Characterization of A Divergent Strain of *Moroccan watermelon mosaic virus* (MWMV) from Tanzania Supports the Existence of Two Major Lineages

By

MAXMILYAND LEIWAKABESSY

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Plant Pathology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

Robert L. Gilbertson

Diane E. Ullman

Joanne B. Emerson

Committee in Charge

2021

Acknowledgements

First, I would like to thank God for the unending blessings in my life.

I would like to express my sincere gratitude to my major advisor, Distinguished Professor Robert L. Gilbertson, for all his support and mentorship. Working at the Gilbertson lab was a life changing opportunity that shaped my perspective and attitude, that prepares me for my future career as a scientist.

I would like to thank Dr. Diane E. Ullman, Dr. Joanne B. Emerson, Dr. Douglas Cook, Dr. Minor R. Maliano, Dr. Maria Rojas and Dr. Tomas A. Melgarejo for their support, advice, and mentoring during my study at UC Davis. I appreciate their guidance during my time at the Department of Plant Pathology at UC Davis. It was a privilege for me to learn from the expert in this field.

I would like to thank all the members of Gilbertson Lab for their support and discussions that helped me through the challenging times. I will always cherish the memories we share. I believe this experience will shape us into a better person in the future. I would like to thank all the students and faculty members of the Department of Plant Pathology at UC Davis who have taught me a lot of lessons, inside and outside of the classroom. Beyond that, I would like to thank all the friends I have met in Davis and Sacramento for the moral support they have given to me during my study at UC Davis.

Most importantly, I would like to thank my parents, Jacobus A. Leiwakabessy and Delly D. P. Matrutty, who are my number one support, now and always. Your love and care are what shaped me into who I am today. Thank you for always believing in me.

Characterization of a divergent strain of *Moroccan watermelon mosaic virus* (MWMV) from Tanzania supports the existence of two major lineages

Abstract

The focus of the research in this thesis is the characterization of a virus isolated from a cucurbit plant showing striking symptoms of virus infection including mosaic/mottle and dark green blisters in a commercial field on the outskirt of Dar es Salaam, Tanzania in 2016. Lateral flow tests were positive for potyvirus infection, and negative for *Cucumber mosaic virus* (CMV) and *Squash mosaic virus* (SqMV). With a degenerate primer pair in RT-PCR, the ~700 bp fragment of the potyvirus cylindrical inclusion (CI) gene was amplified and had highest identities (80-92%) to *Moroccan watermelon mosaic virus* (MWMV). This virus was mechanically transmitted to pumpkin and flexuous filamentous rod-like particles of ~820 nm long were purified from infected leaves. High throughput sequencing (HTS) analysis confirmed these symptoms were only due to the infection of an isolate of MWMV, named MWMV-TZ16. In a host range experiment, MWMV-TZ16 only infected species in the family *Cucurbitaceae*, different from MWMV isolates from North and South Africa and Southern Europe. The complete genome sequence of MWMV-TZ16 was determined with HTS and alignment of overlapping sequences of cloned RT-PCR fragments, with the 5'-end of the genome determined by 5'-RACE PCR. Sequence comparisons and phylogenetic analyses showed that MWMV-TZ16 is a genetically divergent strain that was placed in a strongly supported Central/East African clade, and less closely related to isolates in the North Africa and Southern European clade. General MWMV and East Africa strain-specific primer pairs were developed, and preliminary RT-PCR tests suggest these could be used to further investigate the distribution of these viruses.

Table of Contents

Chapter I

Introduction

Family *Potyviridae*

Potyviridae is the largest family of plant RNA viruses, and is divided into 12 genera: *Arepavirus, Bevemovirus, Brambyvirus*, *Bymovirus, Celavirus Ipomovirus, Macluravirus, Poacevirus, Potyvirus, Roymovirus, Rymovirus,* and *Tritimovirus*, which are distinguished based on host range, genomic features, and phylogeny [1, 3]. Members of this family have single-stranded, positive-sense RNA genomes, encapsidated inside a flexuous filamentous virions that measure 680–900 × 11–20 nm [1]. The virion is composed only of the coat protein (CP), which may be linked to the virus-encoded genome-linked protein (VPg) during the infection [2]. VPg is covalently linked to the 5'-end of the genome, while the 3'-end terminus is polyadenylated [1,5]. Other than the members of the genus *Bymorvirus,* which have bipartite genome, most of viruses in this family are monopartite with genome sizes range from 8.2 (*Macluravirus*) to 11.3 kb (*Bymorvirus*) (Fig. 1.1) [1,4,5]. The genomic RNA of viruses in the family *Potyviridae* are translated into polyprotein, which is subsequently processed by virus-encoded protease into the functional proteins [1,4].

Genus *Potyvirus*

Viruses in the genus *Potyvirus* are transmitted in a non-persistent manner by many species of aphids [1,5]. Potyviruses have a monopartite plus-sense single stranded (ss)RNA genome of ~10 kb [1,4,5] and has a long open reading frame (ORF), that encodes a polyprotein that undergoes a proteolytic processing to produce ten mature proteins: (1) P1 protein involved in translation and modulator of replication, (2) P3 protein involved in virus replication and movement, (3) HC-Pro (helper component proteinase) involved in aphid transmission, cell-to-cell and long distance movement, and function as a silencing suppressor, (4) 6K1 and (5) 6K2 are involved in formation of replication vesicles, (6) CI (cytoplasmic inclusion protein) is a helicase involved in virus movement and replication, (7) VPg (genome-linked protein) involved in translation, (8) NIa-Pro involved in polyprotein cleaving process and (9) NIb is the RNA-dependent RNA

polymerase, whereas (10) CP (coat protein) involved in virus movement and aphid transmission (Fig. 1.1) [5,6]. Another protein, which is involved in the cell-to-cell movement of the virus, is the pretty interesting potyviridae ORF (PIPO) from the P3 coding region via polymerase slippage (Fig. 1.1) [5,7]. P1 is a self-cleaving protein and is a serine protease with a catalytic triad His-(X_{7-11})-Asp-(X_{30-36})-Ser (but Glu rather than Asp for viruses of the *Bean common mosaic virus* [BCMV] subgroup) [4,8]. The Gly-X-Ser-Gly around the P1 cleavage site is strictly conserved [4,8]. HC-Pro is a cysteine protease with a cleavage site of Gly/Gly [4,9]. NIa-Pro is a serine protease with conserved four active site residues of His, Asp, Cys and His, with Gly-X-Cys-Gly around the active cysteine [4].

Plant-to-Plant Transmission of Potyvirus

Potyviruses are transmitted by many species of aphid in a non-persistent manner, which requires a short period of acquisition and inoculation (seconds to a few minutes) [11,13]. The transmission process happens through a helper strategy, in which the non-structural HC-Pro facilitates the binding of virions to receptors associated with the stylet of the aphid vector [12,39]. A DAG motif in the N-terminus of the CP is highly conserved and directly interacts with a PTK motif in the HC-Pro [14]. Importantly, a KITC motif at the N-terminus of HC-Pro is essential for the stylet binding [15]. Some aphid species that are known to transmit potyviruses include *M. persicae, Aphis gossypii*, *A. craccivora*, *A. fabae*, *A. spiraecola*, *A. pisum*, *Macrosiphum euphorbiae*, *Rhopalosiphum maidis*, *R. padi*, and *Lipaphis erysimi* [16]. *Morrocan watermelon mosaic virus* (MWMV) was reported to be efficiently transmitted by *Myzus persicae* and *Aphis gossypii* [21]. The polyphagous nature and high reproduction rates of the aphids, as well as the migrating capacity, facilitate the short and long distance spread of potyvirus [16].

In addition to aphid transmission, some potyviruses are seed-transmitted [17,20]. Potyviruses made up 13% of the 231 plant viruses considered to be seed-transmitted. However, the exact mechanism of potyvirus seed transmission is still unclear [17]. Some examples of important seed-transmitted potyviruses are *Lettuce mosaic virus* (LMV), *Zucchini yellow mosaic virus* (ZYMV), and *Soybean mosaic virus* (SMV) [18-20].

Management of Potyvirus

An integrated approach is required as reliance on pesticide application to control the aphid vector is often not successful, costly, and harmful to the environment [17,22]. Pesticides are not effective in controlling potyvirus spread due to the time it takes to acquire and inoculate the virus [17,22]. For example, insecticide application did not impact the spread of *Potato virus Y* (PVY) in potato fields [23]. In contrast, a combination of stylet oil spray, straw mulching, roguing, and intercropping was more effective for the management of PVY [24]. Resistant cultivars, physical barriers, and the removal of inoculum sources also have been effective as part of an integrated strategy for management of plum pox virus (PPV) [22]. Additionally, reflective mulches and crop borders were effective against the spread of PVY and *Papaya ringspot virus* (PRSV) [25,26].

Breeding for resistance is an important approach for managing diseases caused by potyviruses [32]. Eukaryotic translation initiation factor (eIF) 4E and eIF4G and their isoforms are recessive gene that is commonly used to confer resistance to potyviruses, e.g., to protect against PVY and LMV infection of crops [27,28]. Coat protein (CP)-mediated resistance, which is achieved by expressing the CP of the virus in transgenic plants has been used to provide resistance to potyviruses, including to *Watermelon mosaic virus* (WMV) and ZYMV in commercial squash varieties [29,30]. RNA interference (RNAi) based resistance was also used to developed resistance

to potyvirus infections, e.g. *Cowpea aphid-borne mosaic virus* (CABMV) [31]. Most recently, selection for two potential recessive resistance eIF-like genes from *Cucurbita ecuadorensis* conferred resistance to MWMV isolate from Spain [32].

Cucurbit-Infecting Potyviruses

One of the major economically important crops affected by potyviruses are species in the family *Cucurbitaceae* [33]. Some of these viruses are globally distributed, e.g PRSV, WMV, and (ZYMV), whereas others are limited to specific areas, e.g., *Algerian watermelon mosaic virus* (AlgWMV) in Algeria, *Melon vein-banding mosaic virus* (MWBMV) in Taiwan, *Zucchini yellow fleck virus* (ZYFV) in countries in the Mediterranean Basin, and MWMV in Africa and Southern Eurpe [33]. WMV is closely related to SMV (Fig. 1.2), which is a legume-infecting potyvirus, except for the P1 coding region, which was more identical to that of *Bean common mosaic virus* (BCMV), suggesting the role for recombination in the evolution of WMV [34].

For a cucurbit-infecting potyvirus, WMV has a relatively wide host range and infects approximately 170 species of plants, including carrot, cilantro, orchids, pea and cucurbits [33,35]. In contrast, ZYMV has a narrow host, including wild and cultivated cucurbits, and a few ornamental plants [35]. Cucurbit-infecting isolates of PRSV (PRSV-W) also have a narrow host range, infecting most cucurbits, but also *Chenopodium* spp. and cause local lession, whereas other isolates (PRSV-P) infect papaya and caused only mild symptoms on cucurbits [33]. MWMV is an emerging potyvirus of cucurbits that was first identified in Morocco in 1974 and now have been identified in countries throughout Africa and the Mediterranean Basin [21, 36-38].

