Review Article

A Critical Review of Whitefly (*Bemisia tabaci* gennadius) Cryptic Species Associated with the Cotton Leaf Curl Disease

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Abstract | Cotton Leaf Curl Disease (CLCuD) being caused by begomoviruses exclusively transmitted by *Bemisia tabaci* cryptic species comprised of genetic variants which could be differentiated by mitochondrial cytochrome oxidase I (mtCOI-3′) gene. Most numerous cryptic species is Asia II-1recorded all over in Pakistan whereas North Africa-Middle East (NAFME) previously known to be found in the Sindh province but now also reported in the Punjab province. This study revealed that overall diversity of whitefly cryptic species in Pakistan is higher than previous studies. Some whitefly cryptic species cause direct damage to the crops by feeding and indirectly by transmission of plant viruses that reduce the crop yield and quality. Cotton leaf curl virus (CLCuV) management is difficult due to multiple virulent strains and higher recombination rate that is a serious threat to the cotton crop of the last two decades in Pakistan. Alternate host plants belonged to vegetables, weeds, and mixed farming practices helped the evolution of new viral and whitefly species. The CLCuD develops resistant cultivars against CLCuV and screening of new host resistance forms. Applications of DNA markers to induce resistance into the cultivars and editing of genome are some of the good practices contributing to suppress this disease. The study is helpful in understanding population dynamics of whitefly cryptic species interaction with the begomoviruses and alternative host plants which is imperative to devise effective management strategies for CLCuD and its vector whitefly.

Received | August 16, 2022; **Accepted** | December 21, 2022; **Published** | February 16, 2023
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Citation | Afzal, M., S. Saeed, H. Riaz, M. Ishtiaq, M.H. Rahman. 2023. A critical review of whitefly (*Bemisia tabaci* gennadius) cryptic species associated with the cotton leaf curl disease. *Journal of Innovative Sciences,* 9(1): 24-43.
DOI | https://dx.doi.org/10.17582/journal.jis/2023/9.1.24.43
Keywords | Whitefly, Cryptic Species, Begomoviruses, CLCuV, Host plants

1. Introduction

Cotton (*Gossypium hirsutum* L.) is a most outstanding kharif cash crop, fiber producing crop in Pakistan and important oil seed crop in the world (Farooq *et al.*, 2014). It is cultivated on the largest area as compared to the other crops. Pakistan ranked 4th in cotton growing countries world wide and cotton contributes to the export revenue (Ashraf *et al.*, 2013). Major cotton products are being made in Pakistan and stands at 3rd position as exporter of raw cotton in the world market (Oliveira *et al.*, 2001). Cotton related products like as lint, value addition of Agriculture products, 80% national oil

seed production (Rehman et al., 2019). It contributes around 0.6 % to gross domestic product (GDP) and 2.4 % of the value addition in agriculture. The expected area of cotton cultivation is declined 6.8 percent which, is 1937 thousand hectares during 2021-22 as compared 2079 thousand hectares during the last year due to biotic factors including insect pest and diseases (Anonymous, 2022).

CLCuD is a viral infection to the cotton crop and other susceptible alternative hosts. CLCuD is a major limitation for cotton crop which, cause yield losses in Pakistan (Zubair *et al*., 2017). CLCuD first was observed in the Tiba Sultanpur, Multan region in 1967 but was not given significant importance (Hussain and Ali, 1975). In 1989 disease was observed on a new variety S12 cultivated at Khokhran Multan and spread into other areas of Pakistan. First epidemic is observed because of the Multan strain of *Begomovirus* genus and caused $ 5 Billion loss to the national economy (Briddon *et al*., 2000). The attack of this disease was reduced by use of resistant varieties and the cotton resistance was unfortunately broken in 2001. CLCuD appeared in all resistant varieties susceptible to Burewala strain and it was the start of the second epidemic in all cotton growing districts of Pakistan (Mansoor *et al*., 2003). The second epidemic lasted up to 2013 with an annual loss of $ 10 million to the economy (Sattar *et al*., 2015). Therefore, this study is the need of time to identify the whitefly cryptic species and host plants contributing in the dissemination of begomoviruses to cotton crop in order to adopt effective management strategies to mitigate cotton leaf curl disease.

Whitefly is a polyphagous sucking pest of host plant species belonging to crops, vegetables, horticultural plants, fruits, forest trees and weeds in tropics and sub-tropics (Liu, 2007; Zang et al., 2007; Wan *et al*., 2009). Whitefly becomes a serious pest of short-lived herbaceous hosts and dicotyledonous crops of Agricultural and ornamental in the last 2 decades (Ahmed *et al*., 2010). Whitefly damages the crops by sucking the cell sap and excreting honey dew which, develops sooty mold which, ultimately reduce the plants photosynthesis, growth and also transmit 38 plant diseases (Hussain *et al*., 1991).

Whitefly is a species complex which, distributed worldwide. NAFME (B biotypes) and North Africa Mediterranean Middle East (NAFMED) (Q biotypes) in the past three decades spread through trade of ornamental plants (Chu and Zhang *et al*., 2005; Martinez-Carrillo *et al*., 2007). Three species of whitefly (Asia-1, Asia II-1 and NAFME) have been identified in the cotton growing regions of Pakistan. Asia II-1 identified in the Punjab while Asia-I has been observed both in the Sindh and Punjab province. North Africa-Middle East (NAFME) has been observed only in the Sindh province (Ahmed *et al*., 2011). Six cryptic species of whitefly (Asia II-1, NAFME, Asia-1, Asia II-7, Asia II-5 and Asia II-8) were observed in Pakistan that cause serious threat to the crops production. Asia II-1 widespread in all over the country, however NAFME confined to the Sindh province later shifted to the Punjab provinces (Masood et al., 2017; Islam *et al*., 2017). Whitefly population in Pakistan is more likely home-grown and this population has genetic differences from invasive species of whitefly (De Barro *et al*., 2011). Mitochondrial gene cytochrome oxidase I (COI) is used for identification of whitefly species cryptic species. Fully developed COI sequence for identification of species is cost effective and a solution for identification of species (Hebert *et al*., 2003). India and Pakistan shared dominant haplotypes of *Bemisia tabaci* commonly which, concerned with the transmission of CLCuV. *Bemisia tabaci* appeared worldwide in 1980 by invasion of B biotype. It is a vector of many begomoviruses which, created management problems in many countries.

