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P Valarmathi
Scientist, Plant Pathology, ICAR-Central Institute for Cotton Research (CICR), Regional station, Coimbatore, Tamil Nadu, India

Corresponding Author:
P Valarmathi
Scientist, Plant Pathology, ICAR-Central Institute for Cotton Research (CICR), Regional station, Coimbatore, Tamil Nadu, India

Emerging plant viruses in cotton

P Valarmathi

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Abstract
Plants usually suffer from parasitic and non parasitic diseases in the world. One of the major groups that cause infections in plants belong to viruses, which is obligate parasite and replicate only in living cells. They are generally insect or seed transmitted and changes associated with global warming may affect both their incidence and severity. New virus diseases that are threatening the world's agricultural production are called "emerging diseases" which are commonly detected on different crop plants. Recent emerging viruses have RNA or DNA genome and belong to Potyviridae, Bunyaviridae, Geminiviridae, Closteroviridae, Betaflexiviridae and Secoviridae families. Plant virus diseases, like diseases caused by other pathogens, appear to be proliferating at ever increasing rates. Scientific and popular media abound with terms such as new, emerging, re-emerging and threatening plant diseases. In the present paper, the emerging viral diseases reported in cotton were discussed in detail.

Keywords: CLCuD- TSV-Geminiviridae- Bromoviridae- plant viruses-plant diseases

Introduction
Emerging viruses create a major threat to plant, animal and human health. Majority of them have caused catastrophic losses to crop production. An emerging virus considered to be one that has been recently changed or appeared to occupy and spread within a new environment. Emerging viruses can be new, i.e., not previously know however, they are often known viruses that have become more apparent owing to changes in the environment/ecosystem and/or generation of a new variant, thereby providing the virus with an opportunity to expand into new niches. Key factors mediating virus emergence in the case of plant viruses include changes in agricultural practices and long-distance transport of plant materials. Some emerging viruses gain considerable public recognition and attention because of actual economic losses due to disease epidemics in crop plants (Ertunc, 2020) [21]. In other cases, the emergence of a virus or group of viruses may not result in catastrophic disease or economic losses. However, the rate of emergence of plant viruses does not seem to be any less than that for human and other animal viruses. Some common mechanisms underlie the emergence of plant viruses, irrespective of the nature of the host. In the case of plant viruses, the appearance of emergent viruses is usually mediated via an insect vector. However, with increasing global trade, the emergence of a virus in a new geographical region may be initiated by the introduction of infected plant materials (e.g., plants, propagative materials or seeds). Once introduced, the successful emergent virus expands into a new niche via activity of an existing insect vector or less frequently, through spread by physical contact. Finally, new forms of animal, human and plant viruses also emerge through common mechanisms of genetic variability, including mutation, reassortment and recombination (Rojas and Gilbertson, 2008) [60].

What are Some Plant Viruses that presently are considered as Emergent?
Emergent plant viruses can be placed into two broad categories: entire groups of viruses (e.g., genera or families) or individual viruses. Some examples of plant virus groups that are emergent, on a global level, include (1) the whitefly-transmitted begomoviruses (genus *Begomovirus*, family *Geminiviridae*), (2) the thrips-transmitted tospoviruses (genus *Tospovirus*, family *Bunyaviridae*) and (3) the criniviruses (genus *Crinivirus*, family *Closteroviridae*). Some individual viruses that have emerged relatively recently include the potexvirus, *Pepino mosaic virus* (PepMV), an emergent tomato virus; and the sobemovirus, *Rice yellow mottle virus*. In addition, there also can be outbreaks of "new" viruses, such as those causing necrosis-associated diseases of tomato in Mexico, Spain and Guatemala (Verbeek *et al.*, 2007) [68]. Another example is the emergence of a novel whitefly-transmitted potyvirus infecting cucurbits in Florida (Adkins *et al.*, 2007) [1].

Emergence of viruses in cotton

Among the viral diseases infecting cotton, Cotton leaf curl virus (CLCuV) and Tobacco streak virus (TSV) are found to be emerging threat to the crop.

