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Symptomatology and Physiological Changes

2.1 INTRODUCTION

Disease is a process of any malfunctioning of host cells and tissues resulting from continuous irritation by the infection of pathogenic agent or environmental factors which lead to the development of symptoms. An infection is nothing but an establishment of pathogen inside the host after entry into the cells and tissues. The symptoms are the observable and detectable effects of the pathogen on growth, development and metabolism of infected plant, and the external and internal alteration of plant or plant parts as a result of disease. The orderly study of symptoms in infected plant is called symptomatology. In many cases, a plant, which is although infected with virus but could not produce any symptoms, is called symptomless carrier.

Earlier in the plant virology, symptoms were considered the major concern, by which a virus disease was diagnosed and named. Mostly virus name is used to describe important symptoms in the major host from which the virus is first described. Still to date, the important symptoms they cause in the diseased plant are used to name the virus. Besides this, symptoms are still important criteria in the applied plant virology, where in the field condition it gives the first clue to identify the disease caused by the virus. For the farmers, symptoms are the most important aspects in virus infection in the crop, and nature and severity of disease symptoms. Symptoms also determine the economic importance of a particular virus in a particular area and region. Symptoms in infected plants are of different types. A large number of viruses and their strains occur in nature, and the reactions in the host due to their infection vary depending on the strain of the virus, age and growth conditions of the host plant, time of infection, the plant part which is infected by the virus, and primarily the environmental conditions, particularly temperature and light. The presence of more than one symptom in a diseased plant is called a syndrome. If a host plant is affected by more than one type of the virus or mixed infection, the effect may be greater than expected from their individual symptoms. The cumulative symptoms are referred to as synergism, or synergistic effect of the virus in the host plant.

Virus infection does not always cause disease symptoms in properly infected host, because of many reasons: (a) appearance of mild strain; (b) tolerant or resistant

2.2.2 Necrosis

Necrosis is the death of tissues in localized regions. This is a common symptom produced by the infection of virus in the plant. Necrotic tissues are generally demarked from the living tissues by a distinct dark brown border. Necrosis is the primary symptom of many viruses, like *tobacco necrosis virus*. It may be a secondary disease symptoms in mosaic or yellow disease groups, as the older infected tissues may breakdown. Necrosis may remain localized or spread to nearby tissues or remain superficial or move to deeper tissues. Different patterns of necrosis are observed in infected hosts due to infection by different viruses. Top necrosis means the death of apical meristem or young shoots, also called bud blight or bud necrosis (*groundnub bud necrosis virus*). Necrotic ring spot refers to the condition when necrosis occurs in the form of a ring. When the necrosis is superficial, diffuses and appears to be due to corrosion, the symptoms are called etching (*tobacco etch virus*). Many viruses are restricted to or travel in phloem and consequently cause phloem necrosis (*potato leaf roll virus*). This internal necrosis can occur in other tissues as well; it is generally visible to the naked eye as diffused brown or black streaking on the stem and petioles. In the extreme cases the entire stem can turn black. Stem-pitting (citrus tristeza virus) is the depression of stems of the infected plant, and it can be seen after splitting of the bark.

2.2.3 Malformation

Stunting and dwarfing are the most common symptoms in virus infected plants. In severe cases growth of the entire plant is reduced including leaves, internodes, etc. Sometimes on vein or midrib of the lower surface of leaves, large or small, one or more, papillate or spine-like structures are formed. This protruding malformed outgrowth is called enation (*pea enation mosaic virus*, cotton leaf cure begomovirus). Therefore, enation is virus-induced swelling of the plant tissue. The enation looks like leaves, wings, cups, funnels or boats and possesses the same anatomy like normal leaves. Tumors are large and irregular outgrowths of tissue formed by abnormal increase in number and size of cells, which can grow autonomously and may sometimes show a slight differentiation of tissues. Formation of tumors is not common in plant virus diseases. A few viruses, which are particularly phloem-limited (*Wound tumor virus*), may cause such symptoms.

