Diversity, mutation and recombination analysis of *Chickpea chlorotic dwarf virus* infecting lentil

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Abstract

The epidemic of *Chickpea chlorotic dwarf virus* (CpCDV) is of great concern for about few decades on different crops. Analysis of different sequences isolated from various places of the world showed huge diversity of this Mastrevirus. Phylogenetic analysis indicates that four sequences from CpCDV (KC172669, KC172673, KC172666, KC172672) are ancestors and others are descendants of this.These four sequences evolved and different strains and variants came into being. Results indicate that CpCDV is a highly virulent and could be the most devastating for chickpea, lentil and other crops.

Keywords: CpCDV, Diversity, Mastreviruses, Mutation, Phylogenetic analysis.

Introduction

Mastrevirus is genus of Geminiviruses and is able to cause disease in a vast variety of cereal crops including wheat and maize. This genus has enormous diversification in DNA sequences and is able to infect both monocotyledons and dicotylrdons plants. Eragrostis curvula streak virus, a Geminivirus which reported recently and have peculiar characteristics. Its transcription activator protein behaves like Begomovirus and coat protein behaves like Mastrevirus. The unusual genomic features occurred because of the mixing of genes and not by the recombination. These features have made virus good identifier for the geminiviral ancestral state. Most of the Mastreviruses which infect the monocot plants are found in Africa with the mild exception as Oat dwarf virus, Wheat dwarf virus, and Barley dwarf virus which were originating from Asia, Europe and Middle East (Koklu et al., 2007; Schubert et al., 2007).

Chickpea chlorotic dwarf virus (CpCDV) causes different diseases in Fabaceae family. It causes blockage of phloem, reddening, shortening of height chlorosis and browning. Depending upon varieties sympotoms may vary in chickpea plants. Some of strains of Mastrevirus have been reported from cotton crop (Manzoor et al., 2014). As far as host range is concerned, Mastrevirus infect huge number of dicotyledonous plants however it is need to be proven at molecular level (Trebicki et al., 2010). They have a couple of ORFs one for movement and other for coat protein and a couple of ORFs in complimentary sense (Ruschhaupt et al., 2013). The RepA protein is responsible for making conducive environment and RepB is responsible for rolling circle replication. The focus of this study was to check the diversity and phylogeny of CpCDV.

Material and Methodology

Data arrangement

Full length sequences were collected from NCBI data bank. BLAST was done to determine the homology between other viruses (at specie level). The phylogenetic tree of all the full length sequences is provided. The tree was designed to describe the diversity of CpCDV. Neighbour joining method was used in tree construction.

Phylogenetic analysis

All full length sequences by using MUSCLE alignment method were aligned in MEGA 6 software. Maximum likelihood method was used to construct the phylogenetic tree. With the help of tree editor of MEGA 6, similar or identical isolates were converted into triangle. The CpCDV contains 16 sequences. To calculate pair-wise identity, sequence demarcation tool (SDT) programme was used.

Results and Discussion

Data bank analysis

Sixteen sequences of CpCDV has been reported (n = 16) from subcontinent and other parts of the world. Sequence having accession number KC172672was the only sequences isolated from Yemen (Data Bank analysis).

Phylogenetic analysis

A total of 16 full length sequences of CpCDV were analyzed. Sequence having accession number KM229810 was isolated from Sudan in 2014. With the passage of time, virus would have evolved. Different factors could be reasons for arrival of new strains and variants. These factors could be evolution

of vectors species, recombination of different variants in same host plant, recombination of different strains belonging to different genus etc. Sequences which were collected from different regions in the subcontinents could be transferred from one place to another via vector movement. There are many chances of agricultural trade between different countries. So it would be a factor for cross continent and cross country transmission of this virus.

In this diversity and distribution, multiple factors could be involved. These factors could be the movement of vector species, trade of infected material between these two countries etc. Some of these viruses are also isolated from different plants. This shows that this viruse has multiple host plants. There is always a reason for evolution when a pathogen has a number of host plants. Due to huge number of host plants, there is always a chance for pathogen to visit different host plants at a same time. Due to different vector species, viruses are able to evolve according to their vectors and become transmissible (Fig. 1)

SDT analysis

Matrix was formed which is indicating that how much sequences are different from each others. It is showing variance score. SDT analysis tells how many species, variants and strain are present in data.

