CHAPTER 2

Literature Review

2.1 Cucurbitaceae

The Cucurbitaceae is a family of frost sensitive, predominantly tendril-bearing vines which are found in subtropical and tropical regions around the globe. The few species that are native to or cultivated in temperate climates are prolific seed producing annuals, perennials that live for one season until killed by frost or xerophytes perennials whose succulent underground parts survive the winter. Ecologically, the family is dichotomous; many genera consist of aggressive climbers which flourish in the humid tropics, particularly in southeastern Asia and the entropic, whereas other genera are native to the arid regions of Africa, Madagascar and North America. Members of the latter group, the xerophytes, typically have large, perennial roots and succulent stems that are clambering and creeping and at least partially subterranean; in some cases, tendrils or leaves are lacking or greatly modified. The Cucurbitaceae, which is not closely related to any other plant family, consists of two well-defined subfamilies, eight tribes representing varying degrees of circumscriptive cohesiveness, and about 118 genera and 825 species. The four major cucurbit crops (watermelon, cucumber, melon, and squash) and five other important crops (Luffa, bottle gourd, chayote, wax gourd, bitter melon) in family belong to the Cucurbitoideae subfamily. Four of these-watermelon, luffa, bottle gourd and wax gourd belong to the tribe Benincaseae (Robinson and Decker-Walters, 1997). All of the following Cucurbitoideae: cucumber, pumpkin, and zucchini, botanical character description are based on Robinson and Decker-Walters (1997).

2.1.1 Cucumber

Botanical characters

Most cucumber plants are indeterminate, producing a small trailing vine 1-3 m long, but determinate cultivars with a compact plant habit have been developed for home growers and for mechanical, once-over harvesting. A single, unbranched tendril develops at each leaf axils, trichomes occur on the angular stems and the triangularly ovate, 3-5 lobed leaves.

Cucumis sativus was originally monoecious; as are many modern cucumber cultivars, but gynoecious and andromonoecious cultivars were subsequently bred. Generally, only one of the clustered staminate flowers at a node is open on a given day. Male and female flowers are typically borne at different nodes, with the female flowers at higher, i.e. more distant, nodes than the male flowers. Female flowers are usually solitary at occasional nodes, but there may be several female flowers at a node if the plant has a multiple pistillate allele, and gynoecious plants have a female flower at every node. The inferior ovary has three united carpels in most cultivars, five in the Lemon cultivar. Immature fruits are green at the edible stage, except in a few cultivars, where they are white or yellow. Fruits are round to oblong or narrowly cylindrical, with small tubercles (warts) and spines at trichome origin on the rind. Spine color is associated with mature fruit color and fruit netting. Fruits of white-spined cultivars are light green to yellow at maturity and not netted. Black-spined fruits become orange or brown when mature and may be netted. Fruit flesh is crisp and usually white, but is pale orange in a few cultivars. Seeds are small, white and flat.

Origin and history

Cucumber is of Asiatic origin: the progenitor may be the closely related, wild *Cucumis sativus* var. *hardwickii*, which was first found in the Himalayan foothills of Nepal. Plants of this variety are highly branched, day length sensitive and prodigious producers of bitter fruits. Cucumbers remain in eastern Iran have been dated to the third millennium BC. Cucumber cultivation goes back at least 3,000 years in India and 2,000 years in China. China is considered as a secondary center of genetic diversification. Today, cucumber is one of the most important vegetable crops in that country, second

only to Chinese cabbage in area cultivated. Early travelers brought cucumber to Mediterranean countries 3,000-4,000 years ago, where the fruits were esteemed by the ancient Romans cultivated in the UK: there, the fruits were known as cucumbers. Portuguese explorers subsequently carried cucumber to West Africa. Columbus introduced this species to the New World, planting it in Haiti in 1494. Today, cucumber is grown throughout the world in small gardens, large commercial farms, and glasshouses.

Uses

The common use of cucumbers is as food. They are most often consumed fresh or pickled. In China, India, Indonesia, Malaysia and some other countries, they may be cooked before eaten. The fruits are used in curries and chutney in India. Cucumber seeds are eaten, particularly in Asia, and they yield and edible oil which is sometimes used in French cuisine. Young leaves and stems are cooked in Southeast Asia. Cucumber cultivars are classified as slicers, usually served fresh in salads, or picklers, which are often fermented. However, in some areas, fruits of pickling cultivars are used as slice in salads. Small-fruited pickling cucumbers are called gherkins in various countries, including India. Generally, pickles have shorter fruits with more prominent warts than slicers. The length to width ratio, usually about three to one, is important for pickling cucumbers. Most slicing cultivars have white-spined fruits, but pickles may have either white or black spines. Pickling cultivars with white spines are becoming more popular because their fruits retain green color longer.

2.1.2 Squash

Botanical characters

In Cucurbita, there are five domesticated (*C. argyrosperma*, *C. ficifolia*, *C. macima*, *C. moschata*, and *C. pepo*) and approximately ten wild species. Most species, including the domesticated species, are mesophytic with fibrous root systems; the remaining taxa are xerophytic perennials with enlarged roots. All produce frost-sensitive, tendril-bearing vines. Wild species and most cultivars have long, trailing vines, but some cultivars, particularly in *C. pepo*, have a compact bush habit in which

the tendrils have been reduced in size and function. The large leaves are palmately lobed to nearly round. Some cultivars of *C. pepo* and C. maxima, as well as many cultivars of *C. moschata* and *C. argyrosperma*, have mottled leaves, with white or silvery areas at the junctions of principle veins.

Species of Cucurbita are monoecious. The unisexual flowers are large, showy and orange (except for the cream-flowered *C. okeedhobeensis*), occurring singly in leaf axils. The five petals are reflexed at their tips and fused at their bases. Calyx lobes are narrow (*C. pepo*) or broad to sometimes leaf-like (*C. moschata*). Petals alternate with sepals, which are also united at their bases and fused with the lower corolla to form a cup-like hypanthium. Although the three filaments are separate, the anthers are more or less united and produce abundant amounts of heavy, sticky pollen. Styles are typically joined together, but they diverge slightly where the stigmas are attached. Nectar is produced in a disc inside and at the base of the hypanthium. The unilocular, inferior ovary has three or five placentae, corresponding to the number of bilobed stigmas. The fruit is a pepo; there is a great diversity in fruits size, shape and color among cultivars. Fruit flesh is bitter in wild taxa and in most ornamental gourds of *C. pepo*. Seeds of the domesticated species are large, measuring up to 3 cm long in *C. argyrosperma*.

Fruits of the wild species have hard, lignified rinds which help to protect the seeds from herbivores. The fruit may remain intact long after the plant has died. After lengthy storage, little is left except for the dried rind, peduncle, and botanists to determine prehistoric species distributions and uses. The intact dried fruits are buoyant, permitting seed dispersal via waterways.

Nomenclature

Different Cucurbita species are known by the same common name, and different common names, such as squash, pumpkin, cushaw and gourd, have been used for the same species. We will use the unqualified term squash to refer to any or all of the domesticated species of Cucurbita.

The word squash is derived from the American aboriginal word askutasquash, meaning eaten raw or uncookedf. Cultivars are classified as summer squash (sometimes called vegetable marrow) or winter squash, depending on whether the fruit is used when

immature or mature. The term winter squash refers to the ability of the fruit to be stored until the winter months. Summer squashes are generally *C. pepo,* but winter squashes may be *C. pepo* (e.g. Acorn), *C. maxima* (Hubbard), *C. moschato* (Butternut) or *C. argyrosperma* (Green Striped Cushaw). In *C. pepo,* the bush habit distinguishes most summer squash cultivars from winter squashes.

Pumpkin comes from the old English word pompin, the Greek pepon and the Latin pepo, which together mean a large, ripe, round melon or gourd. Today, the term pumpkin is used in various ways and has no botanical meaning. It typically refers to any squash used for pies, jack-o-lanterns or stock feed. *Cucurbita maxima* and *C. moshata* cultivars that would be called winter squashes in the USA are often called pumpkins in India and other countries.

Cushaw defines a winter squash cultivar with a curved neck. Its use is not limited to a single species. Thus, Green Striped Cushaw is *C. argyrosperma,* but Golden Cushaw is *C. moschata*.

Gourd often designates a cucurbit not used for food, e.g. wild species of Cucurbita. The cultivated ornamental gourds of *C. pepo,* which have small fruits of a wide assortment of shapes and colors, are used for decoration. Cucurbita gourds have hard shells. And some consider this a distinction between gourd and squash. However, the fruit of some summer squash cultivars also has a hard rind when mature.

Origin and History

Most Cucurbita species originated in Mexico, but a few species, including C. maxima, are native to South America. Since the wild fruits of this genus are bitter and inedible, early gatherers probably first collected the fruits for their edible seeds or to use the durable rinds as containers. These squash, along with corn and beans, became a staple in the diets of the Aztec, Incan and Mayan civilizations of Latin America. Archaeological evidence places wild populations of *C. pepo* in Mexico and eastern USA around 10,000 and 30,000 years ago, respectively. Domestication of these species apparently took place independently in these two areas. The USA cultivars of ssp. ovifera (L.) Decker var. ovifer, which include various summer squashes and most ornamental gourds, were probably selected mainly from wild populations of var.

ozarkana Decker-Walters inhabiting the Mississippi Valley. However, population in Texas (var. texana (Scheele) Decker) and northeastern Mexico (ssp. fraternal (Bailey) Andres) may have contributed to the genetic evolution of these cultivars as well. The wild progenitor of the Mexican lineage of cultivars (ssp. pepo), which include pumpkins and vegetable marrows, is currently unknown and possibly extinct.

