International Journal of Biology Sciences
www.biologyjournal.net
Online ISSN: 2664-9934, Print ISSN: 2664-9926
Received: 04-03-2021; Accepted: 06-04-2021; Published: 08-05-2021
Volume 3, Issue 1, 2021, Page No. 10-17

Phylogenetic analysis of cotton leaf curl CLCuD virus from old world to new world

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DOI: https://doi.org/10.33545/26649926.2021.v3.i1a.21

Abstract
In Pakistan Cotton is one of the mainly significant fiber and cash crop. CLCuD affect the cotton production mainly in Pakistan. The disease of cotton leaf curl is due to multiple viruses from genus begomovirus and geminiviradae family. Viruses transmitted by whitefly and have single stranded DNA genome. CLCD is a synergistic interaction between virus and linked satellite molecules include betasatellites and alphasatellites. Viruses of different plant develop by mutation and recombination. The particular satellite molecule CLCuMuB is pathogenicity determinant to create disease in cotton crop. In this analysis detailed analysis of phylogenetic of cotton leaf curl betasatellite infect cotton was performed. Sequences of Cotton leaf curl betasatellites retrieved from NCBI database then align with the use of software ClustalW and construct phylogenetic tree through neighbor joining method by using MEGA 7 to know evolutionary relations between many strains of cotton leaf curl betasatellite. Percentage identity in column was achieved by run sequences in SDT. At the last part nucleotide BLAST tool used for sequences have similarity lower than 90 was performed to observe similarity and difference among sequences of cotton leaf curl betasatellite.

Keywords: cotton, CLCUD, begomovirus, bioinformatics tools

Introduction
Pakistan most important fiber and cash crop is cotton and it is the second mainly significant oil seed crop of the world (Farooq *et al*., 2014) [10]. G. hirsutum belong to the family Malvaceae and genus Gossypium (Azhar *et al*., 2013) [3]. Pakistan in the cotton production all over the world is on the 4th and in fiber export is on the first number (Aleem Ashraf *et al*., 2013). Due to huge economic significance cotton is also known as "white gold". Cotton has many use and it give us necessary product like meal, linters, oil, hulls and lint (Rabindran *et al*., 2014) [25]. Cotton is growing about sixty countries of the world (Aized *et al*., 2017) [1]. The main cotton cultivation countries are Pakistan, China, India and Uzbekistan.

Cotton Leaf Curl Disease
Geminiviridae families usually refer to as CLCuD linked begomoviruses. Mostly Old World begomovirus are monopartite have genomes that contain only one single stranded circular DNA (ssDNA) particle which are linked with betasatellite (Briddon *et al*., 2008) [11] and a third element known as alpha-satellite (Amrao *et al*., 2010) [2].

Symptoms of CLCuD
Symptom in cotton typically emerges 2–3 weeks of inoculation. Infected plants show a variety of symptoms like curling of leaf, stunted growth and enations on the vein on the underneath of leave that may build up into cup like structure (Singh *et al*., 1997) [31]. Thickening of vein are of two types i.e. thickening of small vein and main-vein thickening. In minor vein thickening on juvenile leaves small green bead formed. The thickening appear first close to the leaf border and then extend interior to form a net of dark green thicken main vein (Mansoor *et al*., 2011) [18].

Gemini viruses
Gemini virus tiny set of plant virus and have circular single strand genome (Kurstak, 1981) [17] which surrounded inside distinctive twin isometric particle (Harrison *et al*., 1977) [12]. Gemini virses transmit through insects and cause disease in both mono and dicots plants (d'Arcy *et al*., 2005) [8]. Gemini viruses that transmit through Bemicia tabaci (white fly) are more than 80% and belongs to the begomo virus genus. They frequently contain bipartite genome nominated DNA A and DNA B and cause disease in dicotyledonous plants (Harrison and Robinson, 1999) [13].

Genera of Geminiviruses
The family Geminiviridae has been lengthened in recent times have nine genera Eragrovirus, Begomovirus, Capulavirus, Mastrevirus, Curtovirus, Becurtovirus, Topocuvirus, Grablovirus Turncurtovirus (Zerbini *et al*., 2017) [36] and Begomovirus genus contain viruses which cause more destruction and transmit through white fly.

