Important of Plant Viruses and TMV Virus

Hamid Kheyrodin

(Assistant Professor, Semnan University, Iran)

Abstract: *Viruses cause many diseases of international importance. Amongst the human viruses, smallpox, polio, influenza, hepatitis, human immunodeficiency virus (HIV-AIDS), measles and the SARS coronavirus are particularly well known. While antibiotics can be very effective against diseases caused by bacteria, these treatments are ineffective against viruses and most control measures rely on vaccines (antibodies raised against some component of the virus) or relief of the symptoms to encourage the body's own defense system.*

Viruses also cause many important plant diseases and are responsible for huge losses in crop production and quality in all parts of the world. Infected plants may show a range of symptoms depending on the disease but often there is leaf yellowing (either of the whole leaf or in a pattern of stripes or blotches), leaf distortion (e.g. curling) and/or other growth distortions. TMV was the first virus to be discovered over a century ago and was the first virus ever purified. It has since yielded fascinating insights into how viruses infect their hosts. Research on TMV has also led to major Nobel prize winning discoveries on general principles of life. TMV can also survive outside the plant in sap that has dried on tools and other surfaces. If a TMV plant is handled and then you open a door with that hand, you have now put TMV on the door handle. The next person to open the door can pick up the TMV and spread it to any plant that they touch.

Keywords: *Viruses, control, plants, TMV virus*

I. INTRODUCTION

The discovery of plant viruses causing disease is often accredited to A. Mayer (1886) working in the Netherlands demonstrated that the sap of mosaic obtained from tobacco leaves developed mosaic symptom when injected in healthy plants. However the infection of the sap was destroyed when it was boiled. He thought that the causal agent was the bacteria. However, after larger inoculation with a large number of bacteria, he failed to develop a mosaic symptom. More recently virus research has been focused on understanding the genetics and molecular biology of plant virus genomes, with a particular interest in determining how the virus can replicate, move and infect plants. Understanding the virus genetics and protein functions has been used to explore the potential for commercial use by biotechnology companies. In particular, viral-derived sequences have been used to provide an understanding of novel forms of resistance. The recent boom in technology allowing humans to manipulate plant viruses may provide new strategies for production of value-added proteins in plants. Viruses are extremely small and can only be observed under an electron microscope. The structure of a virus is given by its coat of proteins, which surround the viral genome. Assembly of viral particles takes place spontaneously. Over 50% of known plant viruses are rod-shaped (flexuous or rigid). The length of the particle is normally dependent on the genome but it is usually between 300–500 nm with a diameter of 15–20 nm. Protein subunits can be placed around the circumference of a circle to form a disc. In the presence of the viral genome, the discs are stacked, then a tube is created with room for the nucleic acid genome in the middle.[5]

Insects

Plant viruses need to be transmitted by a vector, most often insects such as leafhoppers. One class of viruses, the Rhabdoviridae, has been proposed to actually be insect viruses that have evolved to replicate in plants. The chosen insect vector of a plant virus will often be the determining factor in that virus's host range: it can only infect plants that the insect vector feeds upon. This was shown in part when the old world white fly made it to the United States, where it transferred many plant viruses into new hosts. Depending on the way they are transmitted, plant viruses are classified as non-persistent, semi-persistent and persistent. In non-persistent transmission, viruses become attached to the distal tip of the stylet of the insect and on the next plant it feeds on, it inoculates it with the virus.[6] Semi-persistent viral transmission involves the virus entering the foregut of the insect. Those viruses that manage to pass through the gut into the haemolymph and then to the salivary glands are known as persistent. There are two sub-classes of persistent viruses: propagative and circulative. Propagative viruses are able to replicate in both the plant and the insect (and may have originally been insect viruses), whereas circulative cannot. Circulative viruses are protected inside aphids by the chaperone protein symbionin, produced by bacterial symbionts. Many plant viruses encode within their genome polypeptides with domains essential for transmission by insects. In non-persistent and semi-persistent viruses, these domains are in the coat protein and another protein known as the helper component. A bridging hypothesis has been proposed to explain how these proteins aid in insect-mediated viral transmission. The helper component will bind to the specific domain of the coat protein, and then the insect mouthparts — creating a bridge. In persistent propagative viruses, such as tomato spotted wilt virus (TSWV), there is often a lipid coat surrounding the proteins that is not seen in other classes of plant viruses. In the case of TSWV, 2 viral proteins are expressed in this lipid envelope.

