

## First Report of *Cucurbit aphid-borne yellows virus* Infecting Ridge Gourd in Pakistan: An Emerging Threat to Cucurbit Production

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### ABSTRACT

*Cucurbit aphid-borne yellows virus* (CABYV) poses a significant threat to cucurbit crops worldwide. This study provides insight into coat protein and RNA-dependent RNA polymerase regions of CABYV isolates infecting ridge gourd (*Luffa acutangula*) in Pakistan. Field surveys in the Lahore district revealed that ridge gourd plants have severe interveinal yellowing and vein thickening symptoms (usually associated with polerovirus infection) and a 10–40% disease incidence. PCR amplification by polerovirus group-specific primers and sequencing confirmed CABYV infection in 11 of 19 samples. In phylogenetic reconstruction, Pakistani CABYV-isolates clustered with isolates from Taiwan and Indonesia, and the BLAST analysis showed >90% sequence similarity with these isolates, highlighting CABYV's global genetic diversity. These findings underscore the need for comprehensive surveys and genomic studies to develop sustainable management strategies for this destructive pathogen.

**Key words:** Polerovirus, CABYV, Viral diseases in cucurbits, Molecular characterization.

### INTRODUCTION

Cucurbits, a family of fruits and vegetables of economic and nutritional significance, contribute big to global agriculture. In Pakistan, cucurbits are extensively cultivated on an area of 20,433 hectares in subtropical, tropical, and temperate regions with an annual production of 357,064 tons, contributing significantly to the national economy (Pakistan Bureau of Statistics, 2023). Cucurbits are usually infected by biotic stresses, which include viral infections that lead to considerable yield losses of ~\$30 billion each year (Jones and Naidu, 2019). Cucurbits may be threatened by the Cucurbit Aphid-Born Yellows Virus (CABYV). CABYV, belonging to genus Polerovirus in the family *Luteoviridae* family (Igori et al., 2022) but is currently a member of the

*Solemoviridae* family (Vidal et al., 2023) poses a serious production threat to the cucurbit productoin. The genome of the members of Poleroviruses is single-stranded positive-sense RNA that is 5.6 to 5.9kb in size. At the 5' terminus, a genome-linked protein (VPg) is located (Kamran et al., 2018; Tokuda et al., 2023). Its genome contains six open reading frames (ORFs) among which P2 is considered to play a pivotal role on mobility, interactions and replication of CABYV. The P2 genomic region is thought to be essential for this virus's mobility, interaction, and replication (Lecoq et al., 1992). Almost all the members of the *Cucurbitaceae* family are hosts of these viruses, which are transmitted by two aphid species—*Myzus persicae* and *Aphis gossypii*—in the non-propagative circulative manner (Cui et al., 2022; Nagendran et al., 2023).

CABYV was reported from France for the first time in 1982, and afterwards it was reported from several other regions of the world, including Pakistan, where it was found in melon plants in 2020 (Ahsan et al., 2020). The plants infected with CABYV are reported to show mottling, yellowing, chlorosis, and leaf deformation symptoms, which eventually result in reduced yield and compromised quality of fruits. It has been established that it is almost impossible to devise an effective virus management strategy without identification, characterisation and epidemiology and viral propagation and evolutionary patterns.

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This study was focused on identifying and analyzing the evolutionary association of world CABYV isolates with Pakistani isolates based on the CP gene sequence. The aim was to explore the evolutionary patterns and genetic diversity to contribute to the sustainable management of this viral threat.

## MATERIALS AND METHODS

### Sample Collection

In November 2023, a total of 19 ridge gourd leaf samples exhibiting severe interveinal yellowing, chlorosis and thickening of veins and leaf symptoms (prevailing on 10-40% of crops) were collected (in zipper bags stored in ice) from vegetable fields (Saif Town, Mohlanwal and University of the Punjab Research Field) in the Lahore district of Pakistan. Such symptoms are mainly attributed to Poleroviruses (Lecoq et al., 1992). The average disease incidence was observed to range from 10-40%.