References

1. Wylie SJ, Adams M, Chalam C, Kreuze J, López-Moya JJ, Ohshima K, Praveen S, Rabenstein F, Stenger D, Wang A, Zerbini FM (2017) ICTV virus taxonomy profile: Potyviridae. J Gen Virol 98:352-354. https://doi.org/10.1099/jgv.0.000740
2. Torrance L, Andreev IA, Gabrenaite-Verhovskaya R, Cowan G, Mäkinen K, *et al*. (2006) An unusual structure at one end of potato potyvirus particles. J Mol Biol 357:1–8. https://doi.org/10.1016/j.jmb.2005.12.021
3. Adams MJ, Antoniw JF, Fauquet CM (2005) Molecular criteria for genus and species discrimination within the family Potyviridae. Arch Virol 150:459–479. https://doi.org/10.1007/s00705-004-0440-6
4. Adams MJ, Antoniw JF, Beaudoin F (2005) Overview and analysis of the polyprotein cleavage sites in the family Potyviridae. Mol Plant Pathol 6:471–487. dhttps://doi.org/10.1111/j.1364-3703.2005.00296.x
5. Revers F, García JA (2015) Molecular biology of potyviruses. Adv Virus Res https://doiorg/101016/bsaivir201411006
6. Nigam D, LaTourrette K, Souza PFN, Garcia-Ruiz H (2019) Genome-Wide Variation in Potyviruses Front Plant Sci 10:1439. https://doi.org/10.3389/fpls.2019.01439
7. Rodamilans B, Valli A, Mingot A, San León D, Baulcombe D, López-Moya JJ, García JA (2015) RNA polymerase slippage as a mechanism for the production of frameshift gene products in plant viruses of the Potyviridae family. J of Virol 89:6965-6967 https://doiorg/101128/JVI00337-15
8. Merits A, Rajamaki ML, Lindholm P, Runeberg-Roos P, Kekarainen T, Puustinen P, Makelainen K, Valkonen JPT, Saarma M (2002) Proteolytic processing of potyviral

proteins and polyprotein processing intermediates in insect and plant cells. J Gen Virol 83 1211-1221. https://doi.org/10.1099/0022-1317-83-5-1211

9. Carrington JC, Cary SM, Parks TD, Dougherty WG (1989) A second proteinase encoded by a plant potyviral genome. EMBO J 8:365-370
10. Nury S, Hosseini A Gibbs AJ, *et al.* (2020) Poison hemlock virus Y (PHVY), a novel potyvirus from Iranian *Conium maculatum* (Apiaceae). *Australasian Plant Pathol.* 49: 119–126. https://doi.org/10.1007/s13313-020-00681-0
11. Ng JC, Falk BW (2006) Virus-vector interactions mediating nonpersistent and semipersistent transmission of plant viruses. Annu Rev Phytopathol 44:183–212. https://doi.org/10.1146/annurev.phyto.44.070505.143325
12. Froissart R, Michalakis Y, Blanc S (2002) Helper component-transcomplementation in the vector transmission of plant viruses. Phytopathology 92:576-9 https://doi.org/10.1094/PHYTO.2002.92.6.576
13. Ng JC, Perry KL (2004) Transmission of plant viruses by aphid vectors. Mol Plant Pathol 5:505–511. https://doiorg/101111/j1364-3703200400240x
14. Peng Y, Kadoury D, Gal-On A, Huet H, Wang Y, Raccah B (1998) Mutations in the HC-Pro gene of zucchini yellow mosaic potyvirus: effects on aphid transmission and binding to purified virions. J of Gen Virol 79:897-904 https://doi.org/10.1099/0022-1317-79-4-897
15. Blanc S, Ammar ED, Garcia-Lampasona S, Dolja VV, Llave C, Baker J, Pirone TP (1998) Mutations in the potyvirus helper component protein: effects on interactions with virions and aphid stylets. J Gen Virol 79:3119-3122. https://doi.org/10.1099/0022-1317-79-12-3119

16. Gadhave KR, Gautam S, Rasmussen DA, Srinivasan R (2020) Aphid transmission of potyvirus: The largest plant-infecting RNA virus genus. Viruses 12:773 https://doi.org/10.3390/v12070773
17. Simmons HE, Munkvold GP (2014) Seed transmission in the Potyviridae In: Gullino ML Munkvold (Ed) Global perspectives on the health of seeds and plant propagation material Springer Dordrecht pp 3-15
18. Hajimorad MR, Domier LL, Tolin SA, Whitham SA, Saghai Maroof MA (2018) Soybean mosaic virus: a successful potyvirus with a wide distribution but restricted natural host range. Mol Plant Pathol 19:1563-1579. https://doi.org/10.1111/mpp.12644
19. Baldodiya GM, Baruah G, Sen P, Nath PD, Borah BK (2020) Host-Parasite Interaction During Development of Major Seed-Transmitted Viral Diseases. In: Kumar R and Gupta A(Ed) Seed-Borne Diseases of Agricultural Crops: Detection Diagnosis & Management. Springer, Singapore, pp 265-289
20. Chatzivassiliou EK, Papapanagiotou AP, Mpenardis PD, Perdikis DC, Menexes G (2016) Transmission of Moroccan watermelon mosaic virus (MWMV) by Aphids in Greece. Plant disease 100:601–606. https://doiorg/101094/PDIS-07-15-0805-RE
21. Cambra M, Vidal E (2016) Sharka, a vector-borne disease caused by plum pox virus: Vector species transmission mechanism epidemiology and mitigation strategies to reduce its natural spread ISHS ACta Horticulturae 1163:57-68. http://doi.org/10.17660/ActaHortic.2017.1163.10
22. Boquel S, Zhang J, Goyer C, Giguère MA, Clark C, Pelletier Y (2015) Effect of insecticide-treated potato plants on aphid behavior and potato virus Y acquisition. Pest Management Sci 71:1106–1112. https://doiorg/101002/ps3892

23. Dupuis B (2017) The movement of potato virus Y (PVY) in the vascular system of potato plants. Eur J Plant Pathol 147(2) pp365-373 https://doi.org/10.1007/s10658-016-1008-5
24. Pinese B, Lisle AT, Ramsey MD, Halfpapp KH, DeFaveri S (1994) Control of aphid-borne papaya ringspot potyvirus in zucchini marrow (Cucurbita pepo) with reflective mulches and mineral oil-insecticide sprays. Inter J of Pest Management 40:81-87. https://doi.org/10.1080/09670879409371859
25. Boiteau G, Singh M, Lavoie J (2009) Crop border and mineral oil sprays used in combination as physical control methods of the aphid-transmitted potato virus Y in potato. Pest Manag Sci 65:255–259 https://doiorg/101002/ps1679
26. Nicaise V, German-Retana S, Sanjuán R, Dubrana MP, Mazier M, Maisonneuve B, Candresse T, Caranta C, LeGall O (2003) The eukaryotic translation initiation factor 4E controls lettuce susceptibility to the Potyvirus Lettuce mosaic virus. Plant physiology 132:1272–1282 https://doiorg/101104/pp102017855
27. Ruffel S, Gallois JL, Lesage ML, Caranta C (2005) The recessive potyvirus resistance gene pot-1 is the tomato orthologue of the pepper pvr2-eIF4E gene. Mol genetics and genomics 274:346–353 https://doiorg/101007/s00438-005-0003-x
28. Tricoll D, Carney K, Russell P, *et al.* (1995) Field Evaluation of Transgenic Squash Containing Single or Multiple Virus Coat Protein Gene Constructs for Resistance to Cucumber Mosaic Virus, Watermelon Mosaic Virus 2, and Zucchini Yellow Mosaic Virus. *Nat Biotechnol* 13:1458–1465 https://doi.org/10.1038/nbt1295-1458
29. Fuchs M, Gonsalves D (1995) Resistance of transgenic hybrid squash ZW-20 expressing the coat protein genes of zucchini yellow mosaic virus and watermelon mosaic virus 2 to mixed infections by both potyviruses. Bio/technology 13:1466-1473.

30. Hamim I, Borth WB, Marquez J, Green JC, Melzer MJ, Hu JS (2018) Transgene-mediated resistance to papaya ringspot virus: challenges and solutions. Phytoparasitica 46:1-18. https://doi.org/10.1007/s12600-017-0636-4

31. Cruz ARR, Aragão FJL (2014) RNAi-based enhanced resistance to Cowpea severe mosaic virus and Cowpea aphid-borne mosaic virus in transgenic cowpea. Plant Pathol 63:831-837. https://doi.org/10.1111/ppa.12178

32. Miras M, Juárez M, Aranda MA (2019) Resistance to the emerging Moroccan watermelon mosaic virus in squash Phytopathology 109:895-903 https://doiorg/101094/PHYTO-10-18-0395-R

33. Lecoq H, Desbiez C (2012) Viruses of cucurbit crops in the Mediterranean region: an ever-changing picture. Adv Virus Res 84 67–126 https://doiorg/101016/B978-0-12-394314-900003-8

34. Desbiez C, Lecoq H (2004) The nucleotide sequence of Watermelon mosaic virus (WMV Potyvirus) reveals interspecific recombination between two related potyviruses in the 5′ part of the genome. Arch Virol 149:1619-1632 https://doi.org/10.1007/s00705-004-0340-9

35. Lecoq H, Desbiez C (2008) Watermelon mosaic virus and Zucchini yellow mosaic virus. In Mahy BWJ and Van Regenmortel M (Ed) Encyclopedia of Virology. Academic Press, San Diego, pp 433-440

36. Lecoq H, Dafalla G, Desbiez C, Wipf-Scheibel, C Delécolle B, Lanina T, Ullah Z, Grumet R (2001) Biological and molecular characterization of Moroccan watermelon mosaic virus and a potyvirus isolate from Eastern Sudan. Plant disease 85:547-552

37. Read DA, Muoma J, Thompson GD (2020) Metaviromic analysis reveals coinfection of papaya in western Kenya with a unique strain of Moroccan watermelon mosaic virus and a novel member of the family Alphaflexiviridae. Arch Virol 165:1231-1234 https://doiorg/101007/s00705-020-04582-x

38. Lecoq H, Justafré I, Wipf-Scheibel C, Desbiez C (2008) Moroccan watermelon mosaic virus newly reported on zucchini squash in France. Plant Pathol 57:766 https://doiorg/101111/j1365-3059200801848x

39. Webster CG, Pichon E, Van Munster M, Monsion B, Deshoux M, Gargani D, Calevro F, Jimenez J, Moreno A, Krenz B, Thompson JR (2018) Identification of plant virus receptor candidates in the stylets of their aphid vectors. J Virol 92:e00432-18. https://doi.org/10.1128/JVI.00432-18

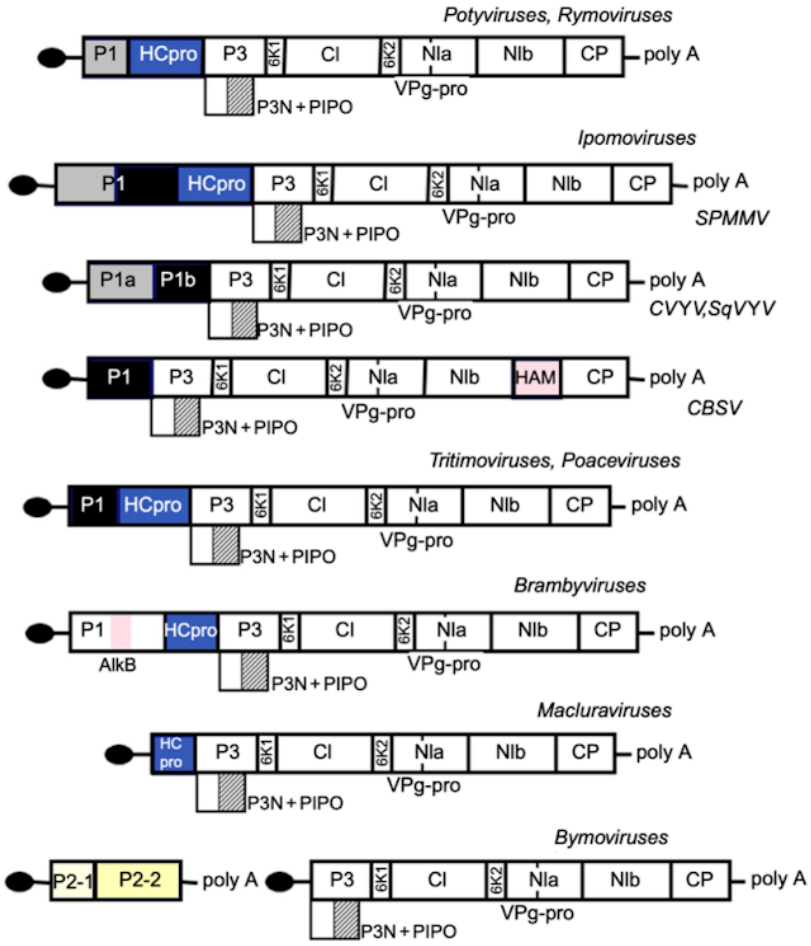


Figure 1.1. Genomic maps of members of the family *Potyviridae* [5].

