Mexican Journal of Phytopathology
ISSN: 2007-8080

Crossref
Open access

Phytopathological Note

Phytopathology and cultural behaviors: putative introduction of Chaya-strain of *Cassava common mosaic virus* to Costa Rica

Mauricio Montero-Astúa*, **Izayana Sandoval-Carvajal**, **Lisela Moreira-Carmona**, Laboratorio de Fitopatógenos Obligados y sus Vectores, Centro de Investigación en Biología Celular y Molecular y Escuela de Agronomía, Universidad de Costa Rica, 11500-2060 San José, Costa Ric; **William Villalobos-Muller**, **Laura Garita-Salazar**, **Sofía Carvajal-Rojas**, Laboratorio de Fitopatógenos Obligados y sus Vectores, Centro de Investigación en Biología Celular y Molecular, Universidad de Costa Rica, 11500-2060 San José, Costa Rica.

***Corresponding Author**:
Mauricio Montero-Astúa
mauricio.monteroastua@ucr.ac.cr

Section:
Special Number

Received:
30 July, 2023
Accepted:
15 December, 2023
Published:
30 December, 2023

Citation:
Montero-Astúa M, Sandoval-Carvajal I, Moreira-Carmona L, Villalobos-Muller W, Garita-Salazar L and Carvajal-Rojas S. 2023. Phytopathology and cultural behaviors: putative introduction of Chaya-strain of *Cassava common mosaic virus* to Costa Rica. Mexican Journal of Phytopathology 41(4): 7. DOI: https://doi.org/10.18781/R.MEX.FIT.2023-3

ABSTRACT

Objective/Background. Leaves of the shrub chaya (*Cnidoscolus aconitifolius*), spinach tree or 'chicasquil' (in Costa Rica), are consumed in the Mesoamerican culinary tradition, having its origin in South Mexico and Guatemala. The objective of this work was to verify the viral nature of the observed in a chaya plant disease and to identify the species of the virus.

Materials and Methods. Plant virus detection and identification was achieved by TEM, RT-PCR using degenerated primers to potexviruses, and sequencing. Pathogenicity tests were done by mechanical inoculation using chaya symptomatic tissue, on *Nicotiana benthamiana* and chaya plants.

Results. We report CsCMV detection in a chaya plant in Costa Rica with mosaic symptoms. Pathogenicity and association of virus and symptoms were demonstrated by mechanical inoculation in *Nicotiana benthamiana* and chaya plants. We hypothesize this infection corresponds to a recent introduction and discussed how cultural traditions impact the distribution of plant viruses.

Conclusion. The findings confirm the presence of a CsCMV-related virus, previously unreported for Costa Rica, in *Cnidoscolus aconitifolius*. The results herein highlighted the need to study its distribution and diversity throughout Latin America.

Keywords: chicasquil, virus spread, *Cnidoscolus aconitifolius*, var. Chayamansa, var. Estrella

Introduction

Chaya, spinach tree or, in Costa Rica known as 'chicasquil' (*Cnidoscolus aconitifolius* spp. *aconitifolius*, Euphorbiaceae; syn. *Cnidoscolus chayamansa*) is a Mesoamerican shrub, probably native to southern Mexico and Guatemala, and distributed from southern Texas through South America. It was introduced to the Caribbean islands and from there to Florida, tropical Africa, Asia, and Oceania. Chaya is cultivated since pre-Hispanic times, it is used in traditional medicine, and in the cuisine for indigenous groups and traditional recipes of several countries, including Costa Rica (Ebel *et al*., 2019; Ross-Ibarra and Molina-Cruz, 2002).

At least four cultivated varieties: 'Chayamansa', 'Redonda', 'Estrella', and 'Picuda' are recognized in Yucatan and Guatemala, in addition to the wild forms of the species. The plant is clonally propagated by stem cuttings and these varieties produce few or no seeds (Ebel *et al*., 2019; Ross-Ibarra and Molina-Cruz, 2002). In Costa Rica, Hammel *et al.* (2010) mentioned that at least two varieties are cultivated, the most common and distributed one, is called 'Chicasquil' and seems to correspond to variety 'Picuda', var. 'Redonda' is very rare, mainly in Baja Talamanca, where it is known as chaya among the indigenous communities.

In March 2020, mosaic symptoms were observed on new growth of a planted cutting of chaya (sample 20.222, Table 1) in a backyard in Moravia, San José (9.964091, -84.041644). Cuttings were given away among family and friends as:

Table 1. Chaya (*Cnidoscolus aconitifolius*) samples evaluated for viral symptoms and sources of stem cuttings for transmission assays.

Sample Code	Canton, Province	Collection Date	Variety	Symptoms	RT-PCR[x]
20.222	Moravia, San José	4/2020	Estrella	Mosaic	Positive[y]
20.439[z]	Montes de Oca, San José	11/2020	Picuda	No	Negative
20.451	Pococí, Limón	11/2020	Estrella	No	Negative
21.030[z]	Vázquez de Coronado, San José	2/2021	Chayamansa	No	Negative
21.031[z]	Alajuela, Alajuela	2/2021	Picuda	No	Negative

[x] Retro-transcription-Polymerase Chain Reaction with primer pair Potex4/Potex5 (Miglino et al., 2006).

