Plant Virus, Vector

Epidemiology and Management

S. Mukhopadhyay

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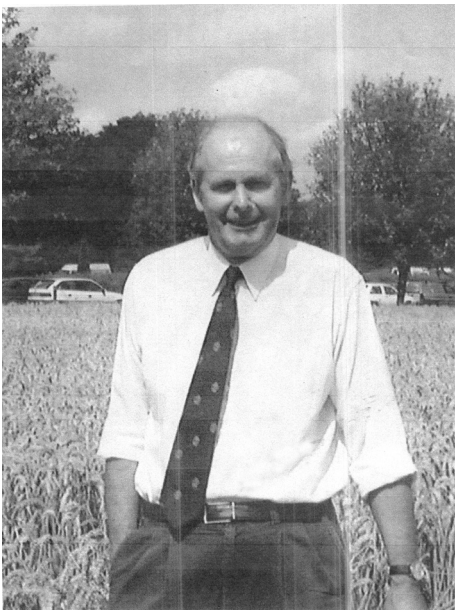

Foreword

More than 25 years ago it was my pleasure to meet Prof Mukhopadhyay at Kalyani where he had established a very effective and productive research group investigating, particularly, the epidemiology of the disease known as Rice Tungro. This disease has an especially interesting aetiology, transmission and dispersal characteristics which his group helped clarify. Even then he was keen to provide a volume that would both demonstrate his enthusiasm and interest, and his view that the practical aspects of plant viruses and their vectors needed study.

Since then much progress has been made in the study of viruses but, arguably, more effort has been devoted to their molecular structures and genetics in the laboratory than to their study in the "real" world where complex interactions between viruses, vectors, plants and the environment occur. While it is clear that molecular methods have the potential to offer much in virus disease control it is equally certain that they will only make a contribution rather than being the solution to control.

Support and encouragement from the British Council-sponsored higher education Link Project with IACR-Rothamsted, now Rothamsted Research, which, as well as stimulating research, has delivered the valuable compendium of "Viruses of Crops and Weeds in Eastern India",

and with the Natural Resources Institute, Chatham, UK, and now part of the University of Greenwich, has helped Prof Mukhopadhyay develop his ideas and discuss them with various interested parties and research groups.

This volume represents Prof Mukhopadhyay's view of the priorities in practical plant virus research and ways in which their control or management should be sought through an understanding of the practical and environmental aspects of the interactions of viruses with their vectors and their environment. Such a view will always be at the heart of virus management and control and I hope this volume will be instrumental in ensuring that others benefit from his knowledge and work.

R.T. Plumb
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Preface

During the current decade two outstanding books on plant viruses have been published, "Matthews Plant Virology" in 2002 and "Principles of Plant Virology: Genomes, Pathogenicity and Virus Ecology" in 2007. Both these are unparallel resource books for plant virologists but these seem to fail to satisfy agricultural practitioners who need more practical information on the ways to combat the ever-increasing problems of virus diseases. Thrust areas of these books mostly appear to be on genes and genetic biotechnology with a view to produce transgenics against viruses in the future. A cursory survey on the research conducted during the last five years also shows that most of the papers published were on gene information technology. In the current perspective, therefore, requirements of agricultural practitioners are not properly addressed.

Virus diseases in fact are the result of interactions between the viruses, hosts, vectors and the environment particularly climate and weather. Vectors play a key role in the spread of virus diseases. Unfortunately very limited number of papers has been published during the last five years on vectors, their dispersal, movement and migration that largely depend upon climatic conditions. Epidemiology of different virus diseases also now needs to be revisited in view of global warming and climate change.

Moreover, transgenics alone may not be the ultimate solution as the virus genomes are continuously reassorting and mutating. Susceptibility of the vectors to the viruses, their transmission efficiencies in various hosts under different circumstances are also changing with time, cropping patterns and crop genetics. Incidence of new viruses is on the rise and no regional package is there for management of virus diseases with respect to climatic zones.

The primary focus of this book is the proper understanding of the vectors, their biology, dispersal, movement and migration, contemporary canvases of epidemiology, and the management of virus diseases keeping in view the globalization of agriculture as also the viruses and their quarantine requirements.

This book starts with a "Prelude", to briefly recapitulate the background knowledge on this unique form of life. Chapter 1 of this book also deals with the recapitulation of the current knowledge on "Nomenclature,

Taxonomy and Classification of Plant Viruses". In this chapter, early records, international consciousness, group system and modern systems of classification have been presented including an Appendix on the updated list of viruses. Chapters 2 & 3 of the book deal with the "Diversities of Physical and Chemical Structures of Viruses". One of the Appendices of Chapter 3 extensively reviews the "Genomic Organization and Expression". The next chapter (Chapter 4) deals with "Diagnostics of Viruses" depending on the differences of shape, sero-diagnosis, molecular diagnosis and diagnosis by biological methods. It also includes an Appendix on methods currently developed for routine diagnosis of some viruses.

Chapter 5 deserves special attention as it gives different unconventional approaches to "Vectors: Their Morphology, Biology and their Relationship with Viruses". Chapter 6 deals with "Dispersal, Movement and Migration of Vectors" including the methodologies to study these aspects particularly flight activity, atmospheric transport and long distance migration. Chapter 7 deals with "Epidemiolgy" in view of all the modern particularly ecological perspectives including the anticipated changes of the conventional approaches due to global warming. Chapter 8 deals with "Virus Diseases Management" including the management of viral sources, sources of resistance and the scope of minimizing the vector build up citing a few cases of field practices.

It is hoped that it may be a good resource book for practical agriculturists to view virus diseases in totality, diagnosis, vectors, and the environment.

May 2010 **S. Mukhopadhyay**

Acknowledgment

Unbound gratitude is accorded to Professor R.T. Plumb, the outstanding Plant virus epidemilogist, for enriching my knowledge on all aspects of viruses and improving the manuscript wherever required. His meticulous observations in general established his acute love for the subject, keen interest for the publication of this book and high spectra of his human values.

Technical support received from Dr. Sukumar Chakraborty, CSIRO Plant Industry, Queensland Bioscience Precinct, Australia, Professor F. Van den Bosch, Biomathematics and Bioinformatics Division, Rothamsted Research, Herpenden, UK and Professor R. Harrington of the same Institute, particularly for providing information on 'Climate Change and Virus and Vector Ecology' and 'Molecular and Ecological Epidemiology' is deeply acknowledged.

Thanks are extended to Dr. B.K. Das, Department of Entomology, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India, for supplying the photographs of insects used in this book. Services provided by Ms Carol Connet of the IACR-Rothamsted to process the Agreement and sending the images of 7 viruses to the Publisher and Mr. Tarun Sen of the Air Bridge Green, West Bengal for partly preparing the photographs in printable forms are also appreciated.

Heartfelt thanks are extended to my wife, Sujata, for sparing me from my duties at home almost for two years, Arnab, my eldest son for his hospitality and initiating the literature search for me through websites during my stay in the UK, Kaustav, my youngest son for playing a key role in the preparatory process by providing his help in computer applications, particularly scanning and setting the figures and looking after the preparation of the final manuscript.

Last but not the least in this line is Master Anish and Arush, my grandsons for carefully glueing the pages together of my first manuscript, perhaps to save it from misplacement or spoilage.

May 2010 **S. Mukhopadhyay**

Contents

Prelude

Etymologically, virus means "Poison, venom, also a rammish smell as of the arm-pits, also a kind of a watery matter, whitish, yellowish and greenish at the same time, which issues out of ulcers and stinks very much; being induced with eating of poison of malignant qualities". Historically, this term has been used to denote the causal agents of a particular type of disease, which differs from those caused by other pathogens such as fungi, bacteria or nematodes. These causal agents, or viruses, were later found to be submicroscopic particulate structures mostly having cubical or helical symmetry being composed of only one type of nucleic acid and protected by a protein coat, some of which could be isolated in purified form and crystallized.

1. VIRUSES: MOLECULES OR ORGANISMS

In any discourse on viruses, an age-old question always arises; is a virus a *Molecule* or an *Organism*? This controversy began during the 1930s and lingers even today. The discovery that some viruses could be crystallized like a chemical molecule and yet retain their ability to cause disease contributed to this controversy and the question of whether viruses are living or non-living? That seemingly sterile fluids from diseased plants, animals or bacteria could be infective was not readily explained by knowledge at that time. At the beginning of the controversy, the *Organism* concept predominated. Pathologists were content to assume that viruses were essentially similar to bacteria as both of them multiply in host organisms and while doing so, occasionally change and produce progeny with new characters. Unusual inclusion bodies were also reported in cells of some virus infected plants and animals and similar inclusions were known in many animal diseases caused by organisms. The discovery of viruses, however, changed the contemporary concept of organisms as systems that possess genetic continuity and an evolutionary independence as viruses possess such continuity and are apparently independent of the host cells for their evolution. Thus, they are independent genetic systems having their own independent movement and survival.

After the successful isolation and purification of many plant viruses, the "*Molecular view*" of the nature of viruses started to gain ground. As distinct

from normal organisms, viruses could be crystallized and reconstituted; they are chemically simple and possess regular internal structures. The chemical simplicity and regular internal structure are constant for all the viruses so far purified, even for those where crystallization was not possible. The *Molecular view* was further strengthened by the apparent homogeneity of the purified virus preparations.

As more and more information is obtained on physical, chemical and biological properties of viruses, the arguments and discussions about the nature of viruses move beyond molecules or organisms, living and non-living, and rather they appear to be at the threshold of life, linking living to the non-living and matter to organism. Viruses are not organisms since they do not possess independent metabolic activities. They are not molecules as they can change and mutate. They are living while within host cells and non-living outside those cells. Thus depending on the phase of their existence, they may either be an *organism* or a *molecule*. The essential organism and molecular features of viruses are summarized in Table P.1.

Table P.1 Molecular and Organism Properties of Viruses

Molecular properties	*Organism behavior*
Non-cellular	Replicates and maintains genetic continuity
Absence of energy releasing systems	Mutates
Uniform size and shape	Intercellular movement in host
Uniform chemical composition	Operates recognition system to select hosts and vectors
Can be crystallized	Show biological relation with vectors
Can be chemically dissociated and reconstituted	Possess own characteristic host range
Synthesis follows specific pathways	Survives beyond the death of parasitized cell

2. DEFINITION OF A VIRUS

The term virus describes a very dynamic entity. With advances in the knowledge of the nature of this entity, scientists started to define it according to their own perceptions and the knowledge at the time. In 1950, Bawden defined a virus as "obligate parasitic pathogen with a dimension of less than 200 mμ". Lwoff and Tournier in 1966 defined viruses as being composed of either deoxyribonucleic acid [DNA] or ribonucleic acid [RNA] and reproducing through the replication of the constituent nucleic acid. They further stated that viruses do not possess any energy producing or releasing system and do not multiply by binary

fission. They further included in their definition that viruses are strictly intracellular and replicate within the host cells, using the host's ribosomes. Fraenkel-Conrat (1969) described viruses as infectious molecules of either DNA or RNA, normally en-coated by protein; after virus entry into the host cell, the nucleic acid takes control of the important organelles of the cell and replicates; in some cases, the viral genome becomes reversibly integrated in the host genome and thereby becomes cryptic or transforms the character of the host cell. Gibbs and Harrison (1976) defined viruses as "transmissible parasites whose nucleic acid genome is less than 3×10^8 daltons in weight and that need ribosome and other components of their host cells for multiplication" and Matthews (1992) defined viruses as "Sets of one or more genomic nucleic acid molecules, normally encased in a protective coat or coats of protein or lipoprotein which is able to mediate its own replication only within suitable host cells. Within such cells, virus replication is (1) dependent on the host's protein synthesizing machinery; (2) derived from pools of the required materials rather than from binary fission; and (3) located at the sites not separated from the host cell contents by a lipoprotein bi-layer membrane".

