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Original Research Paper

Assessment of yield loss caused by cucumber mosaic virus, incitant of infectious chlorosis disease in banana (*Musa* sp.)

Waghrulkar P.S., Nagendran Krishnan, Durga Y., Balasubramanian V. and Selvarajan R.*

ICAR-National Research Center for Banana, Tiruchirappalli - 620 102, India

*Corresponding author Email: selvarajanr@gmail.com

ABSTRACT

A survey conducted during 2022 to 2024 in Maharashtra and Madhya Pradesh recorded cucumber mosaic virus (CMV) incidence in banana crops ranging from 16.81% to 25.40%. Molecular analysis identified the isolates as CMV serogroup IB, showing >98% sequence similarity with Indian isolates. CMV caused an estimated 28% yield loss. Infected plants showed delayed flowering and poor bunch development, and only 3.48% of infected plants produced marketable bunches. Nutrient management with 125% recommended fertilizer dose (RDF) and micronutrient supplementation improved bunch weight of infected plants from 9.87 to 12.8 kg and increased bunch production, although yield remained significantly lower than healthy plants. This is the first report estimating economic loss caused by CMV in banana. The findings emphasize the need for effective disease management strategies to reduce CMV losses in banana cultivation.

Keywords: Aphid, banana mosaic, economic loss, RT-PCR assay, viral disease survey

INTRODUCTION

Banana is the fourth most important food crop after wheat, rice and maize in terms of production and the most consumed fruit by quantity (Pareek, 2016). The global banana industry is valued at approximately USD 25 billion (Crawford & Kueffner, 2020). India is the leading producer of bananas, contributing 26.45% of global production with 39.57 million metric tons annually. Viral diseases are major constraints in banana because of insect vectors, alternate hosts and vegetative method of propagation. Important viral; diseases include banana bunchy top, banana streak, banana bract mosaic and infectious chlorosis, all of which threaten banana cultivation across continents.

Infectious chlorosis caused by cucumber mosaic virus (CMV) is an emerging problem in India. CMV infects more than 1,200 plant species, including bananas (Mochizuki & Ohki, 2012). It belongs to the genus *Cucumovirus* (family Bromoviridae) and is a, positive-sense tripartite single-stranded RNA virus. The virus is transmitted non-persistently by more than 60 aphid species, including *Aphis gossypii*, *A. craccivora*, *Myzus persicae*, etc. (Mali & Rajegore, 1980; Rao, 1980). It also spreads through infected planting material, dodder (*Cuscuta* spp.) and occasionally seeds (Palukaitis & Garcia-Arenal, 2003). In banana, CMV mainly affects young crops, causing chlorosis, mosaic, leaf malformation, stunting, pseudostem necrosis, heart-rot and sometimes plant death (Bouhida & Lockhart, 1990). Symptoms expression varies with host and virus strain (Mochizuki & Ohki, 2012).

CMV in banana was first reported in 1943 from Jalgaon, Maharashtra (Kamat & Patel, 1951). Later reports confirmed its occurrence in Gujarat, Tamil Nadu, Karnataka, Andhra Pradesh, Uttar Pradesh and Kerala (Joshi & Joshi, 1976; Kathirvel et al., 1986; Mohan & Lakshmanan, 1988; Estelitta et al., 1996; Ramesh, 2009; Khan et al., 2011; Ali et al., 2012). Maharashtra and Madhya Pradesh together contribute 20.99% of India's banana production (DA&FW, 2024). Grand Nain is the predominant cultivar in these states, propagated through tissue culture and suckers. Despite its long presence, no resistant banana source has been identified. Therefore, the present study was undertaken to estimate yield loss caused by CMV and to evaluate whether nutrient management can improve productivity in infected orchards.

MATERIALS AND METHODS

Survey for incidence of infectious chlorosis disease

A survey was conducted during 2022 to 2024 in 23 tehsils of 8 districts of Maharashtra and Madhya Pradesh to assess CMV incidence in banana. Per cent incidence (PI) was calculated as the number of infected plants relative to the total plants observed.