Table 1: Recognized genera of the *Geminiviridae*, host details & properties

Genus	Genomic segmentation	Replication site	Transmission	Host details
Begomovirus	Segmented	Nucleus	Whiteflies	Dicotyledonous plants
Curtovirus	Monopartite	Nucleus	Beet leafhopper	Dicotyledonous plants
Becurtovirus	Monopartite	Nucleus	Viral movement; contact	Spinach
Eragrovirus	Monopartite	Nucleus	Treehopper; leafhopper	Plants
Topocuvirus	Monopartite	Nucleus	Leafhopper	Dicotyledonous plants
Mastrevirus	Monopartite	Nucleus	Leafhopper	Monocots
Turncurtovirus	Monopartite	Nucleus	Leafhopper	Turnip
Grablovirus	Monopartite	Nucleus	Alfalfa treehopper	Grapevines
Capulavirus	Monopartite	Nucleus		*Medicago sativa*

Begomovirus

The begomovirus make up the biggest genus of the Geminiviradae family (RybickiEP *et al*., 2000). Most of the begomovirus genome contain two components (Padidam *et al*., 1995) [24]. The first component is DNA A coded every viral function essential for encapsidation, to control gene expression and for replication (Stanley, 1983) [32-33]. The DNA B coded two genes which have role in virus movement among and inside plant cells (Noueiry *et al*., 1994) [23]. Begomovirus have single strand circular genomic component. The earliest begomovirus which is analyzed have bipartite genome known as ACMV (Stanley, 1983; Stanley and Gay, 1983) [33, 33] which is beginning in Old world and TGMV. Two monopartite begomovirus (Dry *et al*., 1993; Kheyr-Pour *et al*., 1991; Navot *et al*., 1991) [9, 15, 22] similar to DNA a component of OW (Old World) begomoviruses which is adequate to produce infectivity in host showed that they fulfill the requirement 'of the movement coded by genes of DNA B. Although some monopartite begomovirus like AYMV (Tan *et al*., 1995) [35] and Multan virus of cotton were not capable to produce disease and they need an additional component (Saunders *et al*., 2001; Saunders and Stanley, 1999; Stanley *et al*., 1997) [28, 29, 34] and also (Mansoor *et al*., 1999) [19] new satellite component known as DNA beta were identified (Saunders *et al*., 2000) [27]. Analysis showed that DNA B have an important role in the upholding of the diseases (Briddon *et al*., 2001) [5]. Begomovirus infect dicotyledonous plant are either mono or bipartite and the biggest genus in the family Geminiviridae (Briddon *et al*., 2010; Fauquet *et al*., 2008) [2, 11]. In addition to this most monopartite begomovirus linked with a symptom producing satellite recognized as betasatellites (Fig 1.2).

Begomoviruses and Associated Satellite Molecules

Satellites are viruses that for replication needs an assistant virus however do not have wide nucleotide sequence characteristics like their helper virus and are not essential for its propagation (Murant and Mayo, 1982) [21]. Most of the satellite comprise of RNA and are linked with virus which have RNA genome and have no effect on the symptom cause by their helper virus in plant (Hu *et al*., 2009) [14].

Betasatellites

Betasatellites are 1350 nucleotide small molecules which are mainly frequently linked with monopartite begomoviruses (Kumar *et al*., 2014). Betasatellites first recognized in 1980 (Saunders *et al*., 2000) [27] from OW (Briddon *et al*., 2008) [4]. Betasatellite need an assistant virus used for transmission, replication as well as movement among host plant (Briddon *et al*., 2003) [6]. Betasatellite could be extremely immoral through interaction with divergent begomovirus in cotton crop and many further host (Mubin *et al*., 2010; Saeed, 2010; Zia-Ur-Rehman *et al*., 2013) [20 , 26 , 37].

Related Work

To analyze the variety of DNA-satellite complexes of begomovirus, plants of okra crop and the weed species (*Ageratum conyzoides*) were studied both of these species have leaf twisting symptom. Complete analyses of the sequence of genome components discovered a distinctive DNA-satellite complex of begomovirus in okra plant and weed species. Practical studies were performed on the betasatellite linked with monopartite begomovirus *Cotton leaf curl Kokhran virus*.Coat protein mutation practices was done (*CP*), *C2*, *V2* and *C4* genes indicated that CP, C2 and *V2* have role in determining pathogenicity and they have role in facilitate movement of virus and upholding of betasatellites.*C4* is involved in create symptom, but not particularly need to retain betasatellites (Sattar, 2012).