Globally 40 diseases caused by Gemini viruses in vegetables and fiber crops (Fauquet and Fargette, 1990). Sixty-one alternative host plants of CLCuV were identified by (Mirza *et al*., 1994). The begomoviruses (family Geminiviridae; genus *Begomovirus*), are little DNA viruses transmitted by insect *Bemisia tabaci* into vegetables, crops and ornamental plants. Begomoviruses spread diseases which are destructive and it is a major limitation for growing crops worldwide. The rates at which, new begomoviruses are appearing to create a serious challenge for the growers in tropics and subtropics areas (Khan *et al*., 2012). Breakdown of resistance in cotton crop against the transmission of CLCuD created hurdles to the cotton growers in the world. CLCuD is appearing in all major cotton growing regions of Pakistan and it may be the appearance of the third epidemic and whitefly is a major vector of begomoviruses transmission. Therefore, a dire need is required for the management of CLCuD and its

vector whitefly.

2. Whiteflies

Whitefly is a serious sucking pest belongs to order Hemiptera (family Aleyrodidae) in tropical and subtropical regions of Central America, North America, South America, China, Central Africa, South Africa, South East Asia, Pakistan and India that alienated to more than 1500 species. All species of Aleyrodidae have character of sexual dimorphism, different size of male and female, ventral abdominal plates, number of antennae (Hodges et al., 2005) and whole body covering with wax like powder (*Bemisia tabaci* and *Trialleurodes vaporariorum* species body completely covering with white wax powder) showed dust like colour appearance which, derive from Greek word 'aleurodes' means flour (Jaeger, 1944). The Aleyrodidae family lays eggs in groups of connecting sacs or parallel arcs onto the leaves of host plants (Richards, 1977). Males develop from unfertilized haploid eggs in the process of arrhenotokous while females develop from fertilized eggs. The female life span is comparatively longer than the male and can also mate with male offspring to produce female (Blackman et al., 1998). The development stages from egg to adult have divided into four larval instars and first instar known as crawler move on the leave to find smaller veins on the same leave of its emergence and settle down on one position for feed while the second and third instar are oval translucent remain sessile (Gill, 1990).

There are four major species in the family Aleyrodidae that have impact on agriculture economy i.e., (1) *Aleurocanthus woglumi,* (2) *Aleyrodes proletella,* (3) *Trialeurodes vaporariorum,* (4) *Bemisia tabaci. Aleurocanthus woglumi* commonly known as blackfly or citrus blackfly because of its greyish-blue colour. Citrus blackfly is mostly found on more than 300 alternate host plant species but citrus plants like orange, lemon and pomelo are heavily infested (University of Florida). *Aleyrodes proletella* is a species of whitefly from family Aleyrodidae, commonly known as cabbage whitefly, which, infested many species of plants families *Brassicaceae, Papaveraceae, Apiaceae, Fabiaceae and Euforbiaceae*. They have 4 gray spots on white forewing covered with wax. The wings are white due to a powdery wax. It is an economic parasitic of plant species because it not a vector of plant viruses which, spread from America, Europe, Australia and East Asia (De Barro et al., 1997). *Trialeurodes vaporariorum* commonly known as greenhouse whitefly (GHWF) or greenhouse whitefly, which, was first reported as *Trialeurodes vaporariorum* (Mound et al., 1978). Like *Bemisia tabaci* it is reported from around the world and it has damaging effects on the vegetables, fruits and ornamental crops like as *Bemisia tabaci* but are causing devastating effects on greenhouse crops. *Trialeurodes vaporariorum* thrives on various plant species families particularly i.e. *Malvaceae, Asteraceae, Solanaceae and Cucurbitaceae.* The viruses of genus *Crinivirus* are transmitted by both *Trialeurodes* and *Bemisia tabaci.* The whitefly *Bemisia tabci* is a complex of forty-six morphological indistinguishable cryptic species and are important pests of industrial and horticultural crops (Rehman et al., 2021).

2.1 Bemisia tabaci (sweet potato whitefly)

Bemisia tabaci, a well-known sweet potato whitefly which, is one of devastating phloem feeding pest around the globe. Sweet potato whitefly thrives around the world in tropical areas and subtropical areas while less predominately in temperate habitats and more than 600 alternate host plants of *Bemisia tabaci* have been found around the world (Greenberg et al., 2000; Oliveira et al., 2001). It belongs to hemiptera order that also include very notorious insect pest like as aphid, psyllids, mealybug, plant hopper, leaf hopper and cicadas which, uses their stylet for sucking of water and nutrient from plant vascular tissues (Martin et al., 1987). Adult whiteflies have paired wings, both male and female can fly. Newly hatched one female can fly up to seventeen minutes while one male can fly up to thirty minutes on one day. Adult is 2-3 mm in length which, covered with white wax powder. It has forewing and hind wings which, are equally in size that helps to quickly escape from danger (Figure 1). Development stages of whitefly intermediate metamorphosis and also including adults it has 5 instars (Phase during development stages between two moulting). In which, 1st instar (mobile phase is crawler) and 5th instar are active can move while 2nd, 3rd and 4th instar stages are sessile (Byrne et al., 1991). *Bemisia tabaci* reproduce by Arrhenotoky which, is type of parthenogenesis in which, male develop from mated and unmated female unfertilized eggs (haploid) while female developed from fertilized eggs (diploid) which, develop into female. Both adults and nymphs of the species can kill in cold temperatures while in suitable environment condition a female

can give up to 50 to 400 eggs (0.10-0.25 mm) on the underside of the leaves during whole life and produce up to fifteen generation in a one year (Blackman et al., 1998; Johnson et al., 2005). Whitefly can produce a sticky substance called honeydew on which sooty molds develop as a secondary infection which almost stops absorption of light by the leaves. It resulted in poor quality of plants, lower growth, and lower yield. Sweet potato whitefly also notorious vector of plant diseases which, transmitted geminiviruses like *Tomato yellow leaf curl virus*, *African cassava mosaic virus* and *Lettuce infectious yellows virus* from years to many continents (Brown *et al*., 1995).

Figure 1: *Bemisia tabaci* (Silver whitefly) adult image generated from https://www.biobestgroup.com/en/biobest/pests-and-diseases/tobacco-whitefly-4973/

3. Study of *Bemisia tabaci* Species Complex

Bemisia tabaci consist of complex cryptic species previously known as biotypes of whitefly (Brown, 2010). Whitefly complex species morphological identification based on nymphs and adult whitefly was identified by the DNA barcoding. For each species primers were developed for recognition of three invasive *Bemisia tabaci* populations obtained from Florida. Three species of whitefly are placed in the phylogenetic tree. Primers were developed for each species to assist the identification (Dickey et al., 2015). The *Bemisia tabaci* biotypes are identified by use of combined primers to amplify the sequence of *mtCOI* more fastly compared to the previous studies. New primers were recognized for amplification at 748bp for 800bp fragment. Additionally, *mtCOI* PCR primers were prepared for fast identification of species complex. Furthermore, these primers were for use of fast PCR, electrophoretic techniques and the *Bemisia tabaci* biotypes can be distinguished within three hours up to ninety-six samples were analyzed once a time (Shatters et al., 2009).

