derived from the pollen that fertilized the flower. In some host–virus combinations the virus is carried in the integument of the seed and infects seedlings as they are wounded on germination.

Pollen Transmission

Virus transmitted by pollen may result in reduced fruit set, may infect the seed and the seedling that will grow from it, and, in some cases, can spread through the fertilized flower and down into the mother plant, which thus becomes infected with the virus (Fig. 14-17). Such plant-to-plant transmission of virus through pollen is known to occur, for example, in sour cherry infected with *prunus necrotic ring spot virus*.

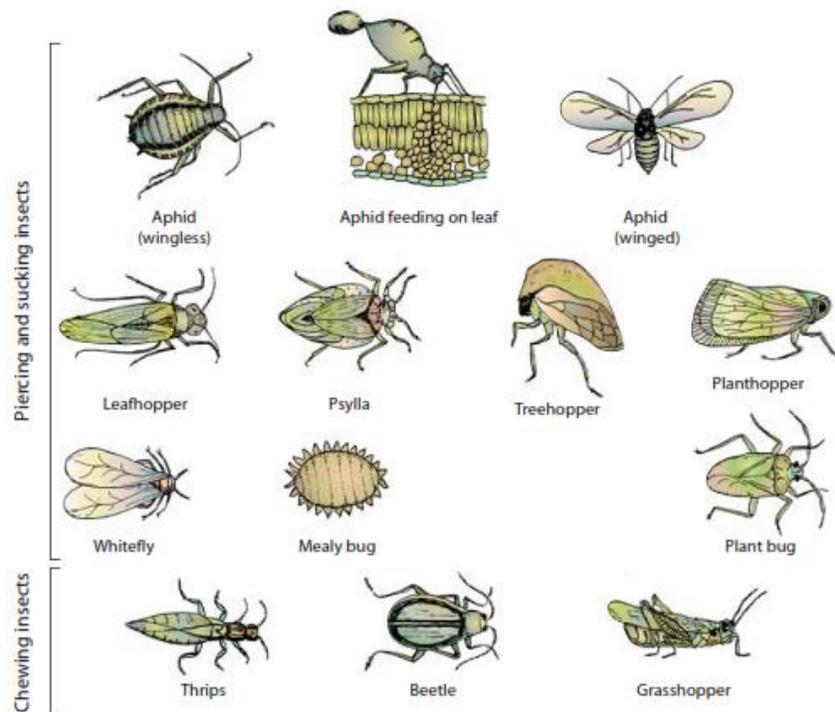


FIGURE 14-18 Insect vectors of plant viruses. Insects in second row from the top also transmit mollicutes and fastidious vascular bacteria.

