The Current Status of Luteovirus and Polerovirus Research in India

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Abstract

The family *Luteoviridae* comprises of three genera *Luteovirus*, *Enamovirus* and *Polerovirus,* of which, only two genera *Luteovirus* and *Polerovirus* are known in India. Though luteovirus and polerovirus infects many crops throughout the world, only a few have been documented in India. Luteoviruses were reported in field crops like barley (barley yellow dwarf virus) and chickpea (chickpea stunt virus), while polerovirus was recorded from potato, jute (potato leaf roll virus [PLRV]) sugarcane (sugarcane yellow leaf virus [SCYLV]) and cotton (cotton leaf roll dwarf virus) crops from India. SCYLV and PLRV are the most important poleroviruses as they are of serious constraints in all the sugarcane and potato growing states of India. SCYLV infection causes 39–43% reductions in plant growth and 30–34% loss in yield in sugarcane. The virus is mainly transmitted through infected seed canes. The secondary spread of the virus in the field is by the aphid vectors. PLRV is reported to cause 50–60% yield losses in potato crop and this virus is tuber borne and transmitted mainly by aphid vectors in a circulative non-propagative manner. The complete genome sequences SCYLV and PLRV isolates from sugarcane and potato are available from India. This book chapter deals with an uptodate information available on distribution, biological properties, identification, serological relationships, genetic diversity and

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transmission of the luteoviruses and poleroviruses reported on different crops from India.

Keywords
Luteovirus • *Polerovirus* • *Sugarcane yellow leaf virus* • *Potato leaf roll virus*

12.1 Introduction

Luteoviridae is a family of plant viruses having common biological properties of persistent transmission by aphid vectors and the induction of leaf rolling, reddening or yellowing symptoms in infected host plants. "Luteo" comes from the Latin luteus, which translates as yellowish. Members of the *Luteoviridae* cause economically important diseases in many food crops, including grains such as wheat and barley, vegetables such as potatoes and lettuce, and other crops such as legumes, sugarcane and sugarbeets. All members of the *Luteoviridae* consist of small (ca. 25–28 nm diameter) icosahedral particles, composed of one major and one minor protein component and a single molecule of messenger sense single-stranded RNA. The family is divided into three genera: *Luteovirus, Polerovirus* and *Enamovirus*. Viruses in *Luteoviridae* are non-enveloped, with icosahedral and spherical geometries of diameter around 25–30 nm. Genomes are linear and non-segmented, around 5.3–5.7 kb in length. Luteoviruses can act as helper viruses for Umbraviruses, providing them with a coat protein (Smith and Barker 1999).

Luteovirus, which resides in phloem tissues of the host plants thereby, inhibits nutrient translocation in the sieve elements. It is a monopartite virus with positive sense single stranded ribonucleic acid (+ssRNA) genome varying in size from 5.6 to 6.0 kb. The geographical distribution of luteoviruses are widespread, with the virus primarily infecting plants via transmission by aphid vectors. The virus only replicates within the host cell and not within the vector .The genus *Enamovirus* (from the type species, *Pea enation mosaic virus 1*) is one of three genera in the family *Luteoviridae* and currently has only one member. Until recently it was considered as an irreversible mixed infection of two autonomously replicating RNAs, but the current taxonomy retains only the old RNA1 (which has some similarity to members of the genus *Polerovirus*) as an enamovirus and places the old RNA2 as a different virus (*Pea enation mosaic virus* 2) in the genus *Umbravirus*. The virions are isometric (polyhedral), not enveloped of two sizes, 25 and 28 nm in diameter. The genome is monopartite, linear, single stranded, positive sense RNA about 5700 nt long. The 3′-terminus has neither a poly(A) tract nor a tRNA-like structure, and the 5′- terminus has a genome-linked protein. The genome (5706 nucleotides) has many similarities to members of the genus *Polerovirus* but lacks the functions for systemic movement and mechanical transmission, which are supplied by pea enation mosaic virus-2. There are currently 17 species in this genus including the

type species *Potato leafroll virus* (Smith and Barker 1999), which is one of the most prevalent viral diseases of potato in India (Mukherjee et al. 2003). The virus is tuber borne, not sap transmitted but transmitted efficiently by aphid in a circulative non-propagative manner. Yield loss normally ranges from 50% to 60% in India (Paul Khurana 2000; Mukherjee et al. 2003). PLRV has small, isometric virions measuring about 24–25 nm in diameter. The genome consists of positive sense single stranded RNA with the viral genome linked protein (VPg) at the 5′ end (Mayo and D'Arcy 1999) and an OH group at the 3′ end.

Out of the three genera of the family *Luteoviridae*, *Luteovirus* and *Polerovirus* are emerging as important plant viruses infecting major crop plants all over the world. Though luteovirus and polerovirus occurrence were reported in many crops throughout the world only few have been reported in India based on symptomatology, host range, genome organization and amino acid sequence similarities in field crops like barley (barley yellow dwarf virus), chickpea (chickpea stunt), potato, jute (potato leaf roll virus), sugarcane (sugarcane yellow leaf virus) and cotton (Cotton leaf roll dwarf virus). We have discussed all the existing information about history, distribution, biological properties, identification, genetic diversity and epidemiology on reported luteo- and poleroviruses on different crops in India.

12.2 Luteoviruses and Disease

Luteoviruses are causing leaf yellowing, reddening and/or rolling symptoms in the infected plants, are mostly confined to the phloem tissues, are transmitted by aphids in the persistent (circulative, non-propagative) manner and possess a monopartite linear ssRNA genome, which is packaged in 25 nm diameter isometric particles. Two special feature of luteoviruses seem to set them apart from all other plant viruses. One is possession of coat protein read through domain which interact with the aphid endo-symbiont product, symbionin, in a way that apparently stabilizes the virus particles when they are in the aphid haemocoel. The second feature is the extent of their capacity to interact with other infectious agents, including umbraviruses, satellite RNA and a viroid, in ways that profoundly affect the survival and spread of one or both partners; a key ingredient in such interactions is the ability of luteovirus coat protein(s) to package non-luteoviral DNA (Harisson 1999).