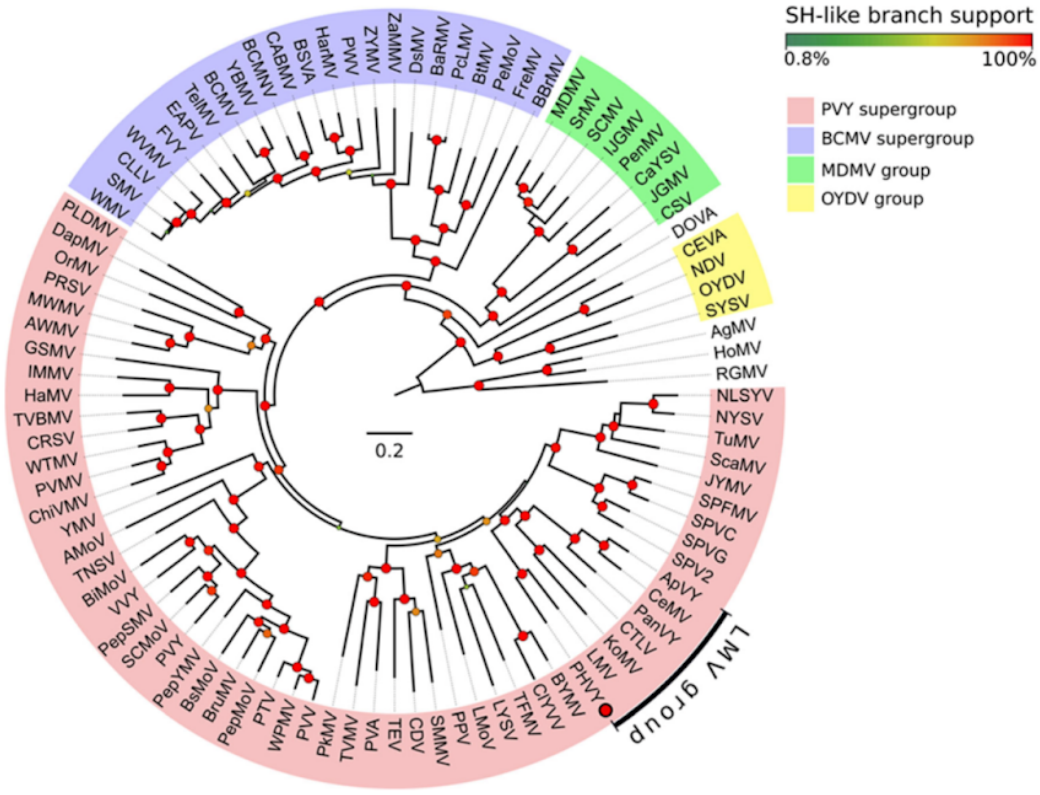


Figure 1.2. Maximum likelihood phylogenetic tree based on the concatenated amino acid sequence of CP, NIb, CI, and HC-Pro of the Potyviruses [10].

Chapter II

Characterization of a divergent strain of Moroccan watermelon mosaic virus (MWMV) from Tanzania supports the existence of two major lineages

M. Leiwakabessy[1], M. A. Macedo[2], M. R. Maliano[3], M. Al Rwahnih[4], T. A. Melgarejo[1], R. L. Gilbertson[1]

[1]*Department of Plant Pathology, University of California Davis, CA, USA*

[2]*Department of Plant Pathology, University of Brasilia, Federal District, Brazil*

[3] *Institute of Molecular Virology and McArdle Laboratory for Cancer Research, University of Wisconsin-Madison, WI, USA*

[4]*Foundation Plant Services, University of California Davis, CA, USA*

Abstract

As part of a disease survey conducted in 2016, striking virus-like symptoms including light green/chlorotic mosaic, distortion and dark green blister were observed in leaves of cucurbit plants in a commercial field on the outskirts of Dar es Salaam, Tanzania. Lateral flow tests with samples of these leaves were weakly positive for potyvirus infection and negative for *Cucumber mosaiv virus* (CMV) and *Squash mosaic virus* (SqMV). In RT-PCR tests with a degenerate potyvirus primer pair, the expected ~700 bp cylindrical inclusion (CI) gene fragment was amplified, and sequence comparisons revealed highest identities (80-92%) with isolates of *Moroccan watermelon mosaic virus* (MWMV). This MWMV-like potyvirus was mechanically transmitted to pumpkin and induced symptoms similar to those observed in the field, and long flexuous filamentous particles measuring ~820 nm were purified from infected leaves. High throughput sequencing (HTS) analysis confirmed no mixed infection with other viruses. The host range of this virus was limited to species in the family *Cucurbitaceae*, unlike MWMV isolates from North and South Africa and Southern Europe. The complete nucleotide sequence of this isolate, MWMV-TZ16,

was determined from overlapping cloned RT-PCR amplified fragments and HTS data, with the 5'-end determined with 5'-RACE-PCR and HTS. Sequence comparisons and phylogenetic analyses showed that MWMV-TZ16 is a divergent strain, which was placed with isolates from Kenya into a strongly supported Central/East African clade, and was less closely related to isolates from the North and South Africa and Southern Europe, which were placed in another clade. General MWMV and East Africa strain-specific primer pairs were developed, and preliminary RT-PCR tests suggest these could be used to further investigate the distribution of these viruses.

Keywords: potyvirus, cucurbit disease, high throughput sequence, virus evolution, virus taxonomy

Introduction

The family *Potyviridae* is a large and diverse group of viruses that are classified into 12 genera: *Arepavirus, Bevemovirus, Brambyvirus*, *Bymovirus, Celavirus Ipomovirus, Macluravirus, Poacevirus, Potyvirus, Roymovirus, Rymovirus,* and *Tritimovirus*) [3,39, 40]. The largest of these, in terms of species, is the genus *Potyvirus*, which currently contain 190 ICTV-approved species [40]. All members are transmitted by aphids species in a non-persisten manner and some are also seed-transmitted [23,24]. These viruses cause mosaic-type disease symptoms in a wide range of crops and non-cultivated plants, including members of the economically important families *Amaranthaceae, Poaceae, Brassicaceae, Solanaceae, Cucurbitaceae,* and *Fabaceae* [1]. Potyviruses possess a positive-sense, single-stranded (ss) RNA genome of ~10 kb, which is encapsidated in long flexuous filamentous virions measuring ~680-900 nm × 11-20 nm [3]. Like other member of the family, potyviruses utilize a polyprotein processing genome strategy [2,38].

These viruses have been extensively studied due to their importance and the diseases they caused [2].

The ~10 kb genome encodes a large open reading frame (ORF), which is translated into a polyprotein of ~3200 amino acids (aa) that is cleaved into 10 mature functional proteins: protein 1 (P1), helper component-protease (HC-Pro), protein 3 (P3), 6-kDa peptide 1 (6K1), cylindrical inclusion protein (CI), 6-kDa peptide 2 (6K2), viral protein genome-linked (VPg), nuclear inclusion A-protease (NIa-pro), nuclear inclusion B (NIb), and coat protein (CP) [2,40]. In addition, a small peptide named pretty interesting potyviridae ORF (PIPO) is translated via an RNA polymerase slippage mechanism [2,4,5]. The genome also possesses 5' and 3' non-translated region (UTRs). The ~60-190 5'-UTR interacts with the VPg, which plays a key role in the viral-host interaction [2,5,6,38]. The 3'-UTR includes nucleotide sequence of variable length followed by the poly(A) tail [2,6].

Cucurbit crops can be severely impacted by diseases caused by potyviruses [41]. Some of these have a worldwide distribution, including *Papaya ringspot virus* (PRSV), *Watermelon mosaic virus* (WMV), and *Zucchini yellow mosaic virus* (ZYMV) [1, 41]. Others have more regional distributions, e.g., *Algerian watermelon mosaic virus* (AlgWMV) in Algeria, *Melon vein-banding mosaic virus* (MWBMV) in Taiwan, *Zucchini yellow fleck virus* (ZYFV) in the Mediterranean Basin, and *Moroccan watermelon mosaic virus* (MWMV) in Africa and Mediterranean Basin [41]. Of this latter group, MWMV has emerged as a major threat to production of cucurbits [15,17].

In the early 1970s, outbreaks of a viral disease of cucurbit in Morocco caused economic losses, especially in squash production (*Cucurbita pepo*). The disease symptoms were chlorotic mottle, vein banding, and erumpent dark green blisters of leaves and malformed fruits [7-9]. Fisher and Lockhart [7] established this disease was caused by a potyvirus based on the purification of

flexuous filamentous rod-shaped particles and aphid transmission. Furthermore, because this virus had serological and host range properties that were similar to those of WMV, it was considered to be a strain of WMV, WMV-Morocco (WMV-Mor). Outbreaks of similar severe viral disease of cucurbit crops were also occurring in South Africa and were associated with a potyvirus with properties similar to those of WMV-Mor [42]. Characterization of an isolate from South Africa, Cu-45, confirmed it had properties similar to WMV-Mor, including a host range of mostly cucurbits, but also inducing chlorotic local lesions in leaves of *Chenopodium amaranthicolor*, *C. album, C. quinoa*, and *Gomphrena globose* plants [45]. Members of *Cucurbita pepo* (squashes and some pumpkins) were highly susceptible [7-9,42], whereas melon also developed necrotic lesion and dieback [7,9,13].

Because of the emerging importance of cucurbit-infecting potyviruses worldwide, especially PRSV (previously WMV1) and WMV (previously WMV2), the properties and relationships among these viruses, including WMV-Mor, were intensively studied [7]. This typically involved comparisons of serological (immunodiffusion and immunosorbent electron microscopy [ISEM]) and biological properties (host range, symptomatology, and aphid transmission) [43,44]. Webb and Scott [44] established that WMV1 and WMV2 are distinct species, PRSV and WMV, respectively, whereas other studies demonstrated that WMV1, WMV2 and WMV-Mor are three distinct potyvirus species [43,45]. For example, antiserum raised against MWMV isolate Cuc 45 only reacted with Cuc 45 and WMV-Mor, whereas antisera to PRSV, WMV and ZYMV did not react with Cuc 45 or WMV-Mor [42].

Definitive evidence that WMV-Mor is a distinct potyvirus species came from peptide profiling and sequencing analyses, which revealed that WMV-Mor had a distinct peptide profile and only 61-68% identity for short peptide aa sequences with other potyvirus, including PRSV,

Soybean mosaic virus (SMV), and WMV [8]. This led to the approval of the name *Moroccan watermelon mosaic virus* (MWMV) by the ICTV in1999. MWMV has now been associated with viral disease outbreaks and economic loses in cucurbits in many African and European countries, e.g., Sudan, South Africa, Kenya, Tunisia, Greece, France, Italy, Portugal and Spain [9-15].