Cotton Leaf Curl disease (CLCuD)

Cotton leaf curl virus (CLCuV) is a group of whitefly transmitted geminiviruses which cause extensive damage to cotton crop in India and Pakistan. Cotton leaf curl disease is caused by a complex of begomovirus species, all of which incite similar symptoms in cotton but are less than 89% identical to each other at the nucleotide level over the entire length of the genome and also vary in geographic distribution. Virus species causing cotton leaf curl disease in India include *Cotton leaf curl Multan virus* (CLCuMV), *Cotton leaf curl Rajasthan virus* (CLCuRV), Cotton leaf curl Bangalore virus. Genetic relationships between the begomoviruses reflect geographic distributions, with those from the same region being most closely related to each other or to other begomoviruses infecting different plant species in the same region. Characteristics of cotton leaf curl disease are from which the following symptom descriptions are derived. The first symptoms of infection in cotton appear within 2-3 weeks of inoculation and are initially characterised by deep downward cupping of the youngest leaves. This is followed by either upward or downward curling of the leaf margins and swelling, darkening and formation of enations on the veins, which frequently (depending on cultivar) develop into cup-shaped, leaf-like structures. All begomovirus species causing cotton leaf curl disease have geminate particles, approximately 18-20 nm in diameter and 30 nm long and a circular, single-stranded DNA genome (Nagrare *et al*., 2013) [46]. The effect of plant viruses on their vector is a key to the understanding geminiviruses epidemiology and developing effective control measures.

Cotton Leaf Curl Virus Disease (CLCuD) is the most destructive disease which causes huge losses to cotton production (Khan and Ahmad, 2005) [37]. Cotton leaf curl disease was first reported in Nigeria (Farquarson, 1912) [22]. Later on reported from Tanzania and Sudan in 1926 and 1934 respectively (Bailey, 1934) [13]. In Pakistan cotton leaf curl virus was reported for the first time in 1967 near Multan (Hussain, 1975) [28]. CLCuVD is transmitted on by its vector whitefly (*Bemisia tabaci*) and belongs to the genus Begomovirus family Geminiviridae, previously known as sub group III (Hameed *et al.*, 1994) [27]. Mahmood *et al.* (1996) reported that CLCuD causes average reduction in plant height (40.6%), boll weight (33.8%) and number of bolls per plant (72.5%) in cotton cultivars. Ahmed (1999) [3] showed that CLCuD can cause decrease in fiber length (3.44%), fiber strength (10%) and elongation percentage up to (10%). Massive losses to crop production in India and Pakistan, experienced two epidemics which involved a virus and satellite which are resistance breaking during last two and half decades. Gemini virus with single stranded ciruclar DNA invaded cotton crop during 1993 in the Indian states of Punjab adjoining to the border of Pakistan and Rajasthan. 4-5 years: North zone. (Monga *et al.*, 2011) [44]. Breakdown of CLCuV occurs during 2010 at Sri Ganganagar which causes yield loss upto 90%.

The ability of the plant to recover from the damage caused by cotton leaf curl virus depends upon the balanced used of fertilizers, which in return reduces the chances of damage from cotton leaf curl virus and increases the seed cotton yield (Pervez *et al.*, 2007) [48]. Beringer and Tolldenier (1978) [14] and Marschner (1995) [43] reported that plants resistance against diseases can be increased by adequate supply of Potassium (K) because of its functions in osmoregulation, synthesis of molecular compounds and in maintaining energy gradient. Potassium (K) has significant effects on certain disease by its specific role in metabolic function that changes the compatibility relationship of host-parasite (Kafkafi *et al.*, 2001) which advocates its possible utility against CLCuD. Adequate Nitrogen: potassium (N: K) ratio should be maintained, as Nitrogen (N) reduces disease resistance whereas Potassium (K) improves it (Chang and Liang, 1978) [19]. The impact of plant spacing and planting time on yield components of cotton and CLCuD incidence studied and showed that a significant interaction of plant spacing and planting time for seed cotton yield its components and CLCuD incidence. Higher seed cotton yield in early planting with high plant spacing and maximum yield with narrow plant spacing in late planting was observed. The disease incidence and intensity increased in late sowing (Iqbal *et al.*, 2008; Iqbal *et al.*, 2010; Tanveer and Mirza, 1996; James *et al.*, 2004) [30, 29, 65, 32]. Iqbal *et al.* (2008) [30] suggested that cotton genotypes those fell prey to severe incidence of CLCuD can be managed to withstand damage by increasing plant population and nitrogen fertilizer application to achieve optimum seed cotton yield.