Cucurbita pepo was the first squash introduced to Europe. Some of the fruits portrayed in ancient European herbals are not unlike those of modern cultivars. Secondary diversification of pumpkin and vegetable marrow cultivars occurred in Asia Minor. Toda, *C. pepo* is grown throughout the world.

Cucurbita moschata was cultivated in Mexico, South America and the southwestern USA in pre-Columbia times. It may have been domesticated independently in Mexico and northern South America; unfortunately, the wild ancestors (s) of *C. moschata* are currently unknown. As a cultivated plant, this species migrated throughout the Caribbean islands, giving rise to various indigenous calabaza landraces. When it reached Florida, Native Americans developed a distinct landrace called Seminole Pumpkin. Additional diversification of cultivars has taken place in several areas of Asia and Africa.

Uses

Summer squash is usually cooked by boiling or frying, and winter squash by baking, boiling or microwaving. Summer squash generally has white flesh and low soluble solids content, but winter squash has been bred to have orange, carotenoid-rich flesh high in soluble solids.

2.1.3 Diseases of Cucurbits

Cucurbits suffer from numerous bacterial, fungal and viral infections. Diseases attack cucurbits at every stage of development, from damping off at the beginning of germination to postharvest fruit rots.

Bacterial diseases: Bacterial wilt is seldom a serious threat to watermelon, but cucumber and melon plants may be killed. *Erwinia tracheiphila*, the pathogen, also

infects squash, often resulting in an exudation and decay of the fruit from secondary soft rot organisms. The bacterium overwinters in cucumber beetles, and these insects disseminate the bacterium the next season when feeding on cucurbits. Additionally, *Pseudomonas marginalis* and *P. viridiflava* cause angular leaf spot have also been reported to produce a bacterial wilt of cucumber in Japan (Ohichi *et al*., 1980 and Shila *et al*., 2013).

Fungal diseases: Damping off can be incited by several pathogens, including *Pythium* spp., *Phytophthora* spp., *Fusarium* spp., *Rhizoctonia solani* and other fungi. The fungi responsible for damping off of seedlings can also cause a root rot in older plants. Roots of infected cucurbits become water-soaked and flaccid, and the foliage may wilt and die. Fungal diseases which reduced yield and fruit quality are powdery mildew caused by *Oidium* sp. and downy mildew caused by *Pseudoperonospora cubensis* are plague of cucurbits and other crops everywhere (Blancard *et al*., 1994).

Viral diseases: Viral diseases of cucurbit crops cause important economic losses. More than 39 viruses are reported to infect these crops around the world, nine of which are seed borne (Provvidenti, 1996; Zitter *et al*., 1996; Yuki *et al*., 2000; Ko *et al*., 2007). Common viruses found infected zucchini are Zucchini yellow mosaic virus (ZYMV), Cucumber mosaic virus (CMV), Squash mosaic virus (SqMV), Watermelon mosaic virus2 (WMV-2) and Papaya ringspot virus-Type W (PRSV-W) [Davis *et al*., 2002; Kemble *et al*., 2005; Jossey and Babadoost, 2008; El-Shamy, 2010]). Cucumber green mottle mosaic virus (CGMMV), melon necrotic spot virus (MNSV), and cucurbit aphid – borne yellow virus (CABYV) were also reported as serious diseases in many cucurbit growing regions (Tomassoli and Barba, 2000). Viruses can be a very serious problem because there are no chemical protectants against them (Choi, 2001).

2.2 Plant viral pathogen

2.2.1 Definition of virus

A virus is a set of one or more nucleic acid template molecules, normally encased in a protective coat or coats of protein or lipoprotein, which is able to organize its own replication only within suitable host cells. It can usually be horizontally

transmitted between hosts. Within such cells, virus replication is (1) dependent on the host's protein-synthesizing machinery, (2) organized from pools of the required materials rather than by binary fission, (3) located at sites that are not separated from the host cell contents by a lipoprotein bilayer membrane, and (4) continually giving rise to variants through various kinds of change in the viral nucleic acid (Hull, 2002).

Viruses are obligate parasites of submicroscopic size, with one dimension smaller than 200 nm. Virus particles, or virions, consist of segments of double- or single-stranded RNA or DNA encased in protein structures, in some cases with lipid and additional substances. Most plant viruses have single-stranded RNA genomes. Viruses, unlike microbial parasites, utilize the processes of the living cell to affect their own multiplication. Viruses also lack the machinery for the production of energy through respiration, and for at least some viruses the isolated nucleic acid genome is infective (Waller, 2002).

2.2.2 Virus taxonomy

The International Committee on Taxonomy of Viruses (ICTV) approved 16 groups of plant viruses in 1970. Following considerable controversy regarding the taxonomy of viruses, ICTV later classified viruses into 233 genera (Pringle, 1999) using four criteria: (1) the general nature of the viral genome; (2) the stranded nature of the viral genome; (3) the facility for reserve transcription; and (4) the polarity of the virus genome. Recently in 2005, Mayo and Brunt reported that ICTV had come up with new approvals for plant virus taxonomy, which include 18 virus families, 82 genera and 17 unassigned genera. The families include viruses that infect cucurbits, i.e. families geminiviridae, bunyaviridae, potyviridae, bromoviridae, closteroviridae, and luteoviridae.

2.2.3 Composition and Structure

Plant virus consists of at least a nucleic acid and a protein. Some viruses consist of more than one size of nucleic acid and proteins, and some of them contain enzymes or membrane lipids. The nucleic acid makes up 5 to 40 percent of the virus, protein making up the remaining 60 to 90 percent. The lower nucleic acid percentages are

found in the elongated viruses, whereas the spherical viruses contain higher percentages of nucleic acid. The total mass of the nucleoprotein of different virus particles varies from 4.6 million to 73 million Daltons. The nucleic acid of most plant viruses consists of RNA, but at least 80 viruses have been shown to contain DNA. Both RNA and DNA are long, chain like molecules consisting of hundreds or, more often, thousands of units called nucleotides. Each nucleotide consists of a ring compound called the base attached to a five-carbon sugar [ribose (I) in RNA, deoxyribose (II) in DNA], which in turn is attached to phosphoric acid. The sugar of one nucleotide reacts with the phosphate of another nucleotide, and this is repeated many times, thus forming the RNA or DNA strand. In viral RNA, only one of four bases, adenine, guanine, cytosine, and uracil, can be attached to each ribose molecule. The first two, adenine and guanine, are purines, and interact with the other two, uracil and cytosine, the pyrimidines (Agrios, 2005; Astier *et al*., 2007).

2.2.4 Economic losses due to plant viruses

Viruses are responsible for far greater economic losses than is generally recognized. This lack of recognition is due to several factors, especially their insidious nature. Virus diseases are frequently less conspicuous than those caused by other plant pathogens and last much longer. This is especially true for perennial crops and those that are vegetatively propagated. One problem with attempting to assess losses due to virus diseases on a global basis is that most of the data are from small comparative trials rather than wide scale comprehensive surveys. Even the small trials do not necessarily give data that can be used for more global estimates of losses. This is for several reasons including: (1) variation in losses by particular virus in a particular crop from year to year; (2) variation from region to region and climatic zone to climatic zone; (3) differences in loss assessment methodologies; (4) identification of the viral etiology of the disease; (5) variation in the definition of losses and complications with other loss factors. In addition to the obvious detrimental effects such as reduced yields and visual product quality, virus infections often do not induce noticeable disease but affect on plants in a variety of more subtle ways. Table 1, identifies some of the ways in which viruses can damage crop plants. From this it can be seen that the effects of virus infection extend into areas far beyond the actual reduction in yield and quality. Loss

estimates do not take account of these indirect factors. In spite of all these limitations, there have been various collections of loss data.

Table 2.1 Some types of direct and indirect damage associated with plant virus infections (Hull, 2002).

Reduction in growth
Yield reduction (Including symptomless infection)
Crop failure
Reduction in vigor
Increased sensitivity to frost and drought
Increased predisposition to attack by other pathogen and pests
Reduction in quality or market value
Defects of visual attraction: size shape, color
Reduced keeping quality
Reduced consumer appeal; grading, taste, texture composition
Reduced fitness for propagation
Cost of attempting to maintain crop health
Cultural hygiene on farm including vector control
Production of virus-free propagation materials
Checking propagates and commodities on export-import (quarantine programs)
Eradication programs
Breeding for resistance
Research, extension and education

2.2.5 Symptoms caused by plant viruses as described based on Hull (2002)

Viruses that produce local lesions when inoculated mechanically onto leaves may not do so when introduced by other means. For example, beet yellow virus (BYV) produces necrotic local lesions on *Chenopodium capitatum*, but does not do so when the virus is introduced by the aphid *Myzus persicae* feeding on parenchyma cells. However, alfalfa mosaic virus (AMV) does produce local lesions following aphid transmission.