RDP (Recombination Detection Programme) analysis

RDP is done to check the recombination between or within sequences of CpCDV infecting lentil. Recombination is detected in three sequences (RDP analysis). These sequences are showing major and minor parents. Some recombinant sequences showing unknown parents.

This investigation has some extraordinary focus when contrasted with past examinations. This investigation showing dynamically in chickpea chlorotic infection as species and strains. Results are indicating immense variety of Begomoviruses as these are disconnected from Indo-Pak subcontinent. Results of this study demonstrating changes and recombination as well as major and minor guardians of modified groupings. This could be useful in anticipating about new infection. This investigation can give progress to future so that scientist may disconnect CpCDV from different harvests which are not influenced from this infection yet are helpless against assault by this microorganism. The significance of this examination is, that the investigation is showing the variety of Begomovirus among various harvests. This variety is expanding the quantity of microbes to farming harvests. The real reason for this investigation is to discover how Begomoviruses are developing so quickly. Recombination among Begomoviruses and trade of DNA parts with other infections is answerable for

this colossal advancement. This examination would additionally open the door for new exploration in this angle. Scientists would have the option to segregate further successions of Begomovirus on various yields.

Hereditary variety is significant for huge populace sizes to exist (Biebricher and Eigen, 2006). The RNA infections transform quickly when contrasted with DNA infections (Holland et al., 1982). This is because of RNA infections give more locales to recombination (Eigen et al., 1988; Worobey and Holmes, 1999). Notwithstanding, if there should be an occurrence of single-abandoned DNA infections, advancement is bound to be happened all the more quickly when contrasted with RNA infections (Shackelton and Holmes, 2006; Duffy et al., 2008). Infections have a place with Geminivirideae family show tremendous hereditary variety. This is because of recombination and change (Ge et al., 2007; Grigoras et al., 2010). Studies have been shown that infections utilizing DNA polymerase show more hereditary variety. In any case, transformation is the key in hereditary variety (Duffy and Holmes, 2009). Studies revealed that the recombination is liable for development in plant infections particularly in Geminiviruses (Heath et al., 2006; Varsani et al., 2006). For instance, tomato yellow leaf twist, cassava mosaic infection, Maize streak infection and cotton leaf twist infection appear to be advanced because of the recombination (Sanz al.. 2000). All things considered this et recombination is because of their reliant replication component (Jeske et al., 2001). Recombination is happened because of commitment of DNA sections from other infections (Zhou et al., 1997; Monci et al., 2002). This recombination brings about hereditary changeability and variety (Silva et al., 2014). Results exhibited that trade of parts between infections produce another strain which add to a huge variety of viral populace. The hereditary variety of Begomovirus is because of two factors: their nucleotide replacement at extremely high rates (Duffy and Holmes, 2009) and continuous recombination which brings about development (Padidam et al., 1999; Pita et al., 2001). Thus, change and recombination assume a fundamental part in hereditary variety and changeability in Begomoviruses. Lima et al. (2013) dissected bipartite Begomovirus tomato extreme rugose infection (ToSRV) add Macroptilium yellow spot (MaYSV). Here 93 successions of Begomovirus were broke down. For a long time, a few investigations demonstrated that Begomo infections recombined at high rate (Martin et al., 2011). This is because of quality of break focuses which are utilizing moving circle replication system for replication and for enhancement (Prasanna and Rai, 2007). The cotton hosts of these infections are simply become conceivable because of the mix in viral arrangements. The coat protein designs of

