4. Cassava Mosaic Disease and Its Management

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Abstract:

Cassava (Manihot esculenta Crantz) is one of the important tuber crops and holds significant importance as a staple food and industrial crop across Asia, Africa and South America. But Cassava cultivation faces significant challenges due to various insect pests and pathogens. Amongst, cassava mosaic disease (CMD), attributed to the Cassava mosaic geminivrus, poses a substantial obstacle to its cultivation resulting in significant annual crop losses. In Asia, CMD is primarily caused by Indian cassava mosaic virus and Sri Lankan cassava mosaic virus. To mitigate the impact of the disease, various strategies including phytosanitation, vector control, breeding, and genetic engineering have been extensively employed. This chapter provides a comprehensive knowledge of its occurrence, host range, symptomatology, genomic organization and transmission of virus and its management.

Keywords:

Cassava mosaic disease, occurrence, management, Cassava mosaic virus

4.1 Introduction:

Cassava (*Manihot esculenta* Crantz) traces its origins to the southern interface of the Brazillian Amazon and was first cultivated by native South Americans approximately 12000 to 17000 years ago (Oslen and Schaal, 1999; Hareesh *et al.*, 2023). The starchy root of this plant serves as fundamental food source for over 8 hundred million people worldwide and is also a vital commodity of industries. Its ability to thrive in poor soil and withstand relatively dry weather conditions make it particularly appealing to smallholder farmers. Cassava has garnered global recognition as a "famine crop" with production escalating from around 124 to 621 million tonnes between 1980 and 2022. It ranks as the world's fourth most crucial staple food after rice, wheat, and maize, contributing to a global caloric intake of 2.6% (Tafesse *et al.*, 2021; Hareesh *et al.*, 2023). In India, this crop is mostly grown in Tamil Nadu, Kerala, Karnataka and Andhra Pradesh and also in a few north-eastern states on an area of 1.72 mha with an annual production of 6.21 mt (FAOSTAT, 2022).

Cassava cultivation faces significant challenges due to various insect pests and pathogens. Among these, *Cassava mosaic geminivirus* (CMG) and *Cassava brown streak virus* (CBSV) are particularly detrimental (Bock *et al.*, 1978; Lennon *et al.*, 1986). Cassava mosaic disease (CMD), caused by CMG (family: Geminividae, genus: *Begomovirus*),

stands out as a major obstacle to cassava cultivation, resulting an estimated annual crop loss of USD 1.9-2.7 billion (Patil and Fauquet, 2009). Symptoms of CMG-infected cassava plants include leaf curling and distortion, yellowing, mosaic patterns, and stunted growth, all of which lead to reduced photosynthesis and yield. The virus transmitted by whitefly (Fauquet and Stanley, 2003). In Africa, several viruses were found associated with CMD, including African cassava mosaic virus (ACMV) (Stanley and Gay, 1983), East African cassava mosaic Malawi virus (EACMMV) (Zhou et al., 1998), East African cassava mosaic Cameroon virus (EACMCV) (Fondong et al., 2000), South African cassava mosaic virus (SACMV) (Berrie et al., 2001), East African cassava mosaic virus (EACMV) (Pita et al., 2001), East African cassava mosaic Zanzibar virus (EACMZV) (Maruthi et al., 2004), East African cassava mosaic Kenya virus (EACMKV) (Bull et al., 2006), Cassava mosaic Madagascar virus (ACMBFV) (Tiendrébéogo et al., 2012), and African cassava mosaic Burkina Faso virus (ACMBFV) (Tiendrébéogo et al., 2012). In Asia, CMD is caused by Indian cassava mosaic virus (ICMV) and Sri Lankan cassava mosaic virus (SLCMV) (Hong et al., 1993; Sunders et al., 2002).