Viruses are very small obligate parasites that contain one to several hundred genes of their own which can mutate. As more knowledge on viruses is acquired, its definition continues to change.

3. VIRUSES AND OTHER INTRACELLULAR ORGANISMS

Viruses have several properties that are exclusive to them and several that they share with other intracellular obligate parasites. The exclusive properties are: (i) the constituent nucleic acids are either DNA or RNA; (ii) nucleic acids may be single or double stranded; (iii) a mature particle may contain other poly-nucleotides in addition to its own genomic ones; (iv) the genomic and/or other nucleotides may be distributed in one or more particles; (v) specific enzymes may be present in the particle for the replication of the genomic nucleic acid; (vi) many viruses usually multiply in the virus-induced area of the host cell; (vii) multiplication of some viruses requires the presence of other viruses.

The properties that viruses share, include (i) size; (ii) nature and quantity of genomic nucleic acid; (iii) presence of RNA/DNA; (iv) presence of envelope; (v) intracellular multiplication; (vi) absence of energy producing system; (vii) dependence on host cell's amino acid pool. There are several intracellular organisms of a similar size to viruses but the poxvirus is larger than the *Chlamydiae*. The nature, quantity and properties of the nucleic acids are more or less similar in both types of parasites, but intracellular

organisms normally contain both types of nucleic acids. There are several viruses (complex viruses) that contain envelopes/membranes, as do intracellular organisms. Both viruses and other intracellular organisms multiply within cells. There are some intracellular microorganisms, such as *Chlamydiae,* which, like viruses, do not have any energy producing system and many bacteria also depend upon the amino acid pool of the host cells for their multiplication. Properties of viruses not found in any intracellular microorganism are that virus particles do not remain separated from the organelles of a cell at the time of their multiplication; viruses do not have any protein synthesizing system; viruses never multiply by binary fission.

4. INTRACELLULAR MICROORGANISMS ALLIED TO VIRUSES

The intracellular microorganisms that are often considered allied to viruses include *viroids, phytoplasmas, spiroplasmas, rickettsia* and *chlamydiae*. *Viroids* are normally called mini-viruses and found only in plants; Diener (1971), while searching for the pathogen of potato spindle tuber disease, discovered that free ribonucleic acid is the infectious agent for this disease and called it a *viroid*. Subsequently similar causal agents were found as the causal agents in several diseases that were previously thought to be virus disease. The RNA of the *viroids* so far known is single stranded and circular.

Doi *et al* (1967) while searching, by electron microscopy, for the causal agents of the so-called "yellows" and "little leaf" type of diseases, saw mycoplasma-like organisms in infected cells. *Mycoplasmas* are wall-free prokaryotic organisms, surrounded by an exterior membrane and contain both RNA and DNA and can be cultured in a cell-free medium. Mycoplasma-like organisms (MLO) now more commonly known as *Phytoplasmas*, cause a range of "yellows" type diseases in plants and are well known pathogens of animals and human beings. They are simple, pleiomorphic cells, normally 100-400 nm in diameter; some of the filamentous forms may be up to 1700 nm long. Their appearance ranges from simple or budding spheres, dumbbell-shaped, simple or branched filaments, occasionally connected in long chains and covered by a continuous membrane, and confined to the sieve tubes of infected plants. The cells of *mycoplasmas* are non-motile and grow in minute colonies with a central nipple in culture media; each cell is normally surrounded by single tri-laminar lipoprotein membrane, about 10 nm thick. A *mycoplasmal* or *phytoplasmal* cell contains ribosomes, RNA and double stranded circular DNA, the molecular weight of which is usually $4 \times 10^8 - 1 \times 10^9$ Da.

Mycoplasmas usually multiply by binary fission or by budding and may undergo structural changes during the course of the reproduction cycle. They can be distinguished from other pathogens by their sensitivity to tetracycline and resistance to the penicillin group of antibiotics. They are transmitted by insect vectors and can be transmitted by grafting.

Spiroplasmas, Rickettsiae and *Chlamydiae* are mostly found as pathogens of human beings and animals. Fudl-Allah *et al* (1972) and Saglio *et al* (1973) while working to diagnoze the pathogen of the citrus stubborn disease successfully isolated a wall-less prokaryote. Davis *et al* (1972) using dark-field and phase contrast microscopy, saw spiral bodies in the crude sap collected from *stunt* infected corn plants. The culture of the prokaryote collected from the *citrus stubborn* infected plants also showed spiral bodies (Cole *et al* 1973). These spiral bodies were designated as "*Spiroplasma*". Normally *spiroplasmas* are pleiomorphic, commonly helical and measure 150-200 nm in diameter and 3-15 nm long. Under unfavourable conditions, they may divide into small helices, asteroids or coccoid structures. The *spiroplasmas* are normally motile but, like viruses and *phytoplasmas*, are transmitted by vectors.

Rickettsia normally does not infect plants but are occasionally found in the vascular tissue of some plants. They are small rods (0.2-0.5 nm × 1-4 nm) bounded by a tri-laminar membrane and an additional cell wall. They can be cultured *in vivo* and transmitted by grafting and vectors. The number of proteins in these organisms also differs. The number of proteins found in Eubacteria (*Escherichia coli*), *Mycoplasma*, *Chlamydiae* (*Psittacosis*), a large virus infecting vertebrates (*Vaccinia virus*), a large virus infecting angiosperms (*wound tumour virus*), a small virus infecting angiosperms (*TMV*) and one of the smallest known plant viruses (*tobacco necrosis satellite virus*) are 4,100, 820, 660, 260, 12, 4 and 1 respectively (Matthews 1992).

Viruses have three-dimensional structure and their volume may be as large as 6×10^5 nm^3 and as small as 2×10^4 nm^3. The smallest plant virus may be as small as a messenger-RNA (mRNA) and the largest may be of the same size as that of the smallest cell.

5. HISTORICAL PERSPECTIVES OF THE NATURE OF VIRUSES

Although we know that virus diseases of humans, animals and plants have always been present, the science of virology and the understanding of the nature and function of viruses do not have a long history. The human disease called "*smallpox*" was described in China as early as the 10th century BC and "*yellow fever*" ravaged tropical Africa for many centuries. "*Jaundice of silkworms*, "*leaf-roll of potato*" and "*tulip flower breaks*"

were known to people from at least the 16th century, but the history of virology, and plant virology in particular perhaps started in the 17th century through the accidental observation of some horticulturalists in Holland that they could transfer *tulip break* and *jasmine mosaic* by grafting (see McKay and Warner 1933).

Edward Jenner, in 1798, laid the foundation of virus research by successfully establishing the antigenic nature of a virus. It was possible to vaccinate humans against *smallpox* by inoculating them with extracts of *cowpox virus*. Pasteur (1884) made the next break-through in antigen research by culturing *Rabies virus* in tissues of laboratory animals. They were able to immunize human beings against that virus by inoculating the attenuated culture. Animal virologists also took the lead in the understanding the mode of transmission of viruses. The transmission of *Yellow fever* by mosquitoes was first observed as early as 1848 and Reed (1902) established the relationship between mosquitoes and *Yellow fever*. Experimental transmission of a plant virus (*Rice stunt virus*) was made by Hashimoto in 1884 (see Fukushi 1933) and confirmed by Fukushi (1933). While animal virologists remained busy with immunology, transmission and epidemiology, plant virologists concentrated their efforts on understanding the nature of viruses and were handicapped as there was no method for their artificial culture.

Mayer (1886) was the first to start research on the nature of plant viruses. He demonstrated the transfer of the mosaic symptom found in tobacco plants by transferring the juice from an infected to a healthy plant using a capillary tube and discovered the sap transmissibility of *Tobacco mosaic virus* (TMV). Sanarelli (1898) became the first person to successfully transmit *Rabbit Somatosis* virus by inoculating the extract from infected tissues to the healthy animals, but neither Mayer nor Sanarelli could find any pathogen in the sap or extract. Ivanowski (1892) was preoccupied with the supposed organismal nature of TMV and demonstrated that the infectious extract would pass through bacteriological filters. While disappointed by the results he unknowingly established the "Filterable nature" of a virus. Loeffler and Frosch (1898) made a similar observation with the *Foot and mouth disease* of animals but they went further and established the serial transmission of this virus and the multiplication of the virus in the host tissues; they also developed a quantitative assay method for the same virus. Beijerinck (1898), a Dutch scientist working with TMV, also made serial transmissions of TMV and established that TMV multiplied in tobacco plants but could not develop any method for its quantitative assay, as it could not be artificially cultured in tobacco tissues. He described the virus as a "*contagium vivum fluidum*" or an infectious living fluid. At the same time search for a method to quantitatively estimate a plant virus continued

and Holmes (1929) discovered the "*local lesion*" reaction in certain hosts and used this method for the quantitative estimation of sap transmissible plant viruses.

As well as the fundamental research on understanding the nature of viruses, reports of their incidence continued and many viruses were reported from more than one thousand species of plants in many widely different families and genera. These viruses were identified by the symptoms they caused, their host ranges, characteristics of their transmission and several physical properties of the infectious sap and different virus-vector relationships without having any knowledge of the virus structure or organization.

Stanley (1935) made an important breakthrough in understanding of the nature of a virus when he was successful to isolate, purify and crystallize infectious protein from a sap extracted from the TMV infected plants. Bawden and Pirie (1936) discovered that TMV is actually an infectious nucleoprotein containing RNA coated by protein molecules; Schlesinger (1936) observed that Coli-phages contain DNA.

Thus virologists became confident that, whatever the host (animal, plant or bacteria), viruses consist only of infectious nucleoprotein, with either RNA or DNA but did not know how they looked like. Kausche *et al* (1939) used their newly constructed electron microscope to examine sap extracted from a tobacco plant infected by TMV. To their great surprise they found small rod like particles but did not prove that they were the infectious agent. As technology and instrumentation improved, virologists established that viruses are either rods or spheres. The shape of the particles suggested an organized structure. X-Ray crystallography revealed the arrangement of protein molecules in the rod shaped particles of TMV.

Delbruck and Bailey (1946), Hershey and Chase (1952) and Zinder and Lederberg (1952) discovered that viruses transfer genetic characters from one bacterium to another where they may be stabilized and carried through subsequent generations and called the process "*Transduction*". Though genetic studies, particularly on bacterial viruses, were making progress, the infectious principle of the virus remained unknown. In-depth studies on the infection of *Escherichia coli* by T2 phages, Hershey and Chase (1952) showed that it is the DNA of the phage that enters the bacterial cell and causes infection; the protein component of the phage stays on the bacterial cell wall. Fraenkel-Conrat and Williams (1955) also drew the same conclusion on the infectivity of the nucleic acid component through their historic "*Reconstitution of TMV*" by changing the pH and ionic strength of the isolation medium. Recombining the RNA of the one strain of TMV with the protein subunits of another, they obtained a

particle that showed the properties of the strain from which the RNA was obtained. Gierer and Schramm (1956) took a direct approach to prove the infectivity of the RNA of TMV. They isolated the nucleic acid from the infected sap and inoculated it to healthy plants that became infected.