In India luteoviruses have been reported from barley and chickpea which are discussed herein:

12.2.1 Barley yellow dwarf virus (BYDV)

BYDV is the most widely distributed and the most economically important virus disease of wheat and barley. It is caused by a group of luteoviruses called barley yellow dwarf luteoviruses. They are transmitted by aphids in a persistent, circulative but non-propagative manner. Five strains were identified from New York in the

United States based on their transmission phenotypes in an experimental system. The strains and their principal vectors are RPV (*Rhopalosiphum padi*), RMV (*R. maidis*), MAV (*Sitobion avenae*), SGV (*Schizaphis graminum*) and PAV (*R. padi*, *S. avenae* and others). The five strains are also distinguishable serologically (Miller and Rasochová 1997). Barley yellow dwarf luteoviruses symptom includes leaf discoloration from tip to base and from margin to centre, in shades of yellow and sometimes red. Plants are usually stunted, with a decrease in tiller number and biomass and a weak root system. In the field, symptoms usually appear as yellow or red patches of stunted plants. Zhang et al. (1983) reported the strain GPV, DAV and GPDAV from China. It seems that some of the Chinese strains have a serological relation to the US isolates (MAV and PAV) but that they differ slightly in their aphid-transmission patterns. Several strains of BYDV can frequently coexist in the same plant. The resulting symptoms can be more severe when the strains are from different groups, but when from the same group, they may result in the an amelioration of symptoms through the mechanism of cross-protection. Losses in wheat due to BYD can be very serious but differ with the BYDV strains, the growth stage at infection, the wheat varieties and the environmental conditions. Losses of around 11–12% due to natural infection have been reported in Morocco and in Chile. In Australia, yield losses of about 2.2 tonnes/ha in a susceptible wheat and losses of about 1.1 tonnes/ha in tolerant varieties have been reported.

The epidemiology of BYD is influenced by the strains involved, the aphid vectors present in the area, the crop rotation, environmental conditions (temperature and rainfall), and the time of sowing. Barley yellow dwarf alternates from reservoir hosts (grasses, maize, other cereals and volunteer plants) to small grain cereals.

In India, barley and wheat crops are reported to be affected with BYDV. But there are no regular recurrence of this virus disease on wheat in India has been noticed. The first authentic report of the BYD disease occurrence in India was made by Nagaich and Vashistha (1963) from Shimla hills in 1958. The prevalence of BYD on large scale in the hilly regions of Mukteshwar and Bhowali (Uttaranchal) was subsequently recorded by Singh et al. (1979). The disease incidence was also recorded on wheat crop from Karnataka (Kulkarni and Hegde 1980). Later, outbreak of MAV-type BYDV on wheat in the Garhwal Hills in India was reported (Khetarpal et al. 1994). Besides wheat and barley, BYD also infects, oats, triticale and more than 100 other graminaceous hosts. In India, October sown wheat crop is prone to viral infection in hilly regions of Kumaon, whereas December-sown crop remains free from BYD apparently due to lack of vector population (Singh et al. 1979). BYD tolerant wheat genotypes viz., NS 879/4, Arjun, DWR 16, 32, HD 2189, 2278, HW 657 and H-10-5-7 hold great promise in breeding resistant varieties (Singh et al. 1979; Kulkarni and Hegde 1983). Destruction of wheat hosts and volunteer plants may help in reduction of initial inoculums and population of insect vectors.

Not much information on genetic diversity of BYDV isolates from India is available which needs further attention.

12.2.2 Chickpea Stunt Disease

Chickpea (*Cicer arietinum*) is one of the important proteinaceous pulse crop being cultivated in many of the Indian states. Of the several viral diseases, Chickpea stunt is an important viral disease prevalent in all the chickpea growing areas. Internodes shortening, yellowing of leaf (in kabuli types), reddening of leaf (in desi types) and browning in collar region are the general characteristic symptoms of the disease (Nene and Reddy 1987; Nene et al. 1991). The early stage of the infected plants showed the stunted growth and died prematurely, whereas stunting may not be obvious in the infected plants when they are mature. The premature deaths of diseased plants are reducing the chickpea production level in many of the growing areas. It was reported that chickpea plants inoculated with bean leaf roll virus (BLRV) caused cent – percent yield losses (Kaiser and Danesh 1972; Kotasthane and Gupta 1978) with chickpea stunt symptoms. Later, chickpea chlorotic dwarf virus (CCDV) was reported to induce the chickpea stunt symptoms (Horn et al. 1993). Hence, an extensive survey was made to ascertain the exact causal disease in India during 1991 and 1992 in Rajasthan, Madhya Pradesh, Gujarat, Haryana and at Patencheru (ICRISAT Asia Center, Telangana). They were tested positive with polyclonal antisera to BLRV in DAS-ELISA. Simultaneously, luteovirus particles were observed under EM from the symptomatic samples. The polyclonal antiserum was produced from the purified virus particle of chickpea stunt which showed positive reaction with the symptomatic chickpea samples but only few of the samples were reacted with BLRV antiserum. Thus the isolate was referred to as Chickpea luteovirus (CpLV) and the findings of the study confirmed that the existence of two distinct luteoviruses *viz.* BLRV of minor importance and a CpLV as major chickpea viruses in India (Horn et al. 1996). During the same period efforts were initiated on transmissible nature of the viruses.

In order to further confirm the earlier reports of virus association in chickpea stunt disease, chickpea plants showing stunt disease symptoms were collected from the experimental fields at ICRISAT Asia Center (IAC), Hyderabad as well as from different farmers' fields in Junagadh (Gujarat), Khargone (Madhya Pradesh), and Akola (Maharashtra) during 1992–1993 and 1993–1994 growing seasons. Samples were examined Electron microscopically as well with DAS- and TAS-ELISA methods using panel of monoclonal antibodies of potato leaf roll, beet western yellows and barley yellow dwarf (RPV strain) luteoviruses. The results confirmed the presence of virus particles from the ICRISAT field samples. Later, vector transmission work was carried out with different aphid species of which *Myzus persicae* was found to be more efficiently transmitted the virus isolates. Furthermore identification, RT- PCR assay was carried out using universal luteovirus specific primers. The results revealed that one isolate (L) was 94% identical with beet western yellows virus based on their coat protein amino acid sequences whereas the other isolate (IC) was 82% identical to the isolate (L) and 80% or less identical to the coat protein sequences of other luteoviruses (Naidu et al. 1997).