Biotypes B, Q and Cv of whitefly identified on morphological and molecular basis. These biotypes have been differed in bright DNA bands. The genetic identities between the Cv, Q and B was 86.0%, 94.7% and 85.8%, respectively. Q and B biotypes showed phylogenetic affiliation more closely than B and Cv as well as between Q and Cv *Bemisia tabaci* biotypes (Qiu et al., 2009). The whiteflies biotypes were identified by using the gene sequence of *mtCOI*. Analyses indicate eastern Asia harbors three biotypes Nauru, An and B while Taiwan harbors An and B biotypes. However, A biotype was observed as a new one in China. Studies showed that human trade activities led to the invasion of B biotype in Taiwan and was new invasion shown by phylogenetic tree. The biotype B was widely distributed in Taiwan. Human trade and natural movements distributed the An and Nauru biotypes in Taiwan. This study provided the management of *Bemisia tabaci* and relationship between *Bemisia tabaci* cryptic species (Hsieh *et al*., 2006). There was no genetic variation in the population collected from cucumber, eggplant and tomato. The analysis of 42 *mtCOI* sequences was done and compared with the local available database which, proves that the Iranian *Bemisia tabaci* was closely related to the biotype B at nucleotide. The B biotype is dominated in Iran and vital for the management practices which, widely differ in alternative host range, insecticides resistance, interaction with the viruses and some biological characters (Shoorcheh et al., 2008). Whitefly (*Bemisia tabaci*) considered a cryptic species and consists of twenty-four morphologically different species complex. Internationally it become prominent in the 1980s due to the widely distribution of B biotypes in the world (De Barro and Khan, 2007; De Barro *et al*., 2011).

Whitefly, *B. tabaci* complex comprises of many undescribed cryptic species, however there is disagreement existed, how many cryptic species could be accepted. Phylogenetic analysis of 10 whitefly species based on whole genome sequencing of 2184 orthologs was obtained. The automatic barcode gap discovery (ABGD) analysis suggested that at least five cryptic species recognized from nuclear orthologs and mtCOI data analysis. Although mtCOI divergences are high among the cryptic species members while

nuclear divergences were comparatively lower. Whole genome sequencing provides a significant means to support in upcoming research studies of this complex group. However, many whitefly populations remained unknown *B. tabaci* cryptic species due to limited studies. Therefore, careful studies should be recommended for official taxonomic description of these cryptic species to minimize the confusion in the recognition of species complex until consensus is developed for considering the present and future evidence (de Moya *et al.*, 2019). CLCuV not only but *Bemisia tabaci* is also becoming a threat to the heavily feeding on the cotton and vegetables crops in the field. Common methods are using to control the whitefly infestation such as pesticides has becoming failed due to development of resistance in whiteflies against the commercial used pesticides like as Triazophos, Profenofos, Methyl, Butacarboxim and Parathion (Ahmad *et al.*, 2010). This character related to the mitotypes prevails in Pakistan.

3.1 Whitefly cryptic species complex in Pakistan

Whiteflies are a species complex with at least forty morphological indistinguishable but genetically different species which, distributed worldwide (Firdaus et al., 2013; Tang et al., 2019). Middle East-Asia Minor 1 (B biotypes) and NAFMED (Q biotypes) in the past three decades spread through trade of ornamental plants (Chu *et al.*, 2005; Martinez-Carrillo and Brown, 2007). The whitefly (*Bemisia tabaci*) complex species identified on the basis of gene sequence of *mtCOI*-3 and recent research fund six cryptic species complex present in Pakistan i.e., Asia-II-1, Asia-1, NAFME, Asia-5, Asia-7, and Asia-8 were reported. Asia II-1 widespread in all over the country and NAFME mentioned as B biotype confined to Sindh province later shifted to the Punjab provinces while Asia-II-8 was also found first time in the Pakistan (Masood et al., 2017; Islam *et al.*, 2018). Asia II-1 was observed in the Punjab previously but now prevails all over in Pakistan which, created serious problems for management of cotton growing regions in Pakistan. Previously reported that 7 biotypes of *Bemisia tabaci* reported in Pakistan which, are Asia II-1, Asia I, Asia II-7, Asia II-5, Asia II-3, NAFME and Pakistan among these biotypes most predominant is Asia II-1 (Ashfaq *et al.*, 2014). Whitefly population in Pakistan is more likely home-grown and this population has genetic differences from invasive species of whitefly (De Barro *et al.*, 2011). *Bemisia tabaci* samples first time collected from north western areas of the Punjab which, indicate that there is great genetic variability and biodiversity present in *Bemisia tabaci* in Pakistan as compared to past results (Islam *et al.*, 2017). Three whitefly cryptic species (Asia II-1, Asia-1 and NAFME) have been identified in the cotton zones of Pakistan. Asia-II-1 were observed in the Punjab province on cotton crops, similarly Asia-1 biotypes were observed from Sindh and Punjab province while NAFME biotypes were identified from Sindh province only (Ahmed *et al.*, 2011). Asia-II-1 acts as a vector of CLCuV as compared with NAFME which, has fewer incidences. NAFMED biotypes were not found in Pakistan. Identification of genetic variance is necessary for the management of pest species. The association of vector diversity with CLCuV transmission to the field crops was identified (Ahmed *et al.*, 2010). Recent research has conducted to check the resistance against the whitefly by the use of transgenic tobacco that expresses dsRNA against necessary whitefly genes has shown encouraging results in Pakistan. It has revealed a powerful tool used in the future as transgenic cotton against whitefly management (Malik et al., 2016; Raza et al., 2016).

