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Original Research Article

DIVERSITY, MUTATION AND RECOMBINATION ANALYSIS OF CHILLI INFECTING POTYVIRUSES FROM SOUTHEAST ASIA AND CHINA

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Abstract: *The chilli ring spot virus (ChiRSV) is one of the most important viruses of chilli crop in Southeast Asia and China. Chilli ring spot virus (ChiRSV) has been found the most devastating virus not only for chilli crop but also for solanaceous crops. In severe cases, up to 100% yield losses have been observed in chilli crop. A large virus vector population spreads this disease throughout Southeast Asia and China. The diversity of this Potyvirus has been proven after examination of many samples isolated from different locations in China and Vietnam, which have shown the virus has successfully prevalent in that part of the world. The phylogenetic investigation of this potyvirus included 11 full size samples in total. Phylogenetic study of different samples indicates that two sequences (KT633930, KX379001) are ancestors of all sequences and others are descendants. Later on, with the passage of time, these sequences (KT633930, KX379001) evolve and different strains, variants and species arrived. Recombination analysis shows that there is no recombination in any sequence and all sequences are quite unique. We have also determined replacement frequencies for Potyviruses. According to our findings, ChiRSV is very important pathogen for not only Solanaceae family but also for other crops because of its presence on different other host plants.*

Keywords: *chilli ring spot virus*; *potyviruses*; recombination; solanaceae; Transcription

Introduction

Potyvirideae is one of the most important families of plant viruses. This family contains round about 218 species (Olspert *et al*., 2015). Members of this family contain ss-RNA genome and all members are flexuous filamentous and about 11-15nm in diameter. *Potyviruses* are either monopartite or bipartite. The monopartite viruses contain 650-950nm genome size and bipartite viruses contain 200-300nm and 500-600nm genome size. Single molecule of viral RNA has been found surrounded by coat proteins which contains 1700-2000 subunits and are arranged in a helical manner. Several members are quite important as they cause huge economic losses (Olspert *et al*., 2015). Other viruses which are of economically important are *turnip mosaic virus* (TuMV) (Olspert *et al*., 2015) and *plum pox virus* (PPV) (Rodamilans *et al*., 2015). Initially *Potyvirideae* family was contained four genera, *Rhymovirus, Ipovirus, Potyvirus, and Bymovirus* (Rodamilans *et al*., 2015). Now, this family contains 6 genera including two new genera, *Tritimoviruses* and *Macluraviruses*. The genome of five genera (*Ipoviruses, Rhymoviruses, Macluraviruses, Tritimoviruses*, and *Potyviruses*) is monopartite and has only one RNA molecule. Viruses belonging to *Bymoviruses* genera are bipartite and have two RNA molecule RNA-1 and RNA-2. Sequence analysis of these six genera indicates that *Rhymoviruses* show great homology with *Potyviruses* and could be included in this genus (Rodamilans *et al*., 2015). *Potyviruses* consists of positive sense ss-RNA molecule. An interest fact about *Potyviruses* is that they encode eleven (11) proteins out of which ten (10) proteins have same precursor (Rodamilans *et al*., 2015). The sole protein that is left is made from a little transcription start site (Adams *et al*., 2011). This thing makes *Potyviruses*