Assessment of disease pressure

A detailed study was undertaken in three orchards at Chinawal village, Jalgaon district during December 2022 using six-month-old banana plants. A fixed-plot survey method was employed to record the disease incidence and severity. Treatments include, 125% recommended fertilizer dose (RDF, N200: P65: K300) application without CMV disease (T1: treated healthy), 125% RDF application with CMV disease (T2: treated infected), RDF application without CMV disease (T3: untreated healthy) and RDF application with CMV disease (T4: Untreated infected). Disease severity was scored on a 0-5 scale, modified from Murphy et al. (2003). Per cent disease severity (PDS) was calculated using the standard formula,

$$\text{Percent disease severity} = \frac{\text{Sum of individual ratings}}{\text{Maximum disease scale} \times \text{Total number of plants examined}} \times 100$$

Serological detection of CMV

Young leaves were collected from symptomatic plants representing severity grades 1-3 and 4-5 and from asymptomatic plants (5 samples each). Samples were tested for CMV by triple antibody sandwich ELISA (TAS-ELISA) using anti-CMV polyclonal and monoclonal antibodies available at the Molecular Virology Laboratory, ICAR-NRCB, Tiruchirappalli. Samples were tested for CMV by triple antibody sandwich ELISA (TAS-ELISA) using anti-CMV polyclonal and monoclonal available at the Molecular Virology Lab, ICAR-NRCB, Tiruchirappalli.

RT-PCR and sequence analysis

Representative samples were used for total RNA extraction by the lithium chloride method (Asif et al., 2000). First-strand cDNA was synthesized using the RevertAid First Strand cDNA Synthesis Kit. The CMV coat protein gene was amplified using primers RSCMV F (ATGGCAAATCTGAATCAAC) and RSCMV R (TCAAACTGGAGCACCC) (Gayathrie & Selvarajan, 2014), targeting a 657 bp fragment. PCR products were sequenced in both directions and assembled using CLUSTAL W. Pairwise identity was determined using SDT v1.2 (Muhire et al., 2014). Phylogenetic analysis was performed in MEGA 11 using the Neighbor-Joining method with 1000 bootstrap replications (Tamura et al., 2021).

Yield loss assessment

Plants with severity grades 1–3 were tagged with red ribbons and healthy plants with white ribbons. The proportion of infected plants producing bunches and marketable bunches was recorded during the 10th and 11th months after planting.

Nutrient management in infected field

To assess the impact of fertilizer application, half of the infected and half of the healthy Grand Nain banana plants received fertilizer dose of N200: P65: K300 (125% of the recommended - JISL, 2024). The fertilizer was applied in three split doses during the 7th, 8th, and 9th months after planting through soil application. Additionally, plants (infected and healthy) were supplemented with a micronutrient mixture (iron: 4.75%; zinc: 5.25%; boron: 2.50%; manganese: 4.50%; copper: 2.40%) developed by ICAR-NRC Banana, Tiruchirappalli, as a foliar spray. Standard intercultural operations were uniformly followed across all treatments. Banana bunches were harvested between 11 and 12 months after planting, based on harvestable maturity (JISL, 2024). Five randomly selected bunches from each treatment per replication were weighed from three fields and yield were recorded and analysed.

Data analysis

Differences in bunch weight (kg) of the Grande Naine variety among the four treatments were analyzed using the one-way analysis of variance in R software (version 4.5.0). Prior to conducting ANOVA, the assumption of normality was evaluated using the Shapiro–Wilk test, which indicated that the data were normally distributed ($p>0.05$). When significant effects were detected, treatment means were separated using Tukey's Honest Significant Difference (HSD) post hoc test at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

A regional survey conducted during 2022–2024 in 8 districts of Maharashtra and Madhya Pradesh showed variable infectious chlorosis incidence. In Maharashtra, mean PI ranged from 16.81% to 22.93%. In Burhanpur, Madhya Pradesh, PI was 25.40% during 2022–23 and 15.71% during 2023–24 (Table 1). CMV incidence was higher in crops planted during June–July in Jalgaon and Burhanpur.