- **Local symptoms**

Localized lesions which develop near the site of entry on leaves are not usually of any economic significance but are important for biological assay. Infected cells may lose chlorophyll and other pigments, giving rise to chlorotic local lesions. The lesion may be almost white or merely a slightly paler shade of green than the rest of the leaf. In a few diseases, for example in older leaves of tomato inoculated with tomato bushy stunt virus (TBSV), the lesions retain move chlorophyll than the surrounding tissue. For many host-virus combinations, the infected cells die, giving rise to necrotic lesions. These vary from small pinpoint areas to large irregular spreading necrotic patches. In a third type, ring spot lesions appear. Typically, these consist of a central group of dead cells. Beyond this, there develop one or more superficial concentric rings of dead cells with normal green tissue between them. Some ring sot local lesions consist of chlorotic rings rather than necrotic ones. Some viruses in certain hosts show no visible local lesions in the intact leaf, but when the leaf is cleared in ethanol and stained with iodine, starch lesions may become apparent.

- **Systemic symptoms**

Major kinds of the effects produced by systemic virus invasion. This various symptoms often appear in combination in particular diseases, and that the pattern of disease development for a particular host-virus combination often involves a sequential development of different kinds of symptoms.

Effects on plant morphology

Reduction in plant size is the most general symptom induced by virus infection. There is probably some slight general stunting of growth even with masked or latent infections where the systemically infected plant shows no obvious sign of disease. The degree of stunting is generally correlated with the severity of other symptoms, particularly where loss of chlorophyll from the leaves is concerned. Stunting is usually almost entirely due to reduction in leaf size and internodes length. Leaf number may be little affected. In perennial deciduous plants such as grapes, there may be a delayed initiation of growth in the spring (Gilmer *et al*., 1970). Stunting may affect all parts of the plant more or less equally, involving a reduction in size of leaves, flowers, fruits, and roots and shortening of petioles and internodes.

Mosaic patterns and related symptoms

The most common obvious effects of virus infection is the development of a pattern of light and dark green areas giving a mosaic effect in infected leaves. In leaves that are past the cell division stage of leaf expansion when they become infected (about 4-6 cm long for leaves such as tobacco and Chinese cabbage) no mosaic pattern develops. The leaves become uniformly paler than normal. In the oldest leaves to show mosaic, a large number of small islands of dark green tissue usually appear against a background of paler color. In monocotyledons, a common result of virus infection is the production of stripes or streaks of tissue lighter in color than the rest of the leaf. The shades of color vary from pale green to yellow or white, and the more or less angular streaks or stripes run parallel to the length of the leaf. In monocotyledons, a common result of virus infection is the production of stripes or streaks of tissue lighter in color than the rest of the leaf. The shades of color vary from pale green to yellow or white, and the more or less angular streaks or stripes run parallel to the length of the leaf. A variegation or breaking in the color of petals commonly accompanies mosaic or streak symptoms in leaves. The breaking usually consists of flecks, streaks or sectors of tissue with a color different from normal. The breaking of petal color is frequently due to loss of anthocyanin pigments, which reveals any underlying coloration due to plastid pigments.

Yellow diseases

Viruses that cause a general yellowing of the leaves are not as numerous as those causing mosaic diseases, but some, such as the viruses causing yellows in sugar beet, are of considerable economic importance. The first sign of infection is usually a clearing or yellowing of the veins in the younger leaves followed by a general yellowing of the leaves. This yellowing may be slight or severe.

Leaf rolling

Virus infection can result in leaf rolling, which is usually upwards or occasionally downwards. Pronounced epinasty of leaf petioles may sometimes be a prominent feature.

Ring spot diseases

A marked symptom in many virus diseases is a pattern of concentric rings and irregular line on the leaves and sometimes also on the fruit. The lines may consist of yellowed tissue or may be due to death of superficial layers of cells, giving an etched appearance. In severe diseases, complete necrosis through the full thickness of the leaf lamina may occur. With the ring spot viruses, such as tobacco ring spot virus (TRSV), there is a strong tendency for plants to recover from the disease after an initial shock period. Leaves that have developed symptoms do not lose these, but younger growth may show no obvious symptoms in spite of the fact that they contain virus.

Necrotic diseases

Death of tissues, organs or the whole plant is the main feature of some diseases. Necrotic patterns may follow the veins as the virus moves into the leaf. In some diseases, the whole leaf is killed. Necrosis extends fairly with potato virus X and PVY in some varieties of potatoes; necrotic streaks appear in the stem. Necrosis spreads rapidly to the growing point, which is killed, and subsequently all leaves may collapse and die. Wilting of the parts that are about to become necrotic often precedes such systemic necrotic disease.

Developmental abnormalities

Besides being generally smaller than normal, virus-infected plants may show a wide range of developmental abnormalities. Such changes may be the major feature of the disease or may accompany other symptoms. For example, uneven growth of the leaf lamina is often found in mosaic diseases. Dark green areas may be raised to give a blistered effect, and the margin of the leaf may be irregular and twisted. In some diseases, the leaf blade may be more or less completely suppressed, such as in tomatoes infected with CMV and/or TMV (Francki, 1980). Some viruses cause swellings in the stem, which may be substantial in woody plants, such as in cocoa swollen shoot disease. Another group of growth abnormalities is known as enations. These are outgrowths from the upper or lower surface of the leaf usually associated with veins. They may be small ridges of tissue, or larger, irregularly shaped leaf like structures, or long filiform outgrowths.

Wilting

Wilting of the aerial parts frequently followed by death of the whole plant may be an important feature. Many virus-infected plants, for example, cucumber infected with CMV show loss of turgidity due to water deficiency. This happens when loss of water in transpiration exceeds water absorption. Reduced water supply may be due to abnormalities in the xylem vessels, such as necrosis or gum formation. When loss of water is near total, the affected plants desiccate and wither, as can be seen in, for instance, pea plants infected with Pea early-browning virus (Khan and Dijkstra, 2006).

Recovery from disease

Not uncommonly, a plant shows disease symptoms for a period and then new growth appears in which symptoms are milder or absent, although virus may be still present. This commonly occurs with Nepovirus infections. Many factors influence this recovery phenomenon. The environment can also affect recovery from disease as can host species or variety and virus strain.

Reduced nodulation

Nitrogen fixing Rhizobium nodules are reduced by virus infection. With AMV infection in *Medicago*, nitrogen fixation per unit of nodule fresh weight is the same as in healthy plants, but infected plants produce less nodule tissue. Hence, nitrogen fixation per plant is reduced (Dall *et al*., 1989)

Genetic effects

Infection with barley stripe mosaic virus (BSMV) induces an increase in mutation rate in *Zea mays* and also a genetic abnormality known as an aberrant ratio (AR). The AR effect was observed only when the original pollen parent was infected and showing virus symptoms on the upper leaves. The AR effect is inherited in a stable manner, with a low frequency of reversion to normal ratios. It is inherited in plants where virus can no longer be detected.

All symptoms are reflections of a disturbed physiology of the infected plant. Before the appearance of externally visible (macroscopic) symptoms, metabolic disturbances may lead to anatomical (i.e., cytological and histological) aberrations. The deviations may consist of abnormal enlargement of cells (hypertrophy) or reduction in their size (hypoplasia), degeneration of organelles (e.g., chloroplasts), abnormal accumulation of metabolites in phloem vessels, and necrosis of cells. Besides changes in existing cells and tissues, new virus-induced structures, so-called inclusion bodies, may be present in infected cells. They found either in the cytoplasm or in the nucleus. Inclusion bodies may be either (para) crystalline or noncrystalline with a round, oval, and irregularly shaped granular appearance, formerly referred to as X-bodies. The (para) crystalline inclusion consists mainly of regularly arranged virus particles, e.g. the crystals induced by tobacco mosaic virus (TMV). The non-crystalline inclusion, however, contain either a mixture of virus particles, virus-coded proteins and degraded cellular material e.g. the irregularly shaped inclusions of Bean yellow mosaic virus in *Vicia faba*, or viral genome-coded protein e.g. the so-called pinwheel inclusions of potyvirus, or cytoplasmic structures where virus synthesis and assembly take place e.g. the viroplasmic structures of caulimovirus. Many of these inclusions can be seen by light microscopy and are of diagnostic value as they are characteristic of virus groups

and independent of the host plant species (Christie and Edwardson, 1984; Khan and Dijkstra, 2006).

2.3 Transmission of plant viruses

Plant viruses do not have the ability to penetrate the plant cuticle, epidermis, and cell wall. For experimental transmission, viruses are often transferred using mechanical methods. In nature, viruses depend on vectors to breach these defenses and to allow entry to living cells. Vectors may be insects, mites, nematodes, fungi, parasitic seed plants. Each virus evolves a unique and specific relationship with its vector. Viruses are dependent on this complex interaction and have developed many methods for capitalizing on the biology of their vectors.

2.3.1 Plant virus vectors and specificity of transmission

Vectors of plant viruses

Vectors of plant viruses are taxonomically very diverse and can be found among arthropods, nematodes, fungi, and plasmodiophorids (Froissart *et al*., 2002; Hull, 2002). Arthropod vectors that transmit most plant viruses are aphids, whiteflies, leafhoppers, thrips, beetles, mealy bugs, mirids, and mites (Spence, 2001), the most common being aphids with more than 200 vector species identified (Ng and Perry, 2004). More than half of the nearly 550 vector-transmitted virus species recorded so far are disseminated by aphids (55%), 11% by leafhoppers, 11% by beetles, 9% by whiteflies, 7% by nematode, 5% by fungi and plasmodiophorids, and remaining 2% by thrips, mites, mirids, or mealy bugs (Astier *et al*., 2001).