Insect Transmission

Undoubtedly the most common and economically most important means of transmission of viruses in the field is by insect vectors. Members of relatively few insect groups, however, can transmit plant viruses (Fig. 14-18). The order Homoptera, which includes aphids (Aphididae), leafhoppers (Cicadellidae), and planthoppers (Delphacidae), contains by far the largest number and the most important insect vectors of plant viruses. Other Homoptera that transmit plant viruses are whiteflies (Aleyrodidae), which transmit the usually severe geminiviruses and several other viruses, mealybugs (Coccoidae), and certain treehoppers (Membracidae). A few insect vectors of plant viruses belong to other orders, such as true bugs (Hemiptera), chewing/sucking thrips (Thysanoptera), and beetles (Coleoptera). Grasshoppers (Orthoptera) occasionally seem to carry and transmit a few viruses also. The most important virus vectors are aphids, leafhoppers, whiteflies, and thrips. These and the other groups of Homoptera, as well as true bugs, have piercing and sucking mouthparts. Beetles and grasshoppers have chewing mouthparts. Of these, the beetles are quite effective vectors of certain viruses. Insects with sucking mouthparts carry plant viruses on their stylets —**stylet-borne viruses** — and can acquire and inoculate the virus after short feeding periods of a few seconds to a few minutes. Stylet-borne viruses persist in the vector for only a few to several hours. Therefore, they are also known as **nonpersistent viruses**. With some other viruses, the insect vectors must feed on an infected plant from several minutes or hours to a few days before they accumulate enough virus for transmission. These insects can then transmit the virus after fairly long feeding periods of several minutes to several hours. Such viruses persist in the vector for a few (1 to 4) days and are called **semipersistent viruses**. With still other viruses, the insect vectors accumulate the virus internally and, after passage of the virus through the insect tissues, introduce the virus into plants again through their mouthparts; these viruses are known as **circulative** or **persistent viruses**. Some circulative viruses may multiply in their respective vectors and are then called **propagative viruses**. Viruses transmitted by insects with chewing mouthparts (beetles) may also be circulative or may be carried on the mouthparts. Aphids are the most important insect vectors of plant viruses and transmit the great majority (about 275) of all stylet-borne viruses. As a rule, several aphid species can transmit the same stylet-borne virus, and the same aphid species can transmit several viruses. In many cases, however, the vector–virus relationship is quite specific. Aphids generally acquire the stylet-borne virus after feeding on a diseased plant for only a few seconds (30 seconds or less) and can transmit the virus after transfer to and feeding on a healthy plant for a similarly short time of a few seconds. The length of time aphids remain viruliferous after acquisition of a stylet-borne virus varies from a few minutes to several hours, after which they can no longer transmit the virus. In aphids transmitting stylet-borne viruses, the virus seems to be borne on the tips of the stylets, it is lost easily through the scouring that occurs during the probing of host cells and it does not persist through the molt or egg. Styletborne viruses are said to be transmitted in a nonpersistent manner. In the few cases of aphid transmission of circulative viruses, aphids cannot transmit the virus immediately but must wait several hours after the acquisition feeding; however, once they start to transmit the virus, they continue to do so for many days after the removal of the insects from the virus source (persistent transmission). Approximately 55 plant viruses are transmitted by leafhoppers, planthoppers, and

treehoppers, including viruses with double-stranded RNA, rhabdoviruses, small isometric viruses, and some geminiviruses. Leafhopper and planthopper-transmitted viruses cause disturbances in plants that affect primarily the region of the phloem. All such viruses are circulatory; several are known to multiply in the vector (propagative) and some persist through the molt and are transmitted through the egg stage of the vector. Most leafhopper and planthopper vectors require a feeding period of one to several days before they become viruliferous, but once they have acquired the virus, they may remain viruliferous for the rest of their lives. There is usually an incubation period of 1 to 2 weeks between the time a leafhopper or planthopper acquires a virus and the first time it can transmit it.

Mite Transmission

Primarily mites of the family Eriophyidae have been shown to transmit at least six viruses, including wheat streak mosaic and several other rymoviruses affecting cereals. These mites have piercing and sucking mouthparts (Fig. 14-19). Virus transmission by eriophyid mites seems

to be quite specific, as each of these mites is the only known vector for the virus or viruses it transmits. Another virus, *peach mosaic virus*, is transmitted by mites of the family Tetranychidae.

Nematode Transmission

Approximately 20 plant viruses are transmitted by one or more species of three genera of soil-inhabiting, ectoparasitic nematodes (Fig. 14-19). Nematodes of the genera *Longidorus*, *Paralongidorus*, and *Xiphinema* transmit several polyhedral-shaped viruses known as nepoviruses, such as *grape fanleaf*, *tobacco ring spot*, and other viruses, whereas nematodes of the genera *Trichodorus* and *Paratrichodorus* transmit at least two rod-shaped tobnaviruses, *tobacco rattle* and *pea early browning*. Nematode vectors transmit viruses by feeding on roots of infected plants and then moving on to roots of healthy plants. Juveniles as well as adult nematodes can acquire and transmit viruses; however, the virus is not carried through the juvenile molts or through the eggs, and, after molting, the juveniles or the resulting adults must feed on a virus source before they can transmit again.