It has been proposed that the viruses bind via these proteins and are then taken into the insect cell by receptor-mediated endocytosis. Table1 shows the Biological and molecular characterization of an isolate of Tobacco streak virus obtained from soybeans

TABLE 1 - Symptoms induced in plants by *Tobacco streak virus*-Brazilian strain, isolated from soybean (*Glycine max*)

Botanical family	Species	Reaction[1]
Amaranthaceae	*Gomphrena globosa* L.	CLL
	Amaranthus sp.	-
Asteraceae	*Emilia sonchifolia* (L.) DC	M
	Bidens pilosa L.	M
Chenopodiaceae	*Chenopodium amaranticolor* Coste & Reyn.	NLL
	C. quinoa Willd.	CLL
Cucurbitaceae	*Cucurbita pepo* L. 'Caserta'	-
Fabaceae	*Glycine max* L. Mer.	
	c.v. Santa Rosa	SN
	c.v. Davis	SN
	Phaseolus vulgaris L.	
	c.v. Rosinha	NLL
	c.v. Carioca	NLL
	c.v. Tibagi	CLL
	Lupinus albus L.	M
	Crotalaria pallida Ait	-
	C. spectabilis Roth.	-
	Arachis hypogeae L.	M
	Vigna unguiculata (L.) Walp. Piti ba	-
	Pisum sativum L.	-
Gramineae	*Zea mays* L.	-
Solanaceae	*Lycopersicom esculentum* Mill.	M
	N. tabacum L. Sansun NN	WCN
	N. glutinosa L.	WCN
	N. debneyi Domin.	M
	N. benthamiana Domin.	M
	Gossypium hirsutum L.	M

[1]WCN= white cirele necrosis; M=mosaic; NLL= necrotic local lesion; CLL= chlorotic local lesion;

Nematodes

Soil-borne nematodes also have been shown to transmit viruses.[7] They acquire and transmit them by feeding on infected roots. Viruses can be transmitted both non-persistently and persistently, but there is no evidence of viruses being able to replicate in nematodes. The virions attach to the stylet (feeding organ) or to the gut when they feed on an infected plant and can then detach during later feeding to infect other plants. Examples of viruses that can be transmitted by nematodes include tobacco ringspot virus and tobacco rattle virus.

Plasmodiophorids

A number of virus genera are transmitted, both persistently and non-persistently, by soil borne zoosporic protozoa. These protozoa are not phytopathogenic themselves, but parasitic. Transmission of the virus takes place when they become associated with the plant roots. Examples include *Polymyxa graminis*, which has been shown to transmit plant viral diseases in cereal crops [8] and *Polymyxa betae* which transmits Beet necrotic yellow vein virus. Plasmodiophorids also create wounds in the plant's root through which other viruses can enter.

Seed and pollen borne viruses

Plant virus transmission from generation to generation occurs in about 20% of plant viruses. When viruses are transmitted by seeds, the seed is infected in the generative cells and the virus is maintained in the germ cells and sometimes, but less often, in the seed coat. When the growth and development of plants is delayed because of situations like unfavourable weather, there is an increase in the amount of virus infections in seeds. There does not seem to be a correlation between the location of the seed on the plant and its chances of being infected. [5] Little is known about the mechanisms involved in the transmission of plant viruses via seeds, although it is known that it is environmentally influenced and that seed transmission occurs because of a direct invasion of the embryo via the ovule or by an indirect route with an attack on the embryo mediated by infected gametes. [5] [6] These processes can occur concurrently or separately depending on the host plant. It is unknown how the virus is able to directly invade and cross the embryo and boundary between the parental and progeny generations in the ovule. [6] Many plants species can be infected through seeds including but not limited to the families Leguminosae, Solanaceae, Compositae, Rosaceae, Cucurbitaceae, Gramineae. [5] Bean common mosaic virus is transmitted through seeds.