Table 1 : Details of survey conducted during the year 2022-24 on CMV disease in banana from Maharashtra and Madhya Pradesh states of India

Sl. No.	District	Maharashtra					
		2022-23			2023-24		
		Total no. of plants	No. of infected plants	Disease incidence (%)	Total no. of plants	No. of infected plants	Disease incidence (%)
1	Ahilyanagar	3200	1300	40.63			
2	Hingoli	18500	2650	14.32	5000	300	6.00
3	Jalgaon	2102057	470607	22.39	958500	168040	17.53
4	Jalna	6000	0	0.00	5000	0	0.00
5	Pune	7600	304	4.00	3800	500	13.16
6	Satara	3000	400	13.33			
7	Solapur	302230	84785	28.05	59950	4698	7.84
Maharashtra		2442587	560046	22.93	1032250	173538	16.81
Sl. No.	District	Madhya Pradesh					
		2022-23			2023-24		
		Total no. of plants	No. of infected plants	Disease incidence (%)	Total no. of plants	No. of infected plants	Disease incidence (%)
1	Burhanpur	2025500	511535	25.25	1275540	226868	17.79
2	Khaknar	87500	35120	40.14	170000	460	0.27
3	Nepanagar	62000	5750	9.27	4000	400	10.00
Burhanpur district		2175000	552405	25.40	1449540	227728	15.71

Table 2 : Assessment of yield loss caused upon infection of CMV in banana at village Chinawal (Jalgaon district, Maharashtra, India) during year 2022-23

Parameter	Field No.			Total
	1	2	3	
Total plants observed	503	2124	2520	5147
Healthy plants	125	1615	1914	3654
Infected plants (A-B)	378	509	606	1493
% Healthy plants [(B/A)×100]	24.85	76.04	75.95	70.99
% disease incidence [(C/A)×100]	75.15	23.96	24.05	29.01
% disease severity	49.62	21.23	20.83	23.81
Number of infected plants produced bunch	44	28	33	105
% infected plants produced bunches [(G/C)×100]	11.64	5.50	5.45	7.03
Number of marketable bunches from CMV infected plants	19	14	19	52
% of CMV infected plants yielding marketable bunches [(I/C)×100]	5.03	2.75	3.14	3.48
% CMV infected bunch marketable [(I/G)×100]	43.18	50.00	57.58	49.52
% of plants produced CMV infected marketable bunches [(I/A)×100]	3.78	0.66	0.75	1.01
Yield loss [100 –(D+L)]	71.37	23.3	23.3	28

Considerable variation in disease incidence and severity was observed among orchards in Chinawal village during 2022–23 (Table 2). Disease incidence ranged from 23.96% to 75.15%, while severity ranged from 20.83% to 49.62%. About 7.03% of infected plants produced bunches, but only 49.52% of those bunches were marketable. Thus, only 2.75-5.03% of infected plants produced marketable bunches, emphasizing the critical impact of CMV on yield and fruit quality. These results support the need to evaluate timely rouging of infected plants in disease management programs.