[y] GenBank accession number OK642586, RdRp partial sequence.

[z] Codes in Bold. Stakes (stem cuttings) were obtained to stablish plants for inoculation assays (modified Koch's postulates).

"chaya, a medicinal tree from southern Mexico". As the new plant developed a light-green/dark-green mosaic was notorious in all leaves (Figure 1a, b). The plant seems like chaya plant (chicasquil), but leaf morphology had some differences. There is no tradition in recognizing chaya varieties in Costa Rica, and we found discrepancies in the illustrations for the varieties between Ebel *et al.* (2019) and Ross-Ibarra and Molina-Cruz (2002), especially var. 'Chayamansa'. Thus, our identification of the varieties is tentative, based on the authors descriptions and illustrations. The symptomatic plant seems to be var. 'Estrella' (uncommon in Costa Rica). We hypothesized the observed symptoms correspond to a viral infection; therefore, the objective of this work was to verify the viral nature of the plant disease and to identify the virus species.

To test the hypothesis of a putative virus infection, the symptomatic plant was uprooted and transplanted to a nursery plastic pot #3000 (3 gallons vol.) using soil from the site of collection. The plant (20.222) is kept at LaFOV-CIBCM

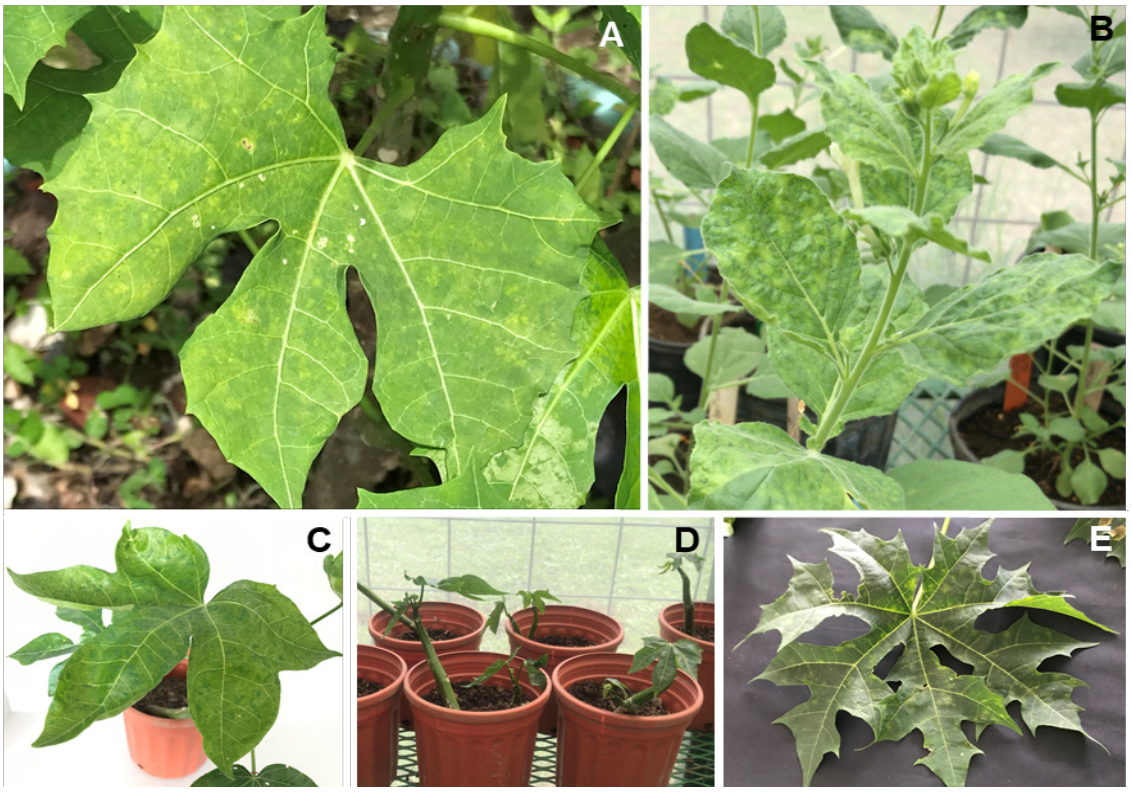

Figure 1. Chaya (*Cnidoscolus aconitifolius*) sample 20.222, tentative var. 'Estrella', with mosaic symptoms and positive for *Cassava common mosaic virus* (CsCMV) (A). Mechanical inoculation of sample 20.222 on *Nicotiana benthamina* (B) and chaya, tentatively var. 'Chayamansa' (C) showing mosaic symptoms. Stablished cuttings from sample 21.030 in LaFOV-CIBCM greenhouse (D). Leaf morphology of var. 'Picuda' (E).