### Total Plant RNA Isolation and Two Step RT-PCR

Total plant RNA was extracted from 100 mg of symptomatic leaves crushed into fine powder by sterilized pestle and mortar using liquid nitrogen by TRIzol® Reagent (Life Technologies, USA) as per manufacturer's protocol. Viral cDNA from the total plant RNA of each sample was synthesized by using CABYV-specific reverse primer (Juarez et al., 2004) by Thermo Scientific RevertAid Reverse Transcriptase as per the given protocol by the manufacturer. A part of the CABYV genome was amplified by PCR using Thermo Scientific DreamTaq

PCR Master Mix and the degenerate primers (PocoN-F 5'-TGGACYTGGTTYGGNTGGAC-3' and PocoN-R 5'-CKRTAYTGRTCRTCCCRTA-3') at PCR conditions: 95°C for 3 min; 35 cycles of 95°C (30s), 55°C (45s), 72°C (60s); final extension at 72°C (10 min) reported by Xiang et al. (2008). The results were further confirmed by CABYV CP-gene-specific primers (Juarez et al., 2004). The QIAquick PCR Purification Kit (Qiagen, Germany) was used to purify the amplicons.

### Sequence Analysis

The purified PCR products were commercially sequenced and analyzed by the NCBI BLAST tool. Highly similar sequences were retrieved from BLAST search results and aligned by CLUSTAL W in MEGA 10. The Tamura-Nei substitution model was used for neighbor-joining tree construction in MEGA 10.

## RESULTS AND DISCUSSION

Despite being a significant crop worldwide, cucurbits are becoming more susceptible to diseases like the *Cucurbit aphid-borne yellows virus* (CABYV). Typical signs of viral infection, such as leaf deformation, curling, and interveinal chlorosis, were observed during field surveys (Fig. 1). Such symptoms have previously been reported to be associated with polerovirus infections in several studies from China, Taiwan, and India (LaTourrette et al., 2021; Knierim et al., 2010; Basavaraj et al., 2023; Rabadan & Gomez, 2023).

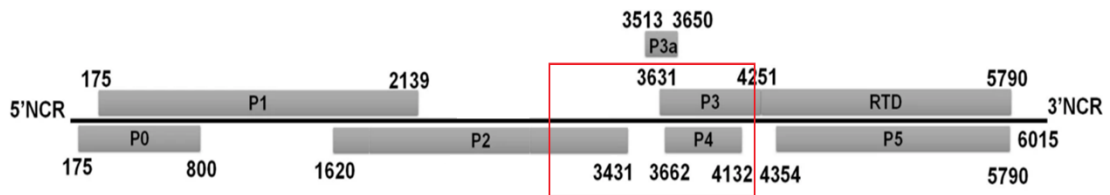


**Figure 1.** Foliar symptoms of Poleroviruses infecting cucurbits observed in farmer's fields.

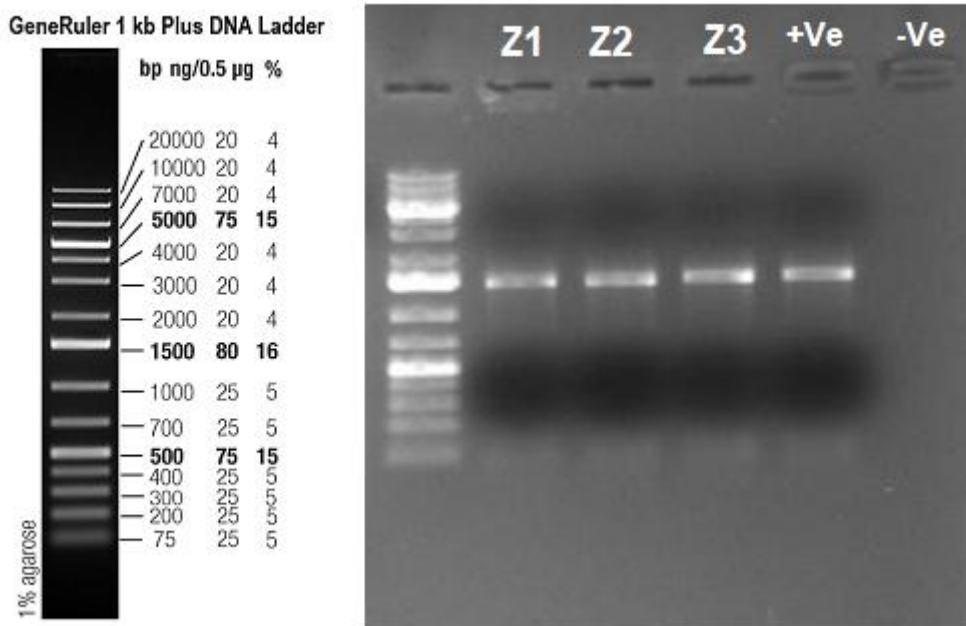
**Sequence Analysis**

The specific primers targeting RdRp and CP genes of the CABYV genome (Fig. 2) amplified ~1400 bp fragments (Fig. 3) by RT-PCR. CABYV was detected in 11 of 19 symptomatic samples. The BLAST analysis revealed that the obtained sequences were 1249 bases of the CABYV genome comprising the P1 gene (partial at the 5' end), the P3-P5 gene (partial at the 3' end), and the CP gene (partial at the 3' end). The nucleotide sequence of a single isolate (CABYV\_PK\_Z3) was submitted to GenBank