In plant and animal cells DNA is double stranded (and infective) whereas normal cellular RNA is single stranded (and non-infective). Bawden and his associates, while laying the foundation of nucleic acid research on TMV in 1936, found that virus RNA was infective and single stranded. The infective DNA of the coli-phages on the other hand, was double stranded (Hershey and Chase, 1952). Subsequent workers proved that there are double stranded infective RNAs in many plant viruses. Shepherd and Wakeman (1971) found double stranded DNA in *cauliflower mosaic virus*. Later, the presence of single stranded DNA in plant viruses was also demonstrated for *Mungbean yellow mosaic virus*. Kassanis (1962) demonstrated that not all viruses are independent in their multiplication; some depend on another virus which he called "satellite virus" (*Tobacco necrosis satellite virus*). It was also discovered that some viruses consisted of separate genomic pieces and these studies were further extended by Kaper and Waterworth (1977) who discovered a fifth piece of nucleic acid in multipartite *cucumber mosaic virus*, which they called "*Satellite RNA*". Recent research on the nature of viruses has mostly concerned with the molecular and genomic properties of the nucleic acid; replication strategies; detection at the molecular level; synthesis of the virus-specific proteins and their assembly into specifically organized structures; their antigenic properties and the genetics controlling their biological properties (Hull 2002). Other recent work with viruses is in their use as vehicles for the transfer of genes from one cellular system to another, the use of virus proteins as promoters for protein synthesis (Porta and Lomonosoff 1996, Scholthop *et al* 1996) and the use of the virus protein shells for the production of vaccines in plant cellular systems (Canizares *et al* 2005). So the wide-angle journey of the virology continues to diversify towards understanding the life at molecular level and its application for human and animal welfare.

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1

Nomenclature and Classification

A. NOMENCLATURE

Before the discovery of virus particles, they were identified on the basis of their biological properties, particularly symptoms, host range, transmission and physical properties of the infectious sap. Viruses used to be denoted by the common name of their main host, or the host on which they were first identified, and the common term specifying the symptoms, as for example *Tobacco mosaic*, *Tobacco ring-spot, Tobacco leaf-curl, Aster yellows, Rice dwarf, Banana bunchy-top* etc. These approaches for identifying and naming a virus served a useful purpose but led to immense confusion and controversies. The same virus can infect many different hosts and produce different symptoms, and a single host may be infected by many viruses that produce different symptoms and is known by different names. *Tobacco mosaic virus* (TMV) produces mosaic symptoms in many plants and is recorded by a large number of different names, for example *Tomato mosaic* when it infects a tomato and *Brinjal streak mosaic* when it infects a brinjal. *Tobacco mosaic virus* and *Cucumber mosaic virus* produce typical mosaic symptoms in tobacco and cucumber respectively. When they simultaneously infect cucumber plants, an altogether different symptom appears in leaves which become horseshoe shaped. So naming a virus and virus nomenclature was, and remains, a serious concern.

1. Early Records

The system that was first proposed for virus nomenclature in 1927 consisted of three parts, the common name of the host on which the virus was first recorded, and the word virus followed by an arabic numeral to denote the chronological order of its detection. According to this system, *Tobacco mosaic virus* is called *Tobacco Virus 1*. It was later modified in 1937 replacing the common name of the host by its Latin name. So *Tobacco Mosaic Virus 1* became *Nicotiana Virus 1*. Subsequently, several attempts were made to introduce a binomial nomenclature using different generic and specific names in Latin, following the methods

used for plants and animals. According to this system, *Nicotiana Virus 1* was renamed as *Marmor tabaci* where *Marmor* means mosaic and *tabaci* reflects the host. But these systems were not widely accepted by virologists and created more confusion.

To give a definitive name to a virus and in the hope of avoiding all future confusion, Gibbs (1968) proposed a 'Cryptogramic' system of nomenclature. According to this system, a cryptogram was to follow the common vernacular name of a virus. Four pairs of characters defined the cryptogram, each pair being the key characters of a virus (Appendix I.1). According to this system, *Tobacco mosaic virus* was referred as '*Tobacco mosaic virus R/1:2/5: E/E: s/x*'. It provides precise information on a few key characters of a virus but it failed to make any ground among the virologists, possibly because of its complexities, but it did stimulate futher discussion and approaches to the issues of virus nomenclature and taxonomy.

2. International Consciousness

The necessity of a scientific and acceptable nomenclature and classification of viruses was realized by the International Organization of Microbiologists as early as 1951 and a Subcommittee was constituted for this purpose at the International Microbiological Congress held in the same year. International efforts continued and the status of the Subcommittee was elevated to that of a full Committee in 1966 and was called the International Committee on Taxonomy of Viruses (ICTV) with different Subcommittees to deal separately with animal, plant, and bacterial viruses. Since then, a number of systems have been proposed but the animal, plant and bacterial virologists could not come to a uniform conclusion.

3. Group System of Nomenclature of Plant Viruses

The Group Concept of Nomenclature of plant viruses was first proposed by Harrison *et al.* (1971). To prepare the concept paper, the authors first collected all the documented data on the 630 viruses collated by Martyn (1968). These were sorted to obtain data useful for analysis. Out of the 630 viruses, 60 were suspected to be due to Mycoplasma-like organisms and the required information was not available for 457 viruses. Therefore only 113 viruses were available for analysis. They were separated into 16 groups by cluster analysis taking the type specimen into account. The characters they considered for analysis and the groups resulting from the analysis are given in Appendices I.2 and I.3. Sixty equally weighted independent qualitative characters were used to give satisfactory and statistically valid groupings. This concept provided a new dimension in the nomenclature of plant viruses, where a virus was denoted by the usual vernacular name followed by the group name. This system was

approved by the ICTV in 1971. These groups are non-hierarchical and did not have any notion of species.

Subsequent workers gave much importance to the physical and chemical properties of the virus particles but the principle for naming a virus remains the same. Francki *et al* (1991) designated 37 groups and these were approved by the ICTV (Appendix I.4). In their groupings, *Tobacco necrosis virus* and *Tomato spotted wilt virus* of Harrison *et al* (1971) was distinctly grouped. Fauquet (1999) proposed to designate the viruses according to their morphology, physical properties of the virion, properties of the genome, including genome organization and replication, properties of proteins and lipids, and their antigenic and biological properties. But this did not significantly change the nomenclature.

Brunt *et al.*(1996) proposed a different nomenclature using the Virus Identification Data Exchange (VIDE) system, which used the Description Language of Taxonomy (DELTA). In their virus species description, the vernacular name comes first, followed by the name which has been used in ICTV publications or listed in Steadman's ICTV words (Calisher and Fauquet 1992). They changed the order of words in some names so that the host's name appears first.

B. CLASSIFICATION

Classification primarily provides proper understanding of organisms. The binomial system of nomenclature and classification in which relationships are determined by similarities and dissimilarities in selected properties or phylogeny has become the most widely used method and systems of classification that may be hierarchical or phylogenetic. The difference being in the use of the available properties of the organisms to separate them into species, genera, families, orders, classes and so on. All characters are considered in natural phylogenetic systems but the hierarchial system is based on only a few key characters. Systems of classification may be monothetic or polythetic depending upon the number of characters considered for systematic arrangement. In polythetic systems all possible characters are taken into consideration.

Attempts to classify plant viruses began as early as 1935 but Holmes (1939) proposed the first internationally accepted classification. This system was further improved in 1948 (Holmes 1948) and included all the viruses irrespective of their hosts using Latin names. All the viruses were placed under the Order 'Virales' divided into three Suborders, 'Phaginae', 'Phytopaginae' and 'Zoopaginae' depending upon the nature of the host (bacteria, plant or animal). Plant viruses were divided into six families on the basis of key symptoms. These were 'Chlorogenaceae' (producing yellows symptoms), 'Marmaraceae' (producing mosaic symptoms), 'Annulaceae' (producing ring-spot symptoms), 'Gallaceae' (Fiji disease of sugarcane), 'Acrogenaceae' (producing potato spindle

tuber disease) and 'Rugaceae' (producing leaf curl diseases). Though, this classification has no relevance today, it is astonishing to see Holmes' vision of differentiating plant viruses simply on the basis of the symptoms.

Subsequently several systems of classification were proposed but none of them was internationally accepted. The next proposal that received approval of the ICTV was put forward by Lwoff *et al* (1962). They divided phylum 'Vira' into two subphyla, 'Deoxyvira' and 'Ribovira' depending on the type of the nucleic acid present. Each sub-phylum was further subdivided into 'Classes', 'Orders', 'Suborders', 'Families', 'Genera' and 'Species' depending on different physical, chemical and biological properties known at that time. This classification also did not differentiate animal, plant and bacterial viruses.

After the establishment of the Group Concept for naming plant viruses, several plant virologists started to propose a separate system of classification for plant viruses. Matthews (1981) proposed a monothetic hierarchical system to classify plant viruses using five key characters: (i) properties of the nucleic acid, (ii) number of nucleic acid strands present in the particle, (iii) presence or absence of envelope around the particles, (iv) number of genomic pieces in the nucleic acid, and (v) structure of the particles. This proposal received the approval of the ICTV and essentially classified all the groups of plant viruses known till that date.

The Plant Virus Subcommittee of the ICTV subsequently advocated the adoption of the 'Family-Genus-Species' concept for the classification of plant viruses. They preferred to rename the so-called 'Groups' and designate them as 'Families' and to put the related members as 'Genera'. Francki *et al* (1991) proposed such a system listing 37 groups described as 'Families/Groups'. Their classification was duly approved by the ICTV. In this classification, they considered the structure of the particles, the presence or absence of an envelope around the particles, the type of nucleic acid, number of nucleic acid strands and the number of genomic fragments as key characters. The approved 'Groups' were systematically arranged and named including two families (Reoviridae and Rhabdoviridae) and 33 groups. They reported 334 members or 'species', 320 probable members or 'deemed species' and the total number of members reported was 655 (Appendix I.4). The major difficulties were in separating one species from another. A virus normally consists of a set of genes that code for functional proteins. But in this perspective defining a 'species' becomes questionable. According to van Regenmortel (1990) a "Species represents a polythetic individual constituting a replicative lineage occupying a particular ecological niche." According to Matthews (1992), a virus 'Species' may be differentiated simply from the information on the coat protein of the particle supplemented by the information on the amino acids of the protein coat, their composition, sequence and immuno-responses. He further postulated possible separation of related strains, variants and pathovars of a 'Species' on the basis of nucleotide sequence of the genome, restriction endonuclease maps and the extent of cross-hybridization.

Fauquet (1999) defined a "Species" mostly on its genomic characters particularly genome rearrangement and sequence homology. He also included in the definition some biological properties, particularly serological relationship, vector transmission, host range, pathogenicity, tissue tropism and geographical distribution. He grouped the 'Species' into 'Genera' on the basis of virus replication strategy, genome organization, size, genome segments, sequence homology, and vector transmission. The 'Genera' were grouped into 'Families', 'Families' into 'Orders' on the basis of biochemical composition, replication strategy, particle-structure and genome organization. A strain may be a set of natural isolates that may have one or several characteristic properties. Strains normally have some stability over time whereas a pathotype is a collection of isolates of a single virus that have a similar behaviour with respect to host resistance.