(Universidad de Costa Rica, San Pedro, San José) greenhouse A (aphid-proof screen house with polycarbonate roof). Fresh leaf tissue was grinded in potassium phosphate buffer (0.05M, pH 7.0) and inoculated by rubbing a cotton swab wet with the leaf extract on the second and third younger leaves (from top to bottom) of 12 *Nicotiana benthamiana* plants sprinkled with carborundum. *N. benthamiana* plants were *ca*. two months old from germination, kept in a LaFOV-CIBCM greenhouse B, and in plastic nursery plant pots #100 with steam-disinfested soil. Additionally, 12 plants were inoculated with buffer as controls. Plants were evaluated once every seven days after inoculation (dai). A piece of the same chaya symptomatic tissue was fixed with Karnovsky solution in 0.05 M cacodylate buffer pH 7 at 6 - 8 °C and afterwards processed for Transmission Electron Microscopy (TEM) following previously described protocols (Montero-Astúa *et al*., 2008).

Total ribonucleic acid (RNA) was extracted from chaya (sample 20.222) and from the symptomatic *N. benthamiana* (i20.222-Nb) following the protocol of the RNeasy® Plant Mini Kit (Qiagen, Germany). cDNA was obtained by retrotranscription with Maxima H minus first strand cDNA kit (Thermo Scientific, Lithuania) following the manufactures protocol. The cDNAs were tested for *Potexvirus* genus presence with degenerate genus specific primers, Potex4 (5'-AGCATGGCGCCATCTTGTGACTG-3') / Potex5 (5'-CTGAAGTCACAATGGGTGAAGAA-3'), 280bp amplicon (Miglino *et al*., 2006) in a final reaction volume of 25 µL, containing 2 µL of the cDNA, 1X DreamTaq PCR Master Mix (Thermo Scientific, Lithuania), and 200 nM each primer. Reactions were run in a thermal cycler (MJMini, Bio-Rad, Singapore) with the profile: 94 ºC x 2 min; 45 x (94 ºC x 30 sec; 60 ºC x 1 min; 68 ºC x 1 min); 72 °C x 10 min. Additionally, PCR reactions were set with potexvirus primer pairs Potex1RC (5'-TCAGTRTTDGCRTCRAARGT-3') /Potex5V (5'-CAYCARCARGCMAARGAYGA-3') or Potex2RC (5'-AGCATRGCNSCRTCYTG-3') / Potex5V; expected amplicons of 735 bp and 584 bp, respectively (van der Vlugt and Berendsen, 2002) with the same PCR conditions as before and with the thermal profile: 95 °C x 5 min; 35 x (92 °C x 30 sec; 51.5 °C x 30 sec; 72 °C x 10 min); 72 °C x 10 min. Negative controls (PCR reaction mixture without cDNA, water instead) were included in all PCR runs and potexvirus positive controls with *Plantago asiatica mosaic virus* (PlAMV) and *Cymbidium mosaic virus* (CymMV) from CIBCM-UCR collection (Montero-Astúa *et al*., 2017). Amplification products were visualized by running a 1% agarose gel in 1X TAE buffer (40 mM Tris base, 20 mM glacial acetic acid, and 1 mM EDTA) at 90 volts, using GelRed® nucleic acid gel stain (10000X in water, Biotium, USA) in sample loading buffer (TriTrack DNA Loading Buffer 6X, Thermo Scientific, Lithuania) and observed on a UV transilluminator (55W, BXT-26.M, Uvitec, France). Amplicons were sent to Macrogen (Macrogen Inc., South

Korea) for purification and Sanger sequencing, in both directions, using the same primer pairs utilized for amplification. Sequences were edited and contigs obtained in BioEdit (v.7.0.5.3). Sequences of CsCMV were downloaded from GenBank and phylogenetic analysis was done with MEGA X (v.10.1.5) with Maximum Likelihood statistical method, using Tamura-Nei (TN93) nucleotide substitution model and modeling non-uniformity of evolutionary rates among sites by a discrete Gamma distribution (+*G*). Test of the phylogeny was done by bootstrap method with 2000 replications.

After identifying a putative virus associated with the symptoms, additionally 13 plants were identified at different locations and visually evaluated for mosaic symptoms. Four plants were sampled (one young fully expanded leaf/plant) for further analysis by RT-PCR with primers Potex4/Potex5 as previously described (Table 1). Modified Kochs postulates for plant viruses were conducted. Stem cuttings from three of those additional plants sampled and negative for potexviruses presence by RT-PCR (20.439, 21.030, 21.031, Table 1) were sown in plastic nursery plant pots #400 with steam-disinfested soil and kept at LaFOV-CIBCM greenhouse B. The stablished plants, four months after sowing were inoculated with sample 20.222 as previously described herein. One cutting from each mother plant was mock inoculated with buffer as controls, thus 13 plants virus inoculated, and three plants mock inoculated. Plants were evaluated once every seven days until 35 dai. Leaf tissue was collected from the symptomatic chaya inoculated plants and a compound sample was obtained per origin of the cuttings (n=3, 20.439, 21.030, 21.031). The samples were stored at -35 °C and processed for RT-PCR with primer pair Potex5/Potex5 as previously described.