The begomo viruses and its linked satellite found in tomato crop which are infected with LCD. The begomovirus selected Burewala cotton virus because it has 99% nucleotide sequence identity. Ninety two percent similarity showed to alpha particle of Burewala virus and is recognized vary of alphasatellite of cotton. Eighty percent similarity found in beta molecules with other existing beta molecules. When healthy tomato infect with Burewala virus along with its betasatellite, in occurrence or lack of alphasatellite, typical leaf curling symptoms produce, although in the absence of betasatellite inoculation with CLCuBuV produced mild symptoms. This confirmed that the betasatellites have role in appearance of disease signs. We give name to the novel found beta molecule To LCHJB (Kumar *et al.*, 2013).

Sixteen viruses were recognized that belonged to begomovirus 16 species affect weeds and crops in Vietnam.Analyses of their Sequences indicate that nine viruses belonged to new specie and six was recognized first time in the Vietnam.In addition to this DNA-b eight molecule were also linked with certain monopartites viruses. Six DNA-b molecule was new. Corchorus golden mosaic virus had many common characteristics with the earlier recognized virus Corchorus virus of New World, proofed that viruses of New World be also found within Old World. Results showed Vietnam given birth to begomovirus diversity(Ha *et al.*, 2008).

Materials and Methods

The research work was performed in the (CABB) Centre of Agricultural Biochemistry and Biotechnology department at Virology Laboratory University of Agriculture Faisalabad (UAF).

Retrieval of sequences from NCBI (National Center for Biotechnology Information)

All sequences of Cotton leaf curl betasatellite were find out in the database of NCBI. Cotton leaf curl betasatellites all complete sequences which is present in GenBank database taken in the FASTA format. First separated all the Cotton leaf curl betasatellite and then prepared a FASTA file of it.

Alignment by use of Clustal W and Phylogenetic study

Cotton leaf curl betasatellite complete sequences which were arranged in FASTA format was now build on MEGA7 for DNA alignment. Following parameters was used in clustal W alignment: 0.5= Transition weight and DNA weight matrix=clustal W 1.6.In clustal W after pairwise and multiple alignment, phylogenetic analysis was performed.

For this purpose opened the option of phylogeny on the main page and selected the option of construct/test neighbour joining tree. After this computed a phylogenetic tree over subsequent analysis preferences: phylogeny test is bootstrap method and number of bootstrap replications was= 1000 and form or technique is highest chance of combined.

Identity percentage by SDT

Previously ready FASTA file was changed into sdt file. SDTv1.2 software was used to open the sdt file. Then cotton leaf curl betasatellite all complete sequences were loaded onSDTv1.2 software. Alignment of loading sequences was completed by using Muscle alignment program. SDTv1.2 software result showed a color coded matrix. In column identity score was obtained for more accurate results. For pairwise comparison of sequences of cotton leaf curl betasatellites present in GenBank database of NCBI score was used for observing the lowest and highest nucleotide percentage.

Nucleotide BLAST

The sequences which have sequence identity percentage below 90% search the sequences in nucleotide BLAST to study the similarities and differences among all complete sequences of cotton leaf curl betasatellites. For this analysis first opened the Nucleotide BLAST, selected the sequences below 90% sequence identity and was copied them in given place and now done the BLAST of sequences. BLAST result showed a sequences list which has identity in genome sequence with the sequences whose BLAST was performed. Now genome alignment of a particular complete sequence of cotton leaf curl betasatellites was opened and checked the differences and similarities of this particular sequence and BLAST sequence was observed.

point of view are very close to each other, climate conditions of both countries are also similar so the cotton species grown in both countries also have similarity. In both countries cotton leaf curl Multan betasatellite have similar field conditions and host. This might be the cause behind occurrence of betasatellites with larger sequence resemblance in their genomes and same origin.