Adopting these principles, van Regenmortel *et al* (2000) classified plant viruses where the viruses were first separated on the basis of nucleic acids (ds DNA-RT, ssDNA, dsRNA, ssRNA–, ssRNA +. They designated 4 orders (on the basis of the nature of the nucleic acids), 14 families and 71 genera. Fifty genera were assigned to families and the rest remained unassigned. They differentiated 657 species and 85 tentative ones. The total number of members reported was 742. The Families and the Groups/Genera are given in Appendix I.5 and Figure 1.1. Brunt *et al* (1996) and Hull (2002) have extensively described the properties of the Groups/Genera. Fauquet *et al* (2005) in the VIIIth Report of the ICTV designated 3 orders, 18 families, 9 sub-families, and 80 genera. They assigned 5450 viruses to 1950 species and classify them at the family and genus levels (Appendix I.6). The list of the currently accepted English names, families and abbreviations of the viruses is given in Appendix I.7.

It is interesting to note that Harrison *et al* (1971) based their groups mostly on biological properties, as information on the virus particles was very limited at that time. van Regenmortel *et al* (2000) and Fauquet *et al* (2005) based their groupings mainly on the properties of the virion (molecular mass, buoyant density, sedimentation co-efficient, stability under different conditions), genome (presence or absence of 5′ cap, presence or absence of 3′ terminal Poly (A) tract, nucleotide sequence, replication strategy, translation process, etc.), proteins (antigenic properties, epitope mapping), tissue tropism etc. However, these critical studies did not substantially change the groupings made by Harrison *et al* (1971) and only increased the number of the groups or genera.

Current studies however suggest the possibility of a phylogenetic classification of plant viruses. It may be presumed that viruses have originated following different evolutionary pathways and gradually take the current forms. The principal points of origin are: (i) pre-biotic RNAs, (ii) escape of plant host genes, (iii) transformation of transposons found in plants, animals and insects and (iv) degeneration of cellular components.

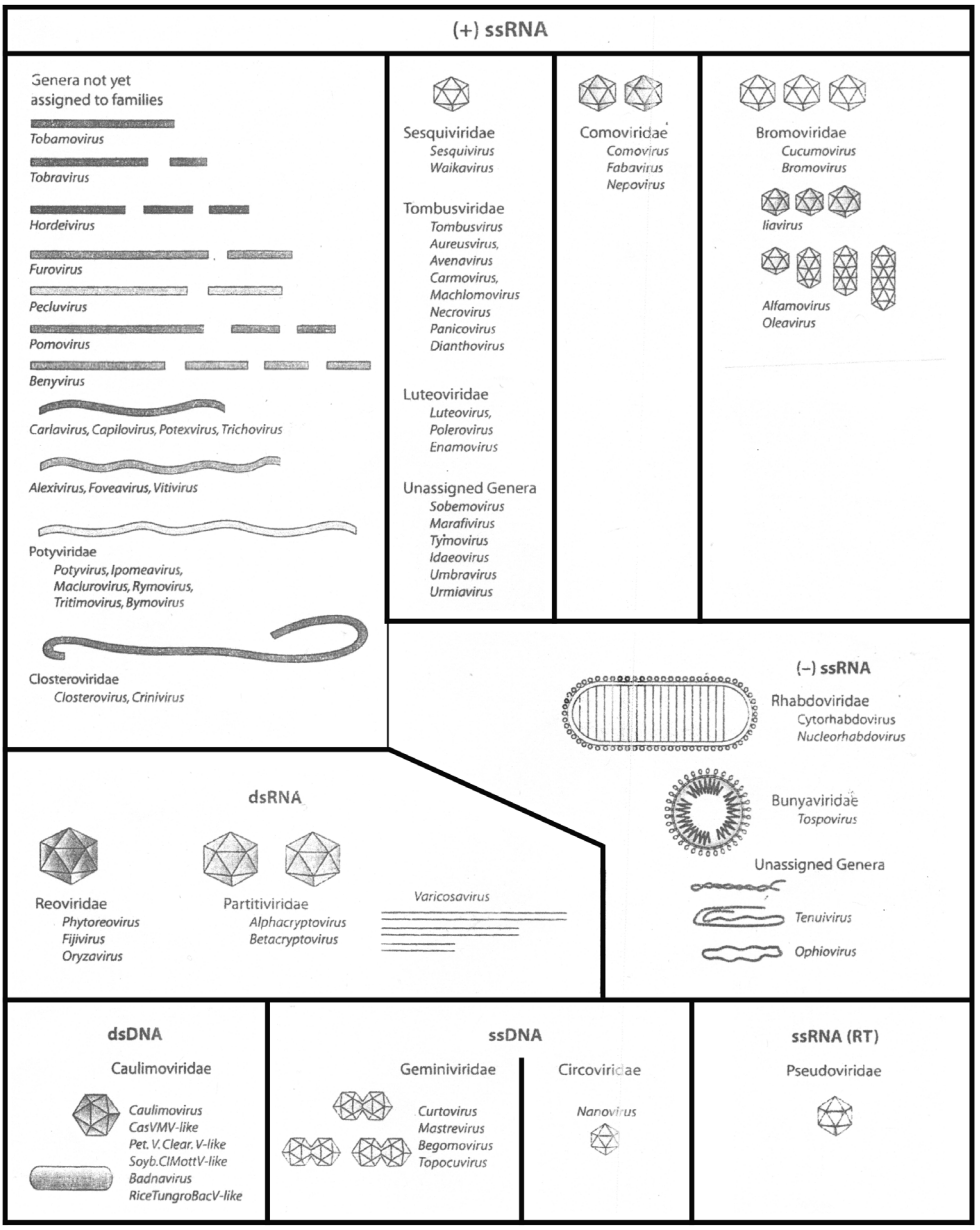

Figure 1.1 Morphological diversity of some genera belonging to different families opted (Agrios 2005 with permission from the Rights Department, Elsevier LTD)

Introns are often found in eukaryotic genes and splicing out the introns from RNA transcripts and legation of the extrons provide RNA the ability to produce new combinations of genes that later parasitize plant cells and undergo changes

to produce new viruses. It is common that some viruses exchange materials with their hosts. Some changed materials may escape the host and transform into new viruses. DNA viruses perhaps originated in this way as the viral DNA can integrate with the host genome. A transposon called *Tnt 1,* 5334 nucleotides long, isolated from plants shows similar nucleotide sequences and open reading frames (ORF) that are found in *Drosophila* and are called *Popia*. Such transposons may be transformed into retroviruses with time that are capable of infecting plants, vertebrates and insects. There is evidence that large DNA viruses have originated due to degenerative processes in host cells. It appears that the scope of the evolution of DNA and dsRNA viruses is very limited. Accordingly limited numbers of DNA (excluding Begomovirus) and dsRNA viruses are found in nature. On the contrary, the scope of changes of ssRNA is enormous. Point-mutations occur very frequently in ssRNA, as do errors in the copying processes during genome replication. There may also be recombination, reassortment of pieces of segmented genes, loss of genetic material or acquisition of nucleotide sequences from unrelated viruses or host genomes. As a result, a large number of ssRNA viruses have evolved.

The classification presented by Fauquet *et al* (2005) reveals very interesting distribution of member (species) in different genera of viruses. Large numbers of members are found in *Begomovirus* (ssDNA) and *Potyvirus* (ssRNA). There are 112 *Begomoviruses* and 100 *Potyviruses*. Next are *Carlavirus* and *Nepovirus* that contain only 33 and 32 members respectively. There are 13 genera each of which contain only one member known so far. These are: 1. *Rice Tungrovirus* (RTV), 2. *Topocuvirus* (TPCTV), 3. *Babuvirus* (BBTV), 4. *Varicosavirus* (LBVaV), 5. *Oleavirus* (OLV-2), 6. *Machlomovirus* (MCMV), 7. *Panicovirus* (PMV), 8. *Enamovirus* (PEMV-1), 9. *Maculavirus* (GFkV), 10. *Mandarivirus* (ICRSV), 11. *Avenavirus* (ACSV), 12. *Petuvirus* (PVCV), and 13. *Cirevirus* (*Citrus leprosis*). Distribution of members (species) in other genera containing 12 to 27 members is given in Table 1.1 and the rest of the genera contain 2-9 members only. Thus, it is apparent that the genomes of monotypic genera are highly conserved. High potential variability of genomes may occur in the

Table 1.1 Genera Containing 12 To 27 Members (Species)

Potexvirus	27
Tobamovirus	22
Tymovirus	21
Alfamovirus	16
Ilarvirus	15
Comovirus	15
Tombusvirus	13
Sobemovirus	11
Pseudovirus	11
Badnavirus	10

genera where the number of species is high and the variation in genomes and evolution of new species/strains/pathotypes may be a continuously occurring natural process.

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2

Diversity of Physical Structure

Viruses show a remarkable diversity in structure from simple nucleic acid threads, particles containing either of the two nucleic acids with structures behaving like organic molecules to highly complex enveloped multi-layered particles. The simplest form is infectious nucleic acid or viroid. True viruses are particulate and structured. Structures are either helically or cubically symmetrical. Helically symmetrical viruses are anisometric particles. They are of different forms and sizes. Forms vary from short or medium length rods, to long or very long flexuous particles (Figures 2.1–2.4). These particles often form liquid crystals in which rods are regularly arrayed in two dimensions. X-ray crystallography is not possible with flexuous rods and complex particles. Cubical particles are isometric with icosahedral symmetry having 20 equal sides. Simple particles form true crystals. They normally do not widely differ in form and size. There is a unique case where particles consist of twinned or geminate icosahedra. Major differences among these viruses lie in the patterns of the assembly of protein subunits and their physical, biochemical, genomic and biological properties.

All types of particles may be mono-partite or multi-partite depending upon the type of the viruses. Some of the mono-partite viruses are: *Dianthovirus, Caulimovirus, Phytoreovirus, Carmovirus, Luteovirus, Potexvirus*, *Capillovirus, Carlavirus, Potyvirus* and *Closterovirus*. *Comovirus*, *Alphacryptovirus, Betacryptovirus, Tobravirus* and *Furovirus* are bipartite. Tri-partite viruses are: *Cucumovirus, Ilarvirus, Pomovirus, Begomovirus* and *Varicosavirus. Alfamovirus*, *Ourmiavirus* and *Benyvirus* are quadric-partite whereas *Tenuiviruses* are penta-partite.

Complex particles are basically isometric, helical or both but the pattern of the assembly of the protein subunits is complex and lipo-protein layers envelope these particles. The basic symmetry in Rhabdoviruses is helical whereas it is isometric in Tospoviruses.