Insects as vectors as described by Andret-Link and Fuchs (2005).

Aphids

Aphids transmit more viruses than any other vector group. Viruses have developed four types of interactions with aphids. These interactions are dependent on the plant tissue infected, the virus association with the vector, and virus replication (or lack of replication) in the vector (Table 2.2)

Non-persistent transmission

Viruses that are transmitted in a non-persistent manner infect the epidermal cells of the host plant. This type of transmission is dependent on the sampling behavior of the aphid that quickly probes in and out of the epidermal cells in order to determine host suitability. The virus forms a brief association with two sites. The first site is at the tip of the stylet, and the second is found just before the cibarial pump at the top of the stylet. This association lasts only as long as the next probe of the aphid when it is flushed from the stylet during the process of egestion. Non-persistent aphid transmission requires only seconds for acquisition and transmission, and is increased by pre-aquisition starvation or by any other condition that increases sampling behavior of the aphid. Evidence suggests that in some non-persistenly transmitted viruses, such as Cucumoviruses, the ability to bind to the aphid is dependent on a property of the coat protein, such as conformation or the binding of metal ions (Ng and Falk, 2006). Non-persistent transmission in some viruses requires the presence of an additional protein called the helper component, which is theorized to assist in the binding of the virus to the aphid stylet. Potyviruses are among the most important viruses transmitted in a non-persistent manner.

Plant viruses transmitted by aphids in a non-persistent manner include members of the genera Alfamovirus (family Bromoviridae), Carlavirus (family Flexiviridae), Cucumovirus (family Bromoviridae), Fabavirus (family Comoviridae), Machlomovirus (family Potyviridae), and Potyvirus (family Potyviridae) (Hull, 2002; Pirone and Perry, 2002) (Table 3). They represent over 130 distinct virus species (Astier *et al*., 2001; Ng and Perry, 2004)

Semi persistent transmission

Semi persistent viruses form an association with the lining of the aphid foregut. The acquisition of these viruses requires phloem feeding, leading to longer acquisition time than no persistently transmitted viruses; transmission requires minutes, and retention of the virus typically lasts for hours. Virus retention does not last through the aphids developmental molts as the lining of the foregut is shed with the rest of the cuticular exoskeleton. Semi persistent transmission may require the presence of a

helper component or a helper virus (Hull, 2002). Cauliflower mosaic virus (CaMV) is semi persistently transmitted by aphids and depends on coat protein and two non-virion proteins in transmission (Ng and Falk, 2006)

Plant viruses transmitted by aphids in a semi persistent manner include members of the genera Ampelovirus (family Closteroviridae), Badnavirus (family Caulimoviridae), Caulimovirus (family Caulimoviridae), Closterovirus (family Closteroviridae), Crinivirus (family Closteroviridae), Ipomovirus (family Potyviridae), Nepovirus (family Comoviridae), Sadwavirus (unassigned family), Sequivirus (family Sequiviridae), Trichovirus (family Flexiviridae), and Waikavirus (family Sequiviridae) (Hull, 2002). They represent over 80 distinct virus species (Astier *et al*., 2001)

Persistent circulative non propagative transmission

All viruses transmitted in this manner must be taken from the phloem of an infected plant and placed in the phloem of a healthy plant. Thus, they are dependent on the phloem probing behavior of aphids. Approximately 20 minutes are required for aphids to establish phloem probes. Thus, the minimum acquisition and transmission time for this type of transmission is 20 minutes. Viruses transmitted in a persistent circulative non propagative manner form a close association with their aphid vectors. The virus moves throughout the intestinal tract to the hindgut where it passes into the aphid's hemolymph and begins to circulate throughout the hemocoel. The virus moves from the hemocoel into the salivary gland by passing through the basal membrane of the salivary gland. The virus is then injected into the healthy plant with the saliva during ejection. Viruses transmitted in this manner are retained for days to weeks. Retention time is correlated with the amount of virus in the hemocoel and the level of virus in the hemocoel is related to the acquisition time during which the vector feeds on the infected host.

Plant viruses transmitted by aphids in a persistent circulative non-propagative manner include members of the genera Begomovirus (family Geminiviridae), Bromovirus (family Bromoviridae), Carmovirus (family Tombusviridae), Comovirus (family Comoviridae), Curtovirus (family Geminiviridae), Enamovirus (family Luteoviridae), Luteovirus (family Luteoviridae), Mastrevirus (family Geminiviridae),

Nanovirus (unassigned family), Polerovirus (family Luteoviridae), Rymovirus (family Potyviridae), Sobemovirus (unassigned family), Tymovirus (family Tymoviridae), and Umbravirus (unassigned family) (Hull, 2002). They represent close to 150 distinct virus species (Astier *et al*., 2001). Vectors in this category are aphids, beetles, leafhoppers, whiteflies, and mirids.

Persistent circulative propagative transmission

Persistent circulative propagative transmission has many characteristics in common with persistent circulative nonpropagative viruses. Viruses transmitted in this manner are phloem-limited viruses. Transmission is dependent on phloem probes and passage of the virus into the hemolymph. However, after entering the hemolymph, the virus infects and replicates in the aphid. Many tissues of the aphid may be infected. The virus can also pass through the ovaries into the offspring, which are viruliferous when they are born. This is termed transovarial passage of the virus.

Plant viruses transmitted by aphids in a persistent circulative propagative manner include members of the genera Cytorhabdovirus (family Rhabdoviridae), Fijivirus (family Reoviridae), Marafivirus (family Tymoviridae), Nucleorhabdovirus (family Rhabdoviridae), Oryzavirus (family Reoviridae), Phytoreovirus (family Reoviridae), Tenuivirus (unassigned family), and Tospovirus (family Bunyaviridae) (Hull, 2002). They represent at least 60 distinct virus species (Astier *et al*., 2001). Vectors in the category include thrips, aphids, leafhoppers, and whiteflies.

Table 2.2 Comparison of Aphid transmission characteristics (Langham, 2008).

	Characteristic types of transmission			
	Non-persistent	Semi persistent	Persistent circulative nonpropagative	Persistent circulative propagative
Tissue infected by transmitted virus	Epidermis	Mesophyll and phloem	Phloem	Phloem
Virus interaction sites	Stylet tip and precibarium	Foregut	Hindgut and salivary glands	Hindgut and salivary glands; also infects many tissues of the host
Type of feeding behavior associated with transmission	Sampling	Phloem probing	Phloem probing	Phloem probing
Acquisition time	Seconds	Minutes to hours	20 min to hours	20 min to hours
Inoculation time	Seconds	Minutes to hours	20 min to hours	20 min to hours
Retention time	Minutes to hours	Hours to days	Days to life	Days to life
Latent period	No	No	Yes (hours)	Yes (days to weeks)
Retained through molt	No	No	Yes	Yes
Found in the hemolymph	No	No	Yes	Yes
Replicates and infects the host tissues	No	No	No	Yes
Transovarial passage-infects offspring	No	No	No	Yes

Table 2.3 Vectors of plant viruses and transmission mode (Andret-Link and Fuchs, 2005; Astier *et al*., 2007).

Vector	Noncirculative		Circulative persistent	
	Non-persistent	Semi persistent	Nonpropagative	Propagative
Aphids	Alfamovirus Carlavirus Cucumovirus Fabavirus Macluravirus Potyvirus	Caulimovirus Closterovirus Sequivirus Trichovirus Waikavirus	Enamovirus Luteovirus Nanovirus Polerovirus Umbravirus	Cytorhabdovirus Nucleorhabdovirus
Beetles	Machlomovirus		Bromovirus (TMV) Carmovirus (?) Comovirus (?) Sobemovirus(?) Tymovirus (?)	
Fungi	Carmovirus Necrovirus Tombusvirus		Benyvirus Bymovirus Furovirus Varicosavirus	
Leafhoppers		Badnavirus Waikavirus	Curtovirus Mastrevirus	Cytohabdovirus Fijivirus Marafivirus Nucleorhabdovirus Oryzavirus Phtoreovirus Tenuivirus Phytoreovirus
Mealybugs		Ampelovirus Badnavirus Trichovirus Vitivirus		
Mirids			Sobemovirus	
Mites		Trichovirus	Rymovirus	
Nematodes		Nepovirus Tobravirus Sadwavirus		
Plasmodiophorids	Aureusvirus Dianthovirus Ophiovirus Varicosavirus			
Thrips	Machlomovirus			Tospovirus
Whiteflies		Crinivirus Ipomovirus	Begomovirus	

(?) Limited information is available on virus-vector interactions, raising some uncertainty on the transmission mode.

Leafhoppers

Leafhopper transmission of viruses highly parallels aphid transmission with one exception. Non-persistent transmission does not occur in leafhoppers. Leafhoppers transmit viruses by semi-persistent transmission, persistent circulative non-propagative transmission, or persistent circulative propagative transmission. Important examples of viruses transmitted by leafhoppers include beet curly top virus (BCTV), potato yellow dwarf virus (PYDV), and maize stripe virus (MSV).