Direct plant-to-human transmission

Researchers from the University of the Mediterranean in Marseille, France have found tenuous evidence that suggest a virus common to peppers, the Pepper Mild Mottle Virus (PMMoV) may have moved on to infect humans.[9] This is a very rare and highly unlikely event as, to enter a cell and replicate, a virus must

"bind to a receptor on its surface, and a plant virus would be highly unlikely to recognize a receptor on a human cell. One possibility is that the virus does not infect human cells directly. Instead, the naked viral RNA may alter the function of the cells through a mechanism similar to RNA interference, in which the presence of certain RNA sequences can turn genes on and off," according to Virologist Robert Garry from the Tulane University in New Orleans, Louisiana.[10]

Translation of plant viral proteins

75% of plant viruses have genomes that consist of single stranded RNA (ssRNA). 65% of plant viruses have +ssRNA, meaning that they are in the same sense orientation as messenger RNA but 10% have -ssRNA, meaning they must be converted to +ssRNA before they can be translated. 5% are double stranded RNA and so can be immediately translated as +ssRNA viruses. 3% require a reverse transcriptase enzyme to convert between RNA and DNA. 17% of plant viruses are ssDNA and very few are dsDNA, in contrast a quarter of animal viruses are dsDNA and three quarters of bacteriophage are dsDNA.[11] Viruses use the plant ribosomes to produce the 4-10 proteins encoded by their genome. However, since many of the proteins are encoded on a single strand (that is, they are polycistronic) this will mean that the ribosome will either only produce one protein, as it will terminate translation at the first stop codon, or that a polyprotein will be produced. Plant viruses have had to evolve special techniques to allow the production of viral proteins by plant cells.

Production of sub-genomic RNAs

Some viruses use the production of subgenomic RNAs to ensure the translation of all proteins within their genomes. In this process the first protein encoded on the genome, and this the first to be translated, is a replicase. This protein will act on the rest of the genome producing negative strand sub-genomic RNAs then act upon these to form positive strand sub-genomic RNAs that are essentially mRNAs ready for translation.

Segmented genomes

Some viral families, such as the *Bromoviridae* instead opt to have multipartite genomes, genomes split between multiple viral particles. For infection to occur, the plant must be infected with all particles across the genome. For instance *Brome mosaic virus* has a genome split between 3 viral particles, and all 3 particles with the different RNAs are required for infection to take place.

Polyprotein processing

This strategy is adopted by viral genera such as the Potyviridae and Tymoviridae. The ribosome translates a single protein from the viral genome. Within the polyprotein is an enzyme (or enzymes) with proteinase function that is able to cleave the polyprotein into the various single proteins or just cleave away the protease, which can then cleave other polypeptides producing the mature proteins.

Symptoms and Signs

Symptoms induced by *Tobacco mosaic virus* (TMV) are somewhat dependent on the host plant and can include mosaic, mottling (Figures 1 and 2), necrosis (Figures 3 and 4), stunting, leaf curling, and yellowing of plant tissues. The symptoms are very dependent on the age of the infected plant, the environmental conditions, the virus strain, and the genetic background of the host plant. Strains of TMV also infect tomato, sometime causing poor yield or distorted fruits, delayed fruit ripening, and nonuniform fruit color (Figure 5).

Figure1

Figure2

Figure 3

Figure 4

Figure 5

Pathogen Biology

Hosts for TMV include tobacco (Figure 1), tomato (Figure 5), and other solanaceous plants. Currently, yield losses for tobacco due to TMV are estimated at only 1% because resistant varieties are routinely grown. In contrast, losses of up to 20% have been reported for tomato. In addition, poor fruit quality may reduce the value of the crop on the commercial fresh market.

TMV is the type member of a large group of viruses within the genus *Tobamovirus*. The rod-shaped virus particles (virions) of TMV measure about 300 nm x 15 nm (Figure 6). A single TMV particle is composed of 2,130 copies of the coat protein (CP) that envelope the RNA molecule of about 6,400 nucleotides (Figure 7). This single-stranded RNA encodes four genes: two replicase-associated proteins that are directly translated from the TMV RNA, and the movement protein and a coat protein that are translated from subgenomic RNAs (Figures 8 and 9).