4.2 Occurrence of CMD:

Cassava mosaic disease (CMD) was initially reported from Tanznania (Warburg, 1894) and later, Zimmermann in 1906 suggested it to be a viral disease (Patil and Fauquet, 2009). Originally identified as *Cassava latent virus*, the virus underwent sequencing, leading to its reclassification as African cassava mosaic virus (Stanley and Gay 1983; Hareesh et al., 2023). In India, CMD was first reported from Kerala. The virus responsible was initially cloned and sequenced from Kerala sample, leading to its designation as Indian cassava mosaic virus (ICMV) (Abraham, 1956; Malathi et al., 1985; Hong et al., 1993; Hareesh et al., 2023). Additionally, another virus species, known as Sri Lankan cassava mosaic virus (SLCMV), was discovered in infected cassava samples from Sri Lanka, Subsequently, SLCMV was also detected in infected cassava material gathered from Kerala (Saunders et al., 2002; Dutt et al., 2005; Hareesh et al., 2023). CMD is prevalent in major cassavacultivating districts of Tamil Nadu, where the disease incidence surpasses 90% (Rajinimala et al., 2011; Hareesh et al., 2023). A survey spanning eighty locations across nine districts of Tamil Nadu unveiled that the majority of samples were infected with SLCMV. Some samples showed mixed infections of SLCMV and ICMV. Genetic analysis revealed minimal variability in these samples, characterized by sporadic single nucleotide alterations (Kushwaha et al., 2018; Hareesh et al., 2023). In a recent survey conducted in West Bengal, CMD incidence ranged from 61% to 92%, with symptoms varying from mild chlorotic patterns to severe mosaic and leaf distortion. Sequence analysis indicated similarity to SLCMV isolates from India (Adhikary et al., 2018; Hareesh et al., 2023). In contrast to Africa, where CMD results in substantial crop losses, this disease has not posed a severe threat to India, with yield losses ranging from only 10% to 15% (Rothenstein et al., 2006; Hareesh et al., 2023).

Since the emergence of CMD in 2015 from Ratanakiri, Cambodia (Wang *et al.*, 2016) SLCMV has been rapidly spreading throughout Southeast Asian nations. Cases of CMD were confirmed in farmers' fields in China and Vietnam in 2017, and in Thailand in 2019 (Uke *et al.*, 2018; Minato *et al.*, 2019; Wang *et al.*, 2020). Field surveys also revealed CMD occurrences in Laos in 2020 (Chittarath *al.*, 2021). All the strains identified thus far in South and Southeast Asia share close genetic similarities with previously characterized SLCMV

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isolates (Hareesh *et al.*, 2023). Minato *et al.* (2019) conducted a study on the geographical expansion of SLCMV in Vietnam and Cambodia during 2016. In Vietnam, comprehensive nationwide survey focused on 15 districts boasting the largest cultivation areas, collecting a total of 2640 samples from 179 fields. Similarly, in Cambodia, a survey was conducted across 16 districts, involving the collection of 3840 samples from 240 fields. Using PCR-based diagnostics, researchers identified 49 positive plants from nine fields, constituting 2% of the total field samples. Notably 14% of these positive plants didn't exhibit visible symptoms, with the majority originating from two provinces in Eastern Cambodia: Ratanakiri and Stung Treng. However, no infections were found in samples from other regions in either country. Within Ratanakiri province, the incidence at the field level ranged from 25% to 37.5% in two fields, while other fields also showed SLCMV incidence. In Stung Treng province, the within-field incidence varied from 6.3% to 56.3% across different fields, notably higher than in Ratanakiri province, where SLCMV was initially detected.

Several factors influence the severity of CMD, such a host age, cultivar, virus strain and climatic conditions. Uke *et al.* (2021) attributed a reduction in cassava root yield to 16-33% when infected cuttings were utilized for planting. Plants aged 1-3 months exhibiting moderate to severe mosaic symptoms experienced more severe effects compared to 5–7-month-old plants, which showed only mild chlorosis. Infections transmitted by whiteflies were generally milder compared to than those from infected cuttings, and in various instances, infected plants showed no symptoms. Furthermore, certain genotypes, such as Ryong 11 and CMR-89, were found to be susceptible to CMD (Saokaham *et al.*, 2021).