Beetles

Beetle transmission is unique among all the other types of virus transmission in that the specificity of virus transmission is not in the ability of the beetle to acquire the virus, but in the interaction of the virus and host after transmission. Beetles can acquire both transmissible and found in the hemolymph of beetles. Other viruses are found only in the gut lumen and mid-gut epithelial cells (Wang *et al*., 1994). However, the specificity of transmission does not depend on these factors. Beetles spread a layer of predigestive material known as regurgitant on the leaves as they feed. This layer contains high concentrations of deoxyribonucleases, ribonucleases, and proteases. When viruliferous beetles spread this layer, they also deposit virus particles in the wound at the feeding site. Beetle transmissible viruses are able to move through the vascular system to an area away from the wound site with its high level of ribonuclease in order to establish infection (Gergerich *et al*., 1986; Gergerich and Scott, 1988). Nontransmissible viruses are retained at the wound site where the ribonuclease levels inhibit their ability to infect the plant (Field *et al*., 1994). Comovirus and Sobemovirus are among the most important viral genera transmitted by beetles.

Whiteflies

The mode of transmission of viruses in whiteflies varies with the genus of virus. Begomovirus is transmitted in a persistent circulative manner resembling aphid transmission of Luteovirus. Howerver, this relationship may be more complex than it appears due to the extended retention lengths and the transovarial passage of some species of this viral genus (Hull, 2002). In contrast, Closteroviruses and Criniviruses

are transmitted in a foregut-borne semipersistent manner (Hull, 2002). Activities of different coat proteins or other proteins may also be necessary for transmission. Lettuce infectious yellow virus (LIYV), a Closterovirus, has a minor coat protein (CPm) that is necessary for transmission (Ng and Falk, 2006). Regardless of which virus is transmitted or what components are required for transmission, whiteflies present constant challenges as virus vectors due to their dynamic population increases, resistance to control, and changes in their biotype.

Thrips

Virus transmission by thrips has been a dynamic area of research in recent years. The rapid increase in the importance of Tospovirus and the diseases that they cause in both greenhouses and fields has stimulated much of this research. Tospoviurses are persistently and propagatively transmitted by thrips. Thrips larvae acquire the virus while feeding on virus-infected tissue and the virus crosses through the midgut barrier and enters the salivary glands. The virus must be acquired by immature thrips because adult thrips cannot acquire the virus (Moyer, 1999); the virus passes from larvae to adult thrips as it undergoes pupation and the changes associated with maturity. This is known as transstadial passage (Whitfield *et al*., 2005). Thrips retain infectivity for their lifetime, and the virus titer has been shown to increase as the virus replicates in the thrips. No evidence of virus passage through the egg (transovarial passage) has been found (Moyer *et al*., 1999), but question of whether or not Tospoviruses are pathogenic to thrips is an active research area (Whitfield *et al*., 2005). Thrips have also been shown to transmit three other viral genera, Ilarvirus, Sobemovirus, and Carmovirus, by movement of virus-infected pollen. The virus from the infected pollen is then transmitted to the host plant through wounds caused by thrips feeding (Hull, 2002).

Other vectors

Mites

Eriophyid mites are tiny arthropods (0.2 mm length) known to transmit several plant viruses, including wheat streak mosaic virus (WAMV). Mites acquire virus during their larval stages. As in thrips, adult mites cannot acquire the virus, but both the

larvae and adults transmit the virus. Mites have been shown to remain infective for over two months (Hull, 2002). WSMV particles have been found in the midgut, body cavity, and salivary glands of the mite (Paliwal, 1980). However, there has been no evidence to prove replication of virus in the mite.

Nematodes

Nematodes that transmit plant viruses are all migratory ectoparasites. Three genera of nematodes, *Longidorus*, *Xiphinema*, and *Trichodorus*, are primarily associated with transmission of viruses. Nematodes feeding on virus infected plants retain virus on the stylet, buccal cavity, or esophagus. When the nematodes are feeding on healthy host plants, the retained virus is release into the feeding site to infect the new host. Viruses in the Tobravirus and the Nepovirus genera are transmitted by nematodes.

Fungi and Fungi-like organisms

The Chytridiomycete, Olpidium, and the plasmodiophoridprot, *Polymyxa*, and thecercozoanprotest, *Spongospora* transmit viruses as they infect the root systems of their hosts. Zoospores released from infected plants may carry virus either externally or internally. Viruses adsorbed on the external surface of the zoospore, such as the Tombusviridae, are released to infect new host plants. Virus adsorbed on the zoospore flagellum can enter the zoospore when its flagellum is retracted to encyst (Hull, 2002). The process through which viruses are carried internally is undefined and remains a topic for future research. Bymovirus and Furovirus are examples of viral genera that are transmitted in this manner. Rhizomania of sugarbeets, caused by beet necrotic yellow vein virus (BNYVV) transmitted by *Polymyxa*, is a good example of a viral disease transmitted in this manner that is a major economic problem.

Dodder

The parasitic seed plant dodder (*Cuscuta* species) forms haustoria into the phloem of the plants that it parasitizes. This connection allows the carbohydrates and other compounds to move into the dodder's phloem. When a dodder plant connects a

healthy and virus-infected host plant, viruses can be transmitted from the infected plant through the dodder to the phloem of the non-infected plant.

Vegetative propagation and grafting

Viruses that systemically infect plants can be transmitted by the vegetative propagation of a portion of the infected plant. This portion can range from leaves, stems, branches, roots to bulbs, corms, and tubers. Grafting is a form of vegetative transmission, a transmission occurs through the newly established vascular system linking the graft and the scion (Hull, 2002)

2.3.2 Seed transmission of viruses

Passage of viral inocula from diseased plants to their offspring via seeds was long thought to be a rare phenomenon. Today, seed transmission is known to occur for about one-seventh of the known viruses in one or more of their hosts (Hull, 2002), and the number is still increasing. Three important effects of seed transmission are: (i) direct injury to the crop and/or indirect injury, infected seeds sown results in numerous randomly scattered foci of infected plant, facilitating early secondary spread in the crop by vectors; (ii) survival of virus inocula from one crop season to the next; and (iii) several viruses has been, and undoubtedly still are, disseminated worldwide through exchange of seed having undetected infection. Viruses may be transmitted in seed by two methods. In the first method, the virus infects the embryo within the seed, and it is already infected when it emerges. This is often referred to as true seed transmission. It should be noted that embryo-transmissible viruses can also occur in an inactivated state in seed parts outside the embryo, e.g. endosperm and testa of seeds, both with and without embryo infection. For example, up to 50% of mature seeds of a pea seed lot infected with pea seed-borne mosaic virus (PSbMV) have been shown to contain inactivated but detectable virus in the seed-coats, but only 2-3% of these seeds carried transmissible virus. The second transmission method is through contamination of the seed, especially the seed coat. As the germinating seedling emerges from the seed, the virus infects the plant through wounds or through microfissures caused through cell maturation. Tobamovirus genera that do not enter the embryo, but are stable enough to survive in or on seeds and from there infect the offspring, for example, Cucumber green

mottle mosaic virus (CGMMV) and Tomato mosaic virus (ToMV) is transmitted to cucumber seedlings and tomato seedlings, respectively by contamination of the seed coat (Albrechtsen, 2005). Hull (2002) listed a number of virus genera, indicating the number of seed-transmitted viruses for each. Among these viruses, 31 belong to the Cryptoviruses (Alphacryptovirus, Betacryptovirus), appearing to be of no economic importance and solely transmitted through seed and pollen. Genera containing relatively high proportions of seed-transmitted members are (no. of seed-transmitted/no. of members): Alfamovirus (1/1), Comovirus (6/15), Cucumovirus (3/3), Ilarviruses (8/17), Nepovirus (17/40), Potyviruses(16/179), Sobemovirus (4/14), Tobamoviruses (7/17), and Tobravirus (3/3). Whereas for genera like Dianthovirus, Luteovirus, Marafivirus and Tenuivirus no seed-transmitted members are reported (Hull, 2002). In the large family Geminiviridae (102 members), no member is assumed to be seed-transmitted.

The present knowledge of seed transmission mechanisms appears to include the following (Albrechtsen, 2005):

1. (a) Most viruses are readily sap-transmissible, indicating an ability to invade parenchymatous tissue.

   (b) Viruses transmitted by certain types of vectors are more often seed-transmitted than those transmitted by other types of vectors. Thus, viruses transmitted by leafhoppers and those transmitted by aphids in a persistent manner (e.g. most members of Luteroviridae) are not seed-transmitted, whereas those transmitted by nematodes, beetles and, in a non-persistent manner, by aphids may be seed-transmitted.
2. There are two ways in which viruses can infect the developing embryo: by indirect invasion, i.e. by infection of the gametes before fertilization, or by direct embryo invasion, i.e. after fertilization. For many virus-host interactions, both modes of embryo infection may result in maximal seed transmission.
3. Virus survival during seed transmission requires its immunity to host ploidy changes (diploid to haploid to diploid) and its intact movement through both vegetative and reproductive tissues. If established in the embryo the virus

must remain stable during seed maturation and storage. Finally, virus is activated during or after seed germination.

4. The difference between viruses in their capacity to enter meristematic tissue and remain viable, or in their incapacity to do so, may determine the specificity of seed transmission.
5. The earlier the mother plant becomes infected, the higher the level of seed transmission, whereas mother plant infection after flowering reduces the probability of transmission through seed. The distribution of virus-infected seeds in infected mother plants tends to be erratic, at least for early infected plants, e.g. lettuce mosaic virus (LMV) in lettuce and some viruses in legumes, where distribution within pods was completely random.