Chlorotic mosaic developed in three *N. benthamiana* plants 28 days after mechanical inoculation with sample 20.222. A second inoculation was done to confirm symptoms, using as inoculum symptomatic *N. benthamiana* tissue obtained from the previous inoculation; and 3 out of 4 *N. benthamiana* showed strong chlorotic mosaic and leaf blistering, 28 dai (Figure 1c).

Chloroplasts showing lack of lenticular shape, alteration or absence of thylakoids, and increased presence of osmiophilic granules were observed by TEM in ultra-thin sections obtained from leaf tissue of the symptomatic chaya (20.222) (Figure 2). Those alterations are similar to the ones described for CsCMV in cassava by Zanini *et al*. (2021). Particles or aggregates associated to potyviruses (pinwheels), isometric particles (e.g. cucumoviruses), bacilliform (badnaviruses) or bullet shaped (plant rhabdoviruses) were not observed. Nuclei showed regular appearance, without inclusions nor viral particles. Banded inclusions were observed in the sample (Figure 2), corresponding to the ones reported for potexviruses and similar to those reported for CsCMV in chaya (Zettler and Elliott, 1986) and cassava (Zanini *et al*., 2014).

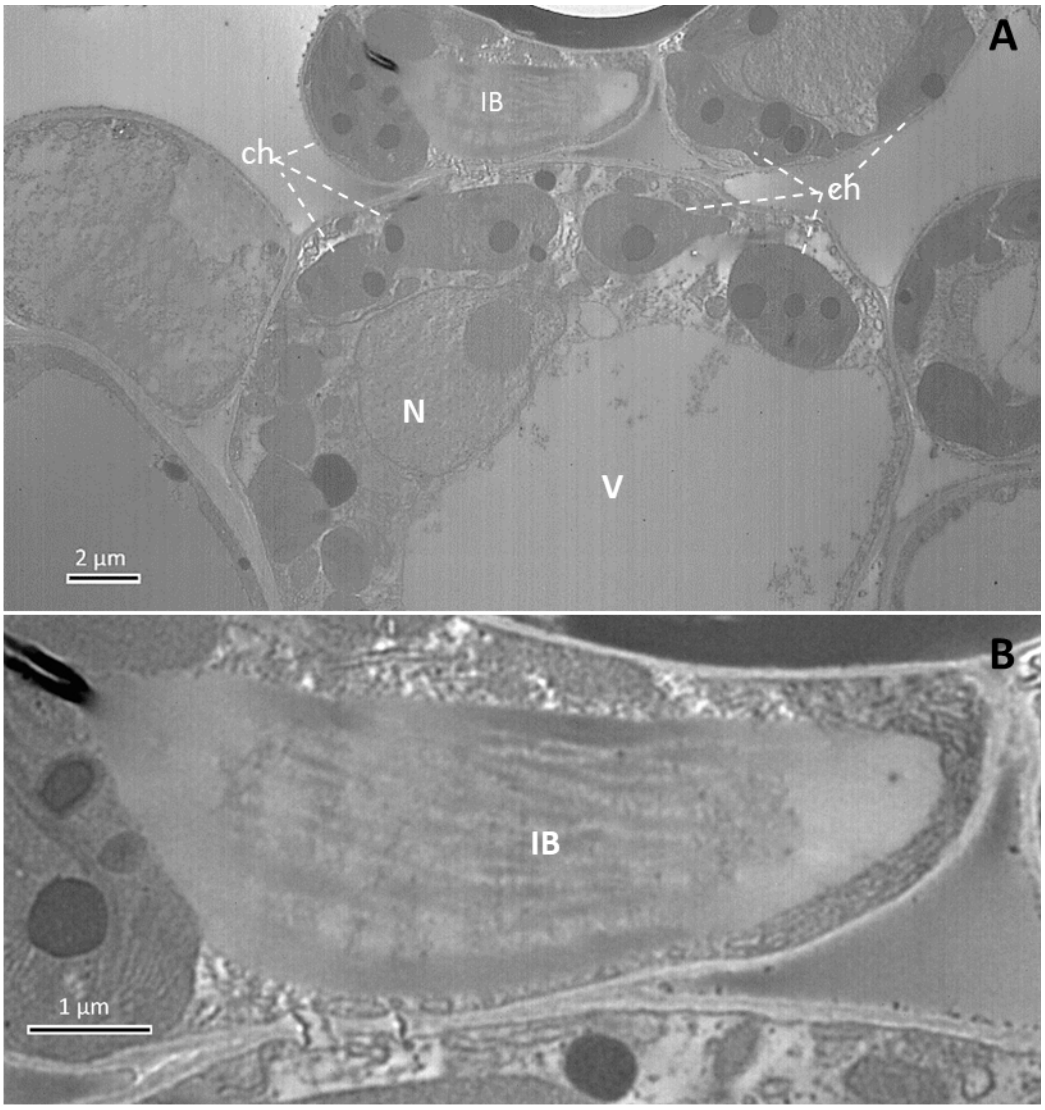

Figure 2. Transmission electron microscope observations of leaf tissue of chaya (*Cnidoscolus aconitifolius*) with mosaic symptoms detected in Costa Rica. Damage observed in chloroplasts (ch), with loss of their typical shape and thylakoids, and a banded inclusion (IB), similar to those associated with potexviruses (A). Detail of IB (B). N: nucleus, V: vacuole.