Geminiviruses give significant data about the hereditary variety and recombination (Liu et al., 1997; Unseld et al., 2001). The coat protein is needed for transmission. A few mistakes in replication may likewise cause transformation at specific focus in arrangements (Zhang et al., 2001). Recombination is the principle, a significant office of hereditary variety and inconstancy in Geminiviruses found in Brazil (Galvao et al., 2003; Inoue-Nigata et al., 2006; Ribeiro et al., 2007). This recombination delivers new strains which cause pandemic on new harvests. Studies shown that weeds are mix station for Begomoviruses. For instance, separates of MaYSV have BGMV and MaYNV as a parent. This affirmed relationship is bv phylogenetic investigation. In Central America, Macroptillium lathyroides advanced and spread to Jamaica and cause plague (Roye et al., 1999). Later it was affirmed that weeds give stage to mix. Begomoviruses gets advanced, different and afterward spread to Jamaica (Paprotka et al., 2010). Some proof shows that pieces of ssDNA of Geminiviruses give change to hereditary material (Harkins et al., 2009). Some infections in Brazil, for example, BGMV showed low degree of hereditary inconstancy (Faria and Maxwell, 1999). Anyway this infection exhibited more noteworthy inconstancy inside an animal groups when investigations were directed by utilizing RCA technique (Ramos-Sobrhino et al., 2010). Phylogenetic examination of cotton leaf twist Geminivirus demonstrates that cotton leaf twist kokhran infection and cotton leaf twist multan infection are answerable for appearance of a few new and destructive strains in cotton crop. Their recombination in nature develops numerous species in nature. Developmental investigation demonstrates that CLcuMuV give Rep protein and CLcuKoV give coat protein during recombination measure. Recombination investigation of accessible successions on NCBI information bank uncovers that CLcuMuV has a replacement rate that is (4.96×10^{-4}) per site) and CLcuKoV has replacement rate (2.706 \times 10⁻⁴ per site). Different investigation shown that cotton leaf twist Geminiviruses detailed from Pakistan as well as from China, Africa and India. Varieties of eleven unique sorts of Geminiviruses infections were contemplated. CLcuMuV segregated from China show 98-99.3% likeness with CLcuMuV secluded from Philippines. CLcuMuV disengaged from Pakistan show 89.4-99.7% homology with CLcuMuV secluded from India. This investigation demonstrates Geminiviruses tremendous variety and pace of advancement. Change pace of CLcuMuV in Rep protein is $(0.821 \text{ gene } 10^{-7} \text{ gualities})$. Transformation pace of CLcuMuV is coat protein is $(1.6 4 \text{ gene } 10^{-7} \text{ qualities})$. Transformation rate CLcuMuB is 0.85 gene 10⁻⁷ qualities. These high paces of transformation empower Begomoviruses to develop quickly and structure new strains, variations and species. Variety of examinations have shown

that CLcuMuB is more conspicuous in Indo-Pak sublandmass. This variety is because of determination transmission of this infection by whiteflies. However, factor of exchange between two nations can't be overlooked. Presence of Begomoviruses in certain pieces of Rajasthan (India) is because of recombination between two genera of Geminiviruses.

Pedilanthus leaf twist infection (PeLCV) is a begomovirus known to contaminate 15 distinctive host plants. Larger parts of these hosts are from Pakistan. A few examinations have shown that it is transformed and spread from Pakistan (Moriones and Navas-Castillo, 2000; Zaidi et al., 2016). Some RNA infections like a cucumber mosaic infection (CMV) displayed to taint a few host plants (Palukaitis and García-Arenal, 2003). Pepper leaf twist infection (PeLCV) is one of best infection among Geminivirus to taint a wide scope of plants (Shakir et al., 2018). Phylogenetic tree of CP and Rep qualities of RaLCuV which confine from India shows closeness to cotton leaf twist khokharan infection (CLCuKoV). CLCuKoV is liable for development and rise of cotton leaf twist sickness (Saleem et al., 2016). Studies have shown that Rep qualities of Begomoviruses are more recombinant and variation than Cp qualities (Lima et al., 2017). Be that as it may, in PeLCV hereditary fluctuation is equivalent among CP and Rep qualities. Cassava mosaic Begomovirus (CMB) are likewise expected microbes in African nations when recombined with other infections (Zhou et al., 1997; Patil and Fauquet, 2009). In South Africa and Angola, EACMV and ACMV are joined together which brings about another variation of Begomovirus in these nations (Kumar et al., 2009). Bean stew leaf twist infection (ChiLCV) is liable for Chili leaf twist sickness across India after Pepper leaf twist Bangladesh infection (PepLCBV) and Tomato leaf twist New Delhi infection (ToLCNDV). Synergistic impacts of ChiLCV and PepLCBV has additionally been seen in eastern spots of India (Kumar et al., 2015). Synergistic impacts of these Begomoviruses were found at six different areas in India (Palampur, Nagpur, Salem, Ghazipur, New Delhi and Chapra). These monopartite (ChiLCV) and bipartite (ToLCNDV) Begomoviruses are a wellspring of advancement of new species (Kumar et al., 2015).