2.2.4 Other Symptoms

Sometimes leaves of the virus infected plant show narrowing. In leaf narrowing the midribs and the main vein of the infected leaves remain almost normal, while the growth of laminar tissues is reduced, which gives rise to narrow leaves. In extreme cases, only the main veins remain normal, and remaining parts of the leaves are almost completely absent. This will form fern leaf (in case of multifoliate leaves of papaya) (*Papaya ring spot virus*) or shoestring in chilli (PVY). When the leaf surface of the virus infected plant becomes rugose (wrinkled), this is called rugosity. Stem grooving is a deformation of the normally smooth surface of a trunk caused by its furrowing (*apple stem grooving virus*). Stem pitting is plant disease symptoms characterized by the formation of large

or smaller depression in the old wood, between the phloem and xylem of the tree trunk (CTV).

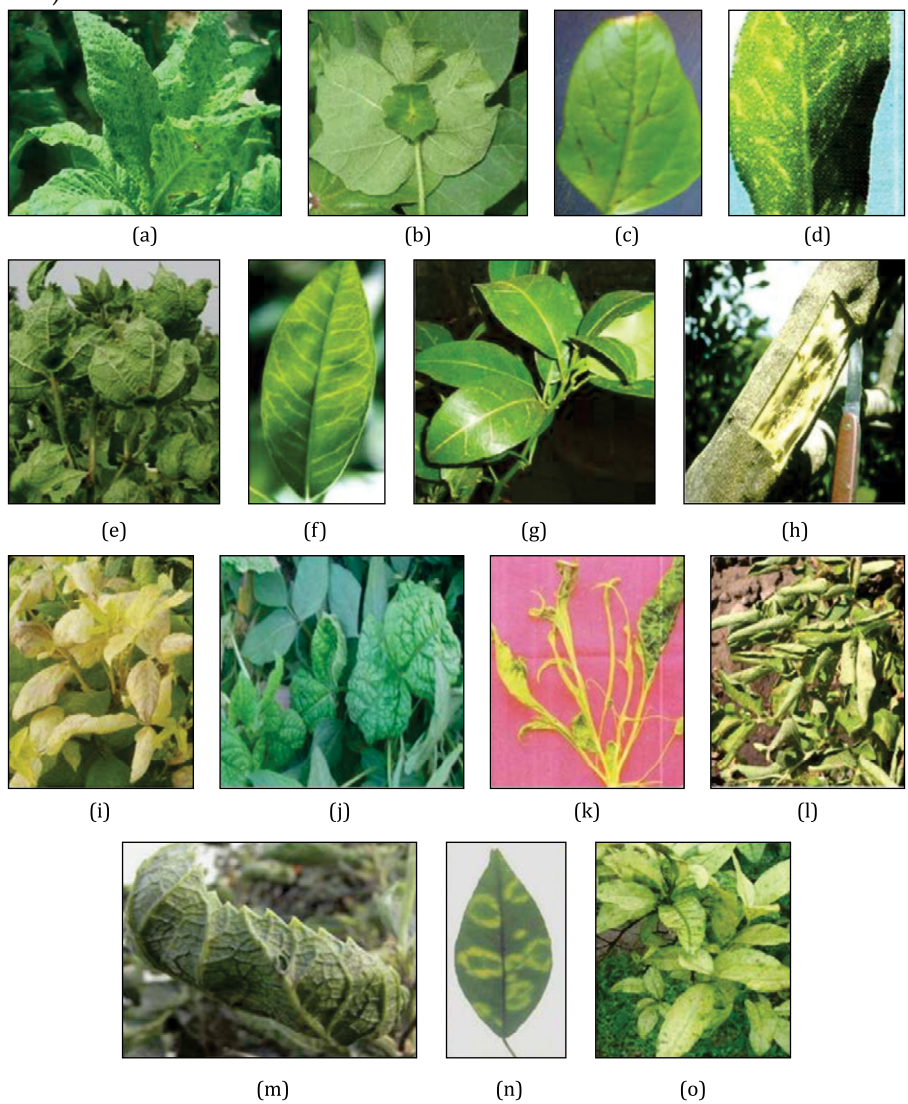

Figure 2.1 Symptoms in different host plants infected by different plant viruses: (a) mosaic caused by *Tobacco mosaic virus* in tobacco; (b) leaf enation caused by Cotton leaf curl begomovirs in cotton; (c) veinal necrosis caused by *Groundnut bud necrosis virus* in cowpea; (d) vein flecking caused by *Citrus tristeza virus* in Kagzilime; (e) upward leaf curl caused by Cotton leaf curl begomovirs in cotton; (f) veinal chlorosis in citrus caused by virus; (g) vein corking caused by *Citrus tristeza virus* in the Darjeeling mandarin; (h) stem pitting caused by *Citrus tristeza virus* in sweet orange; (i) leaf yellowing caused by *Mungbean yellow mosaic India virus* in mungbean; (j) leaf crinkle caused by Urdbean leaf crinkle virus disease complex in urdbean; (k) filiform leaf shoestring symptoms in papaya due to infection of *Papaya ring spot virus;* (l) leaf rolling symptom caused by *Potato leaf roll virus* in potato; (m) vein thickening caused by Cotton leaf curl begomovirus in cotton; (n) ring spot caused by *Indian ring spot virus* in Kinnow mandarin; and (o) chlorosis in mosambi sweet orange caused by *Citrus yellow mosaic virus*.

Leaf crinkling is an extreme form of rugosity and involves a more irregular and extensive wrinkling and furrowing of leaf lamina in urdbean and mungbean (Urdbean leaf crinkling disease complex). Leaf rolling is another common symptom in which leaves roll upward or downward over their entire length (*Potato leaf roll virus*), whereas in leaf curling, the leaves become curled upward or downward (Cotton leaf curl begomovirus). In curly top, the upper part or top of the plant becomes curled (*Beet curly top virus*).

Witches' broom is a special type of malformation in which a large number of slender branches arise from a localized area and run upward more or less parallel to each other, giving the appearance of the broom. This type of symptom appears because of the buds, which normally remain dormant but become activated and result in the development of branches. When an adult plant gets infected, witches' broom is localized at the point of newer growth. When the excessive branching and formation of witches' broom take place only at the top of the plant where newer growth occurs, these plants have bunchy top (*Banana bunchy top virus*). In some cases there is shortening of internodes of virus infected plant and leaves become clumped together at the apices of the branches; this symptom is known as rosette (*Groundnut rosette virus*).