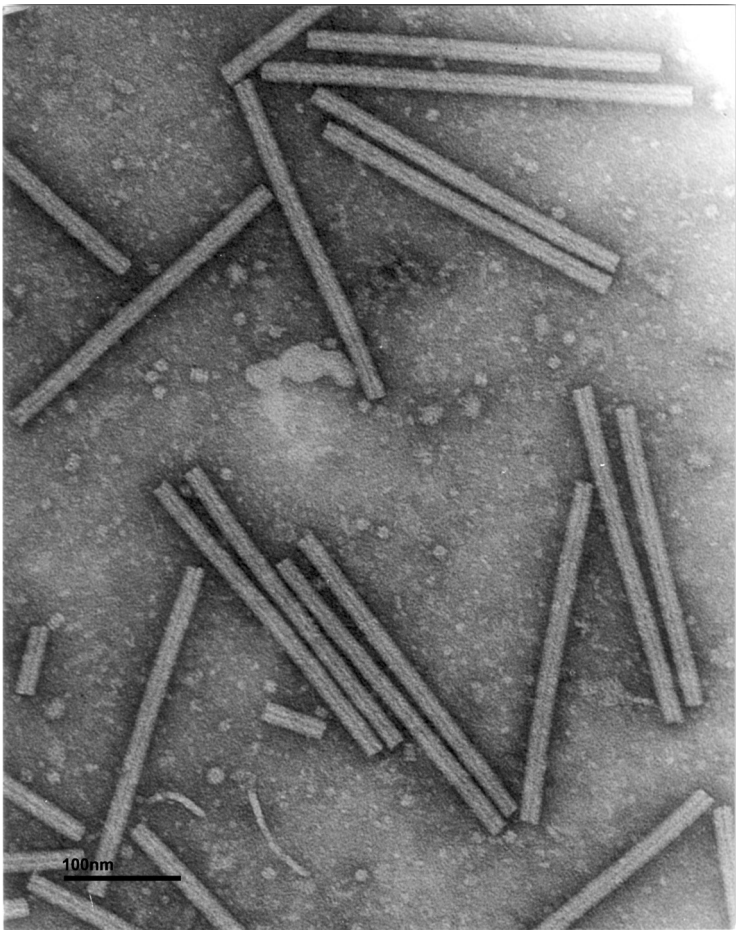

Figure 2.1 Short straight rod-shaped virus particle (*Tobamovirus*). Received from the Archives of the IACR, Rothamsted, UK

A. SIMPLE NUCLEIC ACID THREADS

Infectious nucleic acid threads are commonly known as "Viroids". The type of nucleic acid so far known in viroids is RNA and consists of a single molecular species that may occur in circular or linear forms autonomously replicating using the hosts' polymerase. There are a few groups of these viroids, mostly depending on the sequences of nucleic acid, nucleotide deletions and differences in their symptom expression. These are purely organic molecules but have lineage with viruses indicating the molecular origin of the latter.

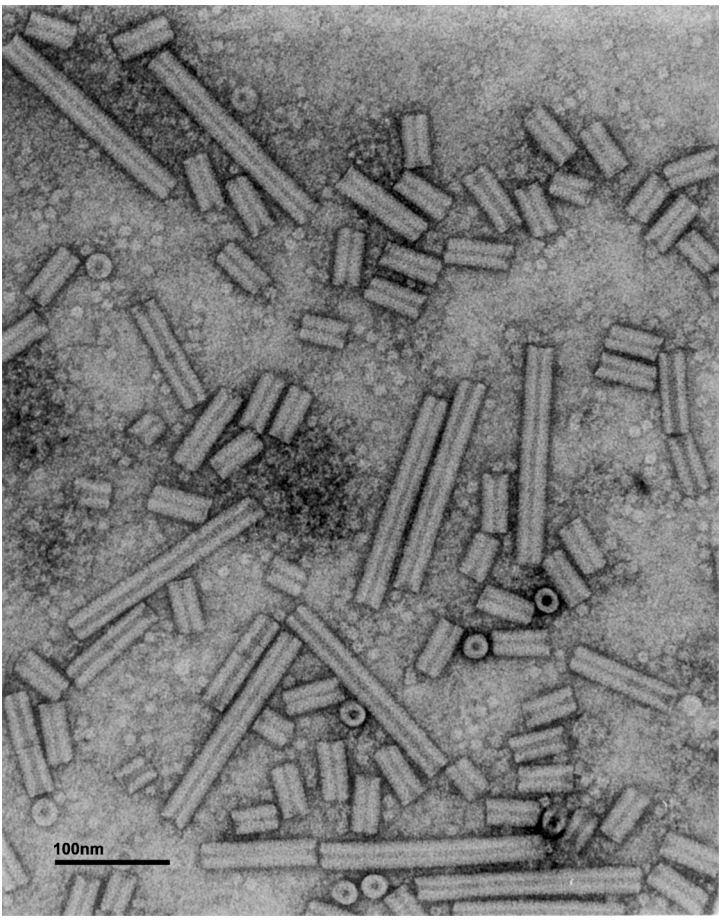

Figure 2.2 Medium straight rod-shaped virus particle (*Tobravirus*). Received from the Archives of the IACR, Rothamsted, UK

Viroid RNA has a series of regions comprising secondary structures separated by single chain loops that gives it *in vitro* the compact conformation of a rod having two loops at the ends.

B. PARTICULATE STRUCTURE

The unique feature of viruses is that their construction follows a general mathematical principle.

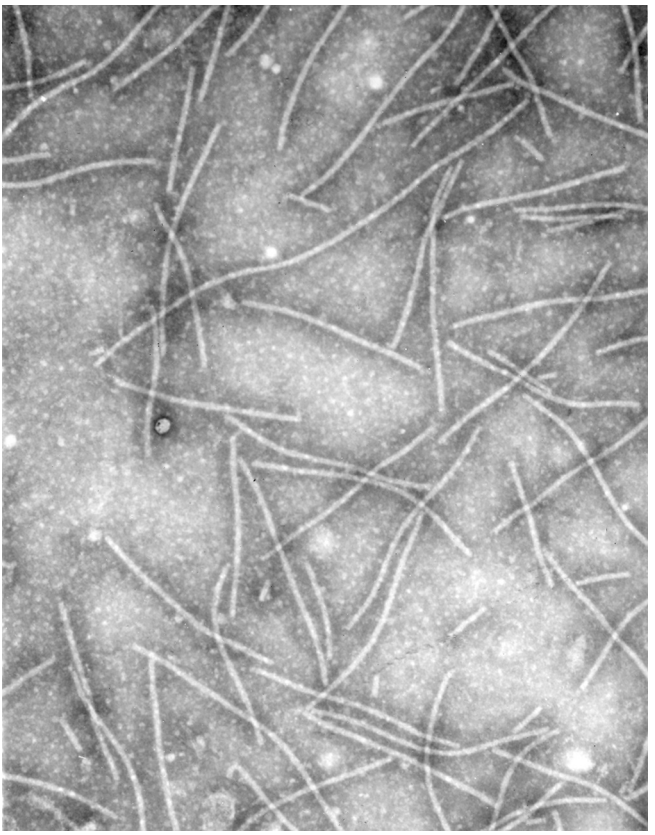

Figure 2.3 Long filamentous virus particle (*Potyvirus*)

1. General Principle of Construction

In most of the viruses infectious nucleic acid is coated by protein that forms a protective structure. The assembly of the protein subunits around the nucleic acid is a process that widely differs in anisometric and isometric viruses. Basically the overall structure of a virus particle is a design of its protein subunits. Normally, a simple virus particle contains only one type of protein (sometimes two or three as in *Comovirus*, *Sequivirus, Phytoreovirus* respectively) or protein subunits also known as "structural units" or "capsids". These subunits are composed of identical protein molecules that are packed together in a regular manner in efficient protective designs. But construction of only a few efficient designs is possible, though many identical molecules are present. Construction of these designs depends upon the type of the assembly process. Self-assembly is a process akin to crystallization and is governed by the laws of statistical

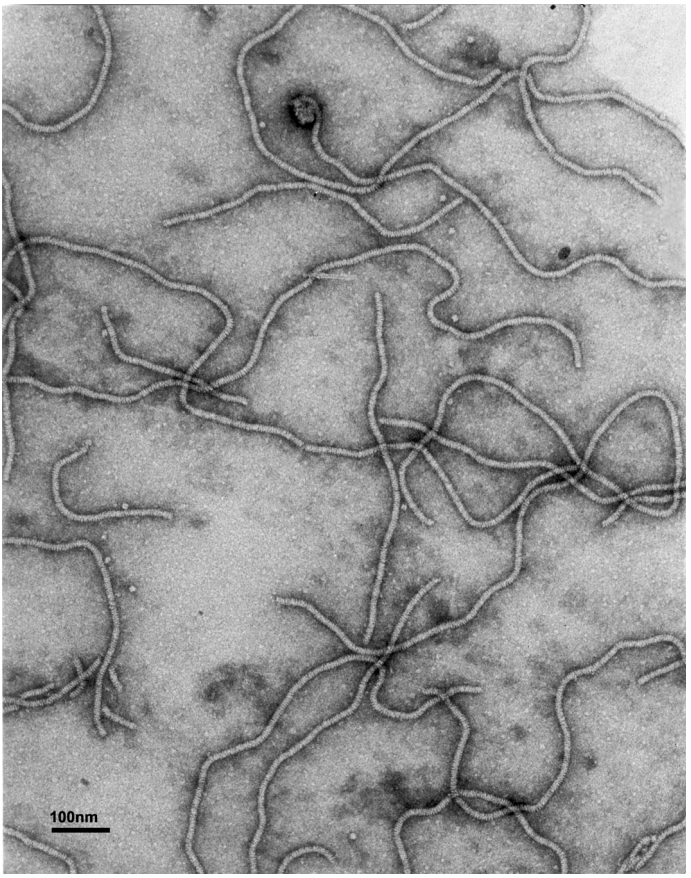

Figure 2.4 Very long filamentous flexuous virus particle (*Closterovirus*). Received from the Archives of the IACR, Rothamsted, UK

mechanics. A system of units with equivalent bonding properties will condense to form an ordered structure or set of ordered structures if the free energy of the ordered state is less than that of all other possible states. The most probable ordered structure is that in which the maximum number of most stable bonds are formed between the units. The necessary physical condition for the stability of any structure is that it is to be in a state of minimum free energy. When changing the environmental conditions of any ordered structure, dissociation can be induced without altering the integrity of the constituent components. Changing the environment back to the conditions that favour bond formation can restore the original organized structure.

An ordered structure built of identical units, always has some well-defined symmetry. Specific bonding between the units necessarily leads to a symmetrical

structure. There will be only a limited number of ways in which any unit can be connected to its neighbours to form the maximum number of stable bonds. Most probable minimum energy designs for surface crystals constructed of a large number of units are tubes with helical or cylindrical symmetry and closed shells with polyhedral symmetry or polyhedral shells with cubic symmetry. Most virus particles, irrespective of their hosts (plant, animal or bacteria), are either helically constructed anisometric rods or cubically arranged isometric icosahedrons.

2. Anisometric Particles

In helical particles, the nucleic acid thread is embedded on the protein subunits in an ordered manner (for figure, see Matthews 1992, Franklin 1955). The structure of *Tobacco mosaic virus* (TMV) shows the ordered arrangement of this process and a more or less similar process is found in other helical viruses.

(a) Structure of TMV Particle

The TMV particle is a hollow cylinder 300 nm long with an inner diameter of 4 nm and an outer diameter of 18 nm. A particle contains 2130 copies of the viral coat protein (17495 daltons) protecting a single strand of RNA of 2×10^6 daltons (~6400 nucleotides). The protein subunits are arranged along the long axis of the particle as a right-handed one-start helix of 2.3 nm pitch and containing 16.34 subunits per turn (in the type strain). The polypeptide chain of the protein subunits is in four α helices. The RNA is embedded between successive turns of the helix at a distance of 4 nm from the axis. There are three contiguous nucleotides of the RNA chain for each successive protein subunit that determine the length of the virus particle. The basic structure repeats every 6.9 nm of its length or, in other words, in every three turns of the helix so there are 49 protein subunits in each repetition and the approximate number of turns in the TMV helix is 130 (for figure, see Franklin 1955).

The domains of the structure result from folding of its polypeptides which involve two types of arrangements: α-helices generated by the rotation of the polypeptide chain on itself, β-sheets in which the chain folds on itself turns and loops that connect the sheets and hence into globular mass. The secondary structures thus formed are not entirely reliable and they need to be confirmed by referring to proteins already known by means of crystallographic methods. Tertiary structures of a polypeptide chain may also be formed from the spatial organization of helices and sheets stabilized by non-covalent interactions between amino acids. A noteworthy feature in the aggregation of protein subunits is that both the N-and C-termini of the polypeptide chains are exposed at the surface of the particle and the protein subunits are tapered at the outside. Each subunit has two groves that together form a furrow and provide space for the embedment of the helical nucleic acid.