Losses due to CLCuD are dependent on infectivity time and variety. The pronounced damage of CLCuD is in early stages but at later stages results minor infections (Brown and Bird, 1992; Akhtar *et al.*, 2003) [4]. CLCuD damage differs on various plant parts and ultimately results in reduction of yield. It can reduce boll weight 33.8%, 73.5% in bolls per plant, GOT% upto 3.93%, seed index 17.0% and yield per plant 64.5% (Ahmed, 1999) [3]. The cotton fiber (lint) is the most important commodity in textile industry and CLCuD also affects fiber quality traits (Ali *et al.*, 1995; Khan *et al.*, 2000; Khan *et al.*, 2001; Kalhoro *et al.*, 2002; Khan *et al.*, 2003) [5, 40, 39, 35, 38]. According to Ahmed (1999) [3] CLCuD can decrease fiber length 3.44%, fiber strength 10% and elongation percentage upto 10%. Akhtar *et al.* 2009 studied the impact of CLCuD on fiber quality traits and their findings depicts that the CLCuD significantly affect traits like GOT%, fiber length, fiber uniformity index, short fiber index, fiber fineness, fiber bundle strength yellowness and maturity ratio. In their studies they observed significant effects of this viral disease on cellulose, protein, wax and pectin which are the major constituent of the fiber.

The genetic resistance in plants is ruined due to the presence of resistance-breaking pathogen genotypes increasing frequently and this phenomenon depends upon the evolutionary potential of the pathogen. The three evolutionary forces i.e. population size, gene or genome flow (i.e. Migration) and reproduction or mating system (i.e. sexual or asexual) is further divided into three categories (1, 2 and 3) of predicted risk of each force and CLCuD comes under category 3 because of its maximum severity reported by García and McDonald (2003) [25]. The possibility of recombination among the Geminiviruses and conducive environmental conditions increases the chances of new more virulent and resistance breaking variants of viruses knocked down the resistance (Shah *et al.*, 1999; Chakrabarty *et al.*, 2010) [61, 18]. Those cultivars showed complete resistance

against Cotton leaf curl Multan virus (CLCuMV) become susceptible to Cotton leaf curl Burewala virus (CLCuBuV) due to the emergence of more virulent new race of virus in this vicinity (Mahmood et al., 2003) [42]. As the phenomenon of recombination is responsible for the evolution of CLCuMV, CLCuBuV and similarly Cotton leaf curl ShahdadPur virus CLCuShV is a new recombinant sequence derived from Begomovirus species that were considered as epidemic of CLCuD in the Punjab during 1990s (Amrao *et al.* 2010; Monga *et al.*, 2011) [8, 44]. Resistance durability is dependant if no resistance breaking has taken place or it is effective for 25 or more years (García and McDonald, 2003) [25]. In case of CLCuD resistance durability is limited and has not been taking place for more than 3 or 4 years as the parents CP-15/2 and LRA5166 used in developing resistant cultivars in Pakistan have a narrow genetic base coupled with the arrival of new strains of the virus (Rahman *et al.* 2005; Padidam *et al.* 1999) [58, 47].

The wild species of *Gossypium* are potential sources of resistance to biotic (insect and diseases) and abiotic (salinity, cold, drought, heat) stresses. *G. anomalum, G. longicalyx, G. stocksii, G, raimondii* and *G. sturtianum* has a source for the improvement of fiber quality characters whereas *G. thurberri, G. anomalum, G. raimondii, G. armourianum* and *G. tomentosum* are the best sources for resistance of insect pests including whitefly which is the main vector for the inoculation of CLCuD (Azhar *et al.*, 2010) [12].

Tobacco streak virus (TSV)

Cotton necrosis disease caused by *Tobacco Streak Virus* (TSV) is an emerging threat in India (Rageshwari *et al.*, 2016, Vinodkumar *et al*., 2017) [52, 69]. The species tobacco streak virus (TSV) belonging to the genus ilarvirus of the family bromoviridae is a multipartite, single-stranded, positive-sense, RNA virus. The host range comprises 200 plant species including, agricultural, horticultural crops and weeds (Fulton, 1948; Fulton, 1985) [23, 24]. *Tobacco streak virus* (TSV) was first identified in tobacco in Brazil (Johnson, 1936; Costa, 1945) [20]. In India, TSV was initially identified in sunflower (Prasada Rao *et al.*, 2000) [50] and peanut (Reddy *et al.*, 2002) [56] causing necrosis disease. In Tamil Nadu, Nakkeeran (AICRP report 2010) [6] first reported the association of TSV in cotton. Tobacco streak virus infecting various crops have been reported to be transmitted through mechanical means, infected seeds and through thrips species (Jagtap *et al.*, 2012; Kaiser *et al.*, 1982; Sharman, 2009) [31, 36, 62].