In floral parts, virus infection causes many types of abnormalities like virescence, phyllody and proliferation. Greening or virescence of floral parts is caused by the development of excessive chloroplast in them. Phyllody is the disease syndrome which is caused by metamorphosis of floral parts into leafy or leaf-like structure. The typical symptoms of phyllody are found in *Sesamum* sp. The big bud symptom appears when the sepals become enlarged, as in virus infected tomato plant. Proliferation is the elongation and transformation of receptacle into sprout or sprouts which give rise to vegetative branches. These sprouts grow out together from a limited space and give rise to witches' broom appearance. Witches' broom in floral parts is the most common and diagnostic symptom of plant diseases caused by mycoplasma-like organisms.

Miscellaneous symptoms are also found in virus infected plant. Some of the plant viruses can induce wilting of the plant parts that result in ultimate death of the whole plant, e.g., in citrus caused by *Citrus tristeza virus*. Premature abscission of leaves such as leaf abscission, leaf drop, leaf fall, defoliation, etc., are common symptoms in many plants after virus infection. Sometimes, pollen is not formed; this is called pollen abortion; or pollen is formed, may be sterile; this is called pollen sterility. Male sterility, that is the absence of viable pollen, is regarded as a symptom caused by virus.

2.2.5 Latent Infection

Sometimes viruses may infect some hosts and multiples in them, but produces no visible symptoms. Such type of infection is called latent infection and this phenomenon is referred to as latency. The latent virus is quite frequent in wild plants or common agricultural weeds. The latent virus can be detected by back inoculation onto a susceptible host plant or by serological assay and nucleic acid-based detection such as PCR. Latent virus infection may be caused due to high level of host tolerance. The cryptoviruses generally cause no visible symptoms. These viruses occur in low concentration in the infected plants, but there is no indication that lower concentration of virus is the cause of symptomless infection.

2.2.6 Factors Influencing Symptom Expression

Several factors are responsible for disease development and severity of the symptoms in the plant infected by viruses.

Host factor: Genetic composition of the host may control the virus infection and replication virus. Some host cultivars may be susceptible to a particular virus or virus strain, while others may be resistant as they carry resistance gene which prevents the virus infection and modify the nature of symptoms produced. The age of the host plant at the time of infection may be the critical factor to determine symptom expression. Generally, the younger the plant, the more susceptible it is to virus infection. The very old leaves or old plants are relatively resistant to virus infection, because in the older plants or leaves, the transport of metabolism is slower than in younger plants or leaves. If the seedlings are infected at the cotyledonary stage, they are more susceptible. When *Mungbean yellow mosaic virus* is inoculated through whitefly at cotyledonary stage in mungbean and urdbean, the symptoms are more severe than when they are inoculated at later stage of plant growth. Similarly, PVY and Cotton leaf curl begomoviruses in potato and cotton, respectively, cause symptoms when younger plants are infected.