12.3 Polerovirus

So far, polerovirus was recorded from potato, jute (*Potato leaf roll virus*), sugarcane (Sugarcane yellow leaf virus, SCYLV) and cotton (Cotton leaf roll dwarf virus) crops from India and are discussed herein:

12.3.1 Sugarcane yellow leaf virus (SCYLV)

Sugarcane is one of the most important commercially grown field crops cultivated in more than 90 countries. In India it is being cultivated in 5.01 million ha of land area. It is a highly industry centric crop with more than 500 sugar industries and many more cottage industries manufacturing *gur* and *khandsari* are being in operation based on the sole crop in the country. Apart from sugar, sugarcane is one of the most viable alternatives for production of bio-fuels and renewable energy in the world. Because of its huge potential, this crop is being seen as an energy cane/bio fuel crop based on growing demand of fuel and energy in the recent years.

Among the viral diseases of sugarcane, yellow leaf disease (YLD) caused by SCYLV has emerged as a major threat across the states in the country (Viswanathan et al. 2008, 2016). It was referred as yellow leaf syndrome (YLS) previously and typical symptoms of the disease were leaf midrib yellowing and laminar discoloration. This disease was first reported in the year 1989 in Hamakua (Hawaii) on variety H65-0782 (Schenck 1990; Schenck et al. 1997) and subsequently from the United States mainland (Comstock et al. 1994) and many other sugarcane growing countries. In India, it was first time reported during the year 1999 (Viswanathan et al. 1999) and later in 2000 (Rao et al. 2000). Initial reports of its occurrences were from major sugarcane growing states viz. Uttar Pradesh, Tamil Nadu, Haryana, Bihar and Uttarakhand on the basis of visual symptoms, electron microscopic observations of virus particles and serological relationship (Rao et al. 2002).

12.3.1.1 Impact of YLD to Sugarcane Cultivation

YLD occurrence is being widely reported in most of the sugarcane growing places of the country and up to 100% disease incidences were recorded on susceptible varieties in the commercial fields (Viswanathan 2002, 2016; Rao et al. 2001) in India and from some major sugarcane growing countries viz. USA (Comstock et al. 1994, 2001), Reunion Islands (Rassaby et al. 2004) and in Thailand (Lehrer et al. 2008). As this crop occupies 5.01 M ha of land area in India, the losses caused by the disease to this crop is significant and it has worked out to be in several million US dollars. Considering its widespread occurrence and the possibility of epidemic outbreak, an intensive work on YLD had been initiated at ICAR-SBI, Coimbatore right from the reporting year 1999 to investigate in all aspects of the disease. The research findings had shown that SCYLV infection reduces the cane thickness, number of millable canes and leaf photosynthetic rate when compared to the disease

free healthy plants, thereby it further reduces yield and sugar potential of the infected crop (Viswanathan 2002; Viswanathan et al. 2006).

Since the virus adversely affects the growth and physiological parameters of the cane, detailed investigation was initiated to access its impacts on physiological efficiency and changes in photosynthates; as both are directly related with source-sink relationship and further by sucrose accumulation in cane. The detailed studies of Viswanathan et al. (2014) had shown that photosynthetic rate, stomatal conductance and SPAD metre values were lower in severely infected YL plants besides it reduced the growth and yield parameters, such as plant height, cane thickness, number of internodes etc. for example the susceptible varieties viz. CoPant 84211, Co 86032, CoC 671 had shown 42.9%, 42.3% and 38.9% reduction in plant growth attributes along with 34.15% reduction in juice yield compared to the disease free healthy plants.

In many susceptible varieties plant growth reduction was noticed when SCYLV was combined with *Leifsonia xyli* sub sp. *xyli* causing ratoon stunting disease and sugarcane leaf yellows associated with phytoplasmas and this suggested that the existence of both these pathogens for a long period in plant crop and subsequent ratoons results varietal deterioration more rapidly, leading to poor performance of the varieties (Viswanathan 2004). Later, planting of infected setts were reported as primary source of the disease in the field and the disease incidence was found more in ratoons and fields with poor maintenance (Viswanathan et al. 2006). Also the latent infection of SCYLV with its asymptomatic stage is considered as an important epidemiological state of the disease and can cause significant yield reduction in susceptible varieties.

12.3.1.2 Biological Properties

YLD symptoms usually appear/visible during 6–8 months stage of the crop in the field. The common YLD symptoms includes intense midrib yellowing on the abaxial surface (Fig. 12.1a), lateral spread of yellow discolouration to the leaf lamina followed by tissue necrosis from the leaf tip spreading downwards along the midrib. In most susceptible varieties, typical yellowing of midribs and laminar region is noticed on upper surface of the leaves. Finally, symptoms of necrosis of discolored laminar region from leaf tip to bottom along the mid rib and subsequent drying of entire leaf is noticed (Fig. 12.1b). The sugarcane varieties showing mild symptoms of midrib yellowing usually records normal cane growth whereas in severely infected clumps cane thickness and stalk height are significantly affected. Severe infection of the disease leads to shortening of internodes on the top. This effect culminates in bunching of leaves at the top. Usually such infection results in drying of leaves in the affected canes (Fig. 12.1b). Usually expression of the symptoms will be more severe in ratoon crops than plant crop (Viswantahan 2002, 2012; Viswanathan et al. 2012).