Tobacco streak virus (TSV) causing cotton necrosis, exhibits various symptoms which includes purplish brown, necrotic lesions in the leaves, squares and petioles. Telangana (India) recorded the highest incidence of TSV with 51.11 PDI in the hybrid RCH659, among locations including Tamil Nadu, Andhra Pradesh, Telangana and Maharashtra states of India (Vinodkumar *et al*., 2017) [69]. Earlier literatures reported TSV infection in crop plants such as, sunflower, groundnut, blackgram, cowpea (Prasada *et al.*, 2009; Ramiah *et al.*, 2001; Ravi *et al.*, 2001; Bhat *et al.*, 2002; Vemana and Jain 2010) [49, 54, 55, 15, 67]. Initial studies on TSV infecting cotton were attempted first by Arutselvan (unpublished, 2013) [10] in Tamil Nadu. Jagtap *et al.* (2012) [31] have reported chlorotic symptoms in the leaves and stunted growth in case of cotton. TSV exhibited different types of symptoms that include chlorotic and necrotic lesions. Most commonly purplish brown, necrotic, spots were observed in the leaves, squares and stem. Natural field infections of TSV in different crop plants have been reported worldwide including sunflower, cotton, chickpea and mungbean- Australia (Sharman *et al*., 2008) [63], cotton - Pakistan (Ahmed *et al.* 2003) [2] and Brazil (Costa and Carvalho, 1961). TSV is widely distributed in the North American and the Pacific regions where it infects *Asparagus officinalis*, *Dahlia spp*., *Glycine max*, *Gossypium herbaceum*, *Melilotus alba*, *Nicotiana tabacum*, *Phaseolus vulgaris*, *Rosa setrgera* and *Trifolium pretense* causing a variety of symptoms (Brunt *et al.*, 1996) [17]. In Tamil Nadu, TSV incidence was found to be severe in cotton growing regions of Coimbatore and Erode districts (Rageshwari *et al.*, 2016) [52]. The study also indicated that hybrids were more susceptible compared to varieties. Environmental factors including, minimum temperature, relative humidity and leaf wetness played major role in the establishment of TSV in cotton. The north west zone of Tamil Nadu was highly susceptible, and the incidence reduced gradually towards south. In the southern zone there was less TSV incidence. Locations with lesser minimum temperature, higher relative humidity and leaf wetness favoured the occurrence of TSV. The germplasm of *Gossypium barbadense* were surveyed for the presence of TSV during the year 2017-2018. The presence of disease affected plants was observed at 90 DAS (Days after sowing). The per cent disease incidence varies from 1.61% (CCB 140) to 26.60% (ICB 71). The symptoms were very distinct with necrotic spots dark purple in colour and also drying of squares. Other symptoms include necrotic streaks on petiole and necrosis on crown region (Valarmathi, 2018) [66].

Presence of TSV has been confirmed through DAC ELISA in onion (Asadhi *et al.*, 2016) [10], soybean (Arun Kumar *et al.*, 2008; Rajamanickam *et al.*, 2016) [9, 53], cotton (Rageshwari *et al.*, 2017) [51], groundnut (Vemana and Jain, 2010; Reddy *et al.*, 2002) [67, 56], blackgram (Ladhalakshmi *et al.*, 2006) [41], lablab (Reddy *et al.*, 2013) [57]. TSV inoculum from strawberry leaves was successfully transferred to *C. quinoa* in Phosphate buffer (0.05 M) amended with 2-mercaptoethanol and poly vinyl pyrolidine (Spiegel and Cohen, 1985) [64]. Kaiser *et al.* (1982) [36] reported that buffer amended with sodium sulphite and 2-mercapto-ethanol successfully transmitted TSV inoculum from cowpea and white mulberry to *C. quinoa*. Sodium phosphate buffer (0.01 M) pH 7.0, amended with 0.1% 2-mercaptoethanol successfully transmitted TSV inoculum from soybean to a variety of test plants including, *V. ungiculata*, *C. quinoa*, *N. tabacum* and *N. glutinosa* (Almeida *et al.*, 2005) [7]. Identification of symptoms due to TSV infection by visual observation of plants often results in misdiagnosis as symptoms produced by this virus can match with those reflecting physiological and nutritional disorders affecting cotton. Development of diagnostic tools with rapidity will have immense role to play in detection and management of the emerging virus. The protocol for rapid diagnosis of TSV infected samples by using Reverse Transcription-Loop Mediated Isothermal Amplification (RT-LAMP) was optimised and this is the first report of its use for diagnosis of TSV on cotton and Soybean. The colorimetric detection for diagnostic shows simplicity of amplified RT-LAMP product by using different dyes lead to enhanced applicability of this technique. The RT-LAMP diagnostic tool can be utilized not only for laboratory research but also for quarantine and field diagnosis of this important emerging pathogen affecting cotton (Gawande *et al.*, 2019) [26].

