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Foorkey disease of Cardamom

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1. Introduction

Large cardamom (*Amomum subulatum*), often referred to as **black cardamom**, is an important spice crop cultivated primarily in the eastern Himalayas of India, Nepal, Bhutan, and parts of Southeast Asia. This aromatic spice is known for its distinct smoky flavor and is a key ingredient in culinary traditions, especially in curries, teas, and desserts. In addition to its culinary use, large cardamom has medicinal properties and is also used in traditional herbal remedies. It is used to flavor rice dishes, stews, meat curries, and beverages like chai (spiced tea). The crop is economically significant for the farmers in these regions, providing income and supporting rural economies. India is the largest producer and exporter, contributing around 70-80% of the global production, particularly in states like **Sikkim, Darjeeling**, and **Himachal Pradesh**.

Large cardamom, like other crops, faces several **biotic stresses** that affect its growth and productivity. Cardamom is prone to two of the major diseases Chirkey and Foorkey disease caused by **Cardamom Bushy Dwarf Virus (CBDV)** (Ramakrishnan *et al*., 2024). CBDV transmitted by aphids, is the most destructive viral disease affecting cardamom. The virus causes a **dramatic decline in yields**, with infected plants showing symptoms like reduced growth, deformation of leaves, and poor flowering. The overall **quality of the cardamom pods** is also reduced, making them less desirable for export. Infected plants may eventually die, leading to substantial economic losses for farmers, particularly in areas where the virus is endemic. In regions heavily affected by CBDV, yield reductions can range from **30% to 70%**, depending on the severity of the infection and the control measures implemented. This viral disease not only causes direct crop losses but also disrupts

the livelihoods of farmers who depend on large cardamom for their income.

2. Foorkey disease

In the Imperial Mycologist's report, Mitra (1936) made the first mention of the foorkey disease. Subsequently, in the Darjeeling area of West Bengal, Varma and Capoor (1953) gathered huge cardamom seeds and rhizomes afflicted by foorkey for the first time, from Kalimpong in 1950. They then researched the nature of the disease and potential preventive strategies. According to Varma and Capoor (1964), the virus that causes foorkey disease is distinct from the virus that causes "Katte" disease and the banana bunchy top virus (BBTV).

2.1 Transmission

The cardamom plants that are impacted develop a lot of vegetative growth from the rhizome's base. The leaves turn pale green color and gently curl, while the stems are drastically decreased in height. The rhizomes shrink and eventually wither away. Nevertheless, the diseased plants endure for a few years despite continuing to be sterile and unproductive (Ghosh *et al*., 2016). The virus causing foorkey disease is not transmitted through plant sap. Varma and Capoor (1964) found that the banana aphid, *Pentalonia nigronervosa*, was a persistent carrier of the foorkey at Poona, spreading it easily. Subsequently, Basu and Ganguly (1968) discovered that the banana aphid did not spread the foorkey in Kalimpong; instead, it was carried by another aphid, *Mycromyzus kalimpongensis* Basu. According to Varma and Capoor (1964), the foorkey virus can infect both big and small cardamom plants, but not *Gladiolus sp*., *Canna indica*, *Zingiberofficinale*, *Triticum aestivum*, *Sorghum vulgare*, or *Zea mays*.

2.2 Origin

In an investigation using electron microscopy conducted in 1981, Alhwat and Raychaudhuri found 37 nm spherical virus particles in a foorkey-infected plant. But no additional research was done to identify the virus that would have been necessary to prove it was the etiological agent of the foorkey disease. In a field study conducted in Sikkim and the Darjeeling Hill regions in 2002 and 2003, it was discovered that 27% of the cardamom plants were afflicted with foorkey disease. Using electron microscopy, it was discovered that a small number of isometric particles between 17 and 20 nm

were found to be associated with the diseased plants. Mandal *et al*. (2004) and Mandal *et al*. (2012) reported for the first time that a nanovirus was linked to the foorkey disease of large cardamom. This association was based on the replication associated protein gene (Rep) nucleotide sequence, which showed 80–82% identity with BBTV and 47.6% to 48.5% identity with other nanoviruses. Subsequent cloning and sequencing of six unique full-length DNA components encoding M-Rep, sRep, CP, Clink, MP, and NSP revealed a phylogenetic relationship with *Babuviruses* (Mandal *et al*., 2008; Barman A.R. 2009).