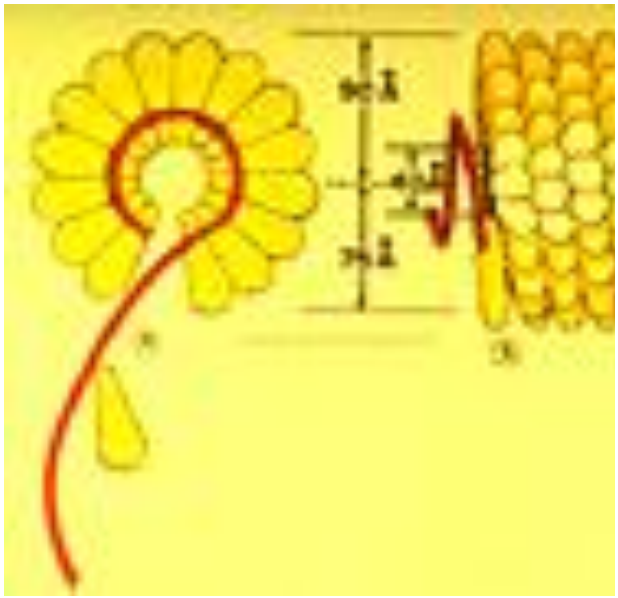

Figure 7

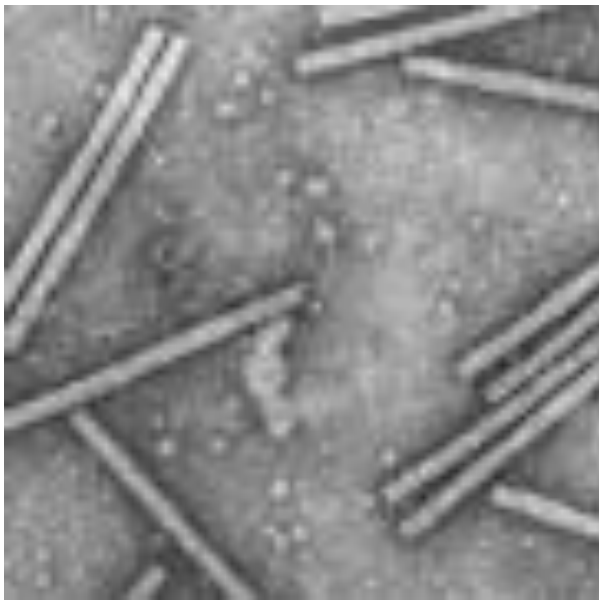

Figure 6

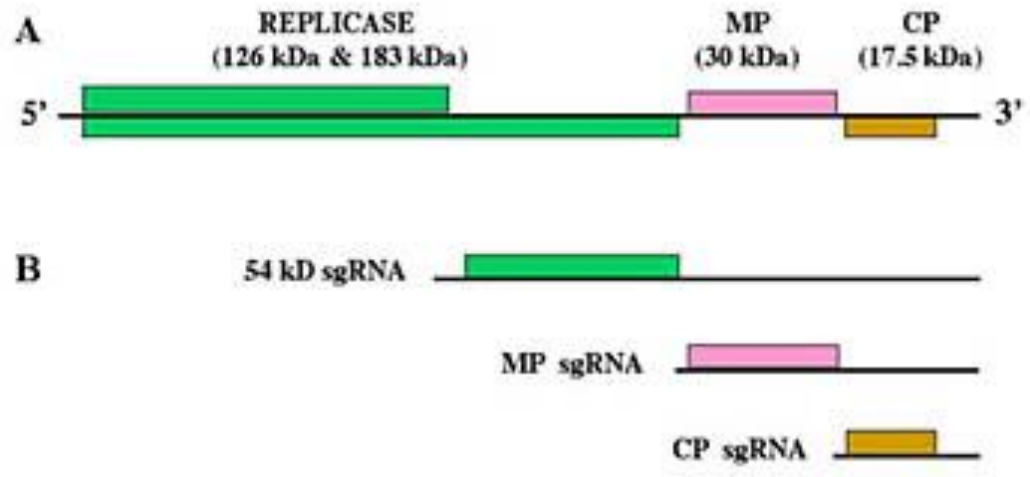

Figure 8

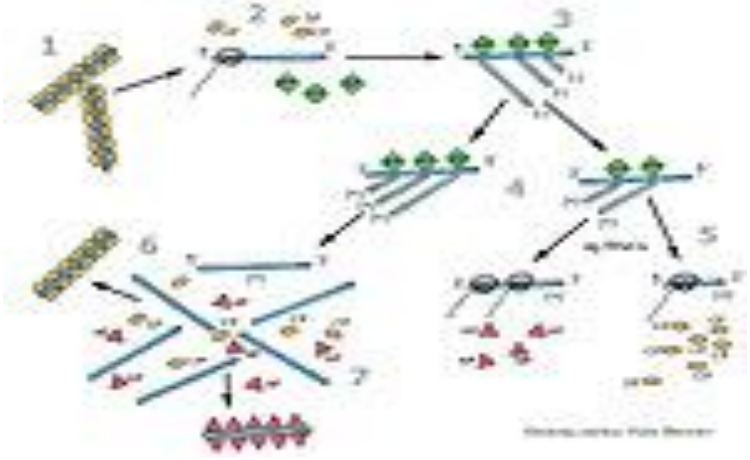

Figure 9

II. DISEASE CYCLE AND EPIDEMIOLOGY

Transmission from plant to plant

TMV is very easily transmitted when an infected leaf rubs against a leaf of a healthy plant, by contaminated tools, and occasionally by workers whose hands become contaminated with TMV after smoking cigarettes. A wounded plant cell provides a site of entry for TMV. The virus can also contaminate seed coats, and the plants germinating from these seeds can become infected. TMV is extraordinarily stable. Purified TMV (Figure 6) has been reported to be infectious after 50 years storage in the laboratory at 4°C/40°F.

Replication

TMV enters the plant cell through minor wounds. Once TMV enters the cell, the virus particles disassemble in an organized manner to expose the TMV RNA. The virus RNA is positive-sense, or "+ sense", and serves directly as a messenger RNA (mRNA) that is translated using host ribosomes. Translation of the replicase-associated proteins (RP) 126- and 183-kDa) begins within a few minutes of infection.