Economic importance of seed-transmitted viruses

When plants become infected with virus, they are infected for life, and the infection normally results in crop losses. Economic losses from seed-transmitted viruses are both direct losses in reduced yield or quality of crops, and indirect losses as costs of control measures. As previously mentioned, even traces of virus-infected seeds sown can, in the presence of efficient vectors, lead to early epidemic spread in the field, resulting sometimes in 100% crop infection. Thus, at a seeding rate of, for example, 250,000seeds per hectare (25seeds/m^2), which is not very high, seed with as little as 0.1% infected seeds results in 250 infected plants/ha. Immediately after emergence, these randomly scattered plants function as reservoirs of inoculum. Examples of yield losses caused by seed-transmitted viruses are shown in Table 4 (Albrechtsen, 2005). Riedle-Bauer *et al*. (2002) reported that seed transmission of ZYMV, potyvirus, a devastating pathogen of cucurbits causing yield losses up to 99% (Table 4), was not considered to play an important role in its epidemiology, as it was only found seed-transmitted in traces, if at all, until 2002, where up to 5% of the seeds of *Cucurbita pepo* var, *styriaca* (all pumpkin) carried transmissible virus.

Table 2.4 Reported yield losses from seed-borne viruses. (Riedle-Bauer *et al*., 2002; Albrechtsen, 2005)

Virus	Crop	Percent yield loss
Bean common mosaic	French bean	35-98
	Mung bean	31-75
Bean yellow mosaic	Broad bean	≤59
Broad bean stain	Lentil	14-61
Cucumber mosaic	Lupine	25-42
Lettuce mosaic	Lettuce	≤30
Pea seed-borne mosaic	Pea	11-36
Peanut mottle	Groundnut	20-72
Peanut stripe	Groundnut	6-79
Soybean mosaic	Soybean	48-99
Tomato mosaic	Tomato	5-50
Zucchini yellow mosaic	Cucurbit	0-99

As previously mentioned, two of the important risks connected with seed transmission are: (i) survival of diseases from one crop season to another, and (ii) seed as a vehicle for the introduction of diseases into new areas via the seed trade.

2.4 Serological diagnostic methods as described by Albrechtsen (2006) and Astier *et al.* (2007)

Serological techniques use the specific interaction of two types of proteins: antigens, proteins of viral origin, and antibodies, proteins specific to these antigens that are elaborated by an animal (generally a rabbit) in response to the injection of the antigen.

2.4.1 Advantages and limitations of serological tests

Serological tests have provided rapid and convenient methods for the identification and estimation of plant viruses, the main advantages being:

- The specificity of the reaction allows virus to be measured in the presence of host material or other impurities.
- Results are obtained in a few hours or overnight compared with days, or even weeks, for infectivity assays.
- The methods give an answer that is directly proportional to viral concentrations.

- Some serological detection and assay procedures are more sensitive than infectivity measurements
- Serological tests are particularly useful with viruses that have no good local lesion host or that are not sap transmissible.
- Antisera can be stored and comparable tests made over periods of years and in different laboratories.

The main limitations of serological tests are as follows:

- They measure the virus protein antigen, not the amount of infective virus. This fact may, of course, be used to advantage in some situations.
- Infectivity measurements can usually detect and measure about one-tenth to one-hundredth the concentration of virus required for the precipitation reaction carried out in tubes. However, certain modifications of the test may require less material than infectivity tests.
- With rod-shaped viruses end-to-end aggregation can markedly affect results with some of the assay methods.

2.4.2 Enzyme-linked Immunosorbent Assay (ELISA)

ELISA introduced in plant virology mainly by Clark and Adams (1977) has become the most widely used serological method for routine detection of plant viruses. ELISA involves use of antibodies linked, or conjugated, with a suitable enzyme. The fact that even a small amount of an enzyme effectively enhances degradation or change of a relatively large amount of a specific compound, called the enzyme substrate, is utilized in ELISA to produce a color reaction indicating the presence of a viral antigen. The method is therefore far more sensitive than earlier serological techniques. To be useful in ELISA, an enzyme should fulfill a number of criteria. It should: (i) have a high turnover; (ii) is highly stable under storage and assay conditions; (iii) are easy to conjugate; (iv) require no noxious substrate; and in plant virus detection should not interfere with or be activated by compounds in the host plant material.

The enzyme best fulfilling these demands and used most widely in plant virus ELISA is alkaline phosphatase (AP), which dephosphorylates the colorless substrate p-nitrophenyl phosphate (pNPP), producing the bright yellow p-nitrophenol (pNP).

The double-antibody sandwich (DAS)-ELISA is a direct ELISA and was the first ELISA procedure developed for plant virus detection. It is still widely used, but requires preparation of an antibody-enzyme conjugate for each virus to be detected. A DAS-ELISA test comprises several stages. The antigen is placed between two layers of specific antibodies. The affinity between the IgG adsorbed to the walls of the well and the virus is so strong that it allows a concentration of the virus on the antibody layer. To reveal the presence of the virus thus retained, the same IgG are used, but conjugated with an enzyme: alkaline phosphatase, for example. After several washes to eliminate all the antibodies not linked to the antigens, the activity of the alkaline phosphatase is revealed by the transformation of a soluble substrate, colorless p-nitrophenyphosphate, into yellow p-nitrophenol. The intensity of the coloration is measured with a spectrophotometer at a wavelength of 405 nm.

2.5 Virus diseases of the Cucurbitaceous

All of the following cucurbit viruses: cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV), papaya ring spot virus type W (PRSV-W), watermelon mosaic virus-2 (WMV-2) and cucumber green mottle mosaic virus (CGMMV) description are based on Brunt *et al*.(1996).

2.5.1 Cucumber mosaic virus (CMV)

2.5.1.1 Nomenclature

Synonyms

Banana infectious chlorosis virus, coleus mosaic virus (Creager, 1945; Holcomb and Valverde, 1991), cowpea banding mosaic virus, cowpea ringspot virus, cucumber virus 1, lily ringspot virus (Brierley and Travis, 1958), pea top necrosis virus, peanut yellow mosaic virus, southern celery mosaic virus (Doolittle, 1916; Price, 1935;

Wellman, 1934), soybean stunt virus (Hanada and Tochihara, 1982), spinach blight virus, tomato fern leaf virus, pea western ringspot virus.

Acronym: CMV

Strains

Numerous, the better known include: A-CMV, E-CMV, L-CMV, N-CMV, P-CMV, Z-CMV and WAI/WAII; there seem to be two antigenic groups, ToRS and DTL (Richard, 1992). .

ICTV decimal code: 10.0.4.0.001

Host range and symptoms

First reported in *Cucumis sativus*; from the U.S.A; by Price (1934).

Natural host range and symptoms

Symptoms persist.

Very many hosts (Kaper and Waterworth, 1981) including:

- *Cucumis sativus* and many other cucurbits - mosaics and stunting, reduced fruit yield.
- *Lycopersicon esculentum* - mosaic, reduction of leaf laminae ("fernleaf") and stunting.
- *Spinacia oleracea* - severe chlorosis and stunting.

Transmission

Transmitted by a vector; an insect; more than 60 spp. including *Acyrthosiphon pisum, Aphis craccivora* and *Myzus persicae*; Aphididae. Transmitted in a non-persistent manner. Virus transmitted by mechanical inoculation; transmitted by seed (in 19 species, but in variable extents).

Geographical distribution

Probably distributed worldwide.

Experimental host range

Many (>9) families susceptible.

Diagnostically susceptible host species and symptoms

- *Chenopodium amaranticolor,*and *C. quinoa* - chlorotic local lesions.
- *Cucumis sativus* - systemic mosaic.
- *Vigna unguiculata* - necrotic local lesions.
- *Lycopersicon esculentum, Nicotiana × edwardsonii, N. glutinosa,*and *N. tabacum* - symptoms depend on virus strain.

Maintenance and propagation hosts

Cucumis sativus, *Nicotiana clevelandii, N. glutinosa,*and *N. tabacum*.

Assay hosts (Local lesions or Whole plants)

Chenopodium amaranticolor (L), *C. quinoa* (L), and *Vigna unguiculata* (L).

2.5.1.2 Physical and biochemical properties

Properties of particles in sap

TIP: 55-70 °C. LIV: 1-10 days. DEP: $\log_{10}$ minus 3-6. Infectivity of sap not changed by treatment with di-ethyl ether. Leaf sap contains few virions, or contains many virions. Electron microscopy: freeze-dry preparations for shadowing, or fix with aldehyde for negative staining with PTA.

Purification method

Lot *et al.* (1972) and modified by Peden and Symons in 1973 (Clark *et al.*, 1974).

Particle morphology

Virions isometric; not enveloped; 29 nm in diameter; rounded in profile; without a conspicuous capsomere arrangement.

Physical properties

One sedimenting component in purified preparations; sedimentation coefficient 99 *S*. Density 1.367 g cm^{-3} in CsCl (after fixation with formaldehyde). Isoelectric point pH 5.5 (Q strain). A_{260}/A_{280} ratio 1.7 (corrected).

Biochemical properties

Virions contain 18 % nucleic acid; 82 % protein; 0 % lipid. Genome consists of RNA; single-stranded; linear. Total genome size 8.621 kb. Genome of three parts; largest (or only) genome part the largest 3.389 kb; the 2nd largest 3.035 kb; the 3rd largest 2.197 kb. Genomic nucleic acid isolated by Gould and Symons (1977). Base composition 24 % G; 23 % A; 23 % C; 30 % U. 5´ terminus of RNA has a methylated nucleotide cap. Infectivity retained when deproteinised with proteases, or decreased when deproteinised with proteases; retained when deproteinised with phenol or detergent. Poly A region absent. Genome has *t*RNA-like activity. Genome accepts tyrosine.