(accession No. PP112273) as all others were identical. This isolate shared a highest of 96% (1199 out of 1249) nucleotide sequence identity with Chinese isolates (EF488997) and 95.11 to 95.92% (1188-1198 out of 1249 bases) with other isolates from Taiwan, China, and Spain. Sequence analysis revealed high nucleotide identity (94.5–98.8%) and amino acid identity (95.8–99.5%), which is in line with earlier studies of CABYV spread (Rabadan et al., 2023; Cui et al., 2022).



**Figure 2.** Position of the amplified fragments (Red Box) in CABYV genome.



**Figure 3.** Amplified fragments of ~1400 bases by PocoN F&R primers (Xiang *et al.*, 2008).

**Phylogenetic Analysis**

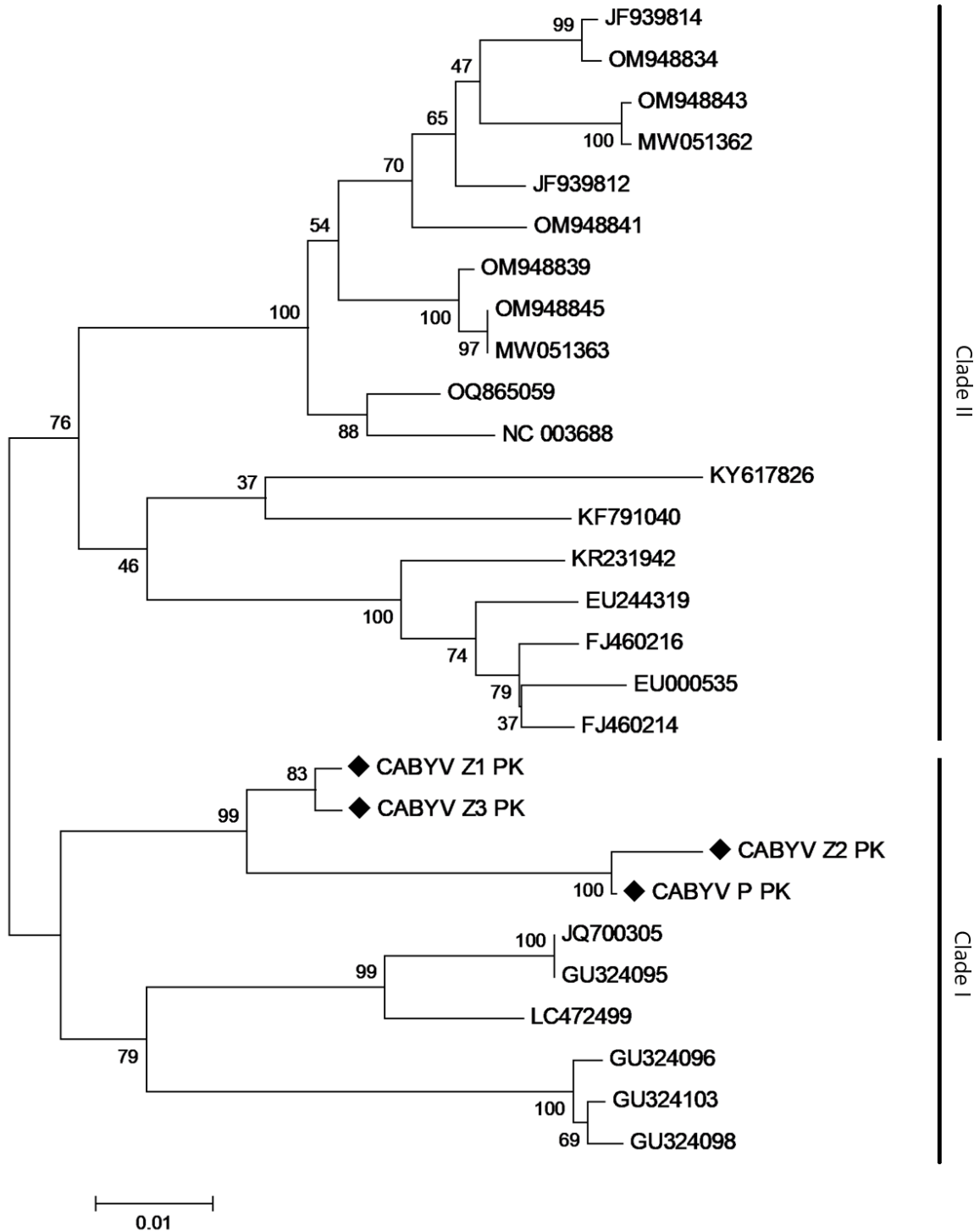
The CP gene nucleotide sequences of Pakistani and other closely related isolates (retrieved from the gene bank given in Table 1) were used for phylogenetic reconstruction. The Pakistani isolates clustered with East Asian strains (Clade I), suggesting potential