The research conducted thus far confirms that SLCMV is the primary virus responsible for CMD in South and Southeast Asia, demonstrating greater pathogenicity compared to ICMV. Despite the coexistence of ICMV and SLCMV in cassava fields in the Indian subcontinent, SLCMV typically suppresses ICMV in mixed infections. However, latent ICMV an re-emerge when the cassava genotypes recover from SLCMV infection, suggesting a dynamic interplay between the two viruses (Karthikeyan *et al.*, 2016).

In Asia, aside from SLCMV and ICMV, no new cassava-infecting geminivirus species have been reported. However, the simultaneous occurrence of SLCMV and ICMV in South Indian cassava fields, along with their presence in other host plants serving as geminivirus reservoirs, underscores the need for vigilance due to their potential for recombination. Long-distance spread of CMD primarily occurs through contaminated plant materials, while local transmission is facilitated by whitefly. The coexistence of African and Asian CMGs in the same host could potentially lead to the emergence of highly virulent strains. Therefore, robust surveillance measures are crucial to mitigate the spread of the disease.

4.3 Host Range:

The host range of viruses is a crucial factor in differentiating geminiviruses and understanding their epidemiology. Each *Cassava mosaic geminivirus* (CMG) exhibits distinct preferences for hosts beyond their natural host, cassava. Previous research has demonstrated that ACMV primarily infects plants within the Solanaceae family, with a particular affinity for genera such as *Nicotiana* and *Datura* (Bock and Woods, 1983).

Both ICMV and SLCMV also infect various species of *Nicotiana*, including *N. tabacum* and *N. glutinosa*. SLCMV, in particular, displays high virulence, demonstrating a wide host range that extends to *Arabidopsis* and even *Ageratum conyzoides* when it is associated with ageratum yellow vein betasatellite (Saunders *et al.*, 2002; Mittal *et al.*, 2008; Patil and Fauquet, 2009).

The host range of *East-African cassava mosaic virus* (EACMV) has not been extensively studied, but it is known to infect *Nicotiana* species, notably *N. benthamiana*, which is commonly used for experimental purposes. This characteristic also applies to *South-African cassava mosaic virus* (SACMV) (Berrie *et al.*, 2001; Patil and Fauquet, 2009).

Consequently, on the basis of their host CMGs was classified into two major groups: ICMV, SLCMV and ACMV form one class, infecting all *Nicotiana* spp. in addition to cassava, albeit with varying virulence levels. In contrast, EACMV-like viruses and SACMV belong to another class, infecting only *N. benthamiana* and cassava. Recent findings have also highlighted an association between ACMV, *East-African cassava mosaic virus* Uganda strain (EACMV-Ug), and *Manihot glaziovii*, a wild cassava species utilized as a resistance source in CMD breeding programs (Sserubombwe *et al.*, 2008; Patil and Fauquet, 2009).

4.4 Symptomatology:

The symptom associated with cassava mosaic disease encompasses reduction in plant size, mottling, distortion of the leaf lamina, and chlorotic mosaic compared to healthy leaves. One of the prominent symptoms observed in infected fields is the presence of a mosaic formation on the leaves, with the coloration ranging from pale green to whitish yellow (Figure 4.1).

The degree of chlorosis across the leaf surface can vary widely, spanning from less than 5% to nearly 100%. Additionally, in cassava fields infected by CMD, common feature is the pronounced narrowing of leaves near the base of the leaflets. It is significant to note that the manifestation of symptoms can depend on various factors such as season and cultivar (Chikoti *et al.*, 2019).



