Chapter - 34
Chilli Leaf Curl Viral Disease and their Management

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Introduction

Chilli belongs to the family of solanaceae and is one of the important widely cultivated crops grown for the value of its green and ripe fruits. India is rich in maximum diversity of chilli varieties with differing habit, size, shape, colour and pungency of fruit. Besides, traditional use of chilli as vegetables, spices, condiments, souces and pickles, it is also used in pharmaceuticals, casmatics and beverages (Tiwari *et al.,* 2005).

Pungency in chilli is produced by the capsaicinoids, alkaloid compounds, that are found only in the plant genus, *Capsicum*. The nature of the pungency has been established as a mixture of seven homologous branded-chain alkyl vanillylamides (Hoffman *et al*. 1983). They often are called capsaicin after the most prevalent one. Dihydrocapsaicin is usually the second most prevalent capsaicinoid, while the other five compounds, norcapsaicin, nordihydrocapsaicin, nornordihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, are considered minor capsaicinoids because of their relative low abundance in most natural products. Capsaicin is a powerful and stable alkaloid that can be detected by human taste buds in solutions of ten parts per million. Capsaicin's composition ($C_{18}H_{27}NO_3$) is similar to peperin ($C_{17}H_{19}NO_3$) that gives black pepper its bite.

In the chilli found following mineral, carbohydrates, vitamins *etc.,* in dry fresh fruits by Kosuge *et al.,* (1961).

Peppers, Hot Chili, Red, Raw	
Nutritional value per 100 g	
Energy	8.8 g
Carbohydrates	166 kJ (40 kcal)
Sugars	5.3 g
Dietary fiber	1.5 g
Fat	0.4 g
Protein	1.9 g

Vitamins	
Vitamin A equiv. beta-carotene	48 µg (6%) 534 µg (5%)
Vitamin B6	0.51 mg (39%)
Vitamin C	144 mg (173%)
Minerals	
Iron	1 mg (8%)
Magnesium	23 mg (6%)
Potassium	322 mg (7%)
Other constituents	
Water	88 g
Capsaicin	0.01g – 6 g

Viral diseases of plants cause economic losses to the tune of 15 billion dollars per annum on golobal basis (Van Fanbing, 1999), particularly in tropical and semitropical regions. These provide ideal conditions for the perpetuation and transmissions of viruses through vectors. Natural occurrence of several viruses have been reported on chilli (*Capsicum annuum* L.) crop by various workers (Martehi and Quacquarelli, 1983) and among them leaf curl virus has been reported as most destructive affecting chilli, tomato, potato, okra, cotton, cucumber cultivation in terms of incidence and yield losses (Green, 1992). Leaf curl virus is a member of genus *Begomovirus*, family geminiviridae. The genome of *Begomoviruses* have a single or double stranded circular DNA of c. 2.7kb, which is encapsulated in a quasi-isometric geminate particles of C.20-30nm (Harrison, 1985). During the past two decades, agricultural intensification has resulted in *Begomoviruses* disease out breaks in tropical and subtropical regions, causing 80 per cent yield losses of many crops (Morles and Anderson, 2011). The leaf curl disease was first reported from India on tomato by Vasudeva and Samraj (1948).

Botany of chilli crop

The genus *Capsicum* consists of approximately 22 wild species and five domesticated species: *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens* (Bosland 1994). It is a perennial small shrub in suitable climatic conditions, living for a decade or more in tropical South and Central America. *Capsicum* probably evolved from an ancestral form in the Bolivia/Peru area (Heiser 1976). Chillli fruits are considered vegetables, but are berries botanically. Chilli types usually are classified by fruit characteristics, *i.e.* pungency, color, shape, flavor, size, and their use (Bosland 1992). Despite their vast trait differences most chile cultivars commercially cultivated in the world belong to the species, *C. annuum*.