Variations in pattern of YLD symptom expression was noticed among the susceptible varieties. In order to understand the influence of weather parameters on disease development and symptom expression, a detailed study was undertaken with a set of highly susceptible sugarcane varieties/genotypes viz., Co 419, Co

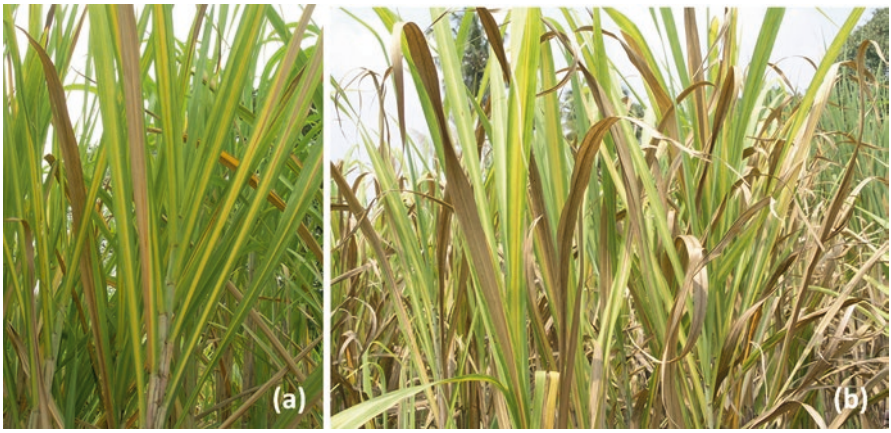

Fig. 12.1 Yellow leaf disease symptoms on sugarcane in the field (**a**) SCYLV-infected sugarcane exhibits intense yellowing of midribs on the abaxial surface. (**b**) Spread of yellow discolouration to adjoining laminar region parallel to midrib and drying of leaf tissue from leaf tip towards bottom

85019, Co 86010, Co 87269, CoBln 9605, CoPant 84211, CoS 767, CoTl 85441, B 38192 and 57 NG 56 in four planting seasons starting from 2009–2010 to 2012–2013.Of these, progressive increase in symptom expression was noticed up to formative stage of the crop and later, a fluctuation in symptom expressions i.e. intensity of yellowing decreases leaving behind the dried laminar region at the tip was observed among the susceptible varieties. The correlation and regression analyses of symptom expression and different weather parameters had shown that prevailing minimum temperature and RH in the afternoon had a partial relation to symptom expression in the virus-infected plants (Chinnaraja and Viswanathan 2015a). However, further studies are required to assess the influence of weather parameters on disease transmitting vectors, host susceptibility and virus titre of the infected plant.

12.3.1.3 Role of Vectors in Virus Transmission

Assumption of possibility of role of vectors in viral disease development is certainly common. Moreover, the virions of luteovirids are not transmitted mechanically as they are restricted in the vascular regions (phloem) of plants. So, plant to plant transmission of virus was reported through vectors (*Melanaphis sacchari*, sugarcane aphid) in many countries (Singh and Rao 2011). Recently, Chinnaraja and Viswanathan (2015b) conducted studies on vector transmission of SCYLV using virus free meristem derived micro propagated plants of cv. Co 86032 with viruliferous aphids and the transmission was confirmed through RT-PCR and the virus titre was analyzed through RT-qPCR. The results had shown the maximum virus targets of 22.3×10^3, 3.16×10^6 and 4.78×10^6copies in the viruliferous aphid inoculated plants after 7, 180 and 300 days respectively and the results confirmed the *M. sacchari* as an efficient vector to transmit SCYLV from one plant to other in fields.

Subsequently, population dynamics of YLD vector *M. sacchari* was assessed during different growth stages in different resources of germplasm, parents and varieties maintained at SBI, Coimbatore. Some of the genotypes recorded maximum aphid population of up to 621 per plant. It was found that aphid population had shown variations from season to season and genotype to genotype. This study revealed that some of the YLD susceptible varieties harbor more aphids and this needs further confirmation (Viswanathan, personal communication).

12.3.1.4 Identification of the Virus

Plant disease diagnosis is important in order to avoid the spread of diseases and disease causing pathogens to new areas and its importance get doubled in case of vegetative propagated crops like sugarcane, where the planting material i.e. setts should be free from disease. As SCYLV primary mode of transmission takes through infected setts and the asymptomatic plants may also harbor the virus, and YL symptom expression can only be seen after 6–8 months of planting, developing YLD diagnosis was felt most important. Taken in to an account, much emphasis had been given ever since it was suspected in sugarcane using different approaches (Rao et al. 2001; Viswanathan 2002, 2004). The results of DAS -ELISA studies using YLD leaf and plant juice samples as antigens showed juice samples contain more viruses compared to leaf samples and it also revealed that plants raised from YLD plants has high virus titre and it was found to be more in the subsequent ratoons (Viswanathan and Balamuralikrishnan 2004; Gaur et al. 2003).

Later, RT-PCR assay was attempted to detect SCYLV due to some drawbacks in serological methods like cross reactivity and limited antisera availability. RT-PCR based diagnosis was standardized with a set of new primers (Viswanathan et al. 2008) which amplified an amplicon of 513 bp of the virus from infected plants and it was confirmed after sequencing. Subsequently, it was validated with different symptom expression stages of YLD in 44 sugarcane varieties and the results had shown that asymptomatic plants too had detectable level of virus infection (Viswanathan et al. 2009). As the RT-PCR assay has been standardized successfully, it was widely used for virus indexing of tissue culture-derived plantlets and in germplasm (Viswanathan et al. 2009). Although it was successful in large scale diagnosis, the virus titre and its relation with disease severity couldn't be assessable under this method which necessitated real time PCR assays to quantify the virus titre. Accordingly the RT-qPCR assay was standardized to quantify the virus titre in the tissue culture raised plantlets through relative standard curve method. The copies of virus from tissue culture derived plantlets and asymptomatic plants were estimated in the ranges of 20,314.58–4,330.87 and 8.96–0.27 million, respectively. The relative expression level of the virus between in vitro plantlets and asymptomatic plants was in the ratio of 73.7: 243,393.1. The assays led to the conclusion that SCYLV population was significantly reduced in the meristem derived tissue culture plants and the copy numbers of the target virus was efficiently detected through relative standard curve method (Chinnaraja et al. 2014).

Apart from these conventional and real time PCR assays, a multiplex RT-PCR was also developed for simultaneous detection of RNA viruses of sugarcane viz.

Sugarcane mosaic virus (SMV), *Sugarcane streak mosaic virus* (SCSMV), *Sugarcane yellow leaf virus* (SCYLV) using coat protein gene specific primers and the PCR conditions were optimized. Furthermore, it was found efficient as equal as to uniplex- PCR in targeted virus amplification (Viswanathan et al. 2010) in a single reaction through which the targets were specifically detected in suspected varieties and it is considered as viable in large scale applications.