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difficult to understand at each infection stage. While other viruses make proteins through a proper channel either by using sub-genomic RNAs (Sztuba-Solin´ ska, 2011) or by using ribosomal frame shifting (Adams *et al*., 2011). Host also shows different types of reactions upon infection. This may include HR to prevent further virus spread. Virus can overcome the host defense. In this way they hijack the host machinery. When a potato plant is inoculated with viral strain, in susceptible varieties transcription was observed while in resistant cultivars host defense genes activation was observed (Adams *et al*., 2011). When *turnip mosaic virus* (TuMV) entered into *Arabidopsis thaliana*, it causes activation of heat shock proteins, lowering the function of chloroplast. This is done due to accumulation of viruses (Adams *et al*., 2011). Proteome analysis has also been found helpful in understanding viral infection (Di Carli, 2012). Host proteins also involve in infection either directly or indirectly. Direct participation involve making of ribonucleoprotein complexes and indirect participation involve signaling mechanisms which is activated when virus into cell. Current scenario tells us that proteome analysis is not so much visible and important because *Potyviruses* are limited to transcriptome level. E.g. *soybean mosaic virus* (SMV) when cause infection, host activate only 28 different proteins (Yang *et al*., 2011). Plant in response to *Potyviruses* infection show such gene expression as show in abiotic stress such as in salinity, drought etc (Adams *et al*., 2011). However, these physiological stresses predispose plant to attack by *Potyviruses*. E.g. when plant is in heat stress condition, this condition will increase the accumulation of *turnip mosaic virus* RNA (Prasch, 2013) and when plant is in salt stress, this will increase up-regulation of PVA gene expression (Suntio, 2012). Plants mostly show Ca^{++} signaling during abiotic stress (Kader, 2010) and ultimately adopt this pathway (Adams *et al*., 2011). Calcium also promotes virus spread locally or systemically. For example, in infection with *pepper yellow mosaic virus*, about 4.9%genes are expressed through Ca^{++} mediated signal transduction pathway. *Potyviruses* are transmitted by aphids and aphids feeding induce calcium signaling pathway (Botha, 2010) and this thing also increase viral infection followed by transmission. Some sequences not only reported from chilli but also from other crops such as Pumpkin and *Solanum xanthocarpum*. Some sequences also reported from different chilli species. In this paper, we present a characterization of *ChiRSV's* mixing and origins as it was isolated from several locations in China and Vietnam. The geographic spread of this virus has also been provided. Additionally, we created an information library that contains the name of the isolated country, the sample accession number, and the submission date.

Material and Methods

Data Arrangement

ChiRSV complete sequences were gathered from the National Center for Biotechnology Information. To compare the homology of different viruses (at the specie level), a BLAST is used (Muhire *et al*., 2014). Evolutionary dendrogram is also given (Figure 1).

Phylogenetic Analysis

Using the MUSCLE alignment approach, every feature length sequences were mapped in MEGA 6 software. The maximum likelihood approach is used to build the dendrogram (Kumar *et al*., 2016). The virus has 11 genomes (Table 1). Using the Sequence Demarcation Tool (SDT) application, bilateral similarity was calculated (Muhire *et al*., 2014).

Recombination Analysis

The exchange was discovered using the recombination detection programme (RDP-4). MEGA 6 software was used to match these genomes before being sent to the RDP-4 tool for analysis (Martin *et al*., 2015). To ascertain the recombination, certain events were used, including GENECONV, MAXchi, SiScan, 3SEQ, and BOOTSCAN. In this test, the p-value is set at 0.05. Meg Align has also verified it. These events were verified using several techniques for improvement and validity.

Results

Data bank Analysis

Eleven various sequences have been collected (n=11) from China and Pakistan. Nine (9) sequences have been reported from China and two (2) sequences from Vietnam (Table 1).

Table 1. Presence of *ChiRSV* in various locations

Count	Virus	Accession No.	China	Vietnam	References
1	ChiRSV	KX379001	Yes	No	(Liu *et al*., 2016)
2	ChiRSV	KX258620	Yes	No	(Dong, 2016)
3	ChiRSV	DQ925439	No	Yes	(Ha, 2008)
4	ChiRSV	KT633930	Yes	No	(Liu *et al*., 2016)
5	ChiRSV	KP310866	Yes	No	(Jian, 2014)
6	ChiRSV	NC_016044	Yes	No	(Gong *et al*., 2011)
7	ChiRSV	JN387066	Yes	No	(Zhang *et al*., 2001)
8	ChiRSV	JQ23492	Yes	No	(Gong

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	SV	2			*et al.*, 2011)
9	ChiRSV	DQ925438	No	Yes	(Ha, 2008)
10	ChiRSV	JN387065	Yes	No	(Zhang *et al.*, 2001)
11	ChiRSV	JN008909	Yes	No	(Gong *et al.*, 2011)

Total two sequences (DQ925438 and DQ925439) have been isolated from Vietnam and all other sequences isolated from China (Table. 1)

Phylogenetic Analysis

Total eleven (11) sequences of *ChiRSV* were examined. There were three (3) species and six (6) strains were found in these eleven (11) sequences. Strain 1 has two (2) sequences, strain 2 has five (5) sequences and remaining strains has single sequence each (SDT analysis data).