The record of accession numbers of each sequence is provided. On node values represent bootstrap values which are 1000 replicates. All CLCuMuB of China were on the same clade which showed that they have in close proximity. This represent that sequences of betasatellite from China contain larger resemblance in their genetic material and it is possible they are from the similar origin. Multan betasatellite of China sequences also presented resemblance with Multan betasatellite of India and Pakistan. India one betasatellite was very nearer to China and their genome has almost 98% sequence resemblance. Betasatellite of china may have their origin from india. Gezira betasatellite sequences of four countries Burkina Faso, Niger, Sudan, and UAE showed much resemblance with each other. It means their genome show much resemblance with each other. They also show resemblance with Multan betasatellite.

The result showed that betasatellite molecule from a particular country like Pakistan, has greater resemblance in their genome and might have evolved from similar origin. In the same way Mutan betasatellite of different countries like IN, PAK and CN also showed sequence resemblance with among them. In addition to this betasatellite sequences from Burkina Faso, Niger, Sudan and UAE showed larger resemblance among Multan betasatellite of Pakistan. It indicated that Pakistan is the center in which Multan betasatellite was initially recognized in the vicinity of Multan although betasatellites from IN, CN, Burkina Faso, Niger, Sudan and UAE might have derived from Pakistan.

Identity proportion by SDTv1.2

The tree of phylogeny does not give data about different strains and accurate proportion of resemblance or divergence among diverse sequences of cotton betasatellite. Now order to know the beatsatellite strains in the world, SDTv1.2 software use which give accurate characteristics proportion among betasatellite sequences.

By processing sequences gave as FASTA file in SDTv1.2 software. We obtained the proportion of Sequence similarity in column. Betasatellite sequences of cotton leaf curl disease pairwise comparisons were made from many countries including Pakistan, India, China, Burkina Faso, Niger, Sudan and UAE to locate highest and lowest similarity proportion values among them.

CLCuB sequences of seven countries pairwise comparison gave following results. The percentage of sequence identity among CLCuMuB of Pakistan was 0.98-0.82%, sequence individuality proportion among Multan betasatellite of PAK and IN was 0.97-0.81%, sequence similarity proportion among Multan betasatellite of PAK and CN was 0.97-0.81%. The proportion of sequence identity among CLCuMuB of IN was 0.96-0.81%, sequence similarity proportion among Multan betasatellite of India and CN was 0.90-0.84%, sequence similarity proportion between Multan betasatellite of China was 0.99-0.97%.

The sequence similarity among Multan betasatellite of Pakistan and CLCuGeB of Burkina Faso was 0.65-0.64%,

sequence identity among CLCuMuB of Pakistan and CLCuGeB of Niger was 0.65-0.64%, sequence identity among CLCuMuB of Pakistan and CLCuGeB of Sudan was 0.67-0.63%, sequence identity among Multan betasatellite of Pakistan and CLCuGeB of UAE was 0.66-0.63%.

Sequence similarity among Multan betasatellite of IN and CLCuGeB of Burkina Faso was 0.67-0.63%, sequence identity among Multan betasatellite of IN and CLCuGeB of Niger was 0.65-0.64%,sequence similarity among Multan betasatellite of India and CLCuGeB of Sudan was 0.67-0.61%, sequence similarity among CLCuMuB of India and CLCuGeB of UAE was 0.64-0.61%.

Sequence identity among Multan betasatellite of CN and CLCuGeB of Burkina Faso was 0.67-0.65%, sequence identity among CLCuMuB of China and CLCuGeB of Niger was 0.65-0.64%, sequence identity among CLCuMuB of China and CLCuGeB of Sudan was 0.67-0.64% sequence identity among CLCuMuB of China and CLCuGeB of UAE was 0.65-0.64%.All SDT results were also described in Table 2 .

Table 2: Maximum and Minimum fraction of Sequence Identity Values for Comparisons of the Cotton Leaf Curl Betasatellite genome available in Genbank Database.