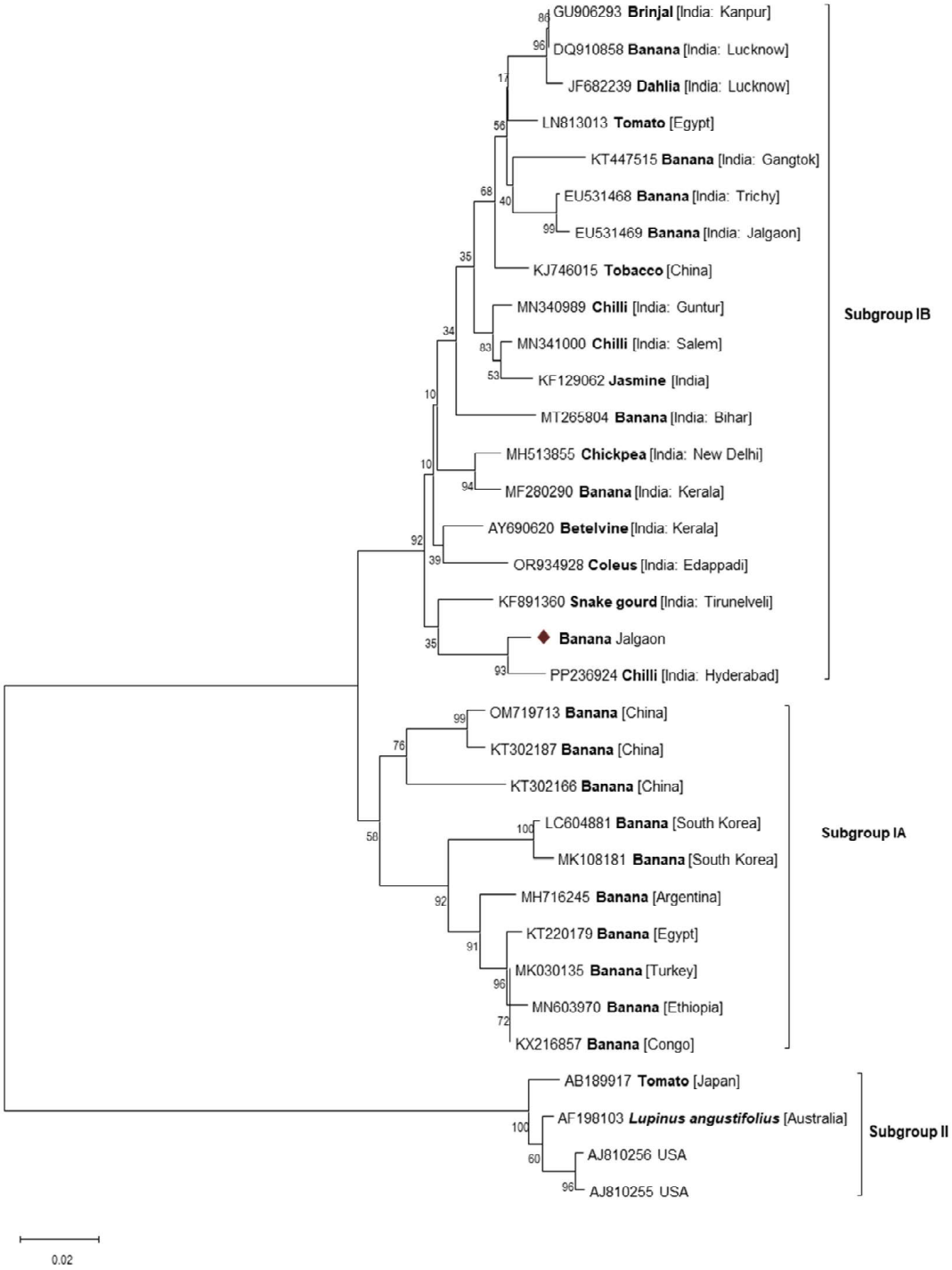

Fig. 1 : Phylogenetic analysis of CMV isolates infecting banana from Jalgaon based on coat protein sequences with other Cucumber mosaic virus isolates across the globe

TAS-ELISA showed high absorbance values (2.17–4.00) in symptomatic plants of severity grades 1–3 and 4–5, whereas asymptomatic plants showed low values (0.25–0.37). However, absorbance values were not clearly related to symptom severity, suggesting effects of host response, environment or isolate variation on symptom expression.

RT-PCR confirmed CMV infection in all symptomatic samples by amplification of the expected 657 bp coat protein fragment, whereas asymptomatic plants were negative. Sequencing results showed >99.3% identity among samples collected from three orchards and showed >95.4% identity with Indian CMV isolates infecting chilli crops (e.g., PP236924, PP236925 and MN340989). Phylogenetic analysis with one representative sequence, grouped within serogroup IB, clustering them with other Indian CMV isolates (Fig. 1), confirming their genetic similarity. CMV isolates belonging to serogroup IB have previously been reported from a wide range of horticultural crops in India (Vinodhini et al., 2020; Nagendran et al., 2018; Kumari et al., 2021; Kumar et al., 2020). Interestingly, CMV isolates infecting banana in other countries such as China, South Korea, Argentina, Egypt, Ethiopia, Turkey, and Congo predominantly belong to serogroup IA, whereas in India it is IB, indicating possible regional.