introduction via aphid vectors or agricultural trade. In contrast, the divergence of European isolates (Clade II) highlights regional evolutionary paths (Fig. 4), which is suggestive of evolutionary stability in important genomic regions of CABYV such as the coat protein (CP) and RNA-dependent RNA

polymerase (RdRp) genes (Khanal and Ali, 2019). The intergenic non-translated region (NTR) between the RdRp and CP genes showed notable sequence differences, including a conserved ACAAAGA motif essential for subgenomic RNA transcription (Chen et al., 2016; Xu et al., 2022).

Table 1. List of *Cucurbit aphid born yellows virus* isolates downloaded from NCBI-Genbank.

Acc. No	Isolate	Country	Year	Host
LC472499	-	Indonesia	2017	Cucumis sativus
JQ700305	CABYV-C-TW20	Taiwan	2008	Bittermelon
GU324095	CABYV-C-TW20	Taiwan	2008	Bittermelon
KY617826	-	Australia	2017	Cucumber
GU324095	CABYV-C-TW20	Taiwan	2008	Bittermelon
KY617826	50AL	Timor-Leste	2015	Cucumber
GU324103	CABYV-C-TW8	Taiwan	2008	Cucurbita moschata
GU324096	CABYV-C-TW27	Taiwan	2008	Squash
KF791040	CABYV-V-TH22	Thailand	2012	Cucumber
GU324098	CABYV-C-TW36	Taiwan	2009	Squash
OQ865059	-	Iraq	2022	Squash
JF939814	-	Spain	2011	
EU244319	Shanxi	China	2007	Squash
OM948839	19.1 M/M	Spain	2015	Melon
OM948834	1.1 M/M	Spain	2014	Melon
NC003688	-			
FJ460216	CABYV-JX	China	2008	Cucurbit
OM948845	22.1 Z/M	Spain	2019	Zucchini
MW051363	LP63	Spain	2019	Watermelon
EU000535	-	China	2007	Cucurbit
JF939812	-	Spain	2011	
FJ460214	CABYV-AH	China	2008	Cucumber
KR231942	CY3	South Korea	2013	Melon
OM948843	2.1M/M	Spain	2013	Melon
OM948841	19.1Z/A	Spain	2019	Zucchini
MW051362	MEC-12	Spain	2014	Melon



**Figure 4.** Phylogenetic tree of Pakistani and world isolates based on CP gene nucleotide sequences of *Cucurbit aphid born yellows virus*.

In Pakistan, the detection, identification (through serological and molecular methods), incidence and distribution have been extensively studied (Reinbold et al., 2003; Moya-Ruiz et al., 2023). However, little

is known about its genomic attributes, since the data about complete genomic characterization is not available. Also, how does the co-infection of CABYV

with other viruses worsen the symptoms and management challenges (Rabadan and Gomez, 2023). In recent studies, CABYV species complex infection was detected, highlighting the possible recombination events and the novel strain formation (Kwak et al., 2018; Vidal et al., 2023).

In the current study, symptomatic plant samples collected from the Lahore district of Punjab were found to be infected with CABYV. The detection of this virus in an area of heavy vegetable production might be a threat as it can spread quickly. To estimate its presence in the crops planted in the region, an extensive survey followed by its molecular characterisation is a dire need of time. This will help to comprehend the development of new strains, its behaviour and their evolutionary patterns. These findings will help in developing a workable strategy to manage the disease in vegetable-growing areas.

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