Table 2. Display of strain information

Serial of strains	Number of genomes	Registration No	Year	Location
1	2	KX379001	2013	China
		KT633930	2015	
2	5	KP310866	2014	China
		NC_016044	2010	
		JN008909	2010	
		JQ234922	2010	
		JN387066	2009	
3	1	KX258620	2008	China
4	1	DQ925439	2004	Vietnam
5	1	JN387065	2009	China
6	1	DQ925438	2004	Vietnam

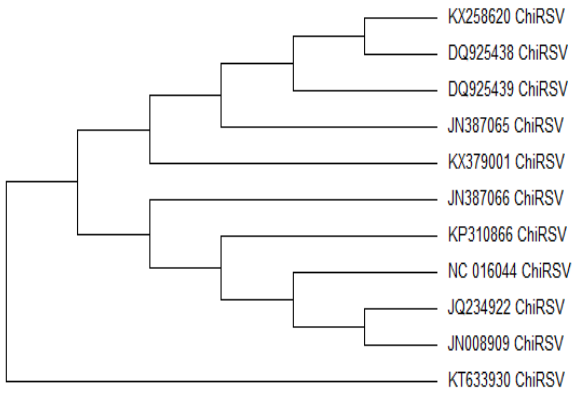

Figure 1. Phylogenetic tree of *Chilli ring spot virus*

Eleven sequences were categorized in a dendrogram. Others are descendants of the sequence with accession number KT633930 (Dendrogram). The rest sequences are identified from China, while the sequences with accession numbers DQ925438 and DQ925439 have been separated from Vietnam (NCBI library). These eleven (11) sequences contained three (3) species and six (6) strains. According to data from the SDT study, strain 1 contains two sequences, strain 2 has five, and the remaining strains each have one (FASTA file and data bank analysis). Numerous variables could be at play within that biodiversity. These elements may include exchange of diseased goods among nations, migration of insect vectors, etc. In addition to chilli, certain of these viruses have been found in other plants. This demonstrates that the virus has a variety of hosts. When a disease has a large number of host plants, there is always a good rationale for diversification. There is frequent an opportunity for a pathogen to visit multiple hosts at once because of the large number of host plants. It is possible for viruses to evolve and become transmissible because there are many distinct species of vector.

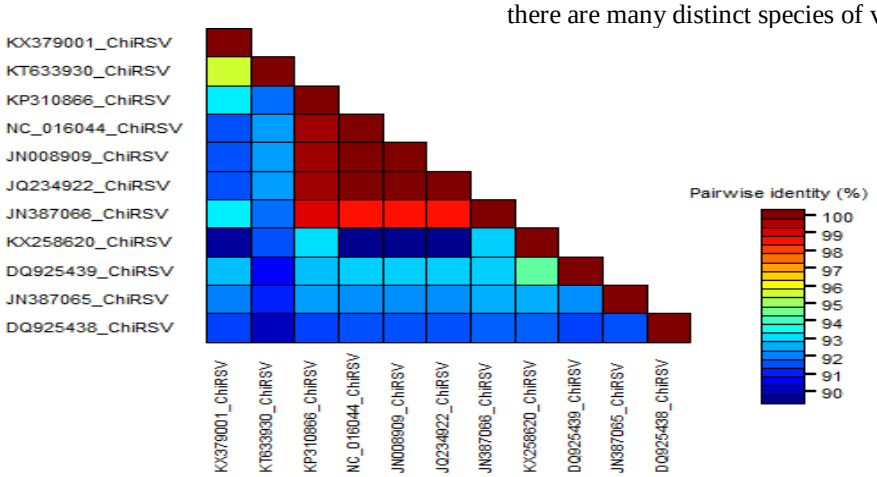

Figure 2. Grid (SDT Analysis) displaying evolution gap among *ChiRSV*. Grid values are maintained around 94 and 91 to identify species (New demarcation rule)

RDP Analysis (Recombination Detection Programme)

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There was no recombination found in any sequence of *Chilli ring spot virus*. All sequences were unique sequences (RDP Analysis results).