From the total number of plants observed in the present study, 70.99% produced healthy bunches, while only 1.01% of plants yielded CMV-infected but marketable-quality bunches (Table 2). The overall yield loss due to CMV infection was estimated as 28%. However, this loss varied based on the crop's growth stage at the time of infection, the severity of symptoms, and the location of cultivation. Tomato yellow leaf curl virus has been shown to cause 20–100% yield losses (Levy & Lapidot, 2008), while Chilli leaf curl virus led to 43–98% reductions depending on infection timing (Ashwathappa et al., 2022). Similarly, Sugarcane mosaic virus and Soybean mosaic virus have caused yield losses ranging from 10–80% (Lu et al., 2021) and 8–25% (Rupe & Luttrell, 2008), respectively.

In chilli, micronutrient application combined with integrated disease management reduced leaf curl incidence and increased yield (Nagendran et al., 2020). Similarly, foliar sprays of zinc sulfate, boric acid, and urea with soil drenching of humic acid reduced papaya ringspot virus symptoms and improved growth (Deepika et al., 2021). In the present study, nutrient treatment influenced bunch weight in CMV-affected banana plants. Treated healthy plants recorded the highest mean bunch weight (27.77 kg), followed by treated infected plants (12.8 kg). Untreated healthy and untreated infected plants produced 24.93 kg and 9.87 kg, respectively (Table 3).

Disease incidence remained similar in treated infected (41.99%) and untreated infected plots (40.15%), indicating uniform disease pressure. However, nutrient treatment had only a limited effect on bunch production in infected plants, increasing bunch-bearing plants from 5.94% to 8.02% (Table 4). Earlier, higher fertilizer doses were also reported to improve growth and yield in banana bract mosaic virus-infected banana plants (Selvarajan et al., 2017). These findings indicate that fertilizer response may vary with the virus–host system and does not necessarily reduce virus impact. Bananas planted during June-July in the Jalgaon-Burhanpur region usually mature in 10–11 months and are harvested in 4–5 rounds. Although, nutrient management slightly improved bunch weight and numbers, CMV delayed flowering and bunch

Table 3 : Effect of 125% RDF supplemented with micronutrients on banana bunch weight (kg) under healthy and CMV-infected conditions

TREATMENT	FIELD 1	FIELD 2	FIELD 3	MEAN ± SD
T1 (Treated healthy)	22.5	33.0	27.8	27.77 ± 3.04[a]
T2 (Treated infected)	10.0	14.6	13.8	12.80 ± 1.46[b]
T3 (Untreated healthy)	21.0	28.0	25.8	24.93 ± 2.04[a]
T4 (Untreated infected)	9.5	9.1	11.0	9.87 ± 1.02[b]
P-value: 0.0001				

Values are expressed as mean ± standard deviation (SD). Means followed by different superscript letters within the column differ significantly according to Tukey's HSD test at p d" 0.05

Table 4 : Effect of treatment (125% RDF with micronutrient mix) on number of bunches produced in CMV infected banana plants

Field	Treated infected		Untreated infected	
	PDI	No. of bunch/No. of infected plants	PDI	No. of bunch/No. of infected plants
1	74.60	27/188	75.70	17/190
2	24.95	15/265	22.98	13/244
3	26.43	21/333	21.67	12/273
Total		63/786		42/707
Average	41.99	21/262	40.15	14/235.67

formation by 20–60 days. As a result, many infected bunches missed the prime harvest window and fetched lower market prices.

CONCLUSION

Infectious chlorosis is transmissible, and infected plants act as reservoirs for further spread. Since only 3.48% of infected plants produced marketable bunches, retaining infected plants in the field is uneconomical. As no CMV-resistant banana variety or effective antiviral treatment is available, farmers should be advised to remove infected plants from fields.

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