Environmental factors: Effect of environmental factors is crucial for virus infection that can greatly influence symptom expression. For some viruses, high temperature may reduce symptom production, for example, CMV in marrow *(Cucurbita pepo)*. High temperature influences the plant to resist virus infection, for example, *Bean common mosaic vius* (BCMV) causes systemic necrotic symptoms in bean (*Phaseshus vulgaris)* at above 30°C temperature.

Light: Light affects production of symptoms in virus infected plant. High light intensity produces hard plants which are less susceptible to virus infection than the plants grown under low light intensities. Therefore, to study virus infection, test plants or seedlings are kept under shade for one or two days prior to virus inoculation. High light intensities may also reduce virus symptoms, for example, CMV in marrow. In temperate regions, virus infected plants produce severe symptoms in greenhouse condition due to combined effect of high light intensity and high temperature.

Plant nutrition: In general, the nutritional conditions that favour plant growth also favour to increase susceptibility to virus infection.

2.3 INTERNAL SYMPTOMS

2.3.1 Histopathological Changes

There are various changes in the histology of virus affected plant parts, especially leaf petioles and stems. Such histological changes or abnormalities can be categorized in three main groups, necrosis, hyperplasia and hypoplasia.

Necrosis: It is the major feature of diseases and is confined to a particular organ and tissues. Necrosis is the death of the infected tissues of the host plant. The phloem cells are found to be killed in potato plant infected by PLRV and tobacco plant by TLCV. Top necrosis, i.e., death of chollenchyma in tobacco plants, is prominent due

to infection of TNV and TLCV. Necrosis of epidermal cells or midrib chollenchyma is caused by LMV in lettuce plant due to infection. Internal necrosis of immature tomato fruits by late infection of TMV is common. Phloem necrosis may be the diagnostic and typical symptom of phloem-limited viruses under the genera *Geminivirus*, *Luteovirus* and *Reovirus*.

Hyperplasia: A malfunction is caused by excessive growth and increased differentiation of cells due to infection of some viruses. The abnormal increase of organ size is called hypertrophy. Tumor and enation in the infected plant are due to abnormal growth of the cambial cells. In vein clearing, the enlargement of cells near the vein and tissue become abnormally translucent due to the presence of little chlorophyll. Due to abnormal division of cambial cells of shoot in cacao plant infected by CSSV causes cacao swollen shoot symptoms. Crimson clover shared abnormal development of phloem cambium cells due to infection of WTV. Development of abnormal sieve elements was found in BCTV infected beet plant.

Hypoplasia: A malformation is caused by reduced growth and decreased differentiation of cells due to infection of some viruses. The abnormal reduction in organ size is called atrophy. A localized retarded growth caused thinner lamina which is associated with mosaic symptoms. Reduction in the number of chloroplasts is the effect of less differentiation of mesophyll cells, where fewer and no intercellular space is observed in yellow sector of mosaic pattern. The pilling or grooving of stem of citrus is due to lack of cambial cell differentiation. Due to infection of stem grooving virus, the cambial cells in the region of groove disappear.

2.3.2 Cytopathological Changes

Various changes have been observed in relation to the alteration in morphology of organelles and formation of inclusion bodies. Abnormal changes occur in nuclei, mitochondria, chloroplast and cell wall. Various virus induced structures are observed in cytoplasm due to infection of some viruses. Accumulation of virus particles, aggregation of virus encoded protein and formation of cytological structures are common in virus infected cells of the host plant.

In mosaic infected leaf, the size and number of starch grains are induced. In sugar beet and potato infected with BCTV and PLRV, accumulation of starch is observed. Cytoplasmic materials are aggregated in *N. glutinosa* infected with TMV. Drastic changes in nucleus have been observed in the plant infected with a specific group of whitefly transmitted geminiviruses, which is an ultrastructural feature of viruses under this group. The nucleoli in tomato infected with TYLCV contain one or more intensely electron opaque ring-shaped structure varying in size from 0.4-3.0 μm. The development of inclusions/inclusion bodies in the virus affected cells is the major cytopathological effect that is found either in the cytoplasm or in the nucleus.