Replication

Replication does not depend on a helper virus.

Cytopathology

Virions found in all parts of the host plant; in cytoplasm. Inclusions present in infected cells; are crystals in the cytoplasm (that are often rhomboidal, hexagonal or roughly spherical and may appear as solid hollow structures); they contain virions.

2.5.1.3 Taxonomy and relationships

Cucumovirus: Bromoviridae (the type species)

Virus(es) with serologically related virions

Peanut stunt and tomato aspermy viruses, but distantly.

Additional comments on relationships

Sequence homologies indicate that the virus is distantly related to brome mosaic virus, and also, in sequences in RNA-1 and RNA-2, to alfalfa mosaic and tobacco mosaic viruses (Rezaian *et al.*, 1985; Davies and Symons, 1987).

2.5.2 Zucchini yellow mosaic virus (ZYMV)

2.5.2.1 Nomenclature

Synonyms

Muskmelon yellow stunt virus (Lecoq *et al.*, 1981; 1983).

Acronym : ZYMV

Strains

Twenty two isolates of the virus have been grouped into three pathotypes by their effect on muskmelon line PI 414723 (Pitrat and Lecoq, 1984; Risser *et al.*, 1981;

Lecoq and Pitrat, 1985). ZYMV isolates differ in the symptoms they cause (Lecoq *et al.*, 1981; Provvidenti *et al.*, 1984), aphid transmissibility (Lecoq, 1986) or virulence towards a resistance gene (Lecoq and Pitrat, 1985).

ICTV decimal code: 57.0.1.0.077

Host range and symptoms

First reported in *Cucurbita pepo*; from Italy; by Lisa *et al.* (1981).

Natural host range and symptoms

Symptoms persist.

- *Cucurbita pepo* (zucchini squash), *Cucumis melo* (muskmelon), *Cucumis sativus* (cucumber) and *Citrullus lanatus* (watermelon) - mosaic, yellowing, shoestring leaves, stunting, and fruit and seed deformation.
- *Melothria pendula* - mosaic, yellowing.

Transmission

Transmitted by a vector; an insect; *Aphis citricola* (Purcifull *et al.*, 1984), *Aphis gossypii; Myzus persicae* (Lecoq *et al.*, 1981; Lisa *et al.*, 1981) and *Macrosiphum euphorbiae* (Lecoq, unpublished data) and *Aphis middletonii, Aphis craccivora, Acrythosiphon pisum, Lipaphis erysimi, Uroleucon* sp. (Adlerz, 1987); Aphididae. Transmitted in a non-persistent manner. Virus transmitted by mechanical inoculation; not transmitted by seed (Lecoq *et al.*, 1981; Dodds *et al.*, 1984).

Geographical distribution

Spreads in Algeria, Australia, Egypt, France, Germany, Israel, Italy, Japan, Jordan, Lebanon, Mauritius, Morocco, Spain, Taiwan, Turkey, the UK, and the USA (Zucchini yellow mosaic virus has been isolated from the wild perennial cucurbit *Melothria pendula* in Florida (Adlerz *et al.*, 1983).

Experimental host range

Several (3-9) families susceptible.

Diagnostically susceptible host species and symptoms

- *Chenopodium amaranticolor,and C. quinoa* - chlorotic local lesions; not systemic.
- *Cucumis melo* - chlorotic local lesions; systemic vein clearing, yellowing, mosaic, leaf deformation, stunting and occasional necrosis.
- *Cucurbita okeechobeensis* - systemic mosaic (N.B. this species is not susceptible to most cucumber mosaic virus isolates).
- *Cucurbita pepo* - chlorotic local lesions; systemic vein netting, yellowing, mosaic and leaf deformation, often lethal.
- *Gomphrena globosa* - local lesions; not systemic (N.B. this species is not infected by squash mosaic virus).
- *Luffa acutangula* - systemic mosaic or latent.
- *Ranunculus sardous* - symptomless systemic infection with most isolates (N.B. this species is not infected by papaya ringspot virus-W or watermelon mosaic virus 2).

Diagnostically insusceptible host species

Lavatera trimestris - but watermelon virus 2 induces necrotic local lesions.

Maintenance and propagation hosts

Cucurbita pepo.

Assay hosts (Local lesions or Whole plants)

Chenopodium amaranticolor (L), and *Cucurbita pepo* (W).

2.5.2.2 Physical and biochemical properties

Properties of particles in sap

TIP: 55-60 °C. LIV: 3-5 days (at room temperature). DEP: $\log_{10}$ minus 4-5. Leaf sap contains few virions.

Purification method

Lisa *et al.* (1981); Lisa and Lecoq (1984); Lesemann *et al.* (1983); Purcifull *et al.* (1984).

Particle morphology

Virions filamentous; not enveloped; usually flexuous; with a clear modal length; of 750 nm; 11 nm wide. Axial canal obscure. Basic helix obscure.

Physical properties

One sedimenting component in purified preparations. Density 1.323 g cm^{-3} in CsCl (at 10ºC).

Biochemical properties

Virions contain 4.5-7 % nucleic acid; 93-95.5 % protein. Genome consists of RNA; single-stranded; linear. Total genome size 9 kb. Genome unipartite; largest (or only) genome part 9 kb.

Replication

Replication does not depend on a helper virus.

Cytopathology

Virions found in all parts of the host plant; in cytoplasm. Inclusions present in infected cells; are pinwheels (but not laminated aggregates (Lisa *et al.*, 1981)); they do not contain virions (endoplasmic reticulum and vesicles containing fibrillar material accumulate (Lesemann *et al.*, 1983)).

2.5.2.3 Taxonomy and relationships

Potyvirus: Potyviridae

Virus(es) with serologically related virions

Watermelon mosaic virus II, bean yellow mosaic virus (Lecoq *et al.*, 1981; Lisa *et al.*, 1981; Adlerz *et al.*, 1983*a*; Lesemann, 1983; Lesemann *et al.*, 1983; Makkouk and Abbasher, 1983; Purcifull *et al.*, 1984) and amaranthus leaf mottle viruses (Lovisolo and Lisa, 1976; V. Lisa and G. D'Agostino, unpublished data), but distantly.

Differences between type strain and others

No serological differences have been detected between Italian and other isolates of the virus (Lecoq *et al.*, 1983; Lesemann *et al.*, 1983; Purcifull *et al.*, 1984; Provvidenti *et al.*, 1984). Different isolates cross-protect in muskmelon (Lecoq *et al.*, 1981).

Best tests for diagnosis

Some isolates of ZYMV cause symptoms similar to those attributed to other cucurbit viruses (cucumber mosaic virus, watermelon mosaic virus 1, strain W of papaya ringspot virus, watermelon mosaic virus 2 and squash mosaic virus), thus a diagnosis based on symptoms is uncertain, and serological tests must be used. ZYMV frequently occurs with other viruses in cucurbits, but can be isolated alone by inoculating, for example, *Cucurbita okeechobeensis* and *Ranunculus sardous*. ZYMV can be separated from squash mosaic by aphid transmission or by inoculation to *Gomphrena globosa*. Zucchini yellow fleck potyvirus, found in the the Mediterranean

region (Vovlas *et al.*, 1981), is not serologically related to ZYMV (M. Russo, unpublished data).

2.5.3 Papaya ring spot virus-W (PRSV-W)

2.5.3.1 Nomenclature

Synonyms

Watermelon mosaic 1, watermelon papaya ringspot.

ICTV decimal code: 57.0.1.0.045

Host range and symptoms

First reported in *Citrullus lanatus*; by Webb (1965).

Natural host range and symptoms

Symptoms persist.

- *Cucurbita pepo* - mosaic, green blistering, leaves and fruit malformed.
- *Citrullus lanatus, Cucumis melo, Melothria pendula,*and *Momordica charantia* - mosaic, mottling, leaves malformed.

Transmission

Transmitted by a vector; an insect; *Myzus persicae, Acyrthosiphon (Aulacorthum) solani, Aphis craccivora* and *Macrosiphum euphorbiae* (Karl and Schmelzer, 1971); Aphididae. Transmitted in a non-persistent manner. Virus transmitted by mechanical inoculation; not transmitted by seed.

Geographical distribution

Spreads in the Middle East and the South and Central American region; Australia, China, France, Germany, India, Italy, Mexico, and the USA.

Experimental host range

Few (<3) families susceptible.

Diagnostically susceptible host species and symptoms

- *Cucurbita pepo* - mosaic, green blistering, leaves and fruit malformed.
- *Cucumis metuliferus* cv. Accession 2459 - systemic mottle or mosaic.
- *Luffa acutangula* - systemic chlorotic mottling or spotting.

Diagnostically insusceptible host species

Carica papaya, *Cucumis metuliferus* plant introduction PI 292190, *Nicotiana benthamiana*, and *Phaseolus vulgaris* cv. Bountiful.

Maintenance and propagation hosts

Cucurbita maxima, *Cucurbita moschata*,and *Cucurbita pepo*.

Assay hosts (Local lesions or Whole plants)

Chenopodium amaranticolor (L) but not for all isolates, *Chenopodium quinoa* (L) but not for all isolates, *Cucumis melo* cv. B633 (L), *Cucumis sativus* (W), *Cucurbita pepo* (W).