Fungus Transmission

Root-infecting fungal-like organisms, the plasmodiophoromycetes *Polymyxa* and *Spongospora*, and the chytridiomycete *Olpidium*, transmit at least 30 plant viruses. Some of these viruses apparently are borne internally in, whereas others are carried externally on the resting spores and the zoospores of the fungi. On infection of new host plants, the fungi introduce the virus and cause symptoms characteristic of the virus they transmit (Fig. 14-19).

Dodder Transmission

Several plant viruses can be transmitted from one plant to another through the bridge formed between two plants by the twining stems of the parasitic plant dodder (*Cuscuta* sp.) (Fig. 14-15). A large number of viruses have been transmitted experimentally this way, frequently between plants belonging to families widely separated taxonomically. The virus is usually transmitted passively through the phloem of the dodder plant from the infected plant to the healthy one.

Q.1 How viruses can be detected by a rapid detection test?

Ans – Virus will be detected by enzyme linked immunosorbent assay (ELISA) as a rapid detection tool.

Q.2. What is viroids?

Ans- Viroids are plant pathogens: small, single-stranded, circular RNA particles that are much simpler than a virus. They do not have a capsid or outer envelope, but, as with viruses, can reproduce only within a host cell. Viroids do not, however, manufacture any proteins. They produce only a single, specific RNA molecule.

Viroids were discovered in 1971 by T.O. Diener, a plant pathologist. It is still not clear how they cause disease, especially since they may cause severe problems in one plant and no particular symptoms in a related species. They encode no proteins. Perhaps they bind to something in the cell and disrupt some regulatory mechanism. The RNA of many viroids contains sections of nucleotide sequence complementary to key regions at the boundaries of RNA introns; maybe that is how they damage cells. They also have nucleotide sequences similar to some seen in transposons and retroviruses.

Q.3 What is prion?

Ans - Prions, so-called because they are proteinaceous, are infectious particles, smaller than viruses, that contain no nucleic acids (neither DNA nor RNA). Historically, the idea of an infectious agent that did not use nucleic acids was considered impossible, but pioneering work by Nobel Prize-winning biologist Stanley Prusiner has convinced the majority of biologists that such agents do indeed exist.

Q.3. What is CRISPR-CAS 9 technique?

Ans - CRISPR-Cas9 is a unique technology that enables researchers to edit parts of the genome by removing, adding or altering sections of the DNA sequence.

It is currently the simplest, most versatile and precise method of genetic manipulation and is therefore causing a buzz in the science world.

Q.4 How CRISPR-CAS 9 works?

Ans - The CRISPR-Cas9 system consists of two key molecules that introduce a change into the DNA. These are:

An enzyme - called Cas9. This acts as a pair of ‘molecular scissors’ that can cut the two strands of DNA at a specific location in the genome so that bits of DNA can then be added or removed.

A piece of RNA-called guide RNA (gRNA). This consists of a small piece of pre-designed RNA sequence (about 20 bases long) located within a longer RNA scaffold. The scaffold part binds to DNA and the pre-designed sequence ‘guides’ Cas9 to the right part of the genome. This makes sure that the Cas9 enzyme cuts at the right point in the genome.

The guide RNA was designed to find and bind to a specific sequence in the DNA. The guide RNA has RNA bases that are complementary to those of the target DNA sequence in the genome. This means that, at least in theory, the guide RNA will only bind to the target sequence and no other regions of the genome.

The Cas9 follows the guide RNA to the same location in the DNA sequence and makes a cut across both strands of the DNA.

At this stage the cell recognizes that the DNA is damaged and tries to repair it.

Scientists can use the DNA repair machinery to introduce changes to one or more genes in the genome of a cell of interest.

Study of phanerogamic plant parasites.