As soon as these proteins have been synthesized, the replicase associates with the 3' end of the + sense TMV RNA for the production of a negative sense, or "- sense", RNA. The - sense RNA is the template to produce both full-length genomic + sense RNA as well as the + sense subgenomic RNAs (sgRNAs) (Figure 8)

The sgRNAs are translated by the host ribosomes to produce the movement protein (MP) (30 kDa) and the coat protein (CP) (17.5 kDa). The coat protein then interacts with the newly synthesized + sense TMV RNA for assembly of progeny virions.

These virus particles are very stable and, at some point when the cells are broken or the leaf dries up, they are released to infect new plants. Alternatively, the + sense TMV RNA is wrapped in movement protein, and this complex can infect adjacent cells.

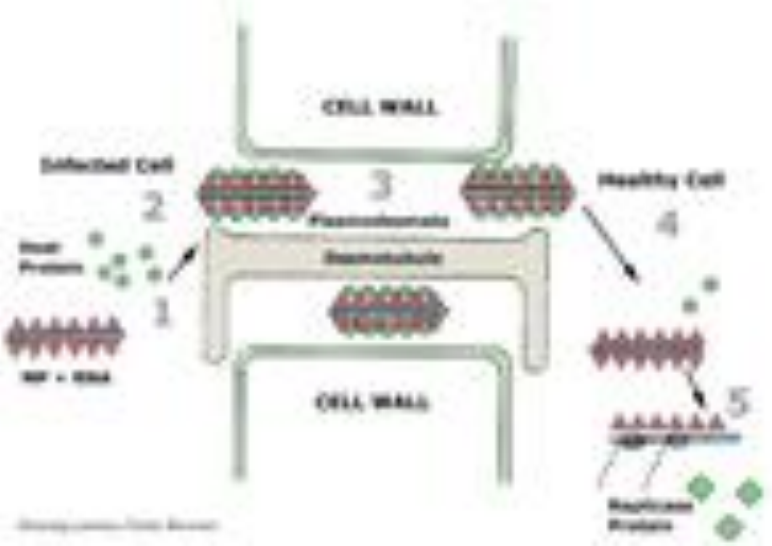

Figure 10

Movement in the infected plant

TMV uses its movement protein to spread from cell-to-cell through plasmodesmata, which connect plant cells (figure 10). Normally, the plasmodesmata are too small for passage of intact TMV particles.