3. Isometric Particles

In isometric particles, there is no direct physical bonding between the protein subunits and the nucleic acids. The subunits organize themselves, following geometrical principles, around the nucleic acid to make the protective cover or capsid. The morphological units of this cover, or the capsomers, can be seen by electron microscopy or X-ray crystallography.

The basic structure of isometric viruses is an icosahedron that has 20 equilaterally placed triangular faces forming among them 12 vertices (Figure 2.5). This solid presents three series of axes of rotation generating symmetries: 5-fold axes pass through the vertices; 3-fold axes are located at the centre of the triangles and are perpendicular to the plane of the triangle; 2-fold axes pass through the midpoint of edges and are perpendicular to the edges. Each face

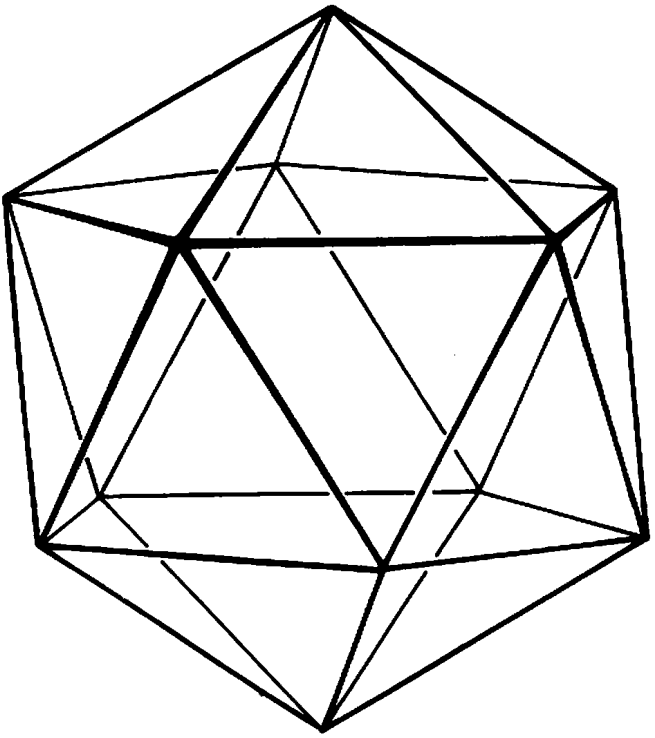

Figure 2.5 Diagrammatic representation of a cubical, isometric and icosahedral virus particle showing 20 equal sides. There are 12 vertices with fivefold rotational symmetry; the centre of each triangular face is on a threefold symmetry axis. Three structural units of any shape can be placed in identical positions on each face, giving 60 structural units (Matthews 1992, with permission from the Rights Department, Elsevier LTD)

is made up of three asymmetrical units. Geometrically, this type of icosahedral structure allows 60 identical subunits, each equilateral triangular face having 3 subunits constituting a closed surface, all having the same environment. These 60 subunits correspond through the 2-fold, 3-fold and 5-fold axes of rotation and in a virus particle they surround at the centre of the particle at fixed internal volume that shelter a polynucleotide.

Such geometrical assembly of 60 protein subunits is found in several viruses, viz. *Nepoviruses* and Satellite viruses. Geometrical clustering of more than 60 protein subunits is also possible. The surface of an icosahedron can be divided into a larger number of smaller equilateral triangles. The degree of such a subdivision is usually denoted by the term *Triangulation Number* (T), i.e. the number of triangles into which each icosahedral face has been divided. Mathematically T can have only certain values. For a simple icosadeltahedron the value of T would be 3. Each face of this structure has 9 and the whole structure would have 180 subunits. The shapes of the subunits are usually designated as banana or β-barrel. Under high-resolution electron microscopy these subunits do not appear identical because of their clustering in certain positions in the triangles. These clusters can be very clearly seen by electron microscopy. Depending on the nature of the viruses, the capsomers show different clustering patterns. Generally the clusters may be of two, three, five and six subunits designated as *dimers, trimers, pentamers* and *hexamers,* respectively.

Normally in icosahedrons, there are 12 vertices with 5-fold symmetry but there may also be 3-fold symmetry. Accordingly, their T values also differ (Figure 2.6)**.** Different clusters of different numbers of subunits are found in the axes of these symmetries (Figure 2.7). As for example in *Tymoviruses* there are

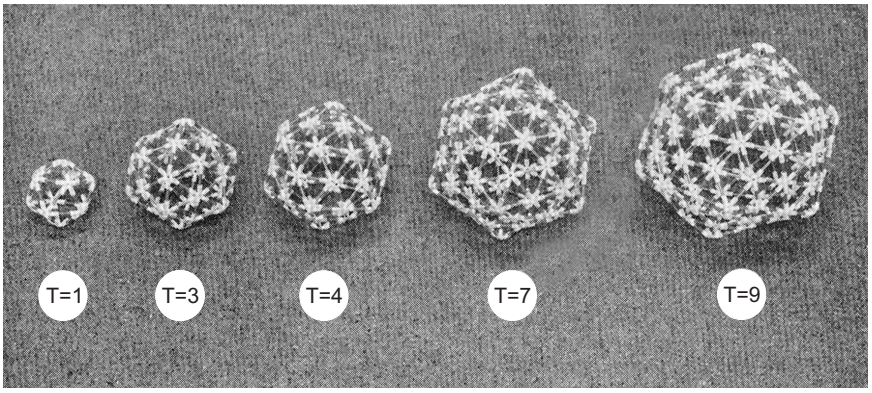

Figure 2.6 Models of icosadeltahedra for the first five triangulation numbers. These icosahedral surface lattices represent possible minimum energy designs for closed shells constructed from identical units (Caspar 1964); reprinted with permission of the University Press of Florida, USA

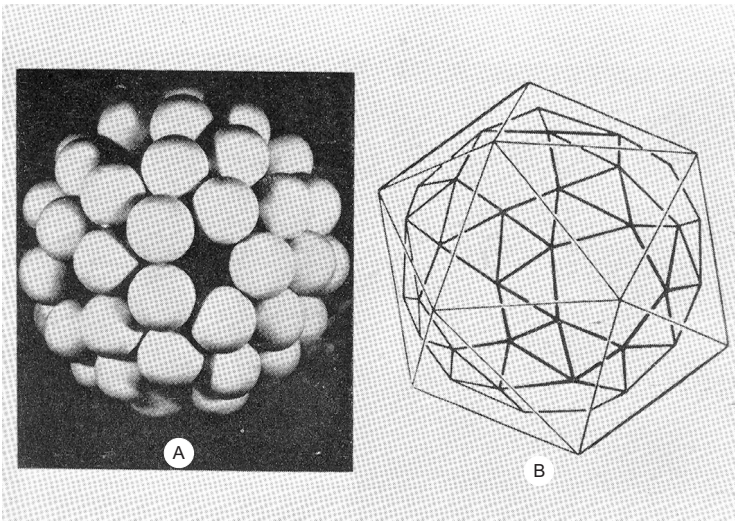

Figure 2.7 Clustering of protein subunits in a particle with icosahedral symmetry to form the protein shell in a spherical virus (Holton *et al* 1959). With permission from Holton C.S. Plant Pathology@1959 by the Board of Regents of the University of Wisconsin System, USA

12 groups of *pentamers* around the 5-fold symmetry and 20 groups of *hexamers* around the 3-fold symmetry axes**.** In more complex icosahedra, there may be twofold symmetry. In such cases the subunits adjust their bonding to adjust different symmetry-related positions in the shell as found in *Tombusvirus*. In viruses where more than one type of protein occurs as in the *Comoviruses* and *Reoviruses*, different polypeptides adjust themselves in a different symmetry environment with the shell. The geometry of these assembly processes has been studied and illustrated by several authors (Klug and Casper 1960, Casper 1964, Gibbs and Harrison 1976, Matthews 1981, Rossmann and Johnson 1989, Matthews 1992, Hull 2002, Astier *et al* 2007).

Protein subunits in capsomers and the capsomers in the capsids are stabilized by the formation of different types of bonds. Usually non-covalent bonds are prevalent in virus particles. There are two types of non-covalent bonds: polar (salt and hydrogen bonds) and non-polar (van der Waal's and hydrophobic bonds). The occurrence of these bonds varies in different viruses. Protein subunits in some viruses are electrovalently linked while in others they are hydrophobically bonded. Electrovalently bonded protein subunits can be easily dissociated and are not very stable. Nucleic acid-free shells are less stable than those containing the nucleic acid. Thus the type of nucleic acid-protein bonding may also contribute to the stability of the particles.

In isometric particles, the arrangement between the capsid and nucleic acid normally does not reflect the stability of the particles but the capsids in many cases are related to the replication of the nucleic acids. The nucleic acids may remain confined to radii inside the particle and there may be penetration of the protein subunits into the region where the nucleic acid is located. There are some spherical viruses the nucleic acids of which remain covered by double capsids made up of more than one type of protein subunits. In others the outer capsids have projecting spikes whereas the inner capsids have hidden spikes as in the *Phytoreoviruses* (for figure, see Hatta and Francki 1977).

The structure of an isometric virus may have simple morphological form as in *Cucumovirus* with round capsid containing 32 capsomers in T=3 (Figure 2.8). In some cases, the isometric particles may take a form of bacilliform particle as in the *Alfamoviruses* (Figure 2.9). The coat protein in this virus behaves as a water-soluble *dimer* stabilized by hydrophobic interactions between the molecules. This *dimer* is the morphological unit out of which the viral shells are constructed. In an extreme case an isometric virus may form twinned or geminate particle (Figure 2.10). There may also be complex arrangement of subunits with 12 pentamers and 60 hexamers in T= 7 as found in *Caulimovirus*. In a *Phytoreovirus* the isometric particle has undeveloped three-layered capsid shell with 260 trimers in T=13; inner shell with 60 dimers in T=1 arrangement.

4. Complex Viruses

The particles of some viruses are complex and cannot be easily differentiated into rods or spheres. The shape of these viruses is normally bacilliform or round. The basic feature of these particles is that they are covered by a lipoproteinaceous membrane; several types of proteins occur in the coat that are complexes with the membrane on the one hand and nucleic acids on the other; the nucleic acids may be helical or spherical and form the core of the particle.

Common complex plant viruses are the *Rhabdoviruses* and the *Tospoviruses*. *Rhabdoviruses* are bullet-shaped and rounded at both ends to give a bacilliform shape. The inner part constitutes the inner capsid formed by an RNA linked to a basic protein. This ribonucleoprotein is arranged in a helix. The outer coat is derived from cellular membranes; it contains two viral proteins, one of which is directed towards the exterior (Figure 2.11). *Tospovirus* particles are round and about 100 nm in diameter. The central core of the particle contains the RNA. A layer of dense material surrounded by a typical lipoprotein bi-layer membrane covers the RNA (Figure 2.12). Jackson *et al* (1999, 2005) reviewed the structure and function of different *Rhabdoviruses*.