PHANEROGAMIC PLANT PARASITES

Most of the diseases are caused by fungi bacteria and viruses. There are few seeds plants called flowering parasites (Phanerogams) which are parasitic on living plants. Some of these attack roots of the host, while some parasites on stem. Some are devoid of chlorophyll and entirely dependent on their host for food supply, while other have chlorophyll and obtain only mineral constituents of food from host by drawing nutrition and water they are called as Holoparasites or complete or total parasite. They have haustoria as absorbing organs, which are sent deep into the vascular bundle of the host to draw nutrients, water and minerals.

Flowering Plant Parasites: There are two types of parasites.

1) Root Parasites:

- i) Striga (Partial root parasite)
- ii) Orobanche (Complete root parasite)

2) Stem Parasites:

- i) Dodder (Cuscuta) (Complete stem parasite)
- ii) Loranthus (Partial Stem parasite)

1. Root Parasites:

1. Total or Complete or Holoparasite: Orobanche(Broomrape/Tokra)

It is annual flashy flowering plant growing to height of about 15-50 cm long, yellow or brownish colour and covered by small thin scaly leaves. Flowers appears in the axil of leaves are white or tubular. Fruits appears in the axil of leaves are white or tubular. Fruits are capsule containing and seeds are very small, black in colour remain viable for several years. The haustoria of parasite

penetrates into the roots of hosts and draw its nourishment. The growth of the plant is retarded, may die some times. It attacks tobacco, tomato, brinjal, cabbage, cauliflower.

2. Hemi Partial or Semi Root Parasite:

Striga (Witch Weed or Turfula or Talop)

Family *Scrophulariaceae*

It is a small plant with bright green leaves grows upto height 20-60 cm leaves bears chlorophylls and developed in clusters of 10-20 % host plant. They are obligate parasites therefore, do not obtain all their nutrient from their host root. Flowers are pink in colour, seed are very minute and produce in grate number 5000 to 100000 seeds plant per years. One flower contain 1200-1500 seeds and remains viable upto 12-40 years. Dissemination takes place with rain water, flood, wind and irrigation water. It cause yellowing and wilting of host leaves. It attacks sugarcane jowar, Maize, cereals and millets.



b. Stem Parasites:

1. Total or Complete or Holoparasite:

Cuscuta or dodder (Amarvel, Lovevine) Family *cuscutaceae*.

Genus – *Cuscuta*

It is non chlorophyllous, leaf less parasitic seed plant.

It is yellow pink or orange in colour and attached to the host. They do not bear leaves but bear minute function less scale leaves is produces flower and fruits. Flower are white, pink or yellowish in colour and found in clusters. Seed are form in capsules. A single plant may be produce 3000 seeds.

The first appearances of parasites is noticed as thread like leaf less stem which devoid of green pigment and twine around the stem or leaves of the host. When stem of parasitic plant comes in contact with host, the minute root like organs. i.e. haustoria penetrates into the host and absorbs. When the relation ship of the host is firmly established, the dodder plant loses the contact from soil.

These affect plant get weakened and yield poorly the seeds spread by animals, water and implements and remain viable when condition are unfavorable.

It attacks berseem alfalfa, clover, flax, onion, potato, ornamental and hedge plants.

2. Partial, Semi or Hemi Stem Parasites: Loranthus

Family-Loranthaceae.

It is a partial parasite of tree trunks and branches with brown stem, dark green leaves but no roots.

1. Stem is thick and flattened of the node, appear in clusters at the point of attack which can be easily spotted on the trees.
2. At the point of attachment with the tree, it shows swellings or tumourous growth where the haustoria are produced. It produces flowers which are long, tubular, greenish, white or red colour and found in clusters. It produces fleshy berries with single seed.
3. The affected host plant becomes stunted in growth and dispersal of seed is mostly through the birds and animals. It attacks mango, citrus, apple, guava.