Discussion

Viral evolution makes viruses enable to adapt new crop plants, new varieties. Virus is a nucleoprotein in which replication play important role in evolution and changes in nucleic acid. During replication sometimes wrong nucleotides are added to synthesis process (Ahmed, 2010). Phylogenetic analysis indicates that two groups of *Potyviruses* are responsible for spread of this virus into North America (Gibbs, 2008). Thirty five species of *papaya ring spot virus* have been reported to cause infection on papaya plants in North America (Goldbach, 1986). Phylogenetic analysis indicates that these 35 species are originated from Middle East. Recombination analysis of *Zucchini yellow mosaic virus* (*ZYMV*) reveals that this *Potyvirus* is actually a recombinant of two different viruses, one is *Chilli ring spot virus* and other is *Papaya ring spot virus* (PRSV) (Adams, 2005b). *Potyviruses* are RNA viruses and evolve more quickly as compared to DNA viruses. As a result of such evolution huge population came into being. Only virulent strains cause infection and less virulent strains play role in recombination (Mayee, 1975). Mutation analysis of *Pea mosaic virus* indicates very high rates of mutation that is ($1.94/10^7$ genes) (Holcik and Sonenberg, 2005). Recombination analysis indicates that *Pea mosaic virus* has nine (9) species, 16 variants and 30 strains. These variants are recombinant of each other. In nature they recombine with each other in a host (Kader, 2010). Gene bank data analysis indicates that *Potyviruses* are evolving very rapidly and drifting towards *Solanaceous* fruits. Several reports on chilli crop indicate its capability to make chilli as a major host plant (Harlan, 1971).

Evolutionary analysis indicates that *BVYMV* evolve from sub-continent. Evolutionary analysis of *Bhendi vein yellow mosaic virus* (*BVYMV*) and its vector (Whitefly) reveals great compatibility, several strains and species emerges which results in great diversity of this virus (Ong *et al*., 1980). *Sugarcane mosaic virus* is one of most important *Potyvirus* of sugarcane crop. Diversity analysis of *SMV* indicates that it is most prevalent in Indian sub-continent and Brazil (NCBI gene bank data analysis). *SMV* transmits through aphid specie. Trade between two countries is also a factor of introduction of *SMV* into new locality (Rajamaki *et al*., 2009). Phylogenetic analysis of *SMV* indicates that it has great diversity. It is RNA virus and RNA viruses and RNA viruses evolve more quickly as compared to DNA viruses (Adams, 2005b).

The existence of huge populations depends on genetic diversity (Biebricher and Eigen, 2006). When contrast to DNA viruses, RNA viruses evolve more quickly (Holland *et al*., 1982). This is because RNA viruses offer more fusion sites (Worobey and Holmes, 1999; Eigen *et al*., 1988). However, compared to RNA viruses, diversification is more likely to have happened more quickly in the case of ss-DNA viruses (Shakelton and Holmes, 2006; Drake, 1991; Shakelton *et al*., 2005; Duffy *et al*., 2008). The *Geminiviridae* family of viruses exhibit a remarkably diverse genetic makeup. Hybridization and evolution are to blame for this (Ge *et al*., 2007; Grigoras *et al*., 2010).

There is increased genetic variety in viruses that use DNA polymerase, according to studies (Duffy and Holmes, 2009). But the essential component of genetic variation is evolution (Balol *et al*., 2010). Researchers have found that recombination, particularly in *Geminiviruses*, is key for diversification in plant pathogens (Fan *et al*., 2007; Lefurve and Moriones, 2015; Heath *et al*., 2006; Varsani *et al*., 2006). Examples of viruses that appear to have mutated as a process of recombination include *CLCuV*, *MSV*, *ToYLCV*, and *CMV* (Varsani *et al*., 2008; Sanz *et al*., 2000). Apparently, their dependent replication process is what causes this recombination (Jeske *et al*., 2001). DNA pieces from different viruses were used to contribute to recombination (Zhou *et al*., 1997; Barrie *et al*., 2001). (Monci *et al*., 2002). Due to this crossover, genetic variety and variability are produced (Silva *et al*., 2014).