	CLCuMuB Pakistan	**CLCuMuB India**	**CLCuMuB China**	**CLCuGeB Burkina FASO**	**CLCuGeB Niger**	**CLCuGeB Sudan**	**Clcugeb Uae**
Clcumub Pakistan	0.99-0.83%	0.99-0.82%	0.99-0.82%	0.65-0.64%	0.65-0.64%	0.67-0.63%	0.66-0.63%
Clcumub India	---	0.99-0.83%	0.90-0.84%	0.67-0.63%	0.65-0.64%	0.67-0.61%	0.64-0.61%
Clcumub China	---	---	0.99-0.97%	0.67-0.65%	0.65-0.64%	0.67-0.64%	0.65-0.64%
Clcurab India	0.94-0.89%	0.90-0.86%	0.89-0.88%	---	---	---	---
Clcubab India	0.84-0.82%	0.85-0.79%	0.84-0.83%	0.66-0.65%	0.66-0.64%	0.66-0.65%	0.67-0.66%
Clcubub Pakistan	0.99-0.91%	0.87-0.86%	0.89-0.88%	---	---	---	---
Clcugeb Burkina Faso	0.65-0.64%	0.67-0.63%	0.67-0.65%	0.99-0.94%	0.94-0.93%	0.95-0.90%	0.87-0.86%
Clcugeb Niger	0.66-0.61%	0.65-0.64%	0.65-0.64%	0.94-0.93%	0.99-0.97%	0.94-0.92%	0.88-0.87%
Clcugeb Sudan	0.67-0.63%	0.67-0.61%	0.67-0.64%	0.95-0.90%	0.94-0.92%	0.99-0.94%	0.89-0.88%
Clcugeb Uae	0.66-0.63%	0.64-0.61%	0.65-0.64%	0.87-0.86%	0.88-0.87%	0.89-0.88%	0.99-0.97

Discussion

Pakistan is at 4th number in cotton production after China and USA. India ranked at the 3rd (Akhtar, 2005). The annual production of cotton is 20 million tones hence considered the major fiber and cash crop. Due to its economic importance it greatly affects the economy of Pakistan (Imran *et al*., 2011).Due to many biotic and abiotic stresses cotton production is at a halt for several years in Pakistan. The main threat to cotton production from past 20 years is cotton leaf curl disease caused severe loses (Farooq *et al*., 2011).

In humidareas the main threat to agricultural crops are the most harmful group of plant viruses known as Begomoviruses (Seal *et al*., 2006) [30] Begomoviruses are dispersed to more geographical areas more over being reported on new hosts caused great yield loses in a large number of crops consists of food, fiber (Mansoor, 2003) [6].

Begomoviruses caused disease in cotton in association with specific satellite molecule known as betasatellite (Cai*et al*., 2010).Betasatellites are broadly spread in Old World. These satellites molecules amplified the disease complexity in association with begomoviruses caused infection in plants (Fiallo-Olive,2010). For the increase of signs in cotton a cotton leaf curl betasatellites are involved. In my research work, cotton leaf curl betasatellites complete phylogenetic analysis was analyzed. For this purpose Cotton leaf curl betasatellite complete genome sequences were downloaded from NCBI present in GeneBank database in FASTA format.

The NCBI houses a chain of databases related to biotechnology like GeneBank database for DNA sequences available. Through clustal W Alignment of sequences were done. Then after Alignment software MEGA7 was used to construct a phylogenetic tree of it. MEGA is the software used for constructing trees. The version 7.0 has the ability for building molecular evolutionary trees scaled to time (time trees). These evolutionary trees were helpful to learn and study associations between diverse betasatellites molecules. The complete betasatellites sequences, in thee volutionary tree are from seven countries; PAK, IN, CN, Burkina Faso, Niger, Sudan and UAE. The tree showed that betasatellites sequence of a particular area like PAK, contain much resemblance among them and it is possible evolved from a common origin. In the same way betasatellite sequence from PAK, IN and CN also showed resemblance among them. In addition to this betasatellite sequence from IN, CNhave much resemblance among Multan betasatellite of PAK. This mean that Multan betasatellite recognized initially in Pakistan in the region of

Multan although betasatellites molecule from IN, CN, Burkina Faso, Niger, Sudan and UAE were possibly derived from Pakistan.