The movement protein (probably with the assistance of as yet unidentified host proteins) enlarges the plasmodesmatal openings so that TMV RNA can move to the adjacent cells, release the movement protein and host proteins, and initiate a new round of infection. As the virus moves from cell to cell, it eventually reaches the plant's vascular system (veins) for rapid systemic spread through the phloem to the roots and tips of the growing plant.

Epidemiology

The TMV disease cycle and its epidemiology are intimately related because the virus is completely dependent on the host for replication and spread. There is wide variation in disease incidence, depending on the time of disease onset in the field and on cropping practices. For example, a few plants could become infected early in the season, either from TMV on the seed coat or by workers contaminating plants. The disease could then spread rapidly throughout the field or greenhouse by TMV-infected plants contacting healthy plants, or by equipment or workers. TMV can also survive or overwinter in infected plant debris or perennial (weedy) hosts and, perhaps, in the soil. Agricultural practices, such as continuous cropping, have the potential to be a

particular problem, especially in greenhouse facilities, where TMV inoculum may increase in more than one plant species.

Disease Management

Greenhouse management

Horticultural practices: To reduce infection of plants with TMV all tools should be washed with soap or a 10% solution of household bleach to inactivate the virus. TMV-contaminated soil should be discarded. To avoid transmitting the virus from an infected plant to healthy plants, the watering hose or watering can should not be allowed to make contact with the plants. Care should be taken to dispose of dead leaves and old plants, because dry, TMV-infected leaves can be blown around the greenhouse as 'dust' which can subsequently infect healthy plants if they are wounded.

Cross protection: Inoculation of a mild strain of the virus onto young plants can protect them from subsequent infection by more severe strains of TMV. This is a well documented control strategy, called "cross protection," that is successfully applied in greenhouse operations. Transgenic plants also offer alternative strategies for virus control (see Biotechnology) (Figure 11).

Figure 11

III. PREPLANTING OPTIONS (GREENHOUSE AND FIELD)

Cultivars

Several tobacco and tomato cultivars have been bred to be genetically resistant to TMV.Biotechnology. Genetic engineering techniques have provided scientists with the ability to express the TMV coat protein gene in transgenic tobacco and tomato plants. This control strategy can safeguard the plants from infection by closely related strains of the virus (Figure 11).

Elimination of inoculum

Under experimental conditions, it has been shown that TMV can be inactivated when workers dip their contaminated hands in milk prior to planting. This inexpensive technique greatly reduces the incidence of disease (Figure 12). Seedlings that are known to be susceptible should not be transplanted into soil that contains TMV-contaminated root or plant debris.

Figure 12

Management in the field

Scouting for disease

During the growing season, infected plants should be dug up, bagged, and removed from the field. Rotation practices that include resistant plants or non host crops also should be employed to reduce the amount of inoculum in the field.

Management at harvest and in storage

TMV can easily overwinter on the seed coat, thus providing an inoculum source for the next planting cycle. Therefore, it is important to treat TMV-contaminated tobacco seed with a 10% solution of trisodium phosphate for 15 minutes. Alternatively, tomato seed contaminated with TMV can be incubated at 70°C/158°F for 2-4 days prior to planting. Both treatments will inactivate the virus that is on the seed coat, but should have little negative effect on seed germination.

Significance

In 1898, Martinus W. Beijerinck, of the Netherlands, put forth his concepts that TMV was small and infectious. Furthermore, he showed that TMV could not be cultured, except in living, growing plants. This report, suggesting that 'microbes' need not be cellular, was to forever change the definition of pathogens. In 1946, Wendall Stanley was awarded the Nobel Prize for his isolation of TMV crystals, which he incorrectly suggested were composed entirely of protein. Research by F.C. Bawden and N. Pirie, in England, during the same period correctly demonstrated that TMV was actually a ribonucleoprotein, composed of RNA and a coat protein. By the mid-1950s, scientists in Germany and the United States proved that the RNA alone was infectious. This discovery ushered in the modern era of molecular virology. TMV is known for several 'firsts' in virology, including the first virus to be shown to consist of RNA and protein, the first virus characterized by X-ray crystallography to show a helical structure (Figure 7), and the first virus used for electron microscopy (Figure 6), solution electrophoresis and analytical ultracentrifugation. TMV also was the first RNA virus genome to be completely sequenced, the source of the first virus gene used to demonstrate the concept of coat protein mediated protection (Figure 11), and the first virus for which a plant virus resistance gene (the N gene) was characterized. Today, TMV is still at the forefront of research leading to new concepts in transgenic technology for virus resistance and developing the virus to act as a 'work horse' to express foreign genes in plants for production of pharmaceuticals and vaccines.