The nucleotide sequence of the 3'-end of the genomic RNAs of WMV-Mor and MWMV-like virus, Su-94-54, from snake cucumber with virus-like symptoms in Eastern Sudan in 1974 were determined by Lecoq *et al.* [9]. Sequence comparisons indicated these were highly divergent strain, with a CP aa identity of 86% and much lower identities with those of other potyviruses (<62%) [9]. Furthermore, although MWMV and Su-94-54 induced similar symptoms in cucurbits, Su-94-54 did not induce local lesions in *C. amaranticolor*, *C. quinoa*, systemically infect *N. benthamiana*, and to induce shoestring in cucurbits and local lesions or tip necrosis in melon [9,13]. Despite substantial genetic, biological, and serological differences with MWMV, Su-94-54 was considered a divergent strain [7,9]. However, due to the molecular differences, Su-94-54 was named a distinct potyvirus species in 2018: *Sudan watermelon mosaic virus* (SuWMV) [50].

In 2008, the complete sequence of a MWMV isolate associated with a severe viral disease outbreak in Tunisia, MWMV-Tn (also referred as MWMV-TN05), was published [13]. Sequence comparisons and phylogenetic analysis showed that MWMV-Tn was an isolate of MWMV-Mor[13]. To investigate the relationship among MWMV isolates from various geographic regions, Yakoubi *et al*. [13] used RT-PCR to amplify an ~480 nt fragment encompassing a small part of the 3' end of NIb and the 5' half of the CP from MWMV isolates from different geographic regions. Comparison of these sequences revealed that i) 23 isolates of MWMV-Tn were nearly identical, ii) MWMV-Tn was placed into a strongly supported clade with isolates from Morocco and Southern Europe (France, Italy and Spain) and iii) isolates from other parts of Africa were

placed in a second less strongly supported clade and, iv) the sequence of the highly divergent MWMV-Su (Su-94-54) had nt identities of 75-80% with other MWMV isolates and was placed on a distinct branch [13]. The clade with the isolates from North Africa and Southern Europe were divided into two strongly supported subclades: one with the isolates from South Africa and the other with isolates from North Africa and Southern Europe. Ibaba *et al*. [10] published complete sequences of two MWMV isolates from South Africa (ZA), and phylogenetic analyses placed these isolates in a strongly supported clade (Southern African lineage) that was placed into the larger strongly supported clade with the isolates from North Africa and Southern Europe (referred to as the Mediterranean lineage). Sequences of isolates from Western and Central Africa were placed in a separate clade, whereas MWMV-Su was placed alone on another branch [10].

In yet another chapter in the MWMV story, two reports have provided evidence that the virus infects papaya (*Carica papaya* L.). The first came from the Democratic Republic of Congo (DRC), where MWMV was detected by RT-PCR and sequencing in leaves of papaya plants affected by a severe viral disease (yellow mosaic, deformation and shoe strings) in 2016 [46]. A partial sequence of the 3' end of one of the papaya isolates revealed 84% nt and 93% CP aa identities with those of MWMV-Mor [46]. This was the first report of MWMV in the DRC and infecting papaya. More recently, MWMV was associated with a severe viral disease of papaya in Kenya. HTS analysis of 29 samples revealed infection with a divergent strain of MWMV. The MWMV isolates from papaya were closely related (~98% identity) and were placed in a different clade than that with isolates from North Africa and South Africa and Southern Europe [11]. These findings are reminiscent of the biotypes described for PRSV, one that infects papaya (PRSV-P) and another that causes severe disease in cucurbits (PRSV-W) [41].

Finally, there is also the potential complication of mixed infections of MWMV with other potyviruses, e.g., WMV and ZYMV, as well as other viruses, such as the carlaviruses, *Cowpea mild mottle virus* (CpMMV) and *Cucumber vein-clearing virus* (CuVCV) [13, 35]. A recent survey showed that ZYMV and WMV were widely distributed in the coastal lowland areas in Tanzania [18].

In the present study, we determined the biological properties and complete nucleotide sequence of an isolate of MWMV from Tanzania (MWMV-TZ16). This virus was associated with a disease outbreak in a commercial field of cucurbits near Dar es Salaam in 2016, in which leaves showed striking chlorotic mottle and vein banding, distortion and dark green blisters. Samples of these leaves were weakly positive for potyvirus infection and negative for *Cucumber mosaic virus* (CMV) and *Squash mosaic virus* (SqMV) in lateral flow tests. RT-PCR amplification and sequencing of a CI fragment revealed highest identities (80-92%%) with isolates of MWMV. This isolate, MWMV-TZ16 was mechanically transmitted to pumpkin plants and induced symptoms similar to those observed in the field. Evidence for a single infection of MWMV-TZ16 came from purification of long flexuous filamentous virions measuring ~800 nm, and high throughput sequence (HTS) analysis. The host range of MWMV-TZ16 was limited to cucurbits and, unlike MWMV isolates from North and South Africa and Southern Europe, did not i) induce local lesions in *Chenopodium amaranticolor* or *C. quinoa*, ii) systemically infect *Nicotiana benthamiana* or iii) induce necrosis or shoestring symptoms in the cucurbits. The complete nt sequence of MWMV-TZ16 is 9,742 nt and has a single large ORF encoding a polypeptide of 3,124 aa. Sequence comparisons revealed highest identities with MWMV isolates infecting papaya in Kenya, and phylogenetic analyses placed MWMV-TZ16 together with these isolates in a strongly supported clade, which was distinct from the clade with isolates from North and South Africa and Southern

Europe. These results are discussed in terms of the evolution and emergence of this important cucurbit-infecting potyvirus.

Materials and Methods

Sample collection and virus detection

During a survey of grower fields on the outskirts of Dar es Salaam, Tanzania conducted as part of a USAID IPM-IL diagnostic workshop held in 2016, virus-like symptoms including chlorotic mosaic/mottle, distortion and dark green blisters were observed in cucurbit plants in one field (Fig. 1A). Leaves with these symptoms were collected from four plants (F4P1-P4) and were tested with Agdia Immunostrips for infection with CMV, SqMV, and general potyvirus. Samples from these leaves were also applied onto Agdia absorption strips [36], which were transported to UC Davis.

Total RNA was extracted from the dried plant sap on the absorption strip pads with the RNeasy Plant Mini Kit (Qiagen, Germantown, MD, USA), and cDNA was generated with Superscript™ II Reverse Transcriptase (Invitrogen). The cDNA was used in RT-PCR tests with a degenerate potyvirus primer pair (CIFor and CIRev [20]), and later with MWMV-specific primer pairs developed in the present study (Supplementary Table S1). RT-PCR-amplified DNA fragments were directly sequenced at the UC DNA Sequencing Facility. To recover the potyvirus, sap was prepared from absorption strip pads with 0.02 M potassium phosphate buffer and mechanically inoculated onto cotylendons of one-week-old pumpkin seedlings (*Cucurbita pepo* cv. Sugar pie).

Virion Purification and Transmission Electron Microscopy (TEM)

Virions were purified from 100 g of infected symptomatic pumpkin leaves collected at 21 to 25 d after mechanical inoculation with MWMV-TZ16. Following homogenization with 0.5 M potassium phosphate buffer containing 0.01 M EDTA and 0.1% sodium sulfate (2 ml/g tissue) [37], and the solution was clarified with butanol as previously described by Lima *et al*. [22]. Following the clarification, 1% (v/v) Triton X-100 was added to solubilize the solution for 20 min [19]. Polyethylene glycol (PEG) 6000 was added to the supernatant (8 g/100 ml) and the mixture was stirred for 3 h at 4 C. After centrifugation at 10,000 × *g* for 10 min to precipitate the virions, the pellet was resuspended in PE buffer (0.01 M potassium phosphate buffer pH 7.0 containing 0.01 M EDTA). The solution was centrifugated at 10,000 × *g* for 10 min, followed by precipitation with PEG (5%) and NaCl (0.3 M), then the resuspended pellet solution was stirred for 30 min at 4 C. The solution then centrifuged at 10,000 × *g* for 10 min and the pellet was resuspended in PE buffer [37]. An aliquot of this preparation was stained with 2% uranyl acetate on a grid for up to 10 min, and examined with a JOEL JEM-123 transmission electron microscopy (TEM) at BioEM Facility at UC Davis. To determine infectivity, 100 µl of this purified virion preparation was diluted in 0.02 M potassium phosphate buffer (pH 7.2) (1:10), and this preparation was mechanically inoculated onto cotyledons of pumpkin seedling as previously described.

Host range experiment

A partial host range experiment was performed by mechanical inoculation of selected cucurbits, including pumpkins (*C. pepo* cv. Sugar Pie and *C. maxima* cv. Big Max), butternut squash (*C. moschata* cv. Waltham Butternut), watermelon (*Citrullus lanatus* cvs. Charleston Gray and Crimson Sweet), cantaloupe (*Cucumis melo* cv. Hales Best Jumbo), honeydew melon (*C. melo*

cv. Sweet Delight), and cucumber (*C. sativus* cv. Marketmore). In addition, following the non-cucurbit species were also inoculated: tomato (*Solanum lycopersicum cv.* Glamour), *Nicotiana benthamiana, N. tabacum cv.* Samsun, *Phaseolus vulgaris cv.* Topcrop, *Chenopodium amaranthicolor, and C. quinoa*. Sap was prepared by grinding symptomatic pumpkin leaf tissue in 0.02 M potassium phosphate buffer (pH 7.2) (1:10 w/v). For cucurbits, sap was mechanically inoculated onto cotyledons that were dusted with celite, whereas for non-cucurbit species, celite-dusted fully expanded leaves were inoculated. Three independent experiments were conducted, each with 5 plants per species/cultivar and representative mock-inoculated plants as the negative control. Symptom development was observed over 30 d post inoculation (dpi), after which leaf samples were collected for detection of MWMV by RT-PCR tests with the general MWMV primer pair (Supplementary Table 1).

Sequencing and phylogenetic analysis

To determine the complete sequence of the genomic RNA of MWMV-TZ16, RT-PCR was used to amplify overlapping fragments covering the genome, which were cloned and sequenced. These sequences were aligned, and a putative full-length sequence was assembled. High throughput sequencing (HTS) was also performed to 1) confirm no mixed infection in MWMV-TZ16-infected pumpkin plants, and ii) to provide additional sequence data. For HTS, total RNA was extracted from 0.1 g of symptomatic pumpkin leaf tissue with the RNeasy Plant Mini Kit (Qiagen, Germantown, MD, USA) and delivered to Foundation Plant Services for HTS as described by Villamor *et al.* [48].

To determine the precise sequence of the 5'-end of the genomic RNA of MWMV-TZ16, rapid amplification of cDNA ends (RACE) was performed. The cDNA for RACE-PCR was synthesized with primer MWMV HCM (Supplementary Table S1), followed by poly-C tailing

with terminal transferase (New England BioLabs). The PCR was then performed with GSP-R2, a specific primer for 5'-end of MWMV-TZ16, targeting P1 gene, and the Abridge Anchor Primer (Supplementary Table S1). The resulting 5'-RACE PCR fragments was cloned into the pGEM-T easy vector (Promega), and sequences of representative clones were determined at the UC DNA Sequencing Facility with the M13F(-21) and M13R sequencing primers (Supplementary Table S1). Additional 5'-end sequences were obtained from the HTS data and the sequencing of cloned RT-PCR fragments. The consensus of the aligned sequences from 15 cloned RACE-PCR fragments, HTS sequences, and the RT-PCR amplified fragments were used to determine the precise 5'-end of the genomic RNA (Supplementary Figure 3).

Open reading frames (ORF) of the MWMV-TZ16 sequence were predicted using ORF Finder (http://www.ncbi.nlm.nih.gov) and by comparison with published MWMV sequences in CLC Sequence Viewer 7.7.1. The sequence of PIPO was identified as described by Chung *et al*. [25]. The alignment of nt and aa sequences of each protein coding region were used to generate an identity matrix with MWMV-TZ16 and other isolates of MWMV in BioEdit 7.2.