Chaya (20.222) and tissue from the inoculated-symptomatic *N. benthamiana* (iNb-20.222) resulted positive to potexviruses by RT-PCR with several *Potexvirus* genus degenerate primers. Sequencing of the amplicons obtained from the chaya sample (20.222) and from *N. benthamiana* (iNb-20.222) rendered partial sequences corresponding to *Cassava common mosaic virus* (CsCMV) and resulted in the identification of the same viral pathogen in both chaya (sample 20.222) and

inoculated-symptomatic *N. benthamina* (iNb-20.222). Amplicons with different primer pairs overlapped, therefore a final contig for the symptomatic chaya sample (20.222) was deposited in GenBank (Accession Number OK642586). A phylogenetic tree with the sequence and several sequences available from GenBank for CsCMV showed the chaya sequence of Costa Rica to group in an independent cluster with CsCMV-chaya sequences from Venezuela (Mejías *et al*., 2015) and separated from CsCMV sequences from cassava, including the one reported as from Costa Rica (Lozano *et al*., 2017) (Figure 3).

We searched mosaic symptoms at several locations with a total of 13 chaya shrubs visually evaluated in addition to the first symptomatic sample (20.222). No mosaic symptoms as in sample 20.222 were observed (Table 1). Four selected additional samples resulted negative to potexviruses by RT-PCR (Table 1).

Only three plants (tentative var. 'Chayamansa' originating from sample 21.030) showed mosaic symptoms 21 dai and were confirmed to be infected with CsCMV by RT-PCR of total 13 chaya plants inoculated with sample 20.222. There were no symptoms 35 dai on 4/4 and 4/4 var. 'Picuda' shrubs propagated by cuttings from samples 20.439 and 21.031, respectively. Thus, modified Koch's postulates were fulfilled, and pathogenicity of the virus to chaya demonstrated. Moreover, a differential reaction was observed among varieties: the Costa Rican var. 'Picuda' seems resistant.

Cassava common mosaic virus (CsCMV) is a plant virus (*Potexvirus, Alphaflexiviridae*) infecting cassava and other hosts in several countries of South America: Brazil, Colombia, Paraguay, Peru and Argentina (Costa and Kitajima, 1972; Di Feo *et al*., 2015; Fernandez *et al*., 2017; Tascon *et al*., 1975); and from China (Tuo *et al*., 2020). The virus is transmitted by asexual reproduction and no vector is known (Costa and Kitajima, 1972). Currently, it is considered a re-emergent pathogen in Argentina (Zanini *et al*., 2018); meanwhile in Colombia it is not associated with high incidence or economic impact (Lozano *et al*., 2017).

A CsCMV strain was reported initially in chaya (CsCMV-Ch) from Florida (USA) and Yucatan (Mexico). The strain CsCMV-Ch is serologically related, but distinct, from the strains infecting cassava of South America (Elliott and Zettler, 1987; Zettler and Elliott, 1986). Currently, CsCMV infection in chaya has been reported from Florida (introduced plant material from Puerto Rico, Zettler and Elliott, 1986), Mexico (Elliott and Zettler, 1987), Tuvalu (Jones *et al*., 1998) and Venezuela (Mejías *et al*., 2015).

A CsCMV strain was reported initially in chaya (CsCMV-Ch) from Florida (USA) and Yucatan (Mexico). The strain CsCMV-Ch is serologically related, but distinct, from the strains infecting cassava of South America (Elliott and Zettler, 1987; Zettler and Elliott, 1986). Currently, CsCMV infection in chaya has been reported from Florida (introduced plant material from Puerto Rico, Zettler and