3.2 Whitefly cryptic species identification by COI genes amplification

Bemisia tabaci is an important pest of Agricultural crops and vector of plant viruses for insecticides resistance, decrease in natural enemies and increased practice of monoculture in the world. Members within the species complex have discussed to as race, strain, biotypes, haplotypes and mitotypes (Brown *et al.*, 1995; Brown, 2010; Gill and Brown, 2009). Populations and molecular studies have confirmed the molecular divergence among *B. tabaci* species worldwide (Brown, 2010; Gill and Brown, 2009; Dinsdale *et al.*, 2010; De Barro *et al.*, 2011). Mitochondrial 16 S (550 bp) and Mitochondrial COI (700 bp) were used as markers for reconstruction of phylogeography for biotypes of *B. tabaci*. 16 S sequences showed less divergence than mtCOI sequences. Phylogenetic analysis determined by maximum-likelihood, neighbour-joining and maximum parsimony methods created almost similar phylogenetic trees on geographical origins based. The data of 16S and COI sequences showed that B biotype emerged from Asia, Africa, Europe (old world) (Frohlich et al., 1999). Mitochondrial gene cytochrome oxidase I (COI) is used for identification of whitefly species and mitotypes. The model COI is used for identification of single specimen from 200 specimens of Lepidopteran and 100% successfully

specimens were identified. Fully developed COI sequence for identification of species is cost effective and a solution for identification of species (Hebert *et al*., 2003). The DNA barcodes method is very effective to identify the species that were difficult to differentiate morphologically (Hebert *et al*., 2003), including whitefly which, is very difficult to distinguish morphologically and few characters in species for identification (Martin, 1987), within species it has great diversity, therefore particular stage is required for identification as 4th instar nymph (Hodges and Evans, 2005). Identification of species by using DNA barcodes that are used in plants, bacteria and animals is an excellent method (Hollingsworth et al., 2009). The gene 3' end for primer easily magnifies DNA in whitefly which, has many species and biotypes as compared to the 5′ end which, is unused as barcode (Shatters et al., 2009). Biotypes of *Bemisia tabaci* are identified by DNA barcodes. India and Pakistan commonly shared dominant haplotypes of *Bemisia tabaci* which, concerned with the transmission of CLCuV. *Bemisia tabaci* appears worldwide in 1980 by invasion of B biotype and 24 morphological indistinguishable different species identified with up to 3.5% genetic divergence (De Barro *et al*., 2011). Number of sequences were reset up to 4% and cryptic species of whitefly were increased to 31 (Lee *et al*., 2013). Forty-five, cryptic species of *B. tabaci* were identified upto 4% genetic divergence globally. It is a vector of many begomoviruses which, created management problems in many countries (Mugerwa *et al*., 2018).

4. Whitefly (*Bemisia tabaci*) role as plants pest

Whitefly is a polyphagous sucking pest which, damages the plants. It affects directly by secreting the honey dew and indirectly damage caused by sooty mold growth on the plant leaves, deteriorates the quality of cotton lint of crops (Byrne et al., 1991); thus reduces the absorption of lights which, is necessary for photosynthesis. First direct damage mechanism was reported in Hawaii (Costa et al., 1993). Most Destructive character of *Bemisia tabaci* is to disseminate large number of plant viruses of genera *Crinivirus* (Closteroviridae), *Carlavirus* (Betaflexiviridae), *Toradovirus* (Secoviridae), *Ipomovirus* (Potyviridae) and largest group of viruses of genera *Begomovirus* (Geminiviridae) which, has more than 300 different reported viruses' species (Navas-Castillo *et al*., 2011).

4.1 Alternate host pant of whiteflies

Alternate host plant species of whitefly belonging to crops, vegetables, horticultural plants, fruits, forest trees and weeds in the tropical and subtropical areas around the world (Liu, 2007; Martin and Mound, 2007; Zang et al., 2007; Wan *et al*., 2009). The alternative hosts and natural enemies were recorded from the different parts of China during survey of 361 alternate host plants recorded from 89 families and preferred host plants families for whitefly were Compositae, Cruciferae, Cucurbitaceae, Solanaceae and Leguminosae, therefore it causes severe damage in these crops due to development of high pest population (Li *et al*., 2011). A few 229 alternate host plants of *Bemisia tabaci* including field crops, vegetables crops, fruits, ornamental crops, forest trees and common weeds have been identified in Pakistan (Attique *et al*., 2003). Whitefly became a serious insect pest of short duration herbaceous hosts, dicotyledonous crops of Agricultural and ornamental in the last 2 decades (Ahmed *et al*., 2010). Previous records indicated that *Bemisia tabaci* collectively colonize 900 host plant species including field crops, vegetables, fruits, ornamental crops, common weeds and forest trees (Perring, 2001, Berry *et al*., 2004). *Gossypium hirsutum* L., among these alternate host plants is an important cash crop in Pakistan. The whitefly biotype Asia-1 development rate was slow on vegetables as compare to the cotton crops whereas NAFME development on different host plants was not significantly different. NAFME population on vegetables and cotton crops were similar while observed more on cotton crop as compared to the vegetable's crops. Asia-1 was best on cotton crop whereas development of NAFME on the entire alternative host plants were similar (Ahmed *et al*., 2014). *Bemisia tabaci* samples were collected from cultivated areas of Rawalpindi, Islamabad and new biotype Asia-II-7 was observed. Previously the biotypes sequences were observed in southern and central Punjab and samples were collected from alternative host plants and cotton crop. The sequences generated by this analysis were arranged into two groups such as Asia-II-1and Asia-II-7. The Asia-II-7 adopted horticultural plants as compared to the vegetable crops. Whitefly samples were collected from vegetables and ornamentals plants however previously indicated more concentration on fiber and food crops instead of horticultural crops (Shah *et al*., 2013).

Bemisia tabaci infest all the cotton varieties but the difference in the infestation of varieties is due to preference of feeding. Whitefly, CLCuD and

environmental factors inter relationship was used for the management of whitefly infestation problem more efficiently. These credible results were used for the forecast of CLCuD and *Bamisia tabaci* epidemiology inclination (Perveen *et al.*, 2010). The whitefly (*Bemisia tabaci*) life cycle duration was 19.8, 21.2 and 22 days observed on soybean, tomato and collard, respectively in laboratory at temperature 25 ºC, RH 70 ± 10%- and 14-hours photoperiod. The hatching of eggs was less on soyabean and tomato as compared to collard crop (Takahashi *et al.*, 2008). The infestation started from the month of March and the highest population of whitefly observed during the month of July. The whitefly showed positive correlation with temperature while negative with relative humidity and these ecological factors were important for its effective management (Selvaraj *et al.*, 2012). Whitefly caused damage through transfer of different types viruses including, single stranded DNA viruses of *Begomovirus* genera into different crops which, caused reduction in crops production and quality (Islam *et al.*, 2017; Navas-Castillo et al., 2011; Brown *et al.*, 2015).