Virus induced inclusions or inclusion body (IB): The virus induced structure that occurs in the cytoplasms or nuclei of infected plant cell is called inclusion body. Inclusion bodies are amorphous, granular, and crystalline, and they can be readily

however, they are responsible for causing a variety of symptoms in the host they infect. Presence of large quantities of virus particles is not always enough to cause disease symptoms. Some plants containing much higher concentration of virus than the other may produce milder symptoms than the latter, or may even not produce any symptoms and infected host remains symptomless. Virus primarily causes depletion of nutrients by diverting the nutrients toward synthesis of the virus. Other most indirect effects of the virus are metabolic changes in the infected host. The metabolic changes are production of virus induced synthesis of new proteins by the host, and biologically active substances like enzyme, which may interfere with the normal metabolism of the host. Physiological changes in the virus infected plants are due to imbalance in all the major metabolic and physiological activities such as respiration, photosynthesis and carbohydrate metabolism, organic acid and nitrogen metabolism.

2.4.1 Photosynthesis

The virus infection usually affects the process of photosynthesis. Decreased photosynthesis has, in some cases, been directly correlated with increased disease severity and increased virus multiplication. However, photosynthetic rate may slightly increase during the earliest stages of infection. A maximum decrease of about 50% in net photosynthesis of leaf canopy took place in ryegrass infected with *Ryegrass mosaic virus* (RGMV). Tops of BYDV infected susceptible wheat cultivar had 50% less of total photosynthesis than that of the healthy tops; half of this reduction was due to stunting and the other half due to reduced photosynthesis.

Reduction in carbon fixation is the most common effect in virus infected leaves showing mosaic or yellowing. This reduction usually becomes detectable within a few days after virus infection. Any reduction in photosynthesis mostly arises from a variety of biochemical and physiological changes. Due to effect of infection by TMV and CymMV in orchid, crassulacean acid activity metabolism (CAM) is significantly reduced and ultrastructural changes occur in chloroplasts. Photosynthetic activity can be reduced by changes in chloroplast structures.

In TYMV infected Chinese cabbage leaves, the hill reaction, and cyclic and non-cyclic photophosphorylation are increased compared to healthy leaves during the period of active virus multiplication. However, at a later stage of infection, the photosynthetic activity was lower than the control plant. In young infected Chinese cabbage leaves, during rapid virus multiplication, there is rapid diversion of the products of photosynthetic carbon fixation away from production of sugars, and shifting that product for synthesis of organic acids and amino acids. This metabolic pathway returns to the normal pattern when virus replication process is reduced or stopped.

Photosynthesis activity is reduced due to decreased amount of photosynthetic pigment. In *Pigeon pea sterility mosaic virus* (PPSMV) infected pigeon pea showing most conspicuous symptom, the mosaic mottling of leaves, the chlorophyll content is reduced and chlorophyllase activity is increased. The chlorophyllase activity is influenced by the age of the diseased hosts. In the diseased leaves, striking increase in chlorophyllase activity occurs when the disease is maximum in progress. It was observed that the

observed, probably indicating a similar increase in mitochondrial numbers.

2.4.3 Nucleic Acids and Amino Acids

Virus infection may have some effect on host cell nucleic acid synthesis, but such effects are likely to be fairly small and difficult to establish. Viruses may affect the transcription process in infected cells of plant, by changing composition, structure and function of the chromatin associated with the cell DNA. Activity of ribonucleases that break down RNA may increase. Several plants resistant to viral diseases may contain higher levels of RNA than the healthy plant, therefore, it is believed that increased transcription takes place, which indicates increased synthesis of substances involved in the defense mechanism of plant cells.