Begomoviruses have elevated incidences of nucleotide substitution (Duffy and Holmes, 2008, 2009) and rapid crossover, which leads to mutation (Padidam *et al*., 1999) both of which contribute to their genetic diversity (Pita *et al*., 2001). Therefore, the genetic diversity and variety of begomoviruses are greatly influenced by evolution and exchange. Bipartite begomovirus *tomato severe rugose virus* (ToSRV) and *Macroptilium yellow spot* were investigated by Lima et al (Lima *et al*., 2013). Here, 11 poty virus genomes were examined.

Numerous investigations have shown for many years that begomoviruses evolved extremely frequently (Martin *et al*., 2011). According to Lefurve et al. (2007a), this is caused by the existence of break points that use rolling circle replication techniques for multiplication and variation (Prasanna and rai, 2007). Geminivirus coat protein architectures reveal significant details regarding genetic diversity and interactions (Unseld, 2001; Liu *et al*., 1997). Transmission requires the coat protein (Hallen and Gafni, 2001). Certain duplication faults could also lead to mutations at particular places in genomes (Zhang *et al*., 2001; Bottcher and others, 2004) According to Galvao et al. (2003) and Inoue-Nigata et al. (2006), recombination is the primary and most significant agent of species variability and variety in Geminiviruses identified in Brazil (Ribeiro *et al*., 2007). Different strains are created by this

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recombination, which spread epidemically on fresh harvests. Grasses are a junction point for begomoviruses, according to studies. For instance, *BGMV* and *MaYNV* are the parents of *MaYSV* isolates. Phylogenetic study verifies this connection (Castillo-Urquiza *et al*., 2008). The epidemic-causing *Macroptillium lathyroides*, which originated in Central America, moved to Jamaica (Roye *et al*., 1999).

It was later established that weeds serve as a foundation for hybridization. Begomoviruses develop, diversify, and then infect Jamaica (Paprotka *et al*., 2010b). Sections of the ssDNA of Geminiviruses appear to cause genetic material to mutate, according to some data (Harkins *et al*., 2009). In Brazil, numerous viruses, including the *BGMV*, exhibit little phenotypic mutations (Faria and Maxwell, 1999). However, when studies were carried out using the RCA method, this virus demonstrated increased heterogeneity within a species (Ramos-Sobrhino *et al*., 2010). Multiple new and aggressive variants of *CLCuV* have entered the cotton crop, according to diversity analysis of *CLCuV*, and *CLCKoV* and *CLCuMuV* are to blame for this (Ahmed, 2010; Ahmad, 2011; Briddon, 2003; Di Carli, 2012; Duffy, 2008; Ahmad, 2011; Bosco, 2004).

A begomovirus known as *PeLCV* infects 15 distinct host, including Pedilanthus. These hosts are primarily from Pakistan. According to certain investigations, it is evolved and originated in Pakistan (Moriones and Castillo, 2000; Zaidi *et al*., 2016). *Cucumber mosaic virus* (CMV), for example, has been found to infect a number of host plants (Palukaitis and Garca-Arenal, 2003). One of the finest viruses from the Geminivirus family to infect a variety of plants is the *pepper leaf curl virus* (PeLCV) (Shakir *et al*., 2018). The development or appearance in leaf curl infection are caused by *CLCuKoV* (Saleem *et al*., 2016).

Research has demonstrated that begomovirus Rep genes are more translational and diverse than Cp genes (Lima *et al*., 2017; Zhou *et al*., 1997; Fondong *et al*., 2000; Patil and Fauquet, 2009). *EACMV* and *ACMV* are joined in South Africa and Angola, resulting in a novel variety of the begomovirus in both nations (Kumar *et al*., 2009; Bisimwa *et al*., 2012; Mabora *et al*., 2008; Cossa, 2010).

Begomoviruses are major cause of leaf curl diseases in India in chilli crop (Kumar *et al*., 2015). Combine attack of *ChiLCV* and *PepLCBV* has also been seen (Kumar *et al*., 2015). This is also found at six different places in India (Nagpur, Palampur, Ghazipur, Salem, New Delhi and Chapra) (Kumar *et al*., 2015).

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Compliance with Ethical Standards

The authors declare no conflict of interest. All members are agreeing to publish this paper in this prestigious Journal.

Conflict of interest

The authors declared absence of conflict of interest.

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