5. Cotton leaf curl disease

Leaf curl viruses belong to the family Geminiviridae and genus *Begomovirus* caused CLCuD to the cotton crop and alternative host plants (Akhtar *et al.*, 2005). Cotton yield is affected by many factors but mainly production is reduced by the diseases, insect pest attack including environmental factors however the most devastating factor is CLCuD that is the major constraint of cotton crop in Pakistan which, cause yield losses (Zubair *et al.*, 2017). Nigeria was found to be attacked by CLCuD in 2012 (Farquarson, 1912). Family Geminiviridae have different groups of single stranded DNA vector transmitted viruses mainly found in the warm and tropical regions of Pakistan (Moffat, 1999; Amudha *et al.*, 2011). Begomoviruses including important strains are cotton leaf curl Burewala virus (CLCuBuV), cotton leaf curl Multan virus (CLCuMuV), cotton leaf curl Kokhran virus (CLCuKoV) which are responsible for CLCV caused huge production losses (Hina *et al.*, 2012; Fauquet *et al.*, 2008; Stanley, 2005). Monopartite begomoviruses require betasatellites that cause mainly cotton leaf curl disease (Briddon *et al.*, 2001; Mansoor *et al.*, 2003).

5.1 Genome organization basis of begomoviruses and associated satellites

Begomoviruses are isolated into monopartite and bipartite groups on the basis of genomic organization. Bipartite begomoviruses are composed of 2 DNA molecules i.e., DNA-A and DNA-B genome, each weighing about 2600 whereas monopartite begomoviruses having single DNA molecule of weighing near about 2800 nt (Fauquet *et al.*, 2008). Monopartite bogomoviruses have circular DNA of weight near about half of the genomic one called alpha satellite or beta satellite. Betasatellite caused pathogenicity in the crops while there is no clear function is known for the alphasatellite regarding the development of symptoms (Mansoor *et al.*, 1999). The beta satellite consist of major three features adenine rich sequence region, satellite conserved region which, help in Gemini viruses replication and a single βC1 gene (Hanley bowdoine *et al.*, 1999). DNA A Component in the Begomoviruses responsible for DNA multiplication, transmission through vectors genetic expression control while DNA B component encoding 2 genes for help of viral movement in plants (Rybicki *et al.*, 2000).

5.2 Structure and function of geminiviruses protein

Begomoviruses are twin quasi-icosahedral virions which could be monopartite or bipartite depending on 1 or 2 genomes (DNA-A and DNA-B) (Zerbini *et al.*, 2017). Bipartite has DNA-A and DNA-B circles of the same size while monopartite has a single genomic circle along with alphasatellite and betasatellite. Betasatellite is responsible for the pathogenicity development in plants (Zhou, 2013). These viruses' species contain of ~2.7-2.8 kb single genomes of monopartite (Consist one genome) and 2.9 kb genomes of bipartite (Consist two genomes) (Figure 2) and disseminating destructive disease in the cash crops like as cotton and vegetables (Rojas *et al.*, 2005). Monopartite of begomoviruses genome is a single stranded circular DNA (ssDNA) ranges from 2.57 to 2.9 kb that has 6 protein encoded regions i.e., replication associated protein (Rep), coat protein (CP), replication enhancer protein (REn) and transcriptional activator protein (TrAP) and C_4 protein while bipartite begomoviruses have two single stranded (ssDNA) genome known as DNA A (like a monopartite genome) and DNA B both of have same size from 2.6 to 2.8 kb. Bipartite begomoviruses DNA-B encoded with movement protein (MP) in complementary sense and nuclear shuttle protein (NSP) in virion sense. Both circular DNA consist

of a highly similar sequence of 200 nt, also known as common region (CR) that is found in the enteric region (IR) (Melgarejo *et al*., 2013).

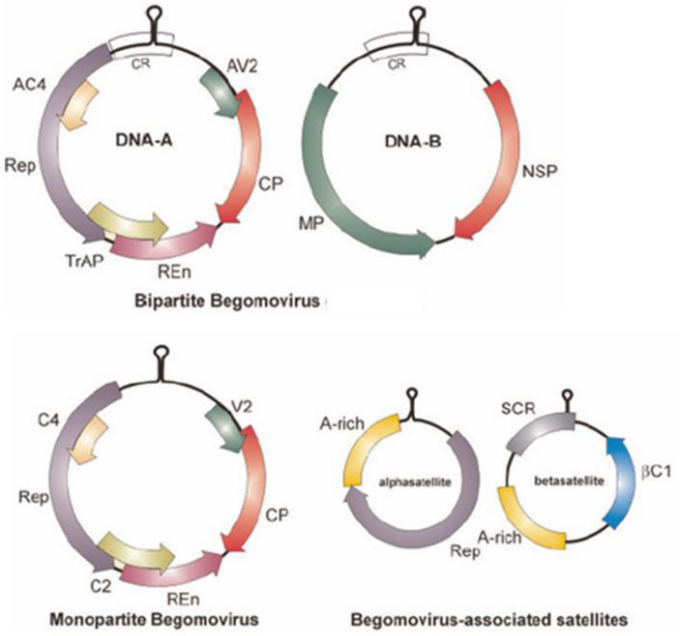

Figure 2: Monopartite (Consist single genome) associated satellite and Bipartite (Consist of two genomes DNA-A and DAN-B) Begomoviruses images were generated from https://www.researchgate.net/figure/Genome-organization-of-Begomoviruses-Begomoviruses-have-either-bipartite-or-monopartite_fig1_234157089

5.3 Study of cotton leaf curl virus (CLCuV) transmission

Geminivirus (2700–3000 nt) generally transmitted through insect pest with more parts of single stranded circular DNA (Moffat, 1999). Geminiviruses mainly spread and infect the field crops mostly due to the frail quarantine measures, poor sanitation and lack of vector control (Gray and Benerjee, 1999). The whitefly sucks the cell sap with viron by stylet and viron passes through the digestive system, haemolymph, then passes into the salivary glands and then it injects into the plant phloem. Virus translocated, replicated in the whitefly, as well as the autophagic system of whitefly activated which, destructs and reduces the replication of viruses. The role of protein cost, different sites required for the begomoviruses to complete translocation, accumulation and immune system of whitefly that destruct the virus replication (Czosnek *et al*., 2017). There are 5 genera of plant viruses within the family, Geminiviridae and mainly genus *Begomovirus* that is transmitted by *Bemisia tabaci* species complex. *Begomovirus* and *Crinivirus* are well known for transmission in contrast to the genera *Torradovirus, Ipomovirus* and *Carlavirus* which, are very little known for the transmission of viruses (Polston *et al*., 2014).