Amino acids, mostly alanine, asparagine, aspartic acid, glutamic acid, glutamine, lysine and serine are most commonly found to increase in the free amino acid pool of the infected plants. There are two possible explanations for increase in concentration of these amino acids in infected leaves. First, amino acids required for viral protein synthesis are provided by the host cells. Any particular amino acid required for virus synthesis is present in limited amounts in the normal amino acid pool of the host. Thus it may act as a limiting factor in virus infection. Therefore, greater amount of this limited amino acid must be available for enhanced and continued virus production for the biosynthesis pathway for greater amount stimulated by virus in the host plant. Therefore, amino acids in excess of the viral requirements are accumulated in the virus infected leaves. The second hypothesis is based on the biosynthesis mechanism of amino acids. The biosynthesis of amino acids is correlated with the TCA cycle. In virus infected tissues, increased activity of TCA cycle may cause enhanced amino acids synthesis by amination. Amination of keto glutamic acid results in the formation of glutamic acid, which is a precursor of many amino acids. While the changes in free amino acids in virus infected plants have been reported.

2.4.4 Low Molecular Weight Compound

In various parts of virus infected plants, there is certain effect on the concentration of low molecular weight compounds. The amides like glutamine, asparagines, and amino acids like pipcolic acid are reported to occur in relatively higher concentration in virus infected tissues. Soluble nitrogen compounds show general deficiency during rapid multiplication of virus-infected tissues. Phosphorus content may be affected in the virus infected plants; there is no clear picture for the affect of infection on host phosphorus metabolism.

In virus infected plant, the resulting yellow mosaic or mottling of leaves is involved, which is due to reduction in leaf pigments. Loss of chlorophylls is a general effect in virus infected plant, but not all viruses cause loss of chlorophyll (turnip infected by CaMV). The plant pigments carotene and xanthophylls are also found to decrease in plants infected by several plant viruses.

Changes in the content of chloroplast pigment are secondary. The reduction of

which showed stunting. Stunting of infected plants in many virus infected plants has been found to be correlated with reduced amount of endogenous gibberellins. It was also found that in all these cases, GA3 application can result in reversion of stunting.

Abscisic acid: Abscisic acid is a natural growth hormone that occurs in retardant and counteracts the plant growth induced by growth promoting hormones, auxins, cytokinin and gibberellins. Abscisic acid keeps balance in growth response of the plants at a particular time in association with growth promoters. There is a marked increase in indigenous abscisic acid content in many virus infected plants, such as CMV infected cucumber, and rice tungro infected rice. In contrast, reduced levels of abscisic acid occur in TMV infected tobacco. Retardation of hypocotyls growth in CMV infected cucumber seedlings is caused due to an increased endogenous abscisic acid content.

Ethylene: Virus infected plants produce increased amount of ethylene. Virus induced necrosis and chlorotic local lesion can stimulate ethylene production. BCTV infected bean, sugar beet and tomato, CPMV infected cowpea and cucumber seedlings and TMV infected hypersensitive tobacco, showed higher amount of ethylene. Epinasty and leaf fall in PVY infected *Physalis floridana* may be due to production of ethylene that causes tissue necrosis. Ethylene has been suggested to play an important role during the formation of necrotic local lesions or in localization of a virus to these necrotic lesions. Enhanced ethylene production has also been associated with the formation of chlorotic lesions by chlorophyll destruction in virus infected cucumber cotyledon.

Growth inhibition in CPMV infected cowpea seedlings has been suggested to be caused due to an interference with auxin transport and auxin metabolism with combined action of ethylene and peroxidase acting as IAA oxidase.

2.4.6 Carbohydrates

Virus infected plants mostly show an effect on carbohydrate metabolism and translocation in the infected leaves, while some viruses can alter little in the carbohydrate synthesis and translocation. Effect of virus infection on mesophyll cells may reduce translocation of carbohydrates out of the leaves. Starch accumulation in the infected leaves is common in virus induced curled leaves. Mosaic and yellowing, for example of *Bean yellows virus* (BYV) in sugar beet, are mainly caused due to degradation of phloem, resulting in inhibition of translocation of photoassimilates. The BYV induces damage to the phloem that results in destruction of translocation of photoassimilates. Although it is not generalized, but a rise in glucose, fructose and sucrose in virus infected leaves is commonly found, a greater rise in these sugars is caused by mild virus strains compared with severe strains.

Curling, mosaic and yellowing type viral diseases cause reduction of total carbohydrates in infected plants. Such types of symptoms are caused due to degradation of phloem resulting in destruction of translocation of photoassimilates. Total carbohydrates including soluble (sugar) and insoluble (starch) may increase or decrease in virus infected tissues. Many systemic viruses cause little or massive accumulation of carbohydrates, particularly as starch, in infected leaves. This is true in case of BYDV