Origin of chilli crop

Capsicum has been known since the beginning of civilization in the Western Hemisphere. It has been a part of the human diet since about 7500 BC (MacNeish 1964). It was the ancient ancestors of the native peoples who took the wild chile piquin and selected for the many various types known today. Heiser (1976) states that apparently between 5200 and 3400 BC, the Native Americans were growing chilli plants. This places chillis among the oldest cultivated crops of the Americas. The wild *Capsicum annuum* var. *aviculare* is harvested and sold in the marketplace alongside the larger-fruited domesticated chillis. *Capsicum* was domesticated at least five times by prehistoric peoples in different parts of South and Middle America. The five domesticated species are *C. annuum* L., *C. baccatum* L., *C. chinense* Jacq., *C. frutescens* L., and *C. pubescens* R. & P. (IBPGR 1983).

Diseases of chilli crop

Chilli crop suffers from a number of fungal (Fusarium wilt, Damping off, Fruit rot and Anthracnose, Powdery mildew, Cercospora leaf spot "frog eye" *etc.*,), bacterial (Bacterial soft rot, Bacterial wilt, and Leaf spot *etc.*,) and nematode (Root knot, Sting nematode *etc.*,) diseases and viral diseases one of them. Viruses are known to cause different symptoms like mosaic, ring spot, curling, yellowing *etc.*, on chilli and these symptoms result in heavy losses.

Symptomology

The symptoms of ChiLCVD consist of abaxial and adaxial curling of the leaves accompanied by puckering and blistering ofinterveinous areas and thickening and swelling of the veins. In advanced stages of the disease, axillary buds were stimulated to produce clusters of leaves which were reduced in size. The whole plant assumed a bushy appearance and stunted growth. Fewer flowers and fruits were developed on the diseased plants, and those that were formed were much reduced in size and curled at the styler end (Mishra *et al*. 1963). Two distinct types of symptoms were observed during a survey of *C. annuum* at IARI (Dhanraj *et al*. 1968). Symptoms in the first type were similar to those earlier reported (Mishra *et al*. 1963). In another type of symptoms, the affected plants became erect and bushy and the leaves were dark green. The leaf tips were curled downwards; the shape of the leaves became oval to round with no upward rolling of the edges as seen in the first type, but ccompanied by severe puckering and leatheriness of the leaves. On closer examination, it was observed that there was pronounced vein thickening and leafy outgrowths or enations on the undersurface of the leaves. The diseased plants developed fewer flowers and fruits. 'Churda Murda' or

malformation disease in chilli was identified (chilli leaf curl disease) in Vidarbha (Maharashtra, India) and visualized three types of symptoms, i.e. upward curling and crinkling, downward curling and mottling, crinkling and puckering (Moghe 1977). Upward curling, puckering and reduced size of leaves were also observed (Senanayake *et al*. 2007). Typical upward leaf curling, crinkling, puckering and reduction in leaf area along with stunting of whole plants were observed (Kumar *et al*. 2012).

Leaf curl disease is characterized by severe stunting of the plants with downward rolling and crinkling of the leaves. The newly emerging leaves exhibit slight yellow colouration and later they also show curling symptoms. Older leaves become leathery and brittle. The nodes and internodes are significantly reduced in size. The infected plants look pale and produce more lateral branches giving a bushy appearance (Capoor, 1967). The infected plants remain stunted (Hussain, 1932). Leaf malformation was reported by Fernando and Pieries (1967). Curling of leaf margin, reduction in leaf size, vein clearing were observed in India, Sri Lanka and USA (Puttarudraih, 1959).Abaxial curling of the leaves accompanied by puckering, thickening and swelling of the veins were observed by Mishra *et al.* (1963) and Muniyappa and Veeresh (1984). The natural occurrence of tobacco leaf curl was observed on chilli in India by Pal and Tandon (1937) and Seth and Dhanraj (1972).