Phylogenetic analyses were performed with i) the complete polyprotein aa sequence and ii) the complete CP aa sequence. The sequences were aligned with the MAFFT algorithm implemented in CIPRES [21]. The phylogenetic tree was generated with Jones–Thornton–Taylor (JTT) model on MEGA X v.10.1.8 with 1,000 bootstrap replications, based on the aligned aa sequences of 19 MWMV isolates, with PRSV and AlgWMV as outgroups. Finally, the nucleotide sequences of the complete open reading frame of the 19 MWMV isolates were used to detect recombination events with RDP4.101.

Designing and testing for MWMV-specific primer pairs

To generate primer pairs for specific detection of MWMV isolates, an alignment of the complete genome sequences of the 19 MWMV isolates, including MWMV-TZ16, was generated as described. This alignment was used as the basis for designing primer pairs specific for 1) all MWMV isolates and 2) isolates from East Africa. The primer pairs were evaluated in RT-PCR tests with total RNA extracts of pumpkin leaves infected with MWMV-TZ16, WMV and ZYMV. Equivalent RT-PCR tests were performed with RNA extracts of *N. benthamiana* leaves infected with *Lettuce mosaic virus* (LMV). The positive control was equivalent RT-PCR tests with these RNA extract and degenerate potyvirus CI primer pair (CIFor and CIRev) and the negative control was equivalent RT-PCR tests with extracts of mock-inoculated plants. Total RNA extraction and cDNA production were as previously described. PCR parameters were 35 cycles of 95 C for 15 sec, 55 C for 15 sec, and 72 C for 60 sec, with a final extension of 72 C for 7 min. PCR products were separated in 1% agarose gels and visualized with ethidium bromide.

Results

During the disease survey conducted on the outskirt of Dar es Salaam in 2016, virus-like disease symptoms were observed in a commercial field of cucurbit with an incidence of ~10%. Leaves of affected plants showed a striking chlorotic mosaic/mottle and raised dark green blisters symptoms (Fig. 2.1A). It was indicated that this was a crop of gem squash (*Cucurbita pepo* var. pepo), which is widely grown as a vegetable in Southern Africa for local and export markets.

Immunostrip tests of the four symptomatic leaves were weakly positive for potyvirus infection and negative for CMV and SqMV. In RT-PCR tests with the degenerate CI primer pairs, the expected-size of ~700 bp DNA fragment was amplified from RNA extracts of all fours

samples, consistent with potyvirus infections. A BLASTx search of the nucleotide sequence of this fragment from sample F4P1 revealed highest identities (80-92%) with isolates of MWMV, indicating that the potyvirus that caused these symptoms was an isolate of this emerging virus of cucurbits. A small number of pumpkin plants inoculated with the sap preparation from absorption strips of sample F4P1 developed chlorotic mottling and dark green blisters in newly emerging leaves, symptoms similar to those observed in the cucurbit field in Tanzania. RT-PCR and sequencing confirmed the infection with an isolate of MWMV, which was named MWMV-TZ16.

The examination of the virus preparation, purified from leaves of pumpkin plant infected with MWMV-TZ16, with the TEM revealed long flexuous filamentous rod-shaped particles, typical of a potyvirus (Fig. 2.2). The average length of these particle was 820 nm, based on measurement of 79 particles, which is within the range for known potyviruses [3]. No other virus-like particles were observed, consistent with no co-infection with other viruses [49]. The development of the chlorotic mottle and dark green blisters symptoms in newly emerging leaves of pumpkin seedlings mechanically inoculated with the diluted preparation of these potyvirus-like particles and RT-PCR detection of MWMV in RNA extract of these leaves confirmed these particles were virions of MWMV.

In the host range experiment, MWMV-TZ16 induced symptoms in most of the inoculated cucurbits tested, including melon, pumpkin, squash, and watermelon; whereas no symptoms were observed in inoculated cucumber and honeydew melon plants or in any of the six non-cucurbit species (Table 2.1). The symptoms that developed in leaves of infected cucurbit plants were the chlorotic mottle, distortion, and green blisters typical of MWMV infection. Furthermore, no symptoms of necrosis or shoestrings were not observed in any of the cucurbit plants infected with MWMV-TZ16. Moreover, the virus was 100% infectious in pumpkin, squash, and watermelon,

whereas only 20% of inoculated melon plants became infected (Table 2.1). Interestingly, leaf samples from all cucumber plants that were symptomless after inoculation tested positive for MWMV-TZ16 infection in RT-PCR tests (Table 2.1), indicating a symptomless infection. All the non-cucurbit species that were symptomless after inoculation tested negative for MWMV (Table 2.1).

The HTS analysis confirmed that these symptoms in the diseased pumpkin leaves were due to infection only by MWMV-TZ16. A *de novo* assembly was created from 14,842,001 clean reads into a 9796 nt virus-like contig. A BLASTx analysis with this contig sequence revealed highest overall identity (88%) with MWMV sequences, and 100% identity with the sequence of the RT-PCR amplified CI fragment of MWMV-TZ16. No other virus-like contigs were detected in the HTS analysis. The HTS assembled sequence was nearly identical (99.5%) to the 9463 nt long full-length MWMV-TZ16 sequence assembled from the alignment of the sequences of cloned RT-PCR fragments, with the exception of the 5'-end.

The RACE PCR to precisely determine the sequence of the 5'-end of the genomic RNA of MWMV-TZ16 generated the expected ~660 bp DNA fragment, which was cloned and sequenced. Sequences of 15 randomly selected cloned fragments were nearly identical, especially for the first 80 nt, and were different from the 5'-end sequences determined with HTS and RT-PCR sequence data (Supplementary Fig. 3). Thus, our results indicated that the consensus sequences of the 5'-end is the motif 5'-AAATTTAAA-3'. This sequence was located upstream from the full-length sequences of the other MWMV isolates, e.g., MWMV-Mur (MH595738), MWMV-Kia (MH595742), MWMV-GR13 (LN810061), and MWMV-Tn (EF579955). Thus, this represents the first determination of the precise 5'-end of a MWMV isolate

The complete genome sequence of MWMV-TZ16 is 9729 nt, excluding the 3'-end poly(A) tail. The 5'-end UTR is 157 nt, whereas the 3'-end UTR is 200 nt. The genome has a single large open reading frame (ORF) starting with an AUG codon at nt 158-160 and ending with a UGA stop codon at nt 9530-9532 (Fig. 2.3A). The polyprotein is 3124 aa, and is predicted to be cleaved into 10 mature functional proteins: P1 (39.2 kDa), HC-Pro (51.4 kDa), P3 (39.8 kDa), 6K1 (6.1 kDa), CI (71.2 kDa), 6K2 (6.5 kDa), VPg (21.6 kDa), NIa-pro (26.9 kDa), NIb (59.5 kDa), and CP (32.4 kDa). The small PIPO ORF is embedded within the P3 gene (nt 3028-3207), and encodes a predicted protein of 60 aa. Thus, the genome organization of MWMV-TZ16 is typical of a member of the genus *Potyvirus*. The putative cleavage sites in the polyprotein were nearly identical to those of MWMV-Tn, slightly divergent from those of PRSV and AlgWMV, the two other species in PRSV group, and substantially more divergent from those of *Bean common mosaic virus* (BCMV), a member of BCMV group (Fig. 2.3A).

The identity matrix data showed that the complete nucleotide sequence of the large ORF of MWMV-TZ16 has 79.8-87.9% identity with those of full-length MWMV sequences (Supplementary Table 4). The highest identities (87.6-87.9%) were with papaya isolates from Kenya, whereas the lowest were with isolates from South Africa (80.1-80.2%) and North Africa and Southern Europe (79.8-80.2%) (Supplementary Table 4). Identities of MWMV-TZ16 with other potyvirus species ranged between 48.1-66.0% (Supplementary Table 3), with highest identities with isolates of PRSV (68.1%) and AlgWMV (66.0%).

Likewise, the full-length polyprotein aa sequence of MWMV-TZ16 had highest identities with those of isolates of MWMV (90.9-94.8%), and much lower identities with those of other potyvirus species (43.4-71.6%). Again, MWMV-TZ16 had highest identities (94.5.-94.8%) with isolates from Kenya, and lowest identities with isolates from South Africa (91.3-91.4) and North

Africa and Southern Europe (90.9-91.0%) (Supplementary Table 4). For the aa sequence of mature proteins, MWMV-TZ16 and isolates from Kenya had higher identities in the P3, CI, and CP genes (90.7-91.6, 91.3-91.9, and 91.6-92.8%, respectively) with lower identities in the NIa and P1 genes (80.4-81.5% and 83.1 and 84.1%, respectively) (Supplementary Table 4). It is also notable that, across the genome, nt identities of individual genes were substantially lower than the corresponding aa identities (Supplementary Table 4).

Based on the comparison of 5'-UTR nt sequence, MWMV-TZ16 had highest identities with those of the isolates from Kenya (80.6-86.5%), and lowest with the isolates from South Africa (72.2%) and North Africa and Southern Europe (69.7-71.4) (Supplementary Table 2). The nt sequence of the 3'-end UTR also had highest identities with those of the isolates from Kenya (90.7-92.4%), and lowest with those of the isolates from South Africa (86.7%) and the North Africa and Southern Europe (87.8-90.1%) (Supplementary Table 2). Finally, the 5'-end and 3'-end UTR sequences of MWMV-TZ16 shared only 40.9% and 70.1% identities, respectively, with those of MWMV Su-94-54, consistent with these being a highly divergent strains of MWMV (Supplementary Table 2).

Phylogenetic analysis performed with the full-length polyprotein aa sequences of potyviruses representating the main groups in the genus *Potyvirus* placed MWMV-TZ16 and MWMV-Tn (EF579955) to strongly supported clade, which was placed in a larger strongly supported clade with AlgWMV (EU410442) and PRSV isolate (X67673) to form the well-established PRSV group (Supplementary Fig. 1). In the phylogenetic tree generated with the full-length polyprotein aa sequences of 19 MWMV isolates, two strongly supported clades were identified. Clade I included MWMV-TZ16 and the papaya isolates from Kenya. Within this clade, MWMV-TZ16 was placed on a strongly supported branch that was distinct from that with the

Kenyan isolates. Clade II contained MWMV isolates from North and South Africa and Southern Europe (Fig. 2.3B). Furthermore, two strongly supported subclades were identified in Clade II, one with the isolates from South Africa and the other with isolates from North Africa (Tunisia) and Southern Europe (Spain and Greece) (Fig. 2.3B).

In order to include a number of available partial MWMV sequences, a phylogenetic tree was generated with the complete CP aa sequence of 24 isolates (the 19 full length sequences and 5 partial sequences; Supplementary Table 2). A similar tree topography was obtained with the strongly supported clades I and II (Supplementary Fig. 2). However, the subclades with isolates from South Africa and those from North African and Southern Europe were placed in different positions in this tree, which were not strongly supported (Supplementary Fig. 2). Although, these different tree topologies suggested a role for recombination in MWMV evolution, no recombination was detected in the analysis of complete nt sequence of full-length polyprotein ORF of the 19 MWMV isolates with The Recombination Detection Program RDP4.101.