Comments on host-range

Many watermelon mosaic virus 2 isolates (Purcifull, Hiebert and Edwardson, 1984) do not infect *Luffa acutangula*, and therefore this species has been used to obtain PRSV isolates free from watermelon mosaic virus 2 (Webb, 1965; Greber, 1978).

2.5.3.2 Physical and biochemical properties

Properties of particles in sap

TIP: 60 °C. LIV: 40-60 days. DEP: $\log_{10}$ minus 4-5. Leaf sap contains many virions.

Purification method

Gonsalves and Ishii (1980); modification of the method described by Purcifull and Hiebert (1979) for a type W isolate. Both purification methods are outlined in the CMI/AAB description No. 292.

Particle morphology

Virions filamentous; not enveloped; usually flexuous; with a clear modal length; of 760-800 nm; 12 nm wide.

Physical properties

One sedimenting component in purified preparations. Density 1.32 g cm^{-3} in CsCl.

Biochemical properties

Virions contain 5.5 % nucleic acid; 94.5 % protein. Genome consists of RNA; single-stranded; linear. Total genome size 11.4 kb. Genome unipartite; largest (or only) genome part 11.4 kb. Genomic nucleic acid isolated by Brakke and van Pelt (1970); de Mejia (1984); Hiebert *et al.* (1984); de Mejia *et al.* (1984). 5´ terminus of RNA has a VPg. Poly A region present; at the 3´ terminus (Nagel and Hiebert, 1985). The 3´ end cloned and expressed in *E. coli*.

Replication

Replication does not depend on a helper virus.

Cytopathology

Virions found in mesophyll, epidermis and phloem; in cytoplasm. Inclusions present in infected cells; are amorphous X-bodies and pinwheels; they do not contain virions.

2.5.3.3 Taxonomy and relationships

Potyvirus: Potyviridae

Virus(es) with serologically related virions

The Guadeloupe isolate of papaya ringspot is very closely related, but distinct (Quiuot-Douine *et al.*, 1986). Furthermore PRV-W reacts with the D-protein of tobacco etch virus (Shepard *et al.*, 1974) and the virion proteins and CI proteins of a potyvirus isolate from cucurbits in Morocco (Fischer and Lockhart, 1974).

2.5.4 Watermelon mosaic virus-2 (WMV-2)

2.5.4.1 Nomenclature as described by Purcifull and Hiebert (1979)

Synonyms

Marrow mosaic virus (Varma, 1988), melon mosaic virus (Iwaki *et al.*, 1984; Komuro, 1962), watermelon mosaic virus.

Acronym: WMV-2

Strains

Soybean mosaic virus (Frenkel *et al.*, 1989; Yu *et al.*, 1989;), indeed it is proposed that all are a single species and should be called soybean mosaic virus.

ICTV decimal code: 57.0.1.0.073

Host range and symptoms

First reported in *Citrullus lanatus*; by Webb *et al.* (1965).

Natural host range and symptoms

Symptoms persist.

- *Cucurbita pepo, Cucumis melo, C. sativus, Citrullus lanatus* and some legumes - mosaic, mottling, leaf shape malformation.

Transmission

Transmitted by a vector; an insect; *Myzus persicae, Aphis craccivora*; at least 29 species of aphids transmit watermelon mosaic 2 potyvirus (Edwardson and Christie, 1986); Aphididae. Virus transmitted by mechanical inoculation; not transmitted by seed.

Geographical distribution

Probably distributed worldwide.

Experimental host range

Many (>9) families susceptible.

Diagnostically susceptible host species and symptoms

- *Chenopodium amaranticolor* - chlorotic local lesions; not systemic.
- *Cucurbita pepo* - systemic mosaic and occasional leaf malformation.
- *Pisum sativum* cv. Alaska - necrotic local lesions; systemic mottling and necrosis.
- *Nicotiana benthamiana* - systemic mosaic.

Diagnostically insusceptible host species

Pisum sativum cv. Little Marvel, and *Nicotiana glutinosa*.

Maintenance and propagation hosts

Cucurbita pepo cvs Small Sugar, Zucchini, and *Nicotiana benthamiana*.

Assay hosts (Local lesions or Whole plants)

Abelmoschus esculentus, Chenopodium amaranticolor, and *C. quinoa, Lavatera trimestris* (L).

2.5.4.2 Physical and biochemical properties

Properties of particles in sap

TIP: 55-65 °C. LIV: 10-50 days. DEP: $\log_{10}$ minus 3-5. Leaf sap contains few virions.

Purification method

Purcifull *et al.* (1979).

Particle morphology

Virions filamentous; not enveloped; usually flexuous; of 730-765 nm. Axial canal obscure. Basic helix obscure.

Physical properties

One sedimenting component in purified preparations; sedimentation coefficient 150s. Density 1.32 g cm^{-3} in CsCl.

Biochemical properties

Virions contain 5 % nucleic acid; 95 % protein. Genome consists of RNA; single-stranded; linear; unipartite. Genomic nucleic acid isolated by Purcifull *et al.* (1984).

Cytopathology

Virions found in mesophyll and epidermis; in cytoplasm. Inclusions present in infected cells; are unusual in shape; scrolls and laminated aggregates (Edwardson's Type III inclusions) in the cytoplasm and thin plates, of unknown nature, in the nucleus; they do not contain virions and they contain virions (in cytoplasmic aggregates associated with membranes).

2.5.4.3 Taxonomy and relationships

Potyvirus: Potyviridae

Virus(es) with serologically related virions

Soybean mosaic and blackeye cowpea mosaic viruses, but distantly.

2.5.5 Cucumber green mottle mosaic virus (CGMMV)

2.5.5.1 Nomenclature

Synonyms

cucumber green mottle mosaic virus, tobacco mosaic virus watermelon strain - W, cucumber virus 3 (Ainsworth, 1935), cucumber virus 4 (Ainsworth, 1935; Hollings *et al.*, 1975), bottlegourd Indian mosaic virus, cucumis virus 2.

Acronym: CGMMV

ICTV decimal code: 71.0.1.0.002

Host range and symptoms

First reported in *Cucumis sativus*; from Great Britain; by Ainsworth (1935).

Natural host range and symptoms

Symptoms persist.

- *Cucumis sativus, Citrullus vulgaris, Lagenaria siceraria* (bottlegourd) - mosaic.

Transmission

Transmitted by means not involving a vector, or a vector; an insect; *Raphidopalpa fevicolis* (Rao and Varma, 1984); Coleoptera. Virus transmitted by mechanical inoculation.

Geographical distribution

Spreads in the Eurasian region; India, Japan, and the UK.

Experimental host range

Few (<3) families susceptible.

Diagnostically susceptible host species and symptoms

- *Cucumis sativus, Citrullus vulgaris* - systemic mosaic.

Diagnostically insusceptible host species

Datura stramonium, Petunia × hybrida.

Maintenance and propagation hosts

Cucumis sativus.

Assay hosts (Local lesions or Whole plants)

Chenopodium amaranticolor (L), and *Cucumis sativus* (W).

2.5.5.2 Physical and biochemical properties

Properties of particles in sap

TIP: 80-90 °C. LIV: more than 240 days (at 20ºC). DEP: $\log_{10}$ minus 6. Leaf sap contains many virions.

Purification method

Tung and Knight (1972); Francki and McLean (1968).

Particle morphology

Virions rod-shaped; not enveloped; usually straight; with a clear modal length; of *c* 300 nm; *c.* 15 nm wide. Axial canal obvious. Basic helix obvious.

Biochemical properties

Virions contain *c.* 5 % nucleic acid; *c.* 95 % protein. Genome consists of RNA; single-stranded; linear. Total genome size *c.* 6.5 kb. Genome unipartite; largest (or only) genome part 6.5 kb. Genomic nucleic acid isolated by Peden and Symons (1973). Base composition 23.2 % G (CGMMV-W, 25.8% (CV4), 25.5% (CV3)); 24.6 % A (CGMMV-W, 25.8% (CV4), 25.8% (CV3)); 20.6 % C (CGMMV-W, 19.3% (CV4), 18.3% (CV3)); 31.6 % U (CGMMV-W, 29.5% (CV4), 30.8% (CV3)). Infectivity decreased when deproteinised with proteases; retained when deproteinised with phenol or detergent. Poly A region absent. Additional factor not required for infectivity. Genome has *t*RNA-like activity. Genome accepts histidine. Nucleotide sequence references: Meshi *et al.* (1983).

Replication

Replication does not depend on a helper virus.

Cytopathology

Virions found in leaves, mesophyll, epidermis, vascular parenchyma, xylem, phloem, companion cells and all parts of the host plant; in cytoplasm and in cell vacuoles. Inclusions present in infected cells; are crystals in the cytoplasm; they contain virions. Other cellular changes: vesiculation of mitochondria.

2.5.5.3 Taxonomy and relationships

Tobamovirus

Virus(es) with serologically related virions

Frangipani mosaic, kyuri green mottle mosaic, ribgrass mosaic, tobacco mosaic, tomato mosaic and tobacco mild green mosaic viruses.

Virus(es) with serologically unrelated virions

Odontoglossum ringspot, sun-hemp mosaic viruses.

Additional comments on relationships

The host ranges of cucumber green mottle mosaic virus and kyuri green mottle mosaic virus are similar, and their coat proteins have greater amino acid sequence similarity than either has to those of other tobamoviruses, which are serologically more distantly related.