History and distribution

In India, ChiLCVD is a major disease of chilli (Chattopadhyay *et al*. 2008). When plant infected in early stage the severity percentage increase of the chilli plants under field conditions. The natural occurrence of chilli leaf curl was first reported to be caused by TLCV (Mishra *et al*. 1963). The occurrence of another type of leaf curl symptoms which produces leaf enations in chilli leaves was also reported. The virus transmitted by whitefly (Bemisia tabaci) (Varma 1962). At that time, begomoviruses were not known, but since 2003, several begomovirus species were reported to cause leaf curl disease of chilli in India (Senanayake *et al*. 2007; Senanayake *et al*. 2012). So far in India and Pakistan, at least four begomovirus species are known to be consociated with leaf curl disease: ToLCNDV, ChiLCV, Cotton leaf curl Multan virus (CLCuMV) and ToLCJV (Hussain *et al*. 2003; Shih *et al*. 2003; Hussain *et al*. 2004; Khan *et al*. 2006). The geographical distribution of the begomoviruses is analogous to occurrence of whitefly in the world (Brown *et al*. 1995). It covers almost entire equatorial regions of the Americas, Africa, Europe, Asia and Australia. It has also been reported from other countries, like the USA, Nigeria, Pakistan, Bangladesh and Indonesia.

Vector and host range

ChiLCV is transmitted by the insect vector *Bemisia tabaci* in a persistent-circulative nonpropagative manner. Transmission of the monopartite genome alone can lead to infection, but the presence of DNA A or DNA B helper viruses plays a large role in symptom development. Association between plant viruses and their insect vectors is very much complicated. Whiteflies are small piercing and sucking insects of the family Aleyrodidae, order Hemiptera, which have been consociated with agriculture and with the transmission of plant viruses for many years

Whitefly *B. tabaci* was first described in (Greece, 1889) as a pest of tobacco known as tobacco whitefly (*Aleyrodes tabaci*) (Gennadius 1889). In 1897, whitefly was known as sweet potato whitefly (*B. inconspicua*) as it was first reported on sweet potato in the New World in the USA (Brown *et al*. 1995. It is a polyphagous pest and is noted on more than 600 plant species spreading more than 60 plant viruses (Rishi 2004). The non-viral aetiology of leaf curl disease complex was experimentally proved, and it was concluded that it was the result of damage caused by thrips (upward curl) and mites (downward curl) (Amin 1979). On the basis of host range (*C. annuum, C. frutescens, C. microcarpum, Solanum lycopersicum* and *Nicotiana tabacum*) and transmission, it was reported that the ChiLCVD has been caused by the agent of tobacco leaf curl (Muniyappa and Veeresh 1984). Whitefly was able to transmit ChiLCV from field samples to 50–100% of chilli test plants, which produced typical disease symptoms (Senanayake *et al*. 2007). ChiLCV was also reported to infect species like Petunia hybrida, Amaranthus spp, Mentha spicata and Mirabilis jalapa (Jaidi *et al*. 2017). In an epidemic of chilli leaf curl disease at Jodhpur (Rajasthan), it was concluded that several isolates were effectually transmitted by whitefly, all of which produced severe leaf curl symptomsin chilli (Senanayake *et al*. 2012). A single whitefly was able to transmit the virus, and eight or more whiteflies per plant resulted in 100% transmission. The minimum acquisition access period (AAP) and inoculation access period (IAP) were 180 and 60 min, respectively. The virus was only able to infect five species, *Viz, C. annuum, Carica papaya, S. lycopersicum, N. tabacum* and *N. benthamiana*, out of the 25 species tested from various families, *Viz*, Asteraceae, Caricaceae, Cucurbitaceae, Euphorbiaceae, Leguminosae, Malvaceae and Solanaceae (Senanayake *et al*. 2012). Infection of ChiLCV and Tomato leaf curl betasatellite on watermelon was reported first time by Shahid and Al-Sadi (2017) at Oman.