An alignment of the MWMV full-length nt sequences was used to design primer pairs to allow detection of 1) all MWMV isolates (general MWMV primer pair) and 2) isolates from East Africa, e.g. MWMV-TZ16. The general MWMV primer pair (MWMV P3-For and MWMV P3-Rev; Supplementary Table 1) was designed to direct the amplification of an ~500 bp fragment of the P3 gene, whereas the East African strain-specific primer pair (MWMV P1F3 and MWMV HCM; Supplementary Table 1) was designed to direct the amplification of an ~1.1 kb fragment spanning the P1 and HC-Pro genes. In control RT-PCR tests with the degenerate CI primer pair, the expected ~700 bp fragment was amplified from total RNA of leaves infected with MWMV, LMV, WMV, and ZYMV, whereas no fragment was amplified from an RNA extract of uninfected leaves (Fig. 2.4A). In the RT-PCR tests with the general MWMV primer pair, the expected ~500

bp targeted fragment was amplified from the RNA extract of MWMV-TZ16-infected leaves, and not from those of leaves infected with the other potyviruses or uninfected leaves (Fig. 2.4B). Similarly, in RT-PCR tests with the East Africa strain-specific primer pair, the expected ~1.1 kb fragment was amplified only from the RNA extract of MWMV-TZ16-infected leaves (Fig. 2.4C). These RT-PCR tests for MWMV detection can now be evaluated as tools for rapid and precise detection of these viruses.

Discussion

MWMV is an emerging threat to cucurbit production in Africa and Southern Europe. Since the first report of MWMV infecting cucurbit crops in Morocco in the early 1970's, this virus has now been detected in countries throughout Africa and Southern Europe [7-15,17]. For example, disease outbreaks in cucurbit crops due to MWMV infection have been reported from Spain, Italy, Greece, Tunisia, Portugal and South Africa [10-14]. Studies of MWMV isolates have revealed substantial genetic diversity, particularly for isolates from different geographic regions. However, the relative importance and relationships of some of these viruses needs further investigation. Here, we present evidence that the symptoms of chlorotic mottle and dark green blisters observed in a commercial fields of cucurbits (gem squash) in Tanzania in 2016 (Fig. 1A) were caused by MWMV-TZ16, a divergent strain of this emerging virus. Potyvirus infection was initially suggested based on the weak positive reaction with the general potyvirus ImmunoStrip, and this was confirmed by the amplification of the ~700 bp CI fragment in RT-PCR tests with the degenerate potyvirus primer pair. Furthermore, the finding that the highest sequence identities for this fragment were 80-92% with those of MWMV isolates indicated the cause virus was a divergent strain of MWMV.

The presence of MWMV in Tanzania was first reported in a watermelon sample with severe viral disease symptoms (mosaic/mottle, deformation, and blistering) collected in Arusha in 2009, and was based on RT-PCR and sequencing results [35]. Furthermore, a mechanical transmission experiment indicated that this MWMV isolate infected a range of cucurbits, but none of the non-cucurbit species, including *Chenopodium quinoa* and *N. benthamiana* [35]. It should also be noted that the watermelon sample was infected with a carlavirus [35]. A host range similar to MWMV-TZ16 was also reported for the divergent Su-94-54 from Sudan [9]. Thus, the findings that MWMV-TZ16 also did not infect *Chenopodium amaranthicolo, C. quiona* and *N. benthamiana* or induce necrosis or shoestring symptoms in cucurbits as reported for isolates from North and South Africa and Southern Europe may reveal an important difference between MWMV isolates from this geographic region. Thus, our result indicating that MWMV caused the chlorotic mottling, vein banding, and dark green blistering symptoms observed in cucurbits around Dar es Salaam in 2016 support and extend those of Menzel *et al*. 2011 [35] that this emerging virus is established in Tanzania.

The accepted species demarcation criteria for potyvirus species are (i) <76% nt and <82% aa identity for the large ORF and polyprotein and (ii) the same value for CP nt and aa sequences [3]. Strains within a potyvirus species generally exhibit >90% aa identity for the full-length coat protein sequence [26,47]. Thus, the nt (79.8-87.7%) and aa (90.9-94.6%) sequence identities determined in comparison of MWMV-TZ16 and other MWMV isolates were consistent with a highly divergent strain. The finding that MWMV-TZ16 shared highest nt and aa sequence identities with isolates from Kenya probably reflects the close proximity of Kenya and Tanzania (Supplementary Fig. 4). However, there also was substantial divergence between the sequence of MWMV-TZ16 and those of the isolates from Kenya, e.g., nt identities of <90% for genes and the

5'-UTR. This suggests that these viruses shared a common ancestor, but also underwent a substantial period of geographic separation and host adaptation. However, host adaptation to papaya alone cannot explain the divergence between isolates, because a similar level of divergence was detected between MWMV-TZ16 and a cucurbit-infecting isolate from Kenya (data not shown). It is tempting to speculate that there are MWMV biotypes that infect cucurbits and papaya, similar to PRSV-W and -P. However, it should be noted that Koch's Postulate for these papaya diseases and the associated MWMV isolates need to be fulfilled, as well as host range determination, to precisely define host adaptation. The greater divergence (~20%) between sequence of MWMV-TZ16 and isolates from North and South Africa and Southern Europe was consistent with the placement of these isolates in a different lineages and suggest a longer period of geographic isolation.

Phylogenetic trees also showed the correlation between genetic diversity of MWMV isolates and geographic origin (Fig. 2.2B). Thus, isolates from North and South Africa and Southern Europe were placed in one lineage (Clade I), whereas isolates from Kenya and Tanzania were placed in another lineage (Clade II) (Fig. 2.2B). Moreover, isolates from North Africa and Southern Europe are very closely related (>98% identities), indicating recent spread from a single origin, e.g., Morocco [13]. In contrast, the greater level of divergence in East African strains suggests a longer periods of local evolution and limited long-distance spread. This clade with East African strains identified in our study corresponds to the 'Central/East African Lineage' described by Ibaba *et al.* 2015 [10], and our results are in agreement with this designation.

A possible exception to the limited long-distance spread concept for MWMV is the relatively close genetic relationship (~90% identities) between MWMV isolates from North and South Africa (Fig. 2.2B). Furthermore, the geographic distribution of Central/East African strains

seems to divide the North Africa/Southern Europe and South African subclades (Supplementary Figure 4). Not only does this suggest a more recent common ancestor, it may suggest a role for long-distance movement in establishing one of these infection sites. In this regard, it is worth noting that van Regenmortel was probably working with MWMV isolates 10-15 years before it was recognized in Morocco [45], perhaps suggesting a South African origin.

Because multiple viruses cause disease in cucurbits and mixed infections are common, it was important that we established the chlorotic mottle and green blisters symptoms in cucurbit leaves were due only to infection by MWMV-TZ16. This was initially established based on (i) purification of only potyvirus flexuous filamentous particles, and (ii) infectivity of the particles and induction of MWMV-like symptoms in inoculated pumpkin plants. HTS data provided conclusive evidence that only MWMV sequences were present, in the infected leaves, consistent with a single infection of MWMV-TZ16. However, a recent study on cucurbit infecting viruses in Tanzania in 2016 showed that ZYMV, WMV, and CMV were prevalent [18], whereas Ibaba et al. reported that MWMV and ZYMV were the prevalent cucurbit-infecting potyviruses in South Africa for the 2011-13 growing seasons and also showed that MWMV isolates formed distinct clades/lineages according to geographic origin [51]. Thus, there is a complex of cucurbit diseases and viruses impacting productions in Tanzania and throughout South Africa, and it will be important to better define the distribution and relative importance of these virus and to identify broad spectrum resistance. Comprehensive surveys could reveal that MWMV is more widely distributed in Tanzania than currently recognized (Arusha and Dar es Salaam). A survey for MWMV could start with alerting stakeholders to the diagnostic symptoms, i.e., chlorotic mottle and dark green blisters, which would be followed by field visits and RT-PCR tests with the general MWMV primer pair to better define the distribution and importance of this divergent strains.

Our results also showed the value of determining the biological properties of MWMV strains, as it can differentiate the two main lineages. These biological differences also may reveal potential for different interactions with resistance genes or induction of different symptoms, such as the top necrosis and shoestrings in melons [9,13], induced by isolates from North and South Africa and Southern Europe. Moreover, two recessive genes from *Cucurbita ecuadorensis,* encoding eukaryotic translation initiation factors (eIFs), were shown to conferred resistance to the isolate of MWMV from Spain [15]. It will be important to evaluate whether these genes confer resistance to the divergent strain of MWMV, e.g., MWMV-TZ16 and MWMV-Su-9454.

In conclusion, we demonstrated that a divergent strain of MWMV, MWMV-TZ16, was the cause of chlorotic mottle, vein banding and green blisters observed in leaves of cucurbits in Tanzania in 2016. In phylogenetic analyses, MWMV-TZ16 was placed into a Central/East African lineage. Consistent with these results, MWMV-TZ16 is genetically and biologically distinct from isolates of the North and South Africa/Southern Europe lineage. RT-PCR tests with primer pairs for all MWMV isolates and specific for MWMV-TZ16 were developed and could be tools to assess the distribution and importance of these viruses. Finally, our results also showed the challenges of classifying potyviruses with sequence identities that are near the species demarcation value, i.e., highly divergent strains and the potential values of biological properties for revealing the relationships. Indeed, Su-94-54, previously considered a divergent strain of MWMV, was recently elevated to a distinct species, *Sudan watermelon mosaic virus* (SuWMV), based on identities beneath the potyvirus species thresholds [39].

References

1. Moury B, Desbiez C (2020) Host range evolution of potyviruses: A global phylogenetic analysis. Viruses. https://doiorg/103390/v12010111
2. Revers F, García JA (2015) Molecular biology of potyviruses. Adv Virus Res. https://doiorg/101016/bsaivir201411006
3. Wylie SJ, Adams M, Chalam C, Kreuze J, López-Moya JJ, Ohshima K, Praveen S, Rabenstein F, Stenger D, Wang A, Zerbini FM (2017) ICTV virus taxonomy profile: Potyviridae. J Gen Virol 98:352-354. https://doi.org/10.1099/jgv.0.000740
4. Rodamilans B, Valli A, Mingot A, San León D, Baulcombe D, López-Moya JJ, García JA (2015) RNA polymerase slippage as a mechanism for the production of frameshift gene products in plant viruses of the Potyviridae family. J Virol. 89:6965-6967. https://doiorg/101128/JVI00337-15
5. Nicolas O, Dunnington SW, Gotow LF, Pirone TP, Hellmann GM (1997) Variations in the VPg protein allow a Potyvirus to overcome va gene resistance in tobacco. Virology 273:452-459. https://doi.org/10.1006/viro.1997.8780
6. Tacahashi Y, Uyeda I (1999) Restoration of the 3′ end of potyvirus RNA derived from poly (A)-deficient infectious cDNA clones. Virology 265:147-152. https://doi.org/10.1006/viro.1999.0027
7. Fischer HU, Lockhart BEL (1974) Serious losses in cucurbits caused by watermelon mosaic virus in Morocco. Plant Dis Reporter 58:143-146.
8. McKern NM, Strike PM, Barnett OW, Ward CW, Shukla DD (1993) Watermelon mosaic virus-Morocco is a distinct potyvirus. Arch Virol 131:467-473. https://doi.org/10.1007/BF01378647

9. Lecoq H, Dafalla G, Desbiez C, Wipf-Scheibel C, Delécolle B, Lanina T, Ullah Z, Grumet R (2001) Biological and molecular characterization of Moroccan watermelon mosaic virus and a potyvirus isolate from Eastern Sudan. Plant disease 85:547-552. https://doiorg/101094/PDIS2001855547
10. Ibaba JD, Laing MD, Gubba A (2016) Genome sequence analysis of two South African isolates of Moroccan watermelon mosaic virus infecting cucurbits. Virus genes 52:896-899. https://doi.org/10.1007/s11262-016-1372-4
11. Read DA, Muoma J, Thompson GD (2020) Metaviromic analysis reveals coinfection of papaya in western Kenya with a unique strain of Moroccan watermelon mosaic virus and a novel member of the family Alphaflexiviridae. Arch Virol 165:1231-1234. https://doi.org/10.1007/s00705-020-04582-x
12. Lecoq H, Justafré I, Wipf-Scheibel C, Desbiez C (2008) Moroccan watermelon mosaic virus newly reported on zucchini squash in France. Plant Pathology 57:766. https://doi.org/10.1111/j.1365-3059.2008.01848.x
13. Yakoubi S, Desbiez C, Fakhfakh H, Wipf-Scheibel C, Marrakchi M, Lecoq H (2008) Biological characterization and complete nucleotide sequence of a Tunisian isolate of Moroccan watermelon mosaic virus. Arch Virol 153:117-125. https://doi.org/10.1007/s00705-007-1074-2
14. Malandraki I, Vassilakos N, Xanthis C, Kontosfiris G, Katis NI, Varveri C (2014) First report of Moroccan watermelon mosaic virus in zucchini crops in Greece, Plant Dis 98:702-702. https://doi.org/10.1094/PDIS-10-13-1100-PDN