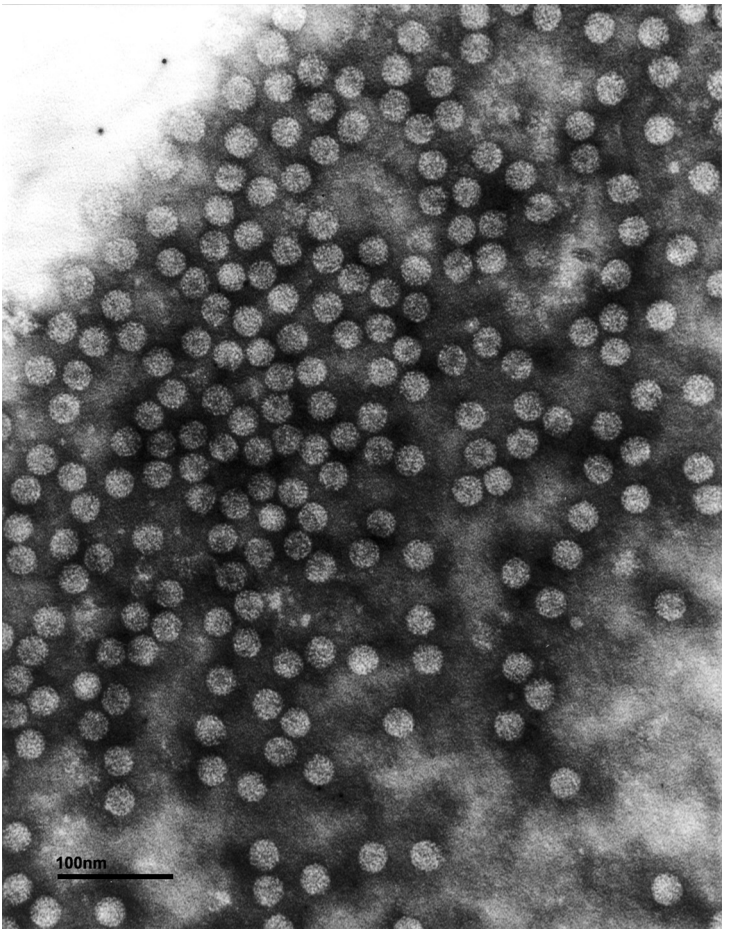

Figure 2.8 An isometric virus with a simple morphological form (round capsid, 32 capsomers, T=3): *Cucumovirus*. Received from the Archives of the IACR, Rothamsted, UK

5. Structural Diversity

Most of the viruses known so far (Fauquet *et al* 2005) are positive sense RNA (RNA+) and a few are negative sense RNA (RNA–), dsRNA, ssDNA and dsDNA. Structurally these viruses are of five types: isometric, geminate, rod, filamentous, and bacilliform. Particles occur as mono-, bi-, tri-, quadripartite and pentads. Approximately 50 viruses contain positive sense RNA, 20 of which are isometric, two are quasi-isometric (*Ilarvirus* and *Oleavirus*) and one is isometric with an envelope (*Tospovirus*). Approximately 16 isometric

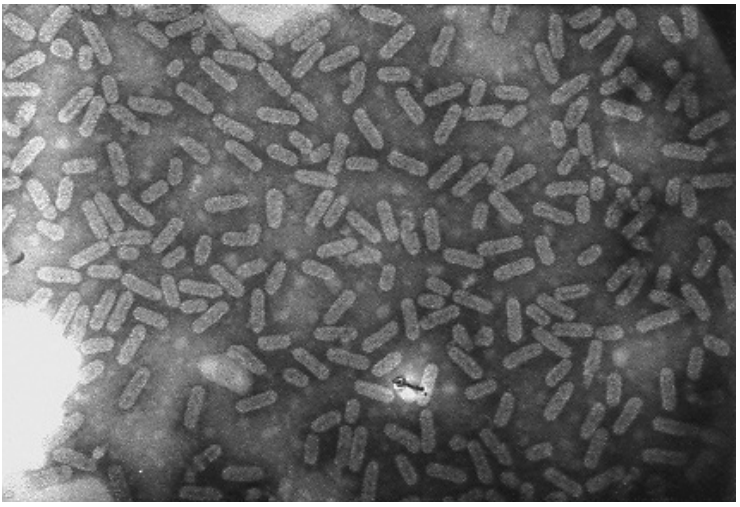

Figure 2.9 Isometric particle taking the form of a bacilliform virus (*Alfamovirus*)

particles are mono-partite, 5 are bi-partite (*Alfacryptovirus, Betacryptovirus, Fabavirus, Nepovirus*, *Idaeovirus*), 2 are tri-partite (*Bromovirus, Cucumovirus*), 1 is quadri-partite (*Oleavirus*) and 1 is a pentad virus (*Nanovirus*). There are 4 mono-partite virus particles that contain dsDNA with the reverse transcriptase enzyme (RT). These are *Caulimovirus, Soymovirus (Soybean chlorotic mottle virus), Cavemovirus (Cassava vein mosaic virus)* and *Petuvirus* (*Petunia vein clearing virus)*. Particles of four viruses are geminated or twinned (*Mastrevirus, Curtovirus, Begomovirus*, *Topocuvirus*); particles of *Mastrevirus* and *Curtovirus* are mono-partite whereas those of *Begomoviruses* are tri-partite; the nature of the particles of *Topocuvirus* has yet to be ascertained. The nucleic acid of all these viruses are ssDNA (circular). Another ssDNA (circular) containing isometric virus, the *Nanoviruses* are pentad viruses that have five particles. Isometric particles of three viruses are enveloped and double shelled with spikes and contain dsRNA (*Fijivirus, Oryzavirus*, *Phytoreovirus*).

There are approximately 8 rod-shaped viruses of which 1 is mono-partite (*Tobamovirus*), 3 are bi-partite (*Furovirus, Pecluvirus, Tobravirus*), 3 are tri-partite (*Varicosavirus*, *Pomovirus, Hordeivirus*) and 1 is quadri-partite (*Benyvirus*). Particles of all these viruses contain ssRNA.

Filamentous viruses are more common than rod-shaped ones. There are approximately 18 filamentous viruses of which 14 are mono-partite (*Closterovirus*, *Ipomovirus*, *Macluravirus*, *Potyvirus*, *Rymovirus*, *Tritimovirus*, *Allexivirus*, *Capillovirus*, *Carlavirus*, *Foveavirus*, *Mandarivirus*, *Potexvirus*, *Trichovirus*, *Vitivirus*), 2 are bi-partite (*Crinivirus*, *Bymovirus*) and 2 are quadri-partite (*Tenuivirus, Ophiovirus*).

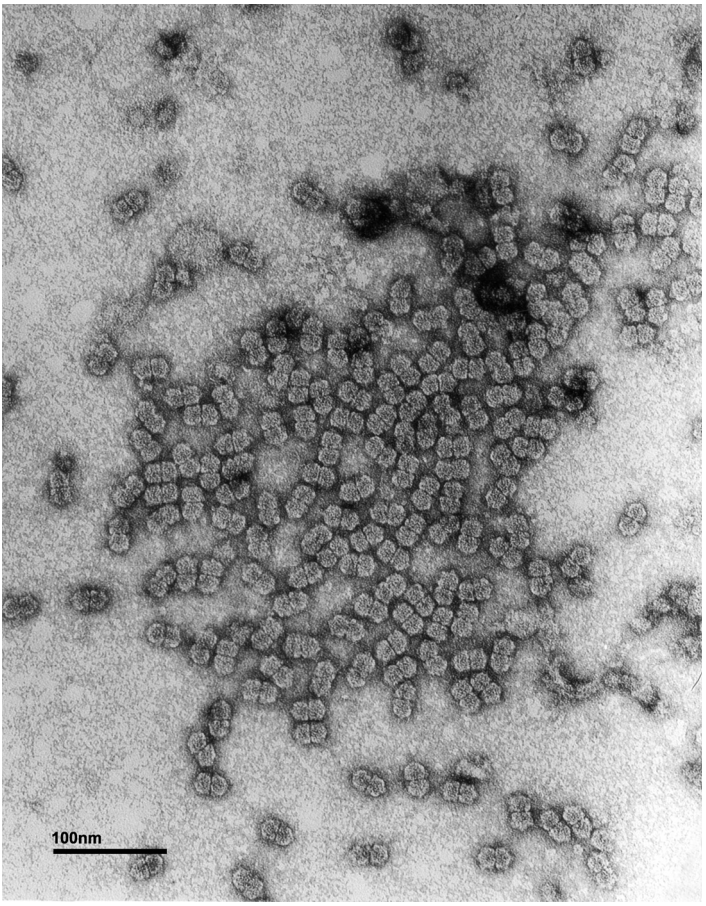

Figure 2.10 An isometric virus with twinned or geminate particle (*Geminivirus*). Received from the Archives of the IACR, Rothamsted, UK

There are 6 bacilliform viruses (*Badna virus, Tungro virus, Ourmia virus* and *Umbra virus*) and 2 are Rhabdo- or bullet/bacilliform viruses (*Cytorhabdovirus* and *Nucleorhabdovirus*. Particles of *Badna* and *Tungro* viruses are mono-partite and do not have an envelope. The particles of Rhabdoviruses are enveloped. The envelope is composed of two layers of lipo-protein membranes. The inner layer contains host-derived lipids penetrated into the G protein of surface layer (Jackson *et al* 1999, 2005).

The majority of the viruses have isometric particles. The particles of 38 viruses are isometric and 4 viruses have geminate particles.

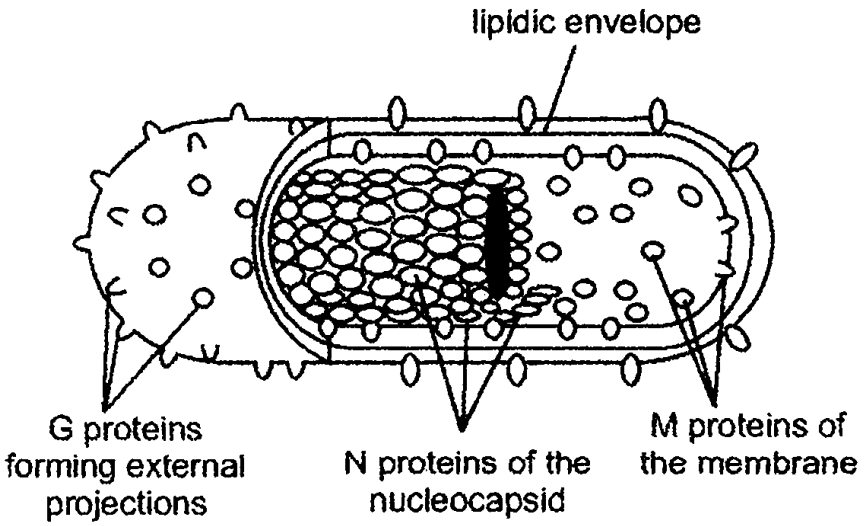

Figure 2.11 Bacilliform complex enveloped particle of *Rhabdovirus* with membrane protein, nucleocapsid proteins and proteins forming external projections (Astier *et al* 2001, source INRA 2001, France)

The structures of the coat protein of most of the viruses are not completely understood, but in general they contain only one type of protein except for *Marafivirus, Fijivirus, Oryzavirus* and *Phytorevirus. Marafivirus* coat protein contains two types of polypeptides whereas the coat protein of *Fiji, Oryza* and *Phytoreoviruses* contains 6-8 types of protein species.

The salient features of the structure of different viruses are given in Appendix II.1. The structures of viruses containing dsDNA-RT are either isometric or bacilliform and mono-partite. The structures of viruses containing ssDNA on the other hand are mostly geminate except one (*Nanovirus*) that is isometric and separated into five particles. The structures of most of the dsRNA viruses are isometric and double shelled; these are mono-partite and enveloped. A few are non-enveloped and bi-partite and one is rod-shaped and tri-partite. The structures of negative sense ssRNA containing viruses are very conservative and found only in rhabdo- or bullet/bacilliform viruses and two other viruses. For three ssRNA-RT viruses (*Metavirus, Pseudovirus* and *Seravirus*), the structural details have yet to be determined.