S. No.	Methods of transmission/ vectors	Total number of inoculated plants	Diseased plants	Healthy plants	Per cent transmission
1.	Mechanical	10	10	0	100.00
2	Seed	10	0	10	0.00
3.	*Bemisia tabaci*	10	10	0	100.00

Role of betasatellites in symptom development

A large number of monopartite begomoviruses are linked with a newly recognized class of ssDNA satellites termed as beta satellites. Satellites are the viruses or nucleic acids that depend upon a helper virus for their replication, but lack extensive nucleotide sequence homology to the helper virus and are not essential for its multiplication (Mayo *et al*. 2005). The size of betasatellite molecules is approximately 1350 bp which is nearly half the size of helper begomoviruses (Briddon *et al*. 2003). Betasatellites have a huge role in inducing symptoms, determination of host range and overcoming host defences (Briddon and Stanley 2006). The symptoms induced by the helper viruses are affected by the presence of satellite RNAs. Satellite RNAs can have an impressive effect on the symptom development, which varies from symptom amelioration to an increase in symptom severity (Roossinck *et al*. 1992). In a study, the authors suggested that the symptoms induced by the virus were enhanced in the presence of the

Beta satellite, even though viral DNA levels in host plants were not affected (Khan *et al*. 2013), whereas high levels of viral DNA were detected from chilli plants which were inoculated with begomoviruses and cognate beta satellites (Kumar *et al*. 2015). Plants inoculated with beta satellites may also show an increase in helper viral DNA A and DNA B levels. It was reported that the DNA B and beta satellites acted contrary to each other, such that the presence of beta satellites leads to 16 times higher amount of DNA B, while accumulation of beta satellites, Cotton leaf curl Multan betasatellite (CLCuMuB) and Luffa leaf distortion beta satellite (LuLDB), was reduced by 60% in the presence of DNA B (Jyothsna *et al*. 2013).

Classification and structure of pathogen

The aetiology of chilli leaf curl disease found in tropical and subtropical areas had proved to be elusive till 1977 when circular single-stranded DNA (ssDNA) containing geminiviruses were reported (Harrison *et al*. 1977). On the basis of the geminate morphology of the virus particles and circular ssDNA as their genomic component, geminiviruses were perceived as a separate group by International Committee on Taxonomy of Viruses (ICTV)

in 1978 (Rishi 2004). The genus begomovirus (Geminiviridae) with 288 species is the largest genus of all viral taxonomy currently recognized by the ICTV (Brown *et al*. 2015). The standards for demarcation of species of the members of family Geminiviridae were revised by ICTV and on the basis of genome organization, distinguished insect vector and host range instead of three, there are four genera distinguished (Mastrevirus, Curtovirus, Begomovirus and Topocuvirus) (Fauquet *et al*. 2003). Recently on the basis of genome organization, nucleotide sequence similarities and biological properties, the geminiviruses are now classified into nine genera—Becurtovirus, Begomovirus, Capulavirus, Eragrovirus, Grablovirus, Mastrevirus, Curtovirus, Topocuvirus and Turncurtovirus. Begomoviruses are described by twin icosahedral particles, approximately 18 9 30 nm in size (Stanley *et al*. 2005). The genomes of begomoviruses commonly contain one or two circular, single-stranded DNA components of 2.5–3 kb in size, known as DNA A and DNA B (Mayo and Pringle 1998) The two components of bipartite begomoviruses share a highly conserved common region (CR), approximately 200 nucleotides (nt) in length which consists of reiterative sequence-specific replicase (Rep) binding motifs called iterons and a nonanucleotide stem-loop structure (TAATATTAC), required for replication of the viral genome (Fauquet and Stanley 2003). Most of the monopartite begomoviruses are consociated with a satellite molecule of approximately 1.4 kb, known as the betasatellite, which is necessary for symptom appearance but depends fully upon the helper virus for its replication, encapsidation and cell-to-cell movement (Mansoor *et al*. 2003; Briddon and Stanley 2006). For the first time, it was substantiated that chilli leaf curl disease (ChiLCD) was caused by a complex consisting of the monopartite, ChiLCV and a betasatellite, Tomato leaf curl Bangladesh betasatellite (ToLCBDB) (Chattopadhyay *et al*. 2008). Later, both bipartite and monopartite begomovirus with alpha and beta satellites have been reported to be associated with ChiLCVD (George *et al*. 2014).