15. Miras M, Juárez M, Aranda MA (2019) Resistance to the emerging Moroccan watermelon mosaic virus in squash. Phytopathology 109:895-903. https://doi.org/10.1094/PHYTO-10-18-0395-R

16. Mumo NN, Mamati GE, Ateka EM, Rimberia FK, Asudi GO, Boykin LM, Machuka EM, Njuguna JN, Pelle R, Stomeo F (2020) Metagenomic analysis of plant viruses associated with papaya ringspot disease in *Carica papaya* L in Kenya. Front Microbiol 11:205. https://doi.org/10.3389/fmicb.2020.00205

17. Loebenstein G, Thottappilly G (2013) Virus and virus-like diseases of major crops in developing countries. Springer Science & Business Media, Dordrecht

18. Sydanmetsa M, Mbanzibwa DR (2016) Occurrence of Cucumber mosaic virus Zucchini yellow mosaic virus and Watermelon mosaic virus in cultivated and wild cucurbits in the coastal areas of Tanzania. Afr J Agric Res 11:4062-4069. https://doi.org/10.5897/AJAR2016.11462

19. Grumet R, Fang G (1990) cDNA cloning and sequence analysis of the 3′-terminal region of zucchini yellow mosaic virus RNA. J Gen Virol 71:1619-1622. https://doi.org/10.1099/0022-1317-71-7-1619

20. Ha C, Coombs S, Revill PA, Harding RM, Vu M, Dale JL (2008) Design and application of two novel degenerate primer pairs for the detection and complete genomic characterization of potyviruses. Arch Virol 153:25-36. https://doi.org/10.1007/s00705-007-1053-7

21. Miller MA, Schwartz T, Pickett BE, He S, Klem EB, Scheuermann RH, Passarotti M, Kaufman S, O'Leary MA (2015) A RESTful API for access to phylogenetic tools via the CIPRES science gateway. Evol Bioinform 11:43-48. https://doi.org/10.4137/EBO.S21501

22. Lima JAA, Purcifull DE, Hiebert E (1979) Purification partial characterization and serology of blackeye cowpea mosaic virus. Phytopathology 69:1252-1258. https://doi.org/10.1094/Phyto-69-1252.
23. Gadhave KR, Gautam S, Rasmussen DA, Srinivasan R (2020) Aphid Transmission of Potyvirus: The Largest Plant-Infecting RNA Virus Genus. Viruses. https://doi.org/10.3390/v12070773
24. Simmons HE, Munkvold GP (2014) Seed transmission in the Potyviridae. In: Gullino ML, Munkvold (Ed) Global perspectives on the health of seeds and plant propagation material. Springer, Dordrecht, pp 3-15
25. Chung BYW, Miller WA, Atkins JF, Firth AE (2008) An overlapping essential gene in the Potyviridae *Proc Natl Acad Sci U S A* 105:5897-5902. https://doi.org/10.1073/pnas.0800468105
26. Shukla DD, Ward CW, Brunt AA (1994) The Potyviridae. CAB international, Wallingford
27. Glais L, Tribodet M, Kerlan C (2002) Genomic variability in Potato potyvirus Y (PVY): evidence that PVY N W and PVY NTN variants are single to multiple recombinants between PVY O and PVY N isolates. Arch Virol 147:363-378. https://doi.org/10.1007/s705-002-8325-0
28. Karasev AV, Hu X, Brown CJ, Kerlan C, Nikolaeva OV, Crosslin JM, Gray SM (2011) Genetic diversity of the ordinary strain of Potato virus Y (PVY) and origin of recombinant PVY strains. Phytopathology 101:778-785. https://doi.org/10.1094/PHYTO-10-10-0284
29. Perotto MC, Pozzi EA, Celli MG, Luciani CE, Mitidieri MS, Conci VC (2018) Identification and characterization of a new potyvirus infecting cucurbits. Arch Virol 163:719-724. https://doi.org/10.1007/s00705-017-3660-2

30. Verchot J, Koonin EV, Carrington JC (1991) The 35-kDa protein from the N-terminus of the potyviral polyprotein functions as a third virus-encoded proteinase. Virology185:527-535. https://doi.org/10.1016/0042-6822(91)90522-d

31. Shiboleth YM, Haronsky E, Leibman D, Arazi T, Wassenegger M, Whitham SA, Gaba V, Gal-On A (2007) The conserved FRNK box in HC-Pro a plant viral suppressor of gene silencing is required for small RNA binding and mediates symptom development. J Virol 81:13135-13148. https://doi.org/10.1128/JVI.01031-07

32. Yakoubi S, Lecoq H, Desbiez C (2008) Algerian watermelon mosaic virus (AWMV): a new potyvirus species in the PRSV cluster. Virus Genes 37:103-109. https://doi.org/10.1007/s11262-008-0237-x

33. Chng CG, Lee KC, Mahtani PH, Wong SM (1997) Sequence and phylogenetic analysis of the cytoplasmic inclusion protein gene of Zucchini yellow mosaic potyvirus: its role in classification of the Potyviridae. Virus Genes 14:41-53. https://doi.org/10.1023/a:1007935423180

34. Lopez-Moya JJ, Wang RY, Pirone TP (1999) Context of the coat protein DAG motif affects potyvirus transmissibility by aphids. J Gen Virol 80:3281-3288. https://doi.org/10.1099/0022-1317-80-12-3281

35. Menzel W, Abang MM, Winter S (2011) Characterization of cucumber vein-clearing virus a whitefly (Bemisia tabaci G)-transmitted carlavirus. Arch Virol 156:2309-2311. https://doi.org/10.1007/s00705-011-1124-7

36. Melgarejo TA, Rojas MR, Gilbertson RL (2019) A bipartite begomovirus infecting Boerhavia erecta (family Nyctaginaceae) in the Dominican Republic represents a distinct

phylogenetic lineage and has a high degree of host specificity. Phytopathology 109:1464-1474. https://doi.org/10.1094/PHYTO-02-19-0061-R

37. Gonsalves D, Ishii M (1980) Purification and serology of papaya ringspot virus Phytopathology 70:1028-1032. https://doi.org/10.1094/Phyto-70-1028
38. Kneller ELP, Rakotondrafara AM, Miller WA (2006) Cap-independent translation of plant viral RNAs. Virus Res 119:63-75. https://doi.org/10.1016/j.virusres.2005.10.010
39. Adams MJ, Antoniw JF, Fauquet CM (2005) Molecular criteria for genus and species discrimination within the family Potyviridae. Arch Virol 150:459–479. https://doi.org/10.1007/s00705-004-0440-6
40. International Committee on Taxonomy of Viruses (2020) Virus Taxonomy: 2020 Release. ICTV Web. https://talk.ictvonline.org/taxonomy/. Accessed on 6 August 2021
41. Lecoq H, Desbiez C (2012) Viruses of cucurbit crops in the Mediterranean region: an ever-changing picture. Adv Virus Res 84 67–126 https://doiorg/101016/B978-0-12-394314-900003-8
42. Van der Meer FW, Garnett HM (1987) Purification and identification of a South African isolate of watermelon mosaic virus–Morocco. J Phytopathology 120:255-70. https://doi.org/10.1111/j.1439-0434.1987.tb04440.x
43. Purcifull DE, Hiebert E (1979) Serological distinction of watermelon mosaic virus isolates. Phytopathology 69:112-6. https://doi.org/10.1094/Phyto-69-112
44. Webb RE, Scott HA (1965) Isolation and identification of Watermelon mosaic viruses 1 and 2. Phytopathology 55:895-900.

45. Van Regenmortel MH, Brandes J, Bercks R (1962) Investigations on the properties of watermelon mosaic virus. J Phytopathology 45:205-216. https://doi.org/10.1111/j.1439-0434.1962.tb02036.x

46. Arocha Y, Vigheri N, Nkoy-Florent B, Bakwanamaha K, Bolomphety B, Kasongo M, Betts P, Monger WA, Harju V, Mumford RA, Jones P (2008) First report of the identification of Moroccan watermelon mosaic virus in papaya in Democratic Republic of Congo. Plant Pathology 57:387 https://doi.org/10.1111/j.1365-3059.2007.01658.x

47. Shukla DD, Ward CW (1989) Identification and classification of potyviruses on the basis of coat protein sequence data and serology. *Arch Virol* 106:171-200. https://doi:10.1007/BF01313952

48. Villamor DEV, Ho T, Al Rwahnih M, Martin RR, Tzanetakis IE (2019) High throughput sequencing for plant virus detection and discovery. *Phytopathology* 109:716-725. https//doi:10.1094/PHYTO-07-18-0257-RVW

49. Martelli GP, Adams MJ, Kreuze JF, Dolja VV (2007) Family Flexiviridae: a case study in virion and genome plasticity. Annu. Rev. Phytopathol. 45:73-100. https://doi.org/10.1146/annurev.phyto.45.062806.094401

50. Desbiez C, Wipf-Scheibel C, Millot P, Verdin E, Dafalla G, Lecoq H (2017) New species in the papaya ringspot virus cluster: Insights into the evolution of the PRSV lineage. Virus Res 241:88-94. https://doi.org/10.1016/j.virusres.2017.06.022

51. Ibaba JD, Laing MD, Gubba A (2015) Incidence and phylogeny of viruses infecting cucurbit crops in KwaZulu-Natal, Republic of South Africa. Crop Protection 75:46-54. https://doi.org/10.1016/j.cropro.2015.04.019