12.3.1.5 Genome Characterization

Molecular research works have been continuing in virology ever since the arrival of PCR and sequencing technologies which make it eases in revealing the genomic structure of an organism; with that more and more information's are being generated by virologists around the world from time to time. In this case, SCYLV has a (+) sense ssRNA genome containing 6- overlapping open reading frames (ORFs) (ORF0 – ORF5) and 3-untranslated regions (UTRs). The genome characterization of SCYLV isolates of different countries has shown the existence of four genotypes (BRA for Brazil, CUB for Cuba, PER for Peru and REU for Reunion Island) based on the geographical locations where they were first detected (Abu Ahmad et al. 2006a, b). Occurrence of fifth genotype of SCYLV i.e. IND from India has been strongly established based on partial sequences of SCYLV encoding for ORF 1 and 2 and complete ORF 3 and 4 sequences other than the existences of CUB and BRA-PER isolates (Viswanathan et al. 2008). Later studies with additional 13 virus isolates from nine states of India also confirmed that the virus population of India has high level of homogeneity and are significantly different from the other reported genotypes (Singh et al. 2011). The coat protein based nucleotide sequences of SCYLV isolates reported across the world were subjected into phylogenetic analysis. The results had shown that all the isolates were separated into three major clusters. The Indian SCYLV isolates from various states viz. Coimbatore, Tamil Nadu, Hyderabad, Andhra Pradesh, Madhya Pradesh, Uttar Pradesh, Pune and Kerala were shown close association with Cuba and Tunisia isolates in cluster one. Majority of the SCYLV isolates from China were grouped in second cluster along with Peru, Hawaii, Reunion, USA, Brazil, Australia, Taiwan, Argentina, Kenya, Guatemala, Colombia and Malaysian isolates. The third cluster consists of South Africa and Mauritius isolates (Fig.12.2).

Recently efforts were made to characterize the full genome of Indian SCYLV isolates for which four SCYLV isolates from Coimbatore were chosen by Chinnaraja et al. (2013) and the complete genome of four virus isolates belonged to the SCYLV-IND genotype (~5875 nt) and has shown close similarity with CHN 1 genotype reported from China. The results of phylogenetic comparison of complete genomic sequences with other isolates and genotypes reported worldwide had shown that IND and CHN 1 originated from Asia grouped together in a cluster and other

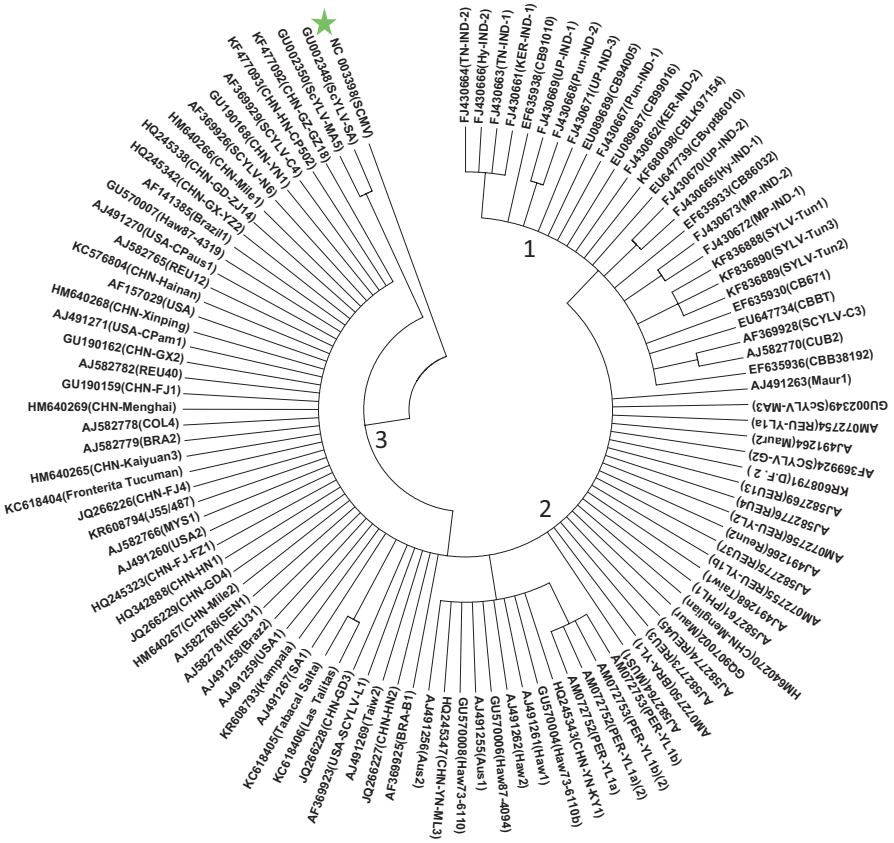

Fig. 12.2 Phylogenetic tree based on coat protein nucleotide sequences of SCYLV isolates (submitted in NCBI) showing evolutionary clustering of Indian isolates with the other globally distributed isolates. Phylograms were generated in MEGA version 4.0.2 using maximum likelihood method Sugarcane mosaic virus (GenBank Accession No. NC 003398) was used as an out group (OG) and is marked with *green color star*

genotypes reported from America and Africa separated in another cluster. These isolates exhibited amino acid sequence differences of 29.2–31.8%, 28.1–34.4% and 30.7–33.4% with REU, HAW-PER and BRA in partial ORF0 sequences, respectively. Further, detailed recombination analyses revealed evidence of recombination in ORF1 to ORF5 with the maximum number of sites occured in ORF2 in one of the four IND isolates (Chinnaraja et al. 2013).