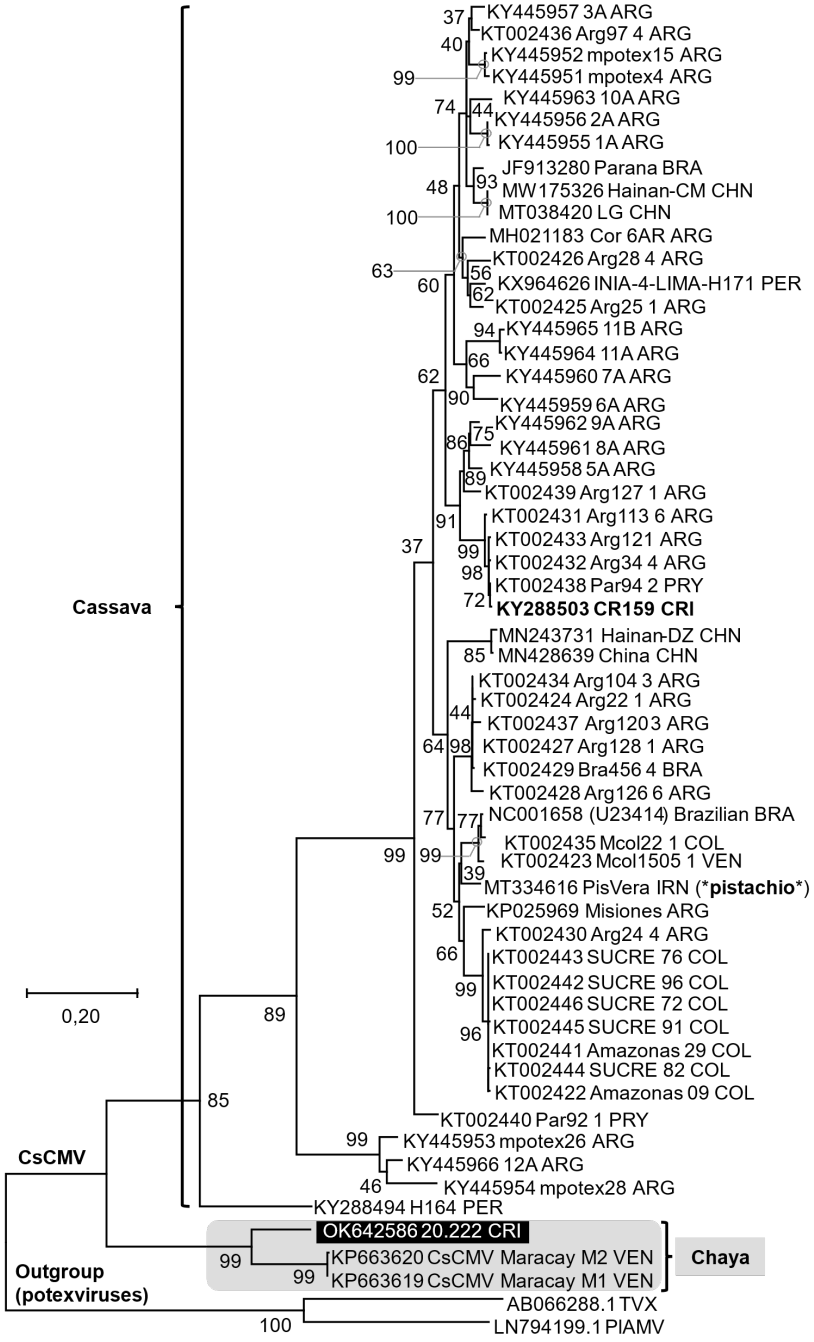

Figure 3. Phylogenetic analysis of partial replicase ORF (618 nucleotides) of *Cassava common mosaic virus* (CsCMV). Gray shaded isolates from chaya (*Cnidoscolus aconitifolius*) host; black shaded the Costa Rica chaya isolate, and in bold the sequence of CsCMV in cassava (*Manihot esculenta*) reported as from Costa Rica. Virus isolates codes: GenBank accession number, isolate identification and three-letter country codes. Maximum likelihood analysis with Tamura-Nei and Gamma distribution model with 2000 replications (bootstrap method) in MEGA X.

Elliott, 1986), Mexico (Elliott and Zettler, 1987), Tuvalu (Jones *et al*., 1998) and Venezuela (Mejías *et al*., 2015).

Presence of CsCMV in Costa Rica is not confirmed locally; neither there are official reports, nor we found local publications, thesis or bulletins with results of its detection. Likewise, the EPPO Global Database (https://gd.eppo.int/) and CABI Compendium (https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium) do not include Costa Rica in the geographical distribution. There is a CsCMV sequence reported from a Costa Rican cassava accession (Lozano *et al*., 2017, supplementary table 1) that was obtained from material collected in 2012 and conserved *in vitro* at the International Center for Tropical Agriculture (CIAT, Colombia). We suggest the need to confirm *in situ* the current presence of the virus in Costa Rica, as with the case of Argentina (Di Feo *et al*., 2015). Moreover, several arguments are justifications to consider more attention to this potexvirus: i) diversity of CsCMV strains, ii) efficient mechanical transmission, iii) re-emergence of the disease in South America, iv) capacity to cause disease without presence of other cassava viruses, v) climate change that may alter the plant-virus interaction, and because vi) cassava is a subsistence and staple food in different regions (Lozano *et al*., 2017; Venturini *et al*., 2016; Zanini *et al*., 2014, 2018).