Cotton blue disease (CBD)

Cotton blue disease (CBD) is caused by cotton leaf roll dwarf virus (CLRDV) (an RNA virus) having positive sense single stranded RNA, transmitted by aphids (*Aphis gossypii*) in a circulative-persistent manner. Cotton plants affected by this disease show stunting, leaf rolling, intense green foliage, vein yellowing, brittleness of leaves, reduced flower and boll size resulting in sterility of plants. The disease is recorded in Maharashtra (Mukherjee *et al.*, 2012) [45].

CBD affected leaves tend to be small, thick, more brittle and leathery than healthy leaves and have an intense green to bluish colour with yellow veins. Reddening of stem petioles and leaf veins can occur in some infections. Leaf edges tend to roll downwards, plants become stunted due to a shortening of the branch internodes and they produce many branches, giving a bunchy zig-zag stem habit. Symptoms are more obvious in plants infected at an early age. Infected plants also produce smaller bolls and boll shed may occur. CBD is often seen as small patches of plants, often just on a single row, with single infected plants occasionally overlooked if overgrown by nearby healthy plants. The susceptibility of different cotton species and commercial varieties to blue disease varies. Some triploid varieties are resistant to the disease, particularly if *G. arboreum* is used as a parent. The longevity of this resistance is unknown, but evidence suggests it is being lowered. There are no known hosts of blue disease outside cotton.

Cotton blue disease (CBD) is suspected to be caused by aphid-transmitted viruses. Although the disease has been reported from Africa, Asia and the Americas, a causal agent has only been identified from Brazil (Cotton leafrolldwarf virus; CLRDV). Although CLRDV is the causal agent of blue disease in Brazil, it is not known if this is the causal agent in other regions. Furthermore, CBD has similarities with other diseases of cotton, such as cotton bunchy top, anthocyanosis and cotton leaf roll. It is not known if the same pathogen causes all these diseases or if there are multiple pathogens causing similar symptoms. However, CLRDV was not detected from Australian cotton plants affected by bunchy top disease. A common feature of all of these diseases is that they are spread by the cotton aphid, *Aphis gossypii*. CLRDV is moved between plants by, the cotton aphid (*Aphis gossypii*). The virus is taken up by the aphid during feeding and remains within the insect for anywhere from a few weeks, to the entire life of the insect. Later, the virus is deposited into other plants when the insect feeds again. Disease spread is favoured by conditions which are suitable for aphid reproduction, feeding and spread. CBD has similar symptoms to cotton bunchy top disease. Both diseases display shortened internodes and can result in stunted plants. However, the discolouration of leaves varies between the two diseases. The typical green-blue colour and yellow veins observed in CBD-affected leaves is absent from those affected by cotton bunchy top and instead there is an angular pattern of pale green margins and darker green centres with the latter disease (https://phys.org/news/2019-10-cotton-blue-disease-states.html)

The role of plant bio security in preventing and controlling emerging plant virus disease epidemics

A number of research strategies have been initiated over the last decade to enhance plant biosecurity capacity at the pre-border, border and post-border frontiers. In preparation for emerging plant virus epidemics, diagnostic manuals for economically important plant viruses that threaten local industries have been developed and validated under local conditions. Contingency plans have also been prepared that provide guidelines to stakeholders on diagnostics, surveillance, survey strategies, epidemiology and pest risk analysis. Reference collections containing validated positive virus controls have been expanded to support a wide range of biosecurity sciences. Research has been conducted to introduce high throughput diagnostic capabilities and the design and development of advanced molecular techniques to detect virus genera. TheZse diagnostic tools can be used by post entry quarantine agencies to detect known and unknown plant viral agents. Pre-emptive breeding strategies have also been initiated to protect plant industries if and when key exotic viruses become established in localized areas. With the emergence of free trade agreements between trading partners there is a requirement for quality assurance measures for pathogens, including viruses, which may occur in both the exporting and importing countries. These measures are required to ensure market access for the exporting country and also to minimize the risk of the establishment of a damaging virus epidemic in the importing country (Rodoni, 2009) [59].

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