A lot of diversity is found in the structures of viruses containing positive sense ssRNA. The structures of most of these viruses are filamentous followed by isometric and rod-shaped particles. These may be the basic structures of viruses; others may arise by changes in the genomes or adaptation of viruses in plants that had altogether different primary hosts.

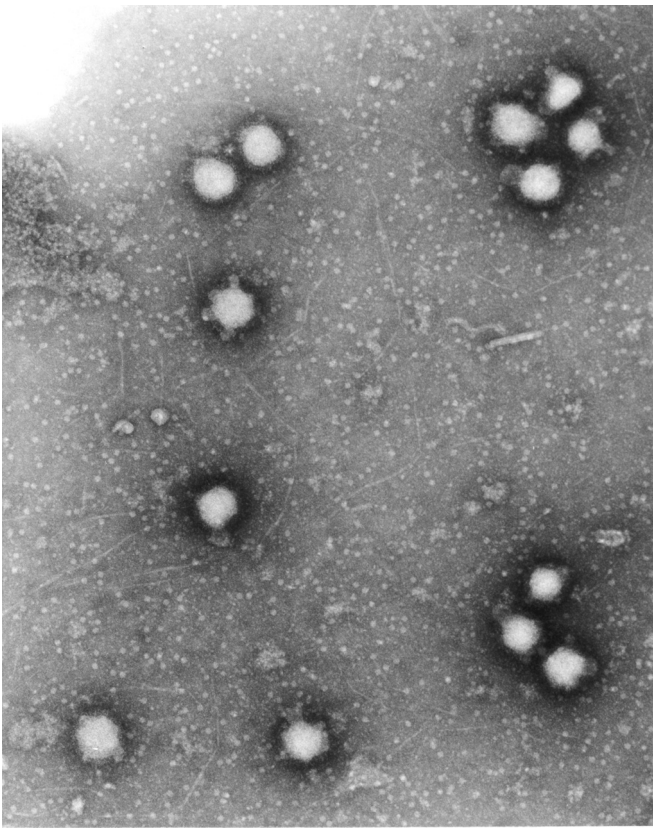

Figure 2.12 Round enveloped complex particle of *Tospovirus*. Received from the Archives of the IACR, Rothamsted, UK

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3

Diversity in Chemical Components and Genomic Structure

A. BASIC COMPONENTS

Virus particles are, in general, composed of two types of biological macromolecules, nucleic acid and protein. In complex viruses an additional component— lipid— also occurs. The amount of these substances present in the particles depends upon their architecture. In a general way it is considered that isometric particles normally contain 15 to 45% nucleic acid whereas the anisometric particles normally contain only 5% nucleic acid. The complex particles normally contain 20% lipid. In addition to these major components, particles of some viruses may contain traces of a few enzymes, polyamines and metallic ions having specific roles in replication and structural stabilization. However wide diversities exist in the nucleic acid and protein contents of the known viruses, but the reasons and effects of such diversity are yet to be explained.

B. DIVERSITIES IN QUANTITATIVE PRESENCE OF IMPORTANT COMPONENTS

The main differences in nucleic acid content are found in particles with different symmetries. In the rod-shaped and filamentous viruses, nucleic acid content normally ranges from 4% to 5%, with the exception of a few that contain a higher percentage of nucleic acid. As for example, *Tenui-* and *Potyviruses* contain 5.2 to 12% and 20-21% nucleic acid respectively. A much greater diversity is observed in the bacilliform viruses with the nucleic acid contents of *Alfamo-, Cytorhabdo-, Nucleorhabdo-* and *Ourmiaviruses* of 16%, 5%, 1-5% and 15-25% respectively. Not all the bacilliform viruses contain lipid, e.g. *Alfamo-* and *Ourmiaviruses*, whereas *Cytorhabdoviruses* and *Nucleorhabdoviruses* contain 25% and 15-37% lipid (Table 3.1).

Table 3.1 Diversities of Important Chemical Components found in Some Bullet, Bacilliform, Rod-shaped and Filamentous Viruses (Brunt *et al.* 1996, Hull 2002, Fauquet *et al* 2005, ICTVdB 2006)

Virus	*Family*	*Shape*	*Nucleic acid content (%)*	*Protein content (%)*	*Lipid content (%)*
1. *Alfamovirus* (ssRNA+)	Bromoviridae	Bacilliform/ Quasi-isometric	16	84	Nil
2. *Allexivirus* (ssRNA+)	Flexiviridae	Filamentous	5	95	Nil
3. *Ampelovirus*	Flexiviridae	Filamentous	–	–	–
4. *Badnavirus* (dsDNA)	Caulimoviridae	Bacilliform	–	–	–
5. *Benyvirus* (ssRNA+)	No family	Rod	–	–	–
6. *Bymovirus* (ssRNA+)	Potyviridae	Filamentous	5	95	Nil
7. *Capillovirus* (ss RNA+)	Flexiviridae	Filamentous	5	95	Nil
8. *Carlavirus* (ssRNA+)	Flexiviridae	Filamentous	5	95	Nil
9. *Closterovirus* (ssRNA+)	Closteroviridae	Filamentous	5-5.17	94.83-95	Nil
10. *Crinivirus* (ssRNA+)	Closteroviridae	Filamentous	5	95	Nil
11. *Cytorhabdovirus* (ssRNA–)	Rhabdoviridae	Bullet/Bacilliform	5	70	25
12. *Foveavirus* (ssRNA+)	Flexiviridae	Filamentous	–	–	–
13. *Furovirus* (ssRNA+)	No family	Rod	4-59	5-96	Nil
14. *Hordeivirus* (ssRNA+)	No family	Rod	3.8-5	95-96.2	Nil
15. *Ipomovirus* (ssRNA+)	Potyviridae	Filamentous	5	95	Nil
16. *Macluravirus* (ssRNA+)	No family	Filamentous	5	95	Nil
17. *Mandarivirus* (ssRNA+)	Flexiviridae	Filamentous	–	–	–
18. *Nucleorhabdovirus* (ssRNA–)	Rhabdoviridae	Bullet/Bacilliform	1-5	68-80	15-37
19. *Ophiovirus* (ssRNA–)	No family	Filamentous	–	–	–
20. *Ourmiavirus* (ssRNA+)	No family	Bacilliform	15-25	75-85	Nil
21. *Pecluvirus* (ssRNA+)	No family	Rod	4	96	Nil

22. *Pomovirus* (ssRNA+)	No family	Rod	–	–	–
23. *Potexvirus* (ssRNA+)	Flexiviridae	Filamentous	5-8	92-95	Nil
24. *Potyvirus* (ssRNA+)	Potyviridae	Filamentous	20-21	78-80	Nil
25. *Rymovirus* (ssRNA+)	Potyviridae	Filamentous	5	95	Nil
26. *Tenuivirus* (ssRNA+)	No family	Filamentous	5.2-12	88-94.8	Nil
27. *Tobamovirus* (ssRNA+)	No family	Rod	5	95	Nil
28. *Tobravirus* (ssRNA+)	No family	Filamentous	5	95	Nil
29. *Trichovirus* (ssRNA+)	Flexiviridae	Filamentous	5	95	Nil
30. *Tritimivirus* (ssRNA+)	Potyviridae	Filamentous	5	95	Nil
31. *Tungrovirus* (dsDNA-RT)	Caulimoviridae	Bacilliform	–	–	Nil
32. *Varicosavirus* (ssRNA–)	No family	Filamentous	–	–	–
33. *Vitivirus* (ssRNA+)	Flexiviridae	Filamentous	5	95	Nil

The nucleic acid content of isometric viruses on the other hand differs widely. Many have nucleic acid contents in the range 14-24%. The *Tospoviruses* contain only 5% nucleic acid. Higher contents of nucleic acid are found in a few viruses ranging from 28% to 46%. These viruses are *Tombusviruses* (28%), *Fabaviruses* (35%), *Comoviruses* (38%), *Tymoviruses* (39%), *Sequiviruses* (40%), *Oryzaviruses* (42%) and *Nepoviruses* (46%). Empty shells without any nucleic acid are also found in a few viruses such as *Faba-, Como-, Oryza-*, and *Nepoviruses*. Among the isometric and geminate viruses only the *Tospoviruses* contain lipid (20%) (Table 3.2).

Table 3.2 Important Chemical Components of Isometric and Geminate Viruses (Brunt *et al* 1996, Hull 2002, Fauquet *et al* 2005, ICTVdB Management 2006)

Virus	*Family*	*Shape*	*Nucleic* acid content (%)	*Protein* content (%)	*Lipid* content (%)
1. *Alphacryptovirus* (dsRNA)	Partitiviridae	Isometric	25	75	Nil
2. *Aureusvirus* (dsRNA)	Tombusviridae	Isometric	18	82	Nil
3. *Avenavirus* (ssRNA+)	Tombusviridae	Isometric	18	82	Nil

4. *Babuvirus* (ssDNA)	Nanoviridae	Isometric	–	–	Nil
5. *Begomovirus* (ssDNA)	Geminiviridae	Geminate or prolate	18-22	78-82	Nil
6. *Betacryptovirus* (dsRNA+)	Partitiviridae	Isometric	24	76	Nil
7. *Bromovirus* (ssRNA+)	Bromoviridae	Isometric	20-23.7	76-79	Nil
8. *Carmovirus* (ssRNA+)	Tombusviridae	Isometric	14-23	77-86	Nil
9. *Caulimovirus* (dsDNA-RT)	Caulimoviridae	Isometric	14.5-17	83-85.9	Nil
10. *Cavemovirus* (dsDNA-RT)	Caulimoviridae	Isometric	–	–	–
11. *Cheravirus* (ssRNA+)	No family	Isometric	–	–	–
12. *Comovirus* (ssRNA+)	Comoviridae	Isometric	0-38.6	62.82-100	Nil
13. *Cucumovirus* (ssRNA+)	Bromoviridae	Isometric	16-21.2	78.8-84	Nil
14. *Curtovirus* (ssDNA)	Geminiviridae	Geminate	–	–	Nil
15. *Dianthovirus* (ssRNA+)	Tombusviridae	Isometric	20-28	72-80	Nil
16. *Enamovirus* (ssRNA+)	Luteoviridae	Isometric	28	72	Nil
17. *Fabavirus* (ssRNA+)	Comoviridae	Isometric	0-35	67-100	Nil
18. *Fijivirus* (dsRNA)	Reoviridae	Isometric	–	– Nil	
19. *Idaeovirus* (ssRNA+)	No family	Isometric	24	76	Nil
20. *Ilarvirus* (ssRNA+)	Bromoviridae	Isometric	12-24	76-88	Nil
21. *Luteovirus* (ssRNA+)	Luteoviridae	Isometric	28-30	70-72	Nil
22. *Machlomo-virus* (ssRNA+)	Tymoviridae	Isometric	18	82	Nil
23. *Macluravirus* (ssRNA+)	Tymoviridae	Isometric	3-4	96-97	Nil
24. *Marafivirus* (ssRNA+)	Tymoviridae	Isometric	25-30	67Nil	
25. *Mastrevirus* (ssDNA)	Geminiviridae	Geminate	19-20	80Nil	
26. *Metavirus* (ssRNA-RT)	Metaviridae	LRT-retrotransposon -like	–	–	–
27. *Nanovirus* (ssDNA)	Nanoviridae	Isometric	16-17	83-84	Nil