An evolutionary tree not gave accurate proportion of resemblance or difference among sequence of betasatellites. Soby SDTv1.2 I did SDT analysis that gives exact identity percentage among sequences of CLCuB. SDTv1.2 allows virus sequences classification based on pairwise sequence identity. It requires a FASTA file as input of aligned or unaligned sequences of DNA or protein and aligns every unique pair of sequences and determines pairwise similarity scores. We gave sequence in the form of FASTA file in SDTv1.2 software. Sequence identity percentage in column was obtained. Pairwise contrast among betasatellite sequences from countries like PAK, IN, CN, Burkina Faso, Niger, Sudan and UAE performed to locate highest and lowest similarity proportion figure.

Then sequence contained sequence similarity proportion lower than 90% Nucleotide BLAST search was completed to examine the resemblance and dissimilarity among complete betasatellite genome sequence. The BLAST tool permits an investigator to evaluate a query sequence by means of sequence present in database and recognize sequences which look like our query sequence. Usually, betasatellite molecule did not permit a large amount of mutation take place in its genetic material. Consequently, less varieties present in betasatellite molecule. Betasatellite Species selection was 78%. Those contained above 78% similarity fraction separated from similar specie. In our study all betasatellites molecules with 78% similarity fraction were present. We can say that all were from the similar specie.

Acknowledgements

I feel highly privileged to pay cordial gratitude to my reverend and zealot supervisor Dr. Muhammad Mubin Assistant Professor, CABB, UAF for his keen personal interest, dynamic supervision. I am also thankful to the members of my supervisory committee, Dr. M. Shah Nwaz ul Rehman Assistant Professor CABB, UAF and Dr. Tahira Iqbal, Department of Biochemistry, UAF for their constant support and help throughout the course of this study.