2.3 Genome organisation

The genome is organized into six primary DNA components, including ORF, SL, CR-M, TATA box, and poly-A signals. The genome components shared two conserved intergenic regions, SL common region (CR-SL) and CR-M, with *Nanoviridae*. The CRSL was 49-70 nt and included the nonanucleotide TATTATTAC in the loop region, which was identical to all components except DNA-sR. DNA-R, -S, and -M loop sequences were identical, however DNA-N, -C, -U3, and -Uf2 diverged by having AC instead of CT. The stem sequences of DNA-R, -S, -M, and -C were identical. However, DNA-N and -U3 diverged with ATG and AGG in place of GCT, respectively.

A major ORF in the DNA-R genome encodes a 33.6 kDa protein with the dNTP binding motif GGEGKT at nt position 639–656, which is shared by members of the *Nanoviridae* family. The Rep ORF included a tiny ORF that encoded a 5.1 kDa protein with an unknown function. In comparison to the other components, the intergenic region of DNA-R was shorter (241 nt). The CBDV DNA-R component's nucleotide sequence was 71.7–78.1% identical to those of ABTV and BBTV, but only 47.2–49.5% identical to those of the *Nanovirus* genus. Potentially, a 19.5 kDa protein was encoded by the CP ORF (513 nt). The 570–571 nt long noncoding intergenic region included the CR-SL and CR-M structures. Only 64.2% of the DNA-S component's nt similarity was shared with ABTV and 59.8% with BBTV, while only 30.8–33.6% was shared with the nanoviruses. Compared to BBTV (75.8%), CBDV's CP was more in line with ABTV (80.5%). An ORF of 354 nt, encoding 13.49 kDa MP, was present in the DNA-M. The length of the intergenic area was 729 nt. The known *Nanoviridae* members and CBDV's

DNA-M were distantly related (22.6–54.1% nt sequence identity). The amino acid sequences of the MP of CBDV were similar to those of BBTV and ABTV by 59.8% and 66.6%, respectively. An ORF encoding an 18.2 kDa NSP was found in the DNA-N genome. Comparing the CR-SL to the other components, it was the shortest at 60 nt. The NSP ORF's 5 terminal contained a TATA box, which was found 57 nt after the initiation codon. An ORF of the Clink protein (160 aa), which codes for an 18.5 kDa protein, was found in the DNA-C genome. The retinoblastoma-binding motif LFCDE (LXCXE) was present in amino acids 111–115 of the CBDV Clink protein. There were no significant ORFs in the DNA-U3. Nonetheless, the U3 had three to four short coding sequences. The component had CR-SL and CR-M structures that were comparable to those of the other main components. The 66-nt CR-SL included the F1, F2, and R iteron sequences (Mandal *et al*., 2013).

3. Management

Management of Foorkey disease in large cardamom, caused by Cardamom bushy dwarf virus (CBDV), primarily involves preventive and control measures. Key strategies include the use of certified disease-free planting material to prevent the introduction of infected plants, as CBDV is spread through infected saplings (Ghosh *et al*., 2016). Insect vectors that may aid in transmission should be controlled using appropriate insecticides (Chen *et al*., 2023). Using resistant varieties like Bebo and moderately tolerant but vulnerable to foorkey illnesses (URL-2) like Ramla is essential for managing chicken disease. It is also important to periodically remove diseased plants and replace them with a large number of certified virus-free plants (Ramakrishnan *et al*., 2024).

3.1 Molecular detection and diagnosis

Biotechnology plays an essential role in the early detection of Cardamom Bushy Dwarf Virus (CBDV), which is crucial for preventing the spread of Forkey disease in cardamom plantations. Molecular techniques such as Polymerase Chain Reaction (PCR) and ELISA (Enzyme-Linked Immunosorbent Assay) can be used to detect the presence of the virus even before visible symptoms appear, by amplifying specific regions of CBDV genome and largescale

screening with antibodies respectively (Biju *et al*., 2010). One possible strategy is to introduce genes for viral resistance, such as RNA silencing genes, which interfere with viral replication within the plant. For example, plants can be engineered to produce small interfering RNAs (siRNAs) that specifically target and degrade viral RNA, preventing the virus from spreading (Kang *et al*., 2023). Another approach could be the transfer of resistance genes from wild relatives of cardamom or other species that have natural resistance to viral diseases, helping to develop genetically modified cardamom plants that are immune to CBDV (Dong *et al*., 2019). Using genomics and bioinformatics tools, researchers can identify regions of the cardamom genome associated with resistance to viral infections, and these markers can be used to accelerate the breeding of resistant varieties (Collard *et al*., 2008).