Figure 4.1: Plant infected with Cassava mosaic virus

4.5 Genomic Organization and Gene Regulation:

Geminiviruses represent a number of plant viruses known for their distinctive geminateshaped particles measuring 30 x 20 nm. These viruses replicate their circular single-stranded DNA (ssDNA) genome, which typically ranges from 2.7 to 2.8 kb, utilizing double-stranded DNA (dsDNA) intermediates. This replication process occurs within the nuclei of infected cells, facilitated by either rolling circle replication or a recombination-dependent mechanism (Hanley-Bowdoin *et al.*, 1999; Jeske *et al.*, 2001). The Geminiviridae family is categorized into four genera based on criteria such as genome organization, host range, and insect transmission (Hull, 2002). Within this family, CMGs (*Curtovirus, Mastrevirus*, and *Topocuvirus*) belong to the genus *Begomovirus*, which primarily consists of geminiviruses transmitted by whiteflies and infecting dicotyledonous plants (Fauquet *et al.*, 2008).

The geminate particles responsible for encapsulating the genomic components of ACMV (*African cassava mosaic virus*) are known to release their genomic DNA through breaks occurring at the top and shoulders of the virus particles (Bottcher *et al.*, 2004; Kittelmann and Jeske, 2008). In terms of genomic structure, the begomovirus components DNA-A and DNA-B share a common region of approximately 200 nucleotides, exhibiting a high nucleotide sequence identity of more than 80% (Harrison and Robinson, 1999).

This common region harbours several regulatory elements, including two TATA motifs, along with multiple copies of *cis*-elements called iterons, which serve as binding sites for the replication-associated protein (Rep) (Hanley-Bowdoin *et al.*, 1999). Iteron sequences found in CMGs can be classified into three distinct groups based on their types:

- 1. The ACMV type, associated with isolates of ACMV, EACMZV (*East-African cassava mosaic Zanzibar virus*), and SLCMV.
- 2. The EACMV type, which encompasses all other EACMV-like viruses as well as SACMV.
- 3. The ICMV type, specific to ICMV isolates alone.

Transcriptional regulation in begomoviruses has been extensively investigated in both transgenic plants and protoplast systems (Frey *et al.*, 2001; Shivaprasad *et al.*, 2005). During rolling circle replication, the double-stranded DNAs formed serve as templates for transcription, and geminivirus genomes are transcribed bidirectionally by bidirectional promoters located in the intergenic regions (Hanley-Bowdoin *et al.*, 1999). Regarding promoter regions, five putative promoter regions have been identified and tested for their transcriptional activity in ACMV genomes. Despite extensive research, significant knowledge gaps persist regarding the structural organization of geminivirus promoters, gene regulation, and transcription.

4.6 Transmission:

Cassava Mosaic Begomoviruses (CMBs) are naturally spread by whiteflies (*Bemisia tabaci*). It is transmitted in a non-propagative and circulative manner (Islam *et al.*, 2018). Given cassava's vegetative propagation, infected cassava stems can also act as a source of disease. While whiteflies primarily serve as disease vectors for short-distance

dissemination, within a range of 20 meters (Maruthi *et al.*, 2017; Uke *et al.*, 2022), but the distribution of cassava planting materials, particularly through the exchange of diseased stem cuttings (Delaquis, 2018), significantly contribute to long-distance dissemination.

It's crucial to note that diseased and healthy stems without leaves are morphologically identical, posing a challenge in the distribution of Cassava Mosaic Disease (CMD) affected vegetative planting materials and potentially leading to the quick transmission of this virus all over Southeast Asia. While the use of insecticides can reduce whitefly density and limit short-distance dissemination, excessive use can promote the displacement of existing *B. tabaci* species by the insecticide-resistant *B. tabaci* Mediterranean (biotype Q) (Horowitz *et al.*, 2014). To address these challenges, implement of integrated pest management (IPM) strategies is necessary in the field conditions to manage whiteflies population and prevent the emergence of insecticide-resistant whitefly populations that could spread to other cassava-cultivating regions.