Conclusion

CpCDV is a very devastating virus for lentil crop. Its diversity shows that it can spread from one country to another. Recombination shows that it has many species and variants. Sequences isolated from subcontinent had showed very much diversity. This diversity enables CpCDV to infect many other crops except lentil. Data bank analysis showed that CpCDV not only present in subcontinent but also in Yemen. Three sequences with recombination would now provide further emergence of new strains. These

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further recombination study would reveal the genetic diversity present in these sequences.

Table1: Analysis of recombinant sequences.

Overview of RDP analysis of CpCDV infecting Lentils											
Event	Found	Recomb.	Major	Minor		Detection Methods					
No	in		parent	parent	R	G	B N	1 C	S	Т	
12	2	KM37761	Unknown	LN86511	+	-	-	+	_	_	+
15	1	KM22980	KM377673	Unknown	_	-	_	—	-	+	-
17	1	KM37763	KM377673	Unknown	+	+	-	-	_	_	-
R = RDP	G = Gen	econv	B = Bootscan	M = MAXch	ni	C = C	Thimer	а	S = S	SiScan	T = 3Sec

Table 2: Recombination results of different strains of CpCDV.

Virus Name	Recombinant				Av.			
		R	G	В	Μ	С	S	Т
CpCDV	KM229810	_	_	-	-	_	1.446*1	0-8 _
CpCDV	KM377673	2.708* 10 ⁻²	4.225* 10 ⁻³	_	_	_	_	_
CpCDV*	KM377673	1.854* 10 ⁻²	_	_	4.366* 10 ⁻⁴			3.73*10 ⁻³
CpCDV*	KM377671	1.854* 10 ⁻²	_	_	4.366* 10 ⁻⁴	_	_	3.73*10 ⁻³

*Samevalues due to same major and minor parents.

R = RDP; G = Geneconv; B = Bootscan; M = MAXchi; C = Chimera; S = SiScan; T = 3Seq. Some strains of CpCDV were quite unique in nature. They had not any kind of adulteration in their genome. Above sequences have some recombinant DNA in their genome.

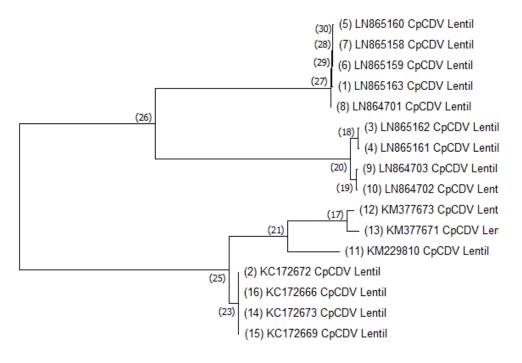


Fig. 1: Phylogenetic tree of Chickpea chlorotic dwarf virus.

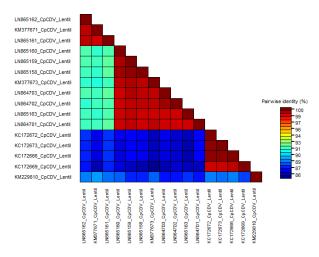


Fig. 2: Matrix showing distance between species of *Pepper leaf curl Llahore virus*. Matrix reading is kept between 94–91 to demark the species (According to new classification scheme).

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