The high incidences of SCYLV recombination suggests that recombination plays a major role in SCYLV evolution and is the driving force in evolution and emergence of new variants of SCYLV. Similarly, the studies conducted from different countries were also suggested that SCYLV genome might have evolved from at least two independent recombination and therefore it is being considered as an

emerging virus evolved from recombination between three ancestors genera *Luteovirus*, *Polerovirus* and *Enamovirus* and now under the family Luteoviridae (Moonan et al. 2000; Smith et al. 2000). However, more data needs to be generated from India and other countries to study the genome dynamics of SCYLV. The findings also revealed ORF3 coding for CP in SCYLV is the most conserved; supporting the strategy of SCYLV management through CP gene derived transgenic resistance. Further, works on developments of SCYLV resistant transgenic plants are in progress at ICAR-SBI, Coimbatore, Tamil Nadu.

Sugarcane Breeding Institute (SBI), Coimbatore houses one of the largest collections of sugarcane germplasm and hybrid collections. Recently Viswanathan et al. (2016) conducted detailed surveys on YLD symptom incidence and severity for five seasons in the germplasm resources totaling ~4066 genotypes/varieties maintained by the Institute at Coimbatore and its research centres, Agali, Kannur (Kerala) and Karnal (Haryana). Among the different centres/collections, Agali centre recorded more severity to YLD followed by National Hybridization Garden (NHG), National Active Germplasm (NAG) and 'Co' canes. However, *Saccharum* sp. clones maintained at Kannur recorded low YLD incidence and least severity for the disease symptoms. Overall, the study indicated that most of the parents used for breeding and hybridization were affected by YLD to varying severities. High incidence of vector population and constitution of varietal/parental materials are suspected for the high disease incidence and intensity in the two collections. The study identified 463 resistant sources in the hybrid clones and 773 in *Saccharum* spp. for the first time. The outcome of the study lays foundation for developing YLD resistance in sugarcane progenies in the country. Further, the newly developed disease scale is being used in 21 research centres under All India Coordinated Research Project (AICRP) on sugarcane to identify YLD resistance in new sugarcane varieties. This programme would identify YLD resistance in promising varieties which are promoted for cultivation and will supplement ongoing disease management approaches through virus-free nurseries.

12.4 Potato Leaf Roll Virus (PLRV)

12.4.1 Occurrence and Significance

PLR is one of the most damaging diseases of potatoes throughout the world. It has long been recognized as a major component of potato degeneration. The disease is caused by PLRV, the type species of the genus *Polerovirus* (Mayo and D'Arcy 1999). All Indian potato varieties are susceptible to this virus. Infected plants produce only a few, small to medium tubers (Singh et al. 2015). The virus is tuber borne, not sap transmitted, transmitted efficiently by aphid in a circulative non-propagative manner (Singh et al. 1982; Paul Khurana and Singh 2000). Yield loss normally ranges from 50% to 60% in India (Paul Khurana and Singh 2000; Mukherjee et al. 2003). However, it is lower in autumn season (7–16%) than in spring season (39–60%) (Paul Khurana and Singh 2000). PLRV has small,

Fig. 12.3 Potato leaf roll virus (PLRV) symptoms in the field (**a**) Primary leaf roll symptoms on infected potato plants (**b**) Leaf roll symptoms due to secondary infection

isometric virions (23–25 nm dia) and are primarily confined to the phloem of the infected plants. It occurs in extremely low concentration in infected plants.

The infected plants show two type of symptoms *viz*., primary or secondary, depending upon the age of infection. The primary symptoms are confined to top young leaves, which usually stand upright, roll and turn slightly pale in certain cultivars (Fig. 12.3a). However, reddish/pink colouration occurs in top leaves starting at the margins, sometimes accompanied with slight rolling of the leaflets in most cultivars. Secondary symptoms develop when the plants are grown from infected seed tubers (Fig. 12.3b). Such symptoms are quite prominent in older leaves, i.e. absent or less pronounced on younger top leaves. Infected plants have characteristic pale, dwarfed, and upright appearance with rolling of lower leaves that turn yellow, brittle and are leathery in texture. In some cultivars, a reddish or purple discolouration develops on the margins and underside of the leaves (Paul Khurana and Singh 2000). In storage, the tubers from the infected plants, in certain varieties develop phloem necrosis but most Indian varieties do not develop this necrosis. In India, occurrence of three groups of PLRV (mild, moderate and severe strain) are reported based on their symptom severity on the test plant, *Physalis floridana* plants as well as on potato varieties/hybrids (Singh et al. 1982). However, these strains did not differ antigenetically (Fig. 12.3).

12.4.2 Genome Sequences and Relations

The genome consists of positive sense single stranded RNA with the viral genome linked protein (VPg) at the 5′ end (Mayo et al. 1982) and an OH group at the 3′ end. The genome is divided into two parts by a small non coding RNA and consists of nine open reading frames (ORFs) numbered from 0 to 8 coding for proteins, P0–P7 and Rap1, respectively. Three 5′-proximal ORFs, which are expressed from the genomic RNA, encode the proteins, P0, P1 and P2. Five other ORFs are expressed by translation from two sub genomic RNAs (sgRNAs). Two structural proteins (P3, P5) and P4 are encoded by sgRNA1 and the sgRNA2 encodes two 3′-proximal

proteins (P6, P7). ORF 1 harbours a small ORF, ORF 8 which encodes Rap 1 (Jeevalatha et al. 2013).P0 is involved in symptom development and acts as a suppressor of RNA silencing (Pfeffer et al. 2002) and has functional motifs like F-BOX motif to overcome PTGS (Pazhouhandeh et al. 2006). P1 is a proteinase containing polyprotein responsible for the release of virus encoded protein (VPg) (Prufer et al. 1999; Sadowy et al. 2001; van der Wilk et al. 1997). P2 is translated by a rarely occurring ribosomal frame shift from ORF1 and carries the conserved motifs typical of RNA-dependent RNA polymerases (RdRp). P3, P4 and P5 correspond to the major coat protein (CP), the putative movement protein (MP) and the read through domain (RTD), which is translated by suppression of the ORF3 stop codon. The P5 of luteoviruses has been proposed to play a role in interaction between the virus particles and receptors in the aphid vectors (Guilley et al. 1994) especially the five-terminal conserved half may be the site of vector specificity (Brault et al. 2005). The functions of P6 and P7 are not known. But P7 is reported to have nucleic acid binding properties (Ashoub et al. 1998). Rap1 is involved in virus replication (Jaag et al. 2003). Almost all types of modulation mechanism (frame shift, initiation bypass, termination suppression, production of subgenomic (sg) RNA and proteolysis of primary translation products) are used during the expression of the different ORFs (reviewed by Sadowy et al. 2001).