4.7 Management:

Various strategies have been employed to manage Cassava Mosaic Disease, like enhanced biocontrol and cultural practices, use of naturally resistant cultivars, and advanced biotechnological interventions. Among the methods utilized to combat CMD, genetic engineering stands out as a rapid means of transferring resistance genes to conventional cultivars, thus circumventing the potential emergence of undesirable traits. An alternative approach, known as antisense RNA technology or RNA interference (RNAi)/gene silencing technology, has shown promising results in conferring resistance to ACMV infection in cassava.

While further enhancements in transgenic approaches for CMD resistance are necessary before their widespread deployment in the field, these strategies hold significant promise. Importantly, they can complement conventional breeding efforts to combat CMD and enable farmers to continue cultivating locally preferred cultivars. This is particularly crucial given the challenges faced in addressing farmer quality preferences in areas affected by the CMD pandemic. This strategy aims to stack different resistance genes to achieve durable resistance against CMD (Patil and Fauquet, 2009).

To control whitefly population, natural predators like lacewig larvae, big-eyed bugs (*Geocris* spp.), ladybird beetles, syrphid fly larvae and predatory mites can be used. They all attack immature stages of whiteflies.

Use of cultural practices is one of the cheap and important methods to manage diseases. Disease free plant cutting should be taken. Rouging of plant should be done as soon as symptoms are seen. Avoid to plant cassava near alternate hosts. After harvest, collect and destroy stems of those plants that showed symptoms of cassava mosaic disease. Don't use them as a source of planting material.

Broad spectrum insecticides like pyrethroids, neonicotinoids and organophosphates should be avoided because whiteflies develop resistance due to their repetitive use. Frequent application of horticultural oil (made from petroleum), white oil (vegetable oil) and soap solution should be recommended because sprays will not kill all of the whiteflies, but will allow predators and parasites to increase and bring the whitefly infestation under control (Jackson, 2022).

4.8 Conclusion:

Over the last twenty years, substantial efforts have been made in our understanding of Cassava Mosaic Geminiviruses (CMGs), including their structural features, genetic variation, movement mechanisms, and interactions with plants. This progress has been fuelled by extensive genomic studies and the analysis of numerous virus sequences. The rapid diversification and transmission of EACMV-like viruses, characterized by their higher recombination rates and synergistic interactions with other CMGs, pose significant threats to cassava cultivation in Africa and the Indian subcontinent. Moreover, the exponential increase in their whitefly vector population exacerbates these challenges. Furthermore, there is a looming threat of Cassava Mosaic Disease (CMD) introduction into South America and Southeast Asia. Therefore, stringent quarantine measures must be implemented to prevent the importation of CMGs and cassava-adapted whitefly biotypes. By enforcing strict quarantine regulations, we can mitigate the risks associated with the introduction and spread of CMGs, safeguarding cassava cultivation and food security in vulnerable regions.

In recent times, there have been reports of dual pandemics involving two significant diseases affecting cassava: one is Cassava Mosaic Disease (CMD) and other one is Cassava Brown Streak Disease (CBSD). CBSD, an RNA virus, suspected to belong to the genus *Ipomovirus* of the family *Potyviridae*, and have been observed particularly in eastern Africa (coastal regions). The swift dissemination of this virus into the Africa (eastern and central regions) has raised concerns within the cassava community (Alicai *et al.*, 2007).

The discovery of the gene silencing mechanism undoubtedly marks a significant milestone in our comprehension of plant-virus interactions and holds immense promise for controlling plant viruses.

It is imperative to leverage this knowledge to enhance the efficacy and sustainability of existing strategies, each of which has its own limitations, while also devising novel approaches to combat the emergence of resistance-breaking geminiviruses and satellites that infect cassava. These innovative approaches for managing CMD demonstrate considerable potential in the near future, warranting continued investment in this technology. Such advancements hold the potential to catalyze another green revolution in cassava cultivation, offering renewed hope for safeguarding cassava production and ensuring food security.

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