Virus replication

Begomoviruses associated with ChiLCVD, like all other members of Geminiviridae, replicates via using a combination of a rolling circle mechanism and recombination mediated replication, which takes place in the nuclei of infected cells (Jeske *et al*. 2001). There is a resemblance in the mechanism by which mammalian DNA tumour viruses activate the host genes required for DNA replication and the ssDNA phages such as / X174 replicates. This type of replication gives rise to multiple copies of long continuous double-stranded (ds) DNA intermediate, the replicative form (RF), which later

changes into genome-sized circular DNA fragments. The proteins required for replication initiation and for recruitment of the host replication machinery are formed from the transcription of dsDNA intermediates. The virus encodes two proteins which are imperative for effective virus replication, i.e. C1 (Rep.) and C3. C1 protein serves as the launching factor and occurs midway the origin detection and DNA cleavage/ligation to begin and to end up the rolling circle replication process. The C3 protein alters the C1 activity and helps in enrolment of host replication enzymes which thus eases the accretion of high levels of viral DNA. Both replication and transcription of the virus occur in the nucleus of the plant cell, so the import of the viral DNA and/or virions into and out of the host plant cell nucleus is necessary for efficient completion of the virus's life cycle. Therefore, in and out movement of the viral genome in the nucleus, as well as from cell to cell and in the whole plant, is very important for viral infection.

Estimation of histochemical changes in chilli leaf curl virus (ChiLCV) infected plants (*Viz*., Chlorophyll, Phenol, Tannin and protein)

Investigations were made on histochemical localization of metabolites *Viz*. starch, lipid, protein, carbohydrates and enzymes in diseased and normal leaf of chilli. Tannins, starch, polyphenol oxidase and peroxidase were observed in enhanced quantity in diseased leaf as compared to healthy leaf. Relatively higher amount of localization of metabolites and activity of enzymes detected in the leaves suggested altered metabolism of the host tissue due to pathogenesis.

The infected plant showed a rapid increase in the quantity of total leaf proteins in mosaic infected plant as compared with healthy leaves. Further the percentage of total leaf protein was decreased.

Management

1. **Cultural practices:** Rouging infected plants and destroying infected fields is also used when necessary.
2. **Resistance breeding:** Three symptoms-less resistant sources, *Viz*, GKC-29, BS-35 and EC-497636, showed no symptoms after challenged by grafting and alternate grafting and were also confirmed by using PCR (Kumar *et al*. 2006). Six genotypes of C. annuum, *Viz*., Punjab Lal, CM-334, CV-2, CV- 1, Kalyanpur Chanchal, VR-339, showed the highly resistant reaction of which Punjab Lal and CV-2 were virtually symptom-less (Kumar *et al*. 2009). Other varieties *Viz*., DLS-Sel-10, WBC-Sel-5, Jowla, and PBC-142
3. **Genetically engineered resistance:** Agrobacterium-mediated

transformation of a pure line variety of hot pepper, RCL 59 M. Other strategies include producing resistant varieties through a transgenic, RNAi-mediated approach.

4. **Physical and chemical measures of control:** The pest is polyphagous in nature, and therefore the cultivated and weed host are the major sources of infestation. A number of synthetic and natural insecticides had been successfully used to control this pest in the past, but it is able to develop resistance at a very rapid rate. Several insect growth regulators and new pyrethroid insecticides may appear promising to manage this pest.
5. **Botanical pesticides:** 2-3 foliar sprays of *Clerodendrum aculeatum* (leaf extract), *Terminalia arjuna* (bark extract), Seed extract of *Sapindus trifoliatus* and *Solanum trilobat* etc.
6. **Chemical pesticides:** Imidacloprid 17.8 SL (0.003%) was most effective than spinosad 48 EC (0.02%), malathion 50 EC (0.05%), acephate 75 SP (0.1%) and methyl-demeton 25EC (0.025%).

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