Mukherjee et al. (2003) reported the coat protein gene sequence of a single PLRV isolate from India. The nucleotide and deduced amino acid sequence of the isolate was 97–99% identities to the other reported PLRV isolates. Recently, Jeevalatha et al. (2013) sequenced complete genome of five Indian PLRV isolates each one from North Western plains, Eastern plains, Northern high hills, North Eastern hills and Southern hills representing the different agro-climatic zones of India. The genome of Indian PLRV isolates comprised of 5883 nucleotides and had nine predicted open reading frames (ORF0 to ORF 8) that were similar to the other PLRV isolates. Except the isolate OTNI-2 in which a single nucleotide substitution (A>G) in the stop codon (at position 5742) of ORFs 5 and 7 was observed. The genome was predicted to contain a non coding sequence of 70 nucleotides at the 5′ end, 144 nucleotides at the 3′ end and 197 nucleotides in between the two blocks of coding sequences. About 97.6–98.7% similarities was observed among the Indian isolates and were more close to European, Canadian, African, American and Czech isolates (Group I) with 95.8–98. 6% identities than to an Australian isolate (Group II, 92.9–93.4%). The five Indian PLRV isolates showed maximum similarity to Poland and Egyptian PLRV isolates (Fig. 12.4). The reason for this may be that the potato was introduced from Europe to India and also the germplasms are being imported from the northern hemisphere countries for breeding purpose.

High level of sequence homology has been observed in geographically distinct strains of PLRV worldwide including Indian PLRV isolates (Jeevalatha et al. 2013), except few key changes in different ORFs. At nucleotide level, ORF 3 and ORF 4, corresponding to coat protein and movement proteins are more conserved than other ORFs. The isolates showed more divergence in the ORF 0 region especially the isolate, PBI- 6 was different from other Indian isolates and had only 94.3–95.1% similarity to other four isolates. Recombination analysis using SISCAN method

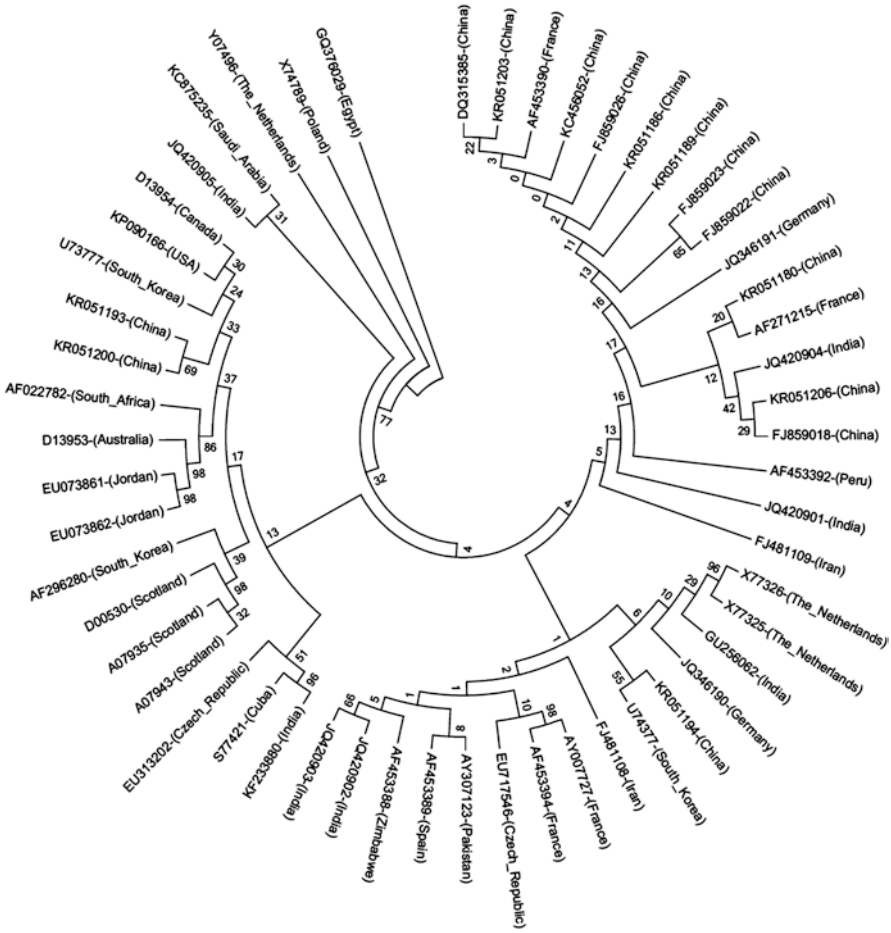

Fig. 12.4 Phylogenetic analysis based on CP gene of seven Indian PLRV isolates with other PLRV isolates reported in NCBI from countries revealed that the Indian isolates were distributed throughout the dendrogram. Two Indian isolates (JQ420904 and JQ420901) were grouped in a single cluster in which most of the Chinese isolate were clustered. Three isolates (GU256062, JQ420902 and JQ420903) formed another cluster along with PLRV isolates from The Netherlands, Germany, South Korea, China, Iran, France, Czech Republic, Pakistan, Spain and Zimbabwe. One isolate (KF233880) grouped with Cuban isolate with 96% bootstrap value and another isolate (JQ420905) was in separate cluster along with Saudi Arabean isolate

showed that the isolate PBI-6 was a recombinant isolate derived from a major parent, EU717546 (Czech isolate) and a minor parent, AF453389 (Spain isolate). The amino acid changes were more in ORF2 region of all five Indian isolates and only few amino acid changes were observed in ORF3 and ORF4 of two isolates. Nucleotide identities of these PLRV isolates with other poleroviruses ranged from 43.7% to 53.1% with a maximum of 52.8–53.1% similarity to CYDV. The identities ranged from 45.3% to 45.6% to CABYV, 45.1–45.2% to MABYV, 46.6–46.9% to

BWYV, 46.4–46.5% to BMYV, 46.8–47.0% to TuYV, 44.2–44.3% to PeVYV, 46.5–46.6% to TVDV, 43.7–44.0% to ScYLV and 44.1–44.3% to CpCSV. The isolates shared 29.1–29.3% similarity to BYDV which belongs to another genus *Luteovirus* of the family *Luteoviridae*.