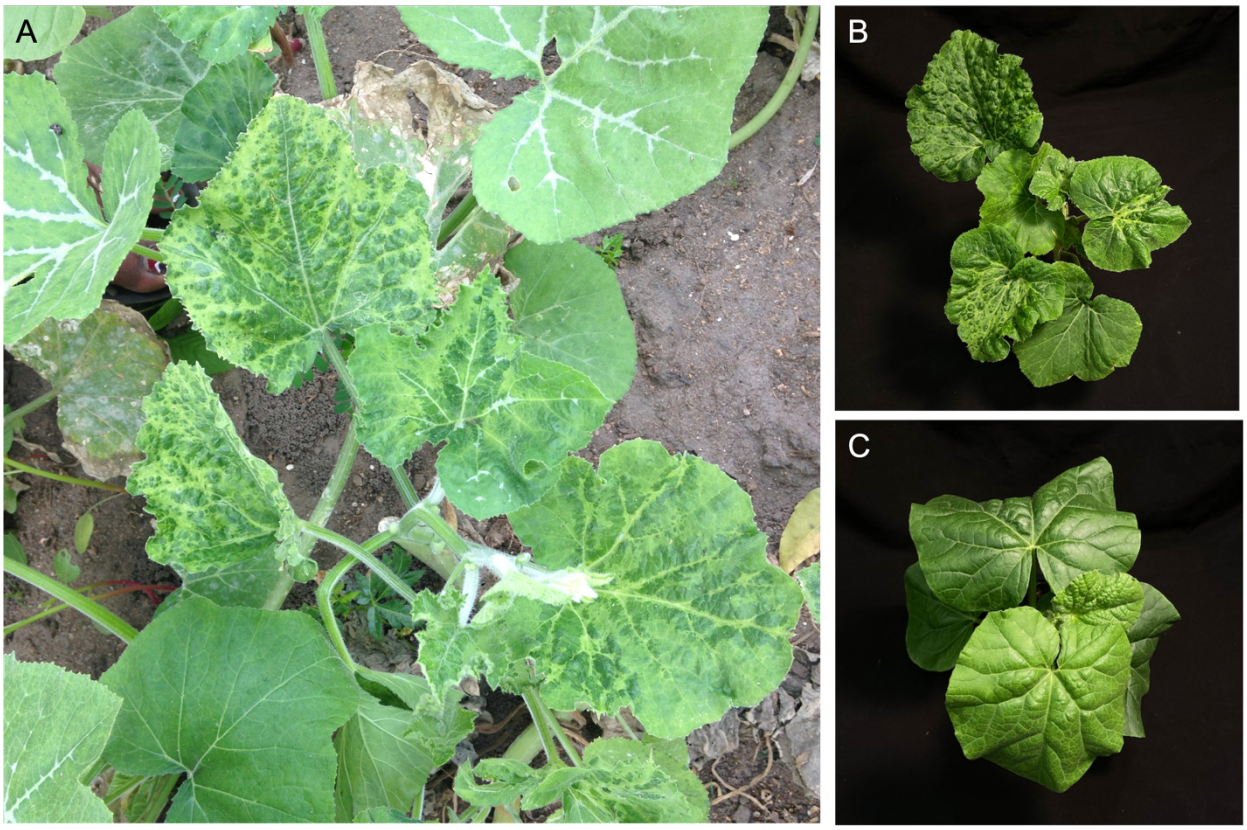


Figure 2.1. (A) Cucurbit plants show striking leaf symptoms of light green to chlorotic yellow mosaic/mottle, distortion, and dark green blistering in a commercial field outside of Dar es Salaam, Tanzania in 2016. (B) Symptoms induced in pumpkin plants (*Cucurbita pepo* cv. Sugar Pie) after mechanically inoculated with sap of an infected leaf sample (F4P1) and (C) mock-inoculated pumpkin plant showing no symptoms. Photographs were taken at 14 d after mechanical inoculation.

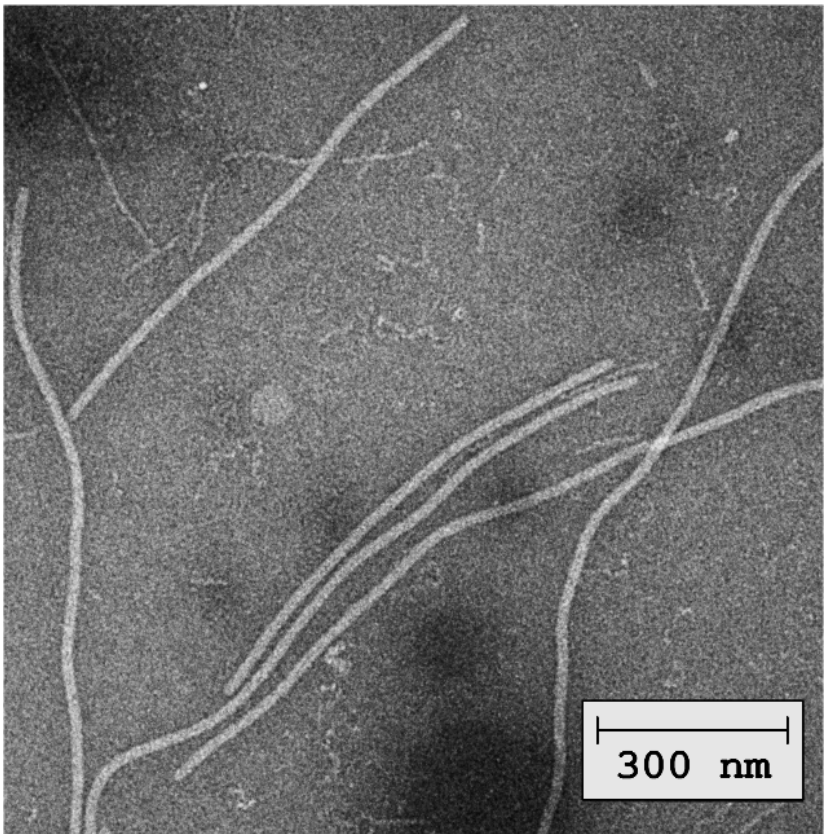


Figure 2.2. Transmission electron microscope image showing long flexuous filamentous potyvirus-like particle observed in a purified preparation from leaf tissue of pumpkin plants infected with a divergent strain of *Moroccan watermelon mosaic virus* (MWMV) from Tanzania, MWMV-TZ16. The average length of these particles was 820 nm based on measurement of 79 particles.

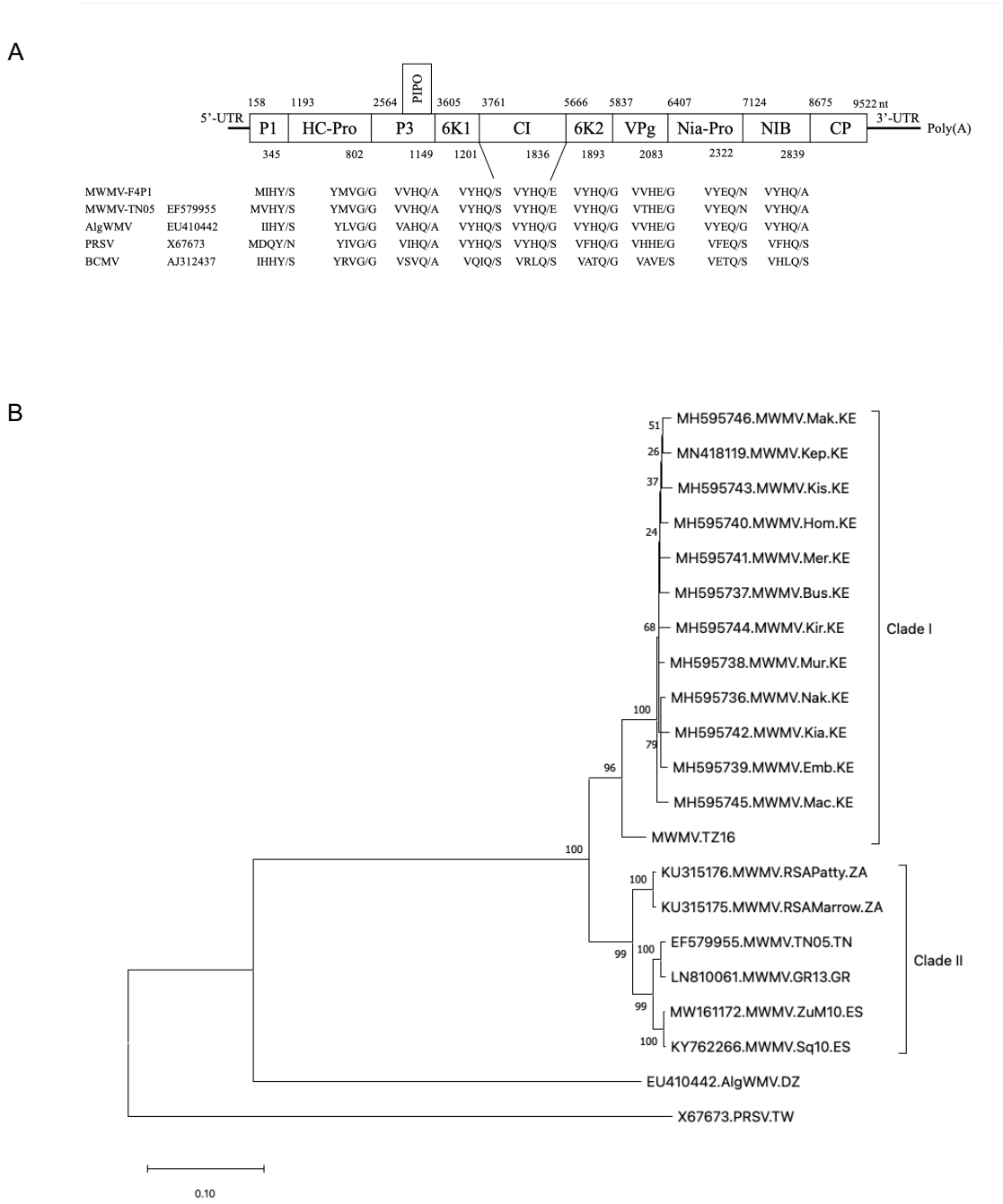


Figure 2.3. (A) Genome organization of an isolate of *Moroccan watermelon mosaic virus* from Tanzania, MWMV-TZ16, with putative cleavage sites of the polyprotein indicated beneath the corresponding coding region, and aligned with those of MWMV-Tn and other closely related potyviruses. (B) A phylogenetic tree generated from an alignment of amino acid sequences of the full-length polyprotein of MWMV-TZ16 and other isolates of MWMV, with those of PRSV and

AlgWMV as outgroups. The tree was constructed with MEGA X v.10.1.8 with the Jones-Taylor-Thomson (JTT) model. Country abbreviation are as follows: Kenya (KE), Tanzania (TZ), South Africa (ZA), Tunisia (TN), Greece (GR), Spain (ES), Algeria (DZ), and Taiwan (TW).

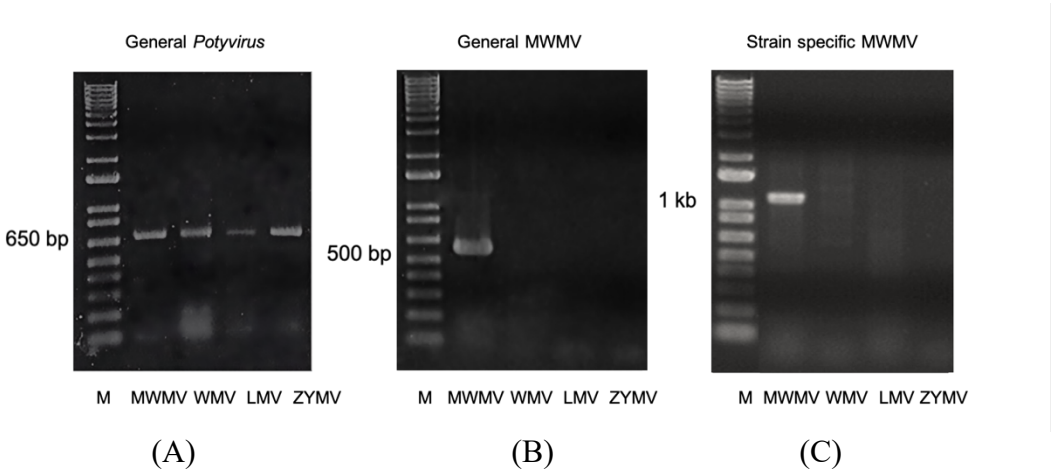


Figure 2.4. Evaluation of primer pairs for specific RT-PCR detection of isolates of *Moroccan watermelon mosaic virus* (MWMV). RT-PCR tests performed with total RNA extracts of leaves infected with MWMV-TZ16, *Watermelon mosaic* virus (WMV), *Lettuce mosaic virus* (LMV), and *Zucchini yellow mosaic virus* (ZYMV) with (A) the degenerate potyvirus CI primer pair (CIFor and CIRev; Ha *et al*. 2008), (B) the general MWMV primer pair predicted to detect all strains (MWMV P3-For and MWMV P3-Rev; Supplementary Table 1), and (C) the primer pair predicted to specifically detect strains of MWMV from East African, e.g., MWMV-TZ16 (MWMV P1F3 and MWMV HCM; Supplementary Table 1).