IV. CONCLUSION

Viruses are very small (submicroscopic) infectious particles (virions) composed of a protein coat and a nucleic acid core. They carry genetic information encoded in their nucleic acid, which typically specifies two or more proteins. Translation of the genome (to produce proteins) or transcription and replication (to produce more nucleic acid) takes place within the host cell and uses some of the host's biochemical "machinery". Viruses do not capture or store free energy and are not functionally active outside their host. They are therefore parasites (and usually pathogens) but are not usually regarded as genuine microorganisms.

Most viruses are restricted to a particular type of host. Some infect bacteria, and are known as bacteriophages, whereas others are known that infect algae, protozoa, fungi (mycoviruses), invertebrates, vertebrates or vascular plants. However, some viruses that are transmitted between vertebrate or plant hosts by feeding insects (vectors) can replicate within both their host and their vector. This web site is mostly concerned with those viruses that infect plants but we also provide some taxonomic and genome information about viruses of fungi, protozoa, vertebrates and invertebrates where these are related to plant viruses.

We also provide information about viroids, which are infectious RNA molecules that cause diseases in various plants. Their genomes are much smaller than those of viruses (up to 400 nucleotides of circular single-stranded RNA) and do not code for any proteins.

Acknowledgements

Thanks are due to Department of Desert Science, Semnan University for supplying soil from Semnan Agriculture Station and Semnan University.

V. REFERENCES

[1] ALMEIDA, A. M.R. & CORSO, I. C. Effect of sowing time on the incidence of bud blight in soybean (Glycine max L. Merr.). Journal of Phytopathology 132:251-257. 1991.

[2] ALMEIDA, A.M.R., BERGAMIN FILHO, A. & AMORIM, L. Disease progress of soybean bud blight and spatial pattern of diseased plants. Journal of Plant Disease and Protection 101:386-392. 1994.

[3] BAER, M., HOUSER, F., LOESCH-FIRES, S.L. & GEHRKE, L. Specific RNA binding by amino-terminal peptides of alfalfa mosaic virus coat protein. EMBO Journal 13:727-735. 1994.

[4] BHAT, A.I., JAIN, R.K., KUMAR, A., RAMIAH, M. & VARMA, A. Serological and coat protein sequence studies suggest that necrosis diseases on sunflower in India is caused by a strain of Tobacco streak virus. Archives of Virology 147:651-658. 2002.

[5] bel, P.P., R.S. Nelson, B. De, N. Hoffmann, S.G. Rogers, R.T. Fraley, and R.N. Beachy. 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science 232:738-743.

[6] Ding, B. 1998. Intercellular protein trafficking through plasmodesmata. Plant Mol. Biol. 38:279-310.

[7] Harrison, B.D. and T.M.A. Wilson. 1999. Tobacco mosaic virus: Pioneering research for a century. Phil. Transact. Royal Soc. London B. 354: 517-685.
[8] Jones, J.B., J.P. Jones, R.E. Stall, and T.A. Zitter. 1991. Compendium of Tomato Diseases. APS Press, St. Paul, MN.
[9] Nelson, R.S. and A.J.E. van Bel. 1998. The mystery of virus trafficking into, through and out of vascular tissue. Prog. Bot. 59:476-533.
[10] Scholthof, K.-B. G. 2004. Tobacco mosaic virus: A model system for plant biology. Annu. Rev. Phytopathol. 42:13-34. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve& db=pubmed&dopt=Abstract&list_uids=15283658&query_hl=5
[11] Scholthof, K-B.G., J.G. Shaw, and M. Zaitlin. 1999. Tobacco mosaic virus: 100 years of contributions to virology. APS Press. St. Paul, MN.
[12] Schumann, G. L. 1991. Plant Diseases: Their Biology and Social Impact. APS Press, St. Paul, MN.
[13] Shew, H.D. and G.B. Lucas. 1991. Compendium of Tobacco Diseases. APS Press, St. Paul, MN.