12.4.3 PLRV Infection in Jute

Jute (*Corchorus olitorius* L.) is a major fiber crop of India grown mainly in west Bengal, Bihar, Assam and Orissa etc. PLRV infection on Jute was first reported from Central Research Institute for Jute and Allied Fibres, Barrackpore (CRIJAF), India, and the disease incidence was less than two percent and diseased plants were stunted in growth and height of the plants were also reduced. Most of the upper leaves showed curling, coiling, puckering and shoe string symptoms on leaf lamina. Stipules and petioles of the infected leaves were exceptionally longer. *Aphis gossypii* vector was often noticed in the field, all the samples were tested by double-antibody sandwich ELISA for common aphid transmitted viruses, e.g., bean common mosaic virus, cucumber mosaic virus, papaya ringspot virus, PLRV, cowpea mosaic virus, potato virus Y and watermelon mosaic virus using commercial ELISA kits available. The symptomatic jute samples showed positive reaction only with PLRV antibody and for further confirmation, reverse transcription PCR was carried out with PLRV CP gene specific primer pair and 627 nucleotide CP gene was sequenced (Accession No. KF233880) that shared 99% sequence identity with the CP gene sequence with PLRV reference strain S77421 (Biswas et al. 2014).

12.5 Cotton leaf roll virus (CLRDV)

Cotton blue disease caused by CLRDV is a serious problem in cotton cultivation in South America causing yield losses up to 80% in susceptible varieties (Silva et al. 2008; Distéfano et al. 2010). This positive-sense, single-stranded RNA virus is transmitted by aphids (*Aphis gossypii*) in a circulative persistent manner. Recently, Mukherjee et al. (2012) reported the occurrence of CLRDV infecting cotton fields at Nagpur, Maharashtra, India with the primers PL4F (5′-GCGACAAATAGTTAATGAATACGGT-3′) and 03R (5′-GTCTACCTATTTBGGRTTNTGGAA-3′). The primers were designed to amplify a region of approximately 600 bp of the capsid protein sequence of CLRDV (Corrêa et al. 2005). Cotton plants affected by this disease show stunting, leaf rolling, intense green foliage, vein yellowing, brittleness of leaves, reduced flower and boll size, sometimes resulting in sterility of plants (Fig. 12.5). PCR from healthy samples did not produce an amplicon. The PCR products were sequenced directly and the resulting sequence was deposited at GenBank. The coat protein sequences derived from the PCR products of Indian

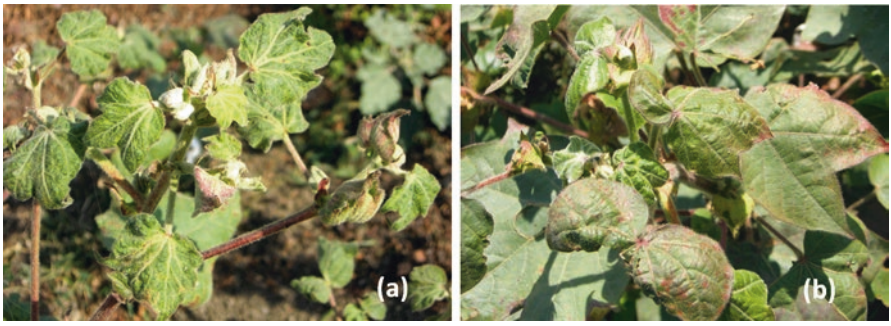

Fig. 12.5 Symptoms of cotton leaf roll virus (**a**) Curling, rolling and vein clearing of leaves; (**b**) leaf vein yellowing and brittleness of leaves (Courtesy: Dr. Arup K. Mukherjee)

isolate of CLRDV (Accession No. JN033875) from symptom-bearing plants showed more than 90% similarity with Cotton leaf roll dwarf virus and chickpea stunt virus (another member of the genus *Polerovirus)* as reported by earlier workers (Corrêa et al. 2005; Silva et al. 2008; Distéfano et al. 2010). This was the first report of the detection of a *Polerovirus* infecting cotton in India. No further report is available for the occurrence and genetic diversity CLRDV in India.

12.6 Concluding Remarks

PLRV and SCYLV are the most established poleroviruses in India but the studies on epidemiology and management of PLRV from potato and sugarcane is limited, which needs a major attention. Similarly, the information on the prevalence, genetic diversity and management of the CLRDV and PLRV is lacking from India**.** SCYLV is the recently reported emerging polerovirus in India. A lot of information that is available regarding symptomatology, morphological, biological and molecular diversity, genome organization, transgenic resistance is required to be utilized for the management of SCYLV as it is has emerged as a serious threat to sugar industry in causing severe economic losses in yield and sugar recovery. In India, research work should be initiated in the area of whole genome SNP identification and genomic selection to identify the genetic basis of resistance to SCYLV. Further, it will contribute to the knowledge and application of molecular mechanisms governing SCYLV resistance in Indian sugarcane cultivars. The diseases like potato leaf roll and sugarcane yellow leaf are mainly responsible for the elimination of many elite commercial varieties in India. Additionally, these diseases contribute to decline in their performance which is referred as 'varietal degeneration' in vegetative propagated crop. Lack of awareness on tuber/seed cane health and ignoring quarantine regulations resulted in introduction of diseases, their epidemics and varietal degeneration in many parts of the country. To increase potato/sugarcane productivity in

India, supply of healthy tuber/seed canes is to be ensured in the field. Research personnel and development workers should be actively involved in creating awareness on supply of healthy seed. In addition to detect poleroviruses in tubers/seed canes, the recent approaches in the disease diagnosis using serological and molecular approaches have applications in the field of developing virus–free seedlings, germplasm exchange and quarantine, disease surveillance and integrated disease management in India.

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