Fig 1 Monopartite and bipartiteBegomovirus

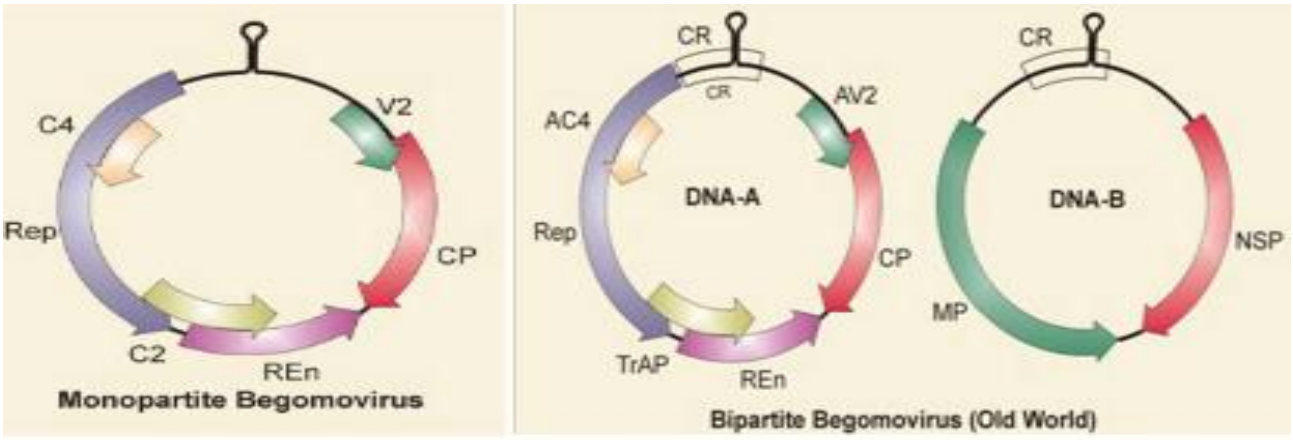

Fig 2: CLCUD disease symptoms on cotton leaf

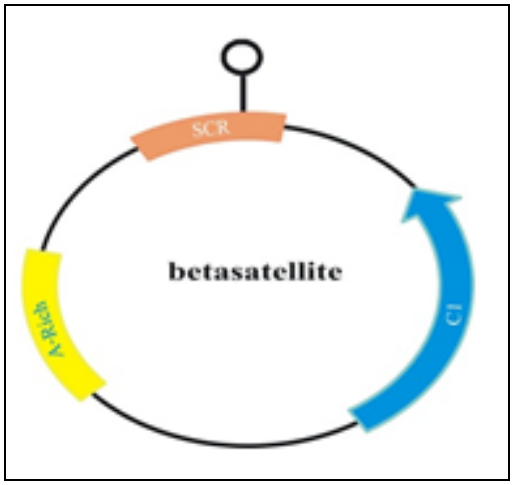

Fig 3: Monopartite begomovirus associated betasatellite molecule

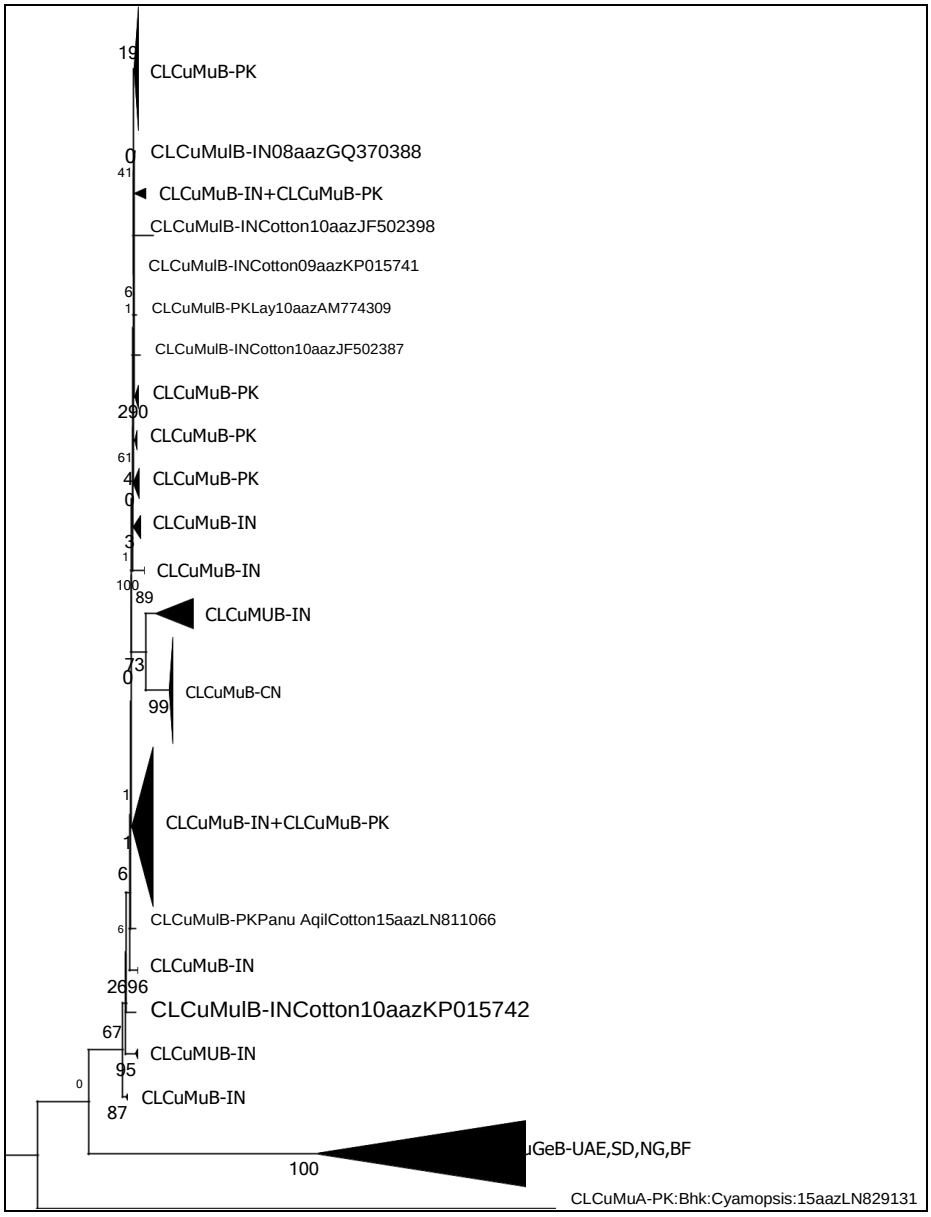

Fig 4: Phylogenetic Tree Base Upon Betasatellite Through Clustal W Available In Genbank Database.

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