Begomoviruses are little DNA viruses that have monopartite and bipartite genomes and undoubtedly monopartite begomoviruses are transmitted by whitefly vector. Mostly crops are infected by *Begomovirus*, *Topocuvirus, Curtovirus* and *Mastrevirus* viruses and genus *Begomovirus* indeed most variable, economically devastating and geographically extremely disseminated genus among viruses in crops (Mansoor *et al*., 2003b; Feehan *et al*., 2013). *Begomovirus* and *Crinivirus* transmission were well studied with specification to the *Bemisia tabaci* and give the relationship between plant virus transmission, etiology and whitefly species. This was the first effort to integrate the virus transmission data record with new observed whitefly species. It is clear that past data for transmission is difficult to match with the framework of current species due to lack of information in the *Bemisia tabaci* diversity (Polston *et al*., 2013). *Cassava mosaic virus* (CMV) and *Sweet potato mosaic virus* (SPMV) in India studied for its transmission on crops. The sweet potato strain was not developed on cassava and the cassava strain was not developed on sweet potato. The common alternative host plants for the both biotypes were eggplant and tobacco. The sweet potato whitefly did not transmit CMV from diseased to healthy cassava plants however cassava whitefly transferred the CMV (Lisha *et al*., 2003). The DNA and RNA viruses in the *Bemisia tabaci* in Florada were investigated and found that there was addition of viruses which, was not identified in the previous study. The metagenomic analyses were used for extraction of DNA from *Bemisia tabaci* which, showed the presence of *Begomovirus*, in addition to RNA viruses but diversity of these viruses is not completely detected. *Cowpea mild mottle virus* (CPMMV) Florida was detected in the whitefly which, indicate the more study is needed for classification of *carlavirus* in whitefly. The whitefly vector was dominant to carry begomoviruses as compared to RNA viruses which, were carried in fewer amounts (Rosario *et al*., 2014). *Bemisia tabaci* is a major vector of CLCuV that should be controlled to manage the CLCuV disease. Whitefly transmitted begomoviruses cause CLCuV disease in the cotton plants. In Pakistan three strains of CLCuV have been identified i.e., Multan strain, Khokhran strain and Burewala strain but unfortunately no resistant cotton

variety have been developed against Burewala strain which, caused 30% decline in the yield of cotton crop in Pakistan each year (Feehan et al., 2013).

5.4 Cotton leaf curl disease (CLCuD) in Pakistan

CLCuD 1st time was observed in Pakistan at Tiba Sultanpur, Multan region in 1967 but was not given importance (Hussain and Ali, 1975). In 1989 disease was observed on a new variety S12 cultivated at Khokhran Multan, and spread into other areas of Pakistan. Whitefly transfer monopartite begomoviruses into cotton plants and emerge as first epidemic during 1990 as *Cotton leaf curl Multan virus, Papya leaf curl virus, Cotton leaf curl Alabad virus, Khokhran leaf curl virus* (Mansoor *et al.*, 2003; Briddon and Markham, 2000) which, could cause almost 50-60 % losses in the cotton. First epidemic started early because of Multan strain of *Begomovirus* which, caused $ 5 Billion loss to national economy (Briddon and Markham, 2000). 97,580 ha of cotton crop area was affected due to CLCuD which, caused 9.1 million bales production loss during 1992-93 and further 7.9 million bales production loss recorded during 1994-95 in the Punjab province of Pakistan (Anonymous, 1997; Aslam and Gillani, 2000). The incidence of disease level was 32.4% in 1997 and disease level gradually started decreasing due to development of resistant genotypes of cotton against this disease. The disease incidence was 16.5% during the year of 1998 and continuously decreased in the year 1999 with disease level of 14.3% and during 2001 it reached a minimum level of about 1.8%. The attack of this disease was reduced by introduction of resistant cotton varieties and the resistance was unfortunately broken in 2001 and CLCuD appeared in all resistant varieties because of emergence of Burewala strain (Amrao *et al.*, 2010). It appears again as the second epidemic during 2000-2001 as recombinant (*Cotton leaf curl khokhran virus Burewala*) cause resistant breaking in cotton varieties. Burewala strain of CLCuD becomes a continuing threat to the cotton yield in Pakistan. CLCuBuV strain is formed by the recombination of CLCuV Khokhran strain and CLCuV Multan strain (Hina *et al.*, 2012) and no tetraploid genotype of cotton has been observed against the Burewala strain (Iqbal *et al.*, 2014). CLCuMV resistant varieties were sown in the field to break the resistance of Burewala strain (Mansoor *et al.*, 2003). The Pakistan has three major group plant viruses CLCuAV (cotton leaf curl Allahabad virus), CLCuMV (cotton leaf curl Multan virus) and CLCuKV (Cotton leaf curl Khokhran virus) (Simón *et al.*, 2003).

The *Begomovirus*e complex under continuous mutational states and during 2015 first time a bipartite Tomato leaf curl New Dehli virus (ToLCuNDV) was also reported for cause of CLCuD (Zaidi *et al.*, 2015). While during the year of 2004 the disease incidence level reached at 40.7% due to emergence of new cotton leaf curl Burewala strain of cotton leaf curl virus and during 2009 it reached at maximum level of incidence 67.7% due to whitefly vector and with favorable environment conditions for this disease. Small disease level controlled by control of infestation of whitefly vector, using of improved techniques and by using of balanced fertilizer reduced the disease level from 62.7 to 35.8% during the year 2013 (PW and QCP). The second epidemic lasted up to 2013 with an annual loss of $ 10 million to the economy (Sattar *et al.,* 2015). About 30% of yield losses caused by the CLCuD in Pakistan every year (Ashraf *et al.*, 2013). Therefore, the present study is the need of the time to identify the whitefly species and host plants contributing to the dissemination of begomoviruses to cotton crop in order to adopt effective management strategies to mitigate Cotton leaf curl disease. Globally 40 diseases produced by geminiviruses in vegetables and fiber crops (Fauquet and Fargette, 1990). Sixty-one alternative host plants of CLCuV were identified by (Mirza *et al.*, 1994). Six viruses infected the pumpkin which, is one of the favorable alternate host plants of whitefly for its development (Costa *et al.*, 1991). Diseases caused by the begomoviruses are destructive and it is a major limitation for growing crops worldwide.