The detection of CsCMV reported herein is not a confirmation of the presence of cassava common mosaic disease (CCMD) in Costa Rica, because phylogenetic analysis with the only partial sequences of the virus from chaya (Costa Rica and Venezuela) clustered independently to the isolates of the virus infecting cassava. A survey in some states of Venezuela did not detect CsCMV in cassava plants (Chaparro-Martínez and Trujillo-Pinto, 2003), but the chaya strain was reported in Venezuela (Mejías *et al*., 2015). We hypothesize the infection reported herein is an independent and probably recent introduction to Costa Rica of the chaya strain of CsCMV (CsCMV-Ch) because i) the name chaya is not widely used in Costa Rica Central Valley. ii) the 'Estrella' variety is unknown in the country. iii) cuttings shared as: "chaya, a medicinal tree from southern Mexico" indicates something foreign or new, not associated with the comestible shrub 'chicasquil'. And iv) the phylogenetic analysis suggested a different strain compared to CsCMV sequences from cassava (Figure 3).

The detection of CsCMV in a chaya shrub in Costa Rica is an example of ease to spread plant viruses by vegetatively propagated plants, and calls attention on important factors to consider. i) High risk of virus introduction to new regions and countries in vegetatively propagated plants, due to human movement, socioeconomic factors, and cultural traditions. In this case, the use of medicinal plants and the tradition to share plant cuttings, runner daughter plants and seeds, among families and friends, helps to propagate and spread the plants and their diseases. Economic speculations or consumer demands may urge producers to trade

plant material within regions of a country or even among countries, without the appropriate phytosanitary surveillance, as was mentioned for cassava case in the north of Argentina (Zanini *et al*., 2018), and for cape gooseberry ('uchuva', *Physalis peruviana*) and several *Passiflora* spp. in Colombia (Rodríguez *et al*., 2016). ii) As a consequence, the phytopathological problem requires a transdisciplinary approach with the participation of phytopathologists, biologists, sociologists, politicians, and social communicators, among other disciplines. iii) On another perspective, local traditions and traditional genetic resources of a particular region may be at risk from introduced plant pathogens toward which the local plants, a genetic reservoir, are naive to the virus.

The findings confirm the presence of a CsCMV-related virus, previously unreported for Costa Rica, in *Cnidoscolus aconitifolius*. The findings, questions, and hypothesis reported herein signal the need to test the susceptibility of different *Cnidoscolus aconitifolius* varieties to CsCMV-Ch and warrants further study of CsCMV occurrence in cassava in Costa Rica.

Acknowledgments

The authors are thankful to Carlos Chacón Díaz and Luis Orlando Barboza Barquero (Universidad de Costa Rica) for providing plant material. This research was funded by Universidad de Costa Rica (grant 801-C0-274 and Research Activity 801-A1801).

Literature Cited

Chaparro-Martínez EI and Trujillo-Pinto G. 2003. Enfermedades virales en el cultivo de yuca (*Manihot esculenta* Crantz) en algunos estados de Venezuela. Revista de la Facultad de Agronomía 20:461-467. http://ve.scielo.org/scielo.php?script=sci_abstract&pid=S0378-78182003000400006&lng=es&nrm=is&tlng=en

Costa AS and Kitajima EW. 1972. *Cassava common mosaic virus*. CMI/ABB Description of Plant Viruses No. 90. Commonwealth Agricultural Bureaux and the Association of Applied Biologists. Wm. Cuiross and Son Ltd., UK. 4p.

Di Feo L, Zanini A, Rodríguez Pardina P, Cuervo M, Carvajal-Yepes M and Cuellar WJ. 2015. First Report of *Cassava common mosaic virus* and Cassava frogskin-associated virus Infecting Cassava in Argentina. Plant Disease 99:733-733. https://doi.org/10.1094/PDIS-10-14-1088-PDN

Ebel R., Méndez Aguilar MJ, Castillo-Cocom JA and Kissmann S. 2019. Genetic diversity in nutritious leafy green vegetable - Chaya (*Cnidoscolus aconitifolius*). Pp. 161-189. In: Nandwani D (eds) Genetic Diversity in Horticultural Plants. Sustainable Development and Biodiversity. Vol 22. Springer, Cham. 297p. https://doi.org/10.1007/978-3-319-96454-6_6

Elliott MS and Zettler FW. 1987. *Cassava common mosaic virus* infections of chaya (*Cnidoscolus aconitifolius*) in Yucatán, Mexico. Plant Disease 71:353-356. https://www.apsnet.org/publications/PlantDisease/BackIssues/Documents/1987Articles/PlantDisease71n04_353.pdf

Fernandez E, Espinoza I, Lozano I, Bolaños C, Carvajal-Yepes M and Cuellar WJ. 2017. First Report of Cassava Common Mosaic Disease and *Cassava common mosaic virus* Infecting Cassava (*Manihot esculenta*) in Peru. Plant Disease 101:1066-1066. https://doi.org/10.1094/PDIS-10-16-1540-PDN

Hammel BE, Grayum MH, Herrera C and Zamora N (eds). 2010. Manual de plantas de Costa Rica. Available at: Manual de plantas de Costa Rica. http://legacy.tropicos.org/Name/12802542?projectid=66

Jones P, Devonshire J, Dabek A and Howells C. 1998. First report of Cassava common mosaic potexvirus infecting chaya (*Cnidoscolus chayamansa*) in Tuvalu. Plant Disease 82:591-591. https://doi.org/10.1094/PDIS.1998.82.5.591B