3.2 Biocontrol measures.

Encouraging natural predators of aphids, such as Ladybird beetles can control the population of aphid species while the parasitoid wasps can lay eggs on aphids and their larvae will consume the aphid from inside leading to aphid mortality (Boivin *et al*., 2012). Fungi like *Beauveria bassiana* and *Metarhizium anisopliae* can be applied as sprays to kill aphids by infecting them with fungal spores. These fungi are host-specific to aphids and can be used safely in the plantation environment (Saif *et al*., 2024). Certain species of nematodes, such as *Steinernema* spp., can parasitize and kill aphids. These nematodes can be applied to the soil or as foliar sprays to target aphids and reduce the vector population (El-Saadony *et al*., 2021). Use of biopesticides, derived from natural sources, can be used to control aphid populations. Neem oil, garlic extract, and chili pepper extract are examples of natural biopesticides that can repel or kill aphids (Daraban *et al*., 2023).

3.3 Development of Resistant Varieties

Researchers can screen cardamom germplasm for resistance to CBDV. Wild relatives or landraces of cardamom that show resistance to aphid infestation or viral transmission can be crossed with cultivated varieties to incorporate these resistance traits into commercial cardamom cultivars (Ghosh *et al*., 2016). Resistance traits from naturally resistant varieties can be bred into high-

yielding cardamom cultivars. However, breeding for virus resistance is challenging because CBDV primarily spreads through aphid vectors, and a plant's immune response to viral infections may not be sufficient to completely prevent the virus (Lecoq *et al*., 2004). Some wild relatives of cardamom may harbor genetic traits that provide resistance to viral infections like Foorkey disease. By incorporating these traits through breeding or genetic modification, it is possible to create new cardamom varieties with enhanced resistance to CBDV (Mandal *et al*., 2013). The process of stacking multiple resistance genes from different sources can be used to create cardamom varieties with robust resistance to Foorkey disease. This could be achieved through both traditional breeding and genetic engineering approaches (Li *et al*., 2023).

3.4 Use of nanotechnology in pest management

Effective and long-lasting solutions have been demonstrated by the application of nanotechnology in pest control. With their own chemical and physical characteristics, nanoparticles open up new possibilities for focused pest management. With substantial mosquitocidal action, nanoparticles have proven to be useful against a variety of insect pest species as toxicants, repellents, and growth retardants. The use of smart pest monitoring where, real-time insect pest detection and monitoring is possible with the development of nanosensors. By recognizing each pest's distinct biomarkers, such as pheromones, enzymes, or particular compounds they release, nanosensors can determine the presence of pests (Sarmah *et al*., 2023).

4. Summary

Foorkey disease, a significant concern in cardamom cultivation, is caused by *Cannabidivarin* (CBDV), a cannabinoid compound. This disease primarily affects the plants' growth, leading to symptoms such as stunted growth, yellowing of leaves, and poor flowering. The presence of CBDV in cardamom plants disrupts normal metabolic and physiological processes, weakening the plants and reducing yield quality. CBDV is thought to disrupt key metabolic and physiological processes in the plant, weakening its defense mechanisms and making it more susceptible to environmental stressors and other pathogens. Despite

ongoing research, the precise molecular mechanisms of CBDV's role in Foorkey disease remain unclear. Effective management strategies are critical to minimize the impact of Foorkey disease, with ongoing efforts focused on breeding resistant varieties and improving cultivation practices. Focusing on deeper molecular studies to understand CBDV's exact pathogenesis and its interactions with cardamom plants. Advances in genomics and molecular breeding could help develop CBDV-resistant cardamom varieties, enhancing plant resilience. Additionally, sustainable agricultural practices, such as crop rotation, pest management, and the use of biocontrol agents, could play a crucial role in mitigating the spread of the disease. Integrating these approaches will be key to ensuring the long-term viability of cardamom farming in affected regions.

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