5.5 Leaf curl disease symptoms and effects in cotton

Cotton leaves showed downward curling, cup-like small leaf formation called leaf enation vein thickening looks two types major and minor vein thickening. Disease starts from the margin of the leaf to inward and thickened veined network formed (Watkins, 1981) (Figure 3). CLCuV infected genotypes chloroplast accumulated tissues gets proliferated and look darker than the healthy one's (Sattar *et al.*, 2013). CLCuD has affected yield of cotton and caused loss in the growth of cotton plant height (40.6%), number of bolls (72.5%), staple length (3.44%), Staple strength (10%), staple elongation and ginning outturn (3.9%) (Ahmed, 1999; Mahmood *et al.*, 1996). In 1994 due to CLCuD, cotton production reduced to 7.9 from 12.4 million bales while cotton production recorded

a maximum 12.4 million bales during 1991 and 1992 (Mahmood *et al.*, 2003). CLCuD were minimized after development of resistant/tolerant varieties against this viral disease and due to the effort of scientists. This production reached to 14.5 million bales during 2004-05 before the start of the second epidemic due to development of new Burewala strain (Amin *et al.*, 2006). Highly devastating cotton on a large scale was recorded due to this new Berewala strain and even tetraploid genotypes were not completely resistant (Iqbal *et al.*, 2014). Unfortunately, resistance was broken in all previously resistant varieties in 2001 that showed CLCuV symptoms and appeared as 2nd epidemic of CLCuD in the cotton growing region in Pakistan (Mansoor *et al.*, 2003; Amrao *et al.*, 2010). The CLCuV complex was rapidly evolving with continuous change to overcome resistance by recombination, mutation and component capture (Sattar *et al.*, 2013). Recently studies identified that ToLCuNDV was associated with CLCuD in Pakistan (Zaidi *et al.*, 2015) and another study identified that CpCDV a *Mastrevirus* associated with development of CLCuD symptoms in cotton crop (Manzoor et al., 2014). CLCuD has captured viruses which may be responsible for breakdown of complete resistant in the cotton resistant varieties that created a serious threat to the cotton crop and industry in the world.

Figure 3: Symptomatic cotton plants.

6. Control strategies for cotton leaf curl disease and its vector

The CLCuD is a major threat to the cotton crop where the whitefly species complex was present. Many short terms and long-term strategies have been developed for the management of whitefly by using conventional and non-conventional methods.

6.1 Short term strategies

A lot of short-term strategies were devised to manage CLCD by minimizing the whitefly population. The early development of whitefly population up to 45 days after sowing (DAS) can be reduced by the seed treatment followed by application of insecticides which, protecting the cotton crop up to 70-90 days after sowing and after this period plants are old which could escape from CLCuV disease. The whitefly can be controlled by using bio pesticides on field crops (Sarwar and Sattar, 2016). Eradication of alternative hosts for whitefly and Cotton leaf curl viruses. Plant health could be improved through nutrition management and application of biological agents (Huseth *et al.*, 2016; Follett, 2017). The management advices of the whitefly in Pakistan are based on threshold level (4-5) whiteflies/leaf regardless of reality a single infected whitefly can transmit CLCuV. Selective application of insecticides should be used for management of whiteflies which, not only save natural enemies and but also decline the resistance development in the whiteflies (Horowitz and Ishaaya, 2014; Roditakis *et al.*, 2017). The CLCuD survives on alternative hosts. Species of crops, vegetables, weeds, ornamental plants and orchards in the fields. Eradication of weeds from cotton crop fields that serve as host plants decreases the chances of disease transmission and it will minimize the sources of inoculum. In Pakistan, cotton farmers grow vegetables, fodders, oil seed crops in neighborhood or as intercropping in the cotton crop and further stress for taking essential control of vector whitefly mitotypes and disease as well. Alternate host plants provide favorable conditions for genetic recombination with the other viruses that result in emergence of new virus strains. Discouraging the cultivation of cotton with surrounding alternate host plants could help to manage whitefly population hence virus transmission will also be reduced. Likewise, avoiding the off-season cultivation of other crops for breaking the whitefly mitotypes lifecycle. Cultural practices and weedicides application should be necessary for significant management of whiteflies and CLCuD development. Likewise, secondary metabolites of plants act as repellent, reported for providing some defense against the whitefly infestation. Highly aromatic plants intercropping like coriander and basil protect the tomato crop from the whitefly infestation (Carvalho *et al.*, 2017). The balanced nutrition can be used to reduce the loss of CLCuD. Potassium application at

recommended doses could enhance the resistance to diseases as it plays role in production of molecular compounds, osmoregulation and sustaining energy gradients. Judicious use of nitrogenous fertilizers is recommended because at high doses it decreases the resistance against diseases. Integrated management of vector and disease could be helpful to enhance the production of cotton crop.

6.2 Long term strategies

Development of resistant varieties through breeding have been recommended for long term management of disease (Rahman *et al.*, 2005).

6.2.1 Leaf curl resistance/tolerance in cotton

The genus, Gossypium belonging to cotton contains fifty domesticated and wild species in which, forty-five are diploid while five are allotetraploid (Fryxell, 1979). Wild species are isolated in 8 genomes (A - G and K) while 4 cultivated species i.e., *Gossypium hirsutum* (tetraploid), *G. barbadense* (tetraploid), *G. herbaceum* (diploid) and *G. arboreum* (diploid). *G. barbadense* and *G. hirsutum* contribute more than 90% of the production of cotton in the world. Two diploid species of cotton of 'A' genome, *G. herbaceous* and *G. arboreum* were found to be free of CLCuV while two tetraploid species of cotton were found to be susceptible of CLCuV. Cotton species *G. hirsutum* has less genetic diversity and is not resistance to the attack of CLCuV while wild diploid species are being resistance and this quality can be used to develop resistance germplasm (Nazeer *et al.*, 2014). Breeders are trying to develop resistant germplasm toward the CLCuV. The cotton cultivated species which, having AD genome is being susceptible toward the CLCuV due to presence of *Begomovirus* and betasatellite (Azhar *et al.*, 2013). Cotton wild species are being a great source of resistance while some cotton's wild species *G. aridum, G. stocksi, G. darwini* and *G. harkensii* are great source of drought tolerance while *G. thurberii* is being a source of frost tolerance (Yik and Birchfield, 1984; Rooney et al., 1991). PCR and hybridization results show that CLCuD viral molecules are not detected in *G. arboreum* and *G. herbaceum*. The PCR detected the presence of *Begomovirus* including betasatellite molecules in species having D genome and also in species having AD genome. The PCR and Southern hybridization techniques were made by RCA techniques for detection of Begomoviruses circular molecules. The RCA results also showed the *Begomovirus* and betasatellite absence in the *G. herbaceum* and *G. arboretum* however CLCuV viral molecules were detected in the species belonging to the D genome (Azhar *et al.*, 2013). The wild cotton species diploids and tetraploids are being utilized in the different hybridization programs due to their resistance characters (Mehetre *et al.*, 2004). Study and survey on large areas showed that the number of *G. arboretum* cotton plants did not detect a single plant which, showing the symptoms of CLCuV disease that means *G. arboretum* have resistant genes against viral molecules (Briddon and Markham, 2000).