Lozano I, Leiva AM, Jimenez J, Fernandez E, Carvajal-Yepes M, Cuervo M and Cuellar WJ. 2017. Resolution of cassava-infecting alphaflexiviruses: Molecular and biological characterization of a novel group of potexviruses lacking the TGB3 gene. Virus Research 241:53-61. https://doi.org/10.1016/j.virusres.2017.03.019

Mejías A, Rodríguez-Román E, Romano M, Zambrano K and Marys E. 2015. New record of cassava common mosaic virus infecting chaya (*Cnidoscolus chayamans*a McVaug) in Venezuela. Plant Disease 99:1190-1190. https://doi.org/10.1094/PDIS-02-15-0132-PDN

Miglino R, Jodlowska A and Van Schadewijk AR. 2006. Detection and identification of a novel potexvirus infecting *Allium* by paramagnetic beads ssRNA isolation and one tube RT-PCR assay with a new potexvirus genus primer set. Acta Horticulturae 722:285-292. https://doi.org/10.17660/ActaHortic.2006.722.35

Montero-Astúa M, Garita L, Vásquez E, Hammond J and Moreira L. 2017. Detection of Plantago asiatica mosaic virus in lily hybrid plants (*Lilium* spp.) in Costa Rica grown from imported bulbs. Australasian Plant Disease Notes 12:57. https://doi.org/10.1007/s13314-017-0281-2

Montero-Astúa M, Chacón-Díaz C, Aguilar E, Rodríguez C, Garita L, Villalobos W, Moreira L, Hartung J and Rivera C. 2008. Isolation and molecular characterization of *Xylella fastidiosa* from coffee plants in Costa Rica. Journal of Microbiology 46:482–490. https://doi:10.1007/s12275-008-0072-8

Rodríguez MH, Niño NE, Cutler J, Langer J, Casierra-Posada F, Miranda, D, Bandte M and Büttner C. 2016. Certificación de material vegetal sano en Colombia: Un análisis crítico de oportunidades y retos para controlar enfermedades ocasionadas por virus. Revista Colombiana de Ciencias Hortícolas 10:164-175. http://dx.doi.org/10.17584/rcch.2016v10i1.4921

Ross-Ibarra J and Molina-Cruz A. 2002. The ethnobotany of chaya (*Cnidoscolus aconitifolius* s.sp. *aconitifolius* Breckon): A nutritious Maya vegetable. Economic Botany 56:350-365. https://doi.org/10.1663/0013-0001(2002)056[0350:TEOCCA]2.0.CO;2

Tascon Â, Kitajima EW and Costa AS. 1975. Microscopia electrônica do vírus do mosaico comum da madioca nos tecidos foliares de diferentes plantas hospedeiras. Bragantia 34:5-10. https://doi.org/10.1590/S0006-87051975000100023

Tuo DC, Zhao GY, Yan P, Li RM, Chen X, Wang WQ, Li XY, Yao Y, Cui HG, Shen WT and Zhou P. 2020. First report of Cassava common mosaic virus infecting cassava in mainland China. Plant Disease 104:997-997. https://doi.org/10.1094/PDIS-08-19-1585-PDN

Venturini MT, Araújo TDS, Abreu EFM, Andrade ECD, Santos VDS, Silva MRD and Oliveira E JD. 2016. Crop losses in Brazilian cassava varieties induced by the Cassava common mosaic virus. Scientia agricola (Piracicaba, Braz.) 73:520-524. https://doi.org/10.1590/0103-9016-2015-0374

van der Vlugt RAA and Berendsen M. 2002. Development of a general potexvirus detection method. European Journal of Plant Pathology 108:367-371. https://doi.org/10.1023/A:1015644409484

Zanini AA, Cuellar WJ, Celli MG, Luque AV, Medina RD, Conci VC and Di Feo LV. 2018. Distinct strains of the re-emergent *Cassava common mosaic virus* (genus: Potexvirus) infecting cassava in Argentina. Plant Pathology 67:1814-1820. https://doi.org/10.1111/ppa.12869

Zanini AA, Di Feo LV, Luna DF, Paccioretti P, Collavino A and Rodriguez MS. 2021. Cassava common mosaic virus infection causes alterations in chloroplast ultrastructure, function, and carbohydrate metabolism of cassava plants. Plant Pathology 70:195-205. https://doi. org/10.1111/ppa.13272

Zanini AA, Rodríguez- Pardina P, Luque A and Di Feo LV. 2014. Identificación y caracterización de Cassava common mosaic virus en cultivos de mandioca en Argentina. Ciencia y Tecnología de los Cultivos Industriales 4:31-38. https://ri.conicet.gov.ar/handle/11336/35246

Zettler FW and Elliott MS. 1986. An antigenically distinct strain of cassava common mosaic virus infecting *Cnidoscolus aconitifolius*. Phytopathology 76:632-638. https://www.apsnet.org/publications/phytopathology/backissues/Documents/1986Articles/Phyto76n06_632.pdf