6.2.2 Biotechnology and genetic diversity a source against CLCuV

Genetic diversity is a great source of safety against diseases and insect pests (Van Esbroeck *et al.*, 1999). Several use exotic and indigenous germplasm, many different varieties with higher productivity have been developed in Pakistan since independence. Scientists are examining any resistant and highly tolerant species because all prevailing ones are mostly susceptible to Burewala strain. Some cotton varieties are least susceptible so they can be recorded against CLCuV disease that provides allelic variation which, can be used to develop new effective gene combinations (Rana *et al.*, 2005). It's very easy by progress in biotechnological techniques to overcome CLCuV disease by cloning of viruses (Farooq *et al.*, 2011). Molecular markers used against this viral disease by markers gene mapping can be easily done and *Gossypium arboreum* has used for isolation of resistance gene and change into susceptible against CLCuV with the help of genetic engineering and some others biotechnological techniques (Farooq *et al.*, 2011).

6.2.3 Transgenic approaches

Genetic Engineering has been used for multiple approaches to develop resistance against CLCuD causal agents. These approaches used for controlling the disease which, mostly depend on using different small or full-length conserved portion of genomes across several same species (pathogen derived resistance) (Goldbach *et al.*, 2003) and genes from related distantly host or non-host genetic sources used to develop resistance against disease (non-pathogen derived resistance) (Castellano *et al.*, 1999).

Strategies for developing resistance by using the conserved genome of virus are; (1) Antisense RNA strategy (Silencing of complementary target mRNA

by the molecule of antisense RNA). (2) RNAi strategy based of transcriptional gene silencing (TGS) and post transcriptional gene silencing (PTGS) and it has been used to create resistance against *Mung bean yellow mosaic virus* (MYMV), *African cassava mosaic virus* (ACMV) and several others viruses (Khalid *et al*., 2017; Chellappan *et al*., 2004). (3) Host Plant hormones and enzymes (Host plant protein interaction with virus e.g. polyubiquitinated proteins suppress the overexpression of βC1 (determinant of pathogenicity) which, associated with the CLCuMuV, (4) DNA binding proteins strategy developed by expressed of trans genetically DNA binding protein that will not bind to the host plant DNA while it binds with virion strand and resultantly inhibits viral replication (Castellano *et al*., 1999; Fontes *et al*., 1992), (5) GroEL-mediated protection develop by a bacterium present in the gut of whitefly which, binds to begomoviruses coat protein that resultantly viruses can be suppressed in the whitefly haemolymph (Rana *et al*., 2012). (6) Cell death induction approach utilized for limiting the geminivirus development in transgenic plants by action of barstar and barnase proteins and this approach used for controlling of the Tomato leaf curl New Delhi virus (ToLCNDV) (Vanderschuren *et al*., 2007). (7) CRISPR/Cas Genome editing strategy due to high level of specificity attracted the attention of biologists for developing resistance to geminiviruses e.g. mutation in the genome of Bean yellow dwarf virus (BeYDV) through the CRISPR–Cass technology in the bean and resultantly reduced symptoms development in the host due to reduce multiplication of the virus (Baltes *et al*., 2015). Likewise, Ali *et al*. (2015) reported that through applying CRISPR/Cas9 approach, the symptoms of TYLCV disease are reduced. Also, Ji *et al*. (2015) using the construct of sgRNA–Cas9 to develop resistance against *Beet severe curly top virus* in the *Nicotiana benthamiana*. It is suggested that as one of the options this approach can be applied to control geminiviruses in the crops (Zaidi *et al*., 2016, 2017).

Conclusions and Recommendations

The *Bemisia tabaci* is a cryptic species group consisting of an unknown number of morphological indistinguishable variants that endemic to tropical, subtropical temperate regions. It causes damage to plant species either directly by sucking cell sap or indirectly by transmitting of plant viruses. Different whitefly species exhibited different behavior and people identified whitefly population based on biological behavior and various molecular features with respect to virus transmission efficiencies, alternate host plants choice, efficiencies to cause damages. Understanding the population dynamics of *Bemisia tabaci* cryptic species composition in agroecosystem is considered important because management practices might cause shifts in the prevalence of different whitefly cryptic species and outbreak of cotton leaf curl disease. *Bemisia tabaci* complex species is a key factor for dissemination of CLCuD in cotton and other alternate host plants. CLCuD has become an epidemic during the 1990s and 2001 in Pakistan and recently there are no single tetraploid cotton variety developed which, resistance to the CLCuD. *Bemisia tabaci* genetic diversity comes under investigation as result of intensive begomoviruses diversification in the cotton crop and other plant species, exotic *Bemisia tabaci* introduction, potential of virus transmission efficiencies and geographic position of the country. Whitefly transmitted plant viruses belong to one to five genera and in these genera *Begomovirus* genus (Geminiviridae) is economically important which, cause important economical plant diseases. Monopartite begomoviruses or strains along with associated betasatellite caused CLCuD and major economic loss in cotton producing areas in Pakistan during past decades. CLCuD is appearing in cotton growing areas in Pakistan that may be the appearance of the third epidemic. Therefore, a dire need is required for the management of CLCuD and its vector whitefly. Distribution of different whitefly cryptic species which, can be differentiated by *mtCOI* gene for development of management strategies, such management strategies enhance the understanding of host range, resistance of pesticides and along with the biological strategies associated with the different cryptic species that transmit the begomoviruses and strains which, responsible for cotton leaf curl disease.

Acknowledgements

We sincerely thank Prof. Dr. Shafqat Saeed for supervision and guidance to review the *Bemisia tabaci* cryptic species and cotton leaf curl viruses.

Novelty Statement

Bemisia tabaci cryptic species and associated cotton leaf curl viruses is an advanced knowledge in the agriculture sector particularly for whitefly and cotton

leaf curl disease management across the world as well as in Pakistan.

Author's Contribution

Muhammad Afzal wrote the manuscript. Shafqat Saeed, Hasan Riaz, Muhammad Ishtiaq and Habib-ur-Rehman provided the technical support and gave fruitful suggestions. All authors read and approved the final manuscript for final submission.

Conflict of interest

The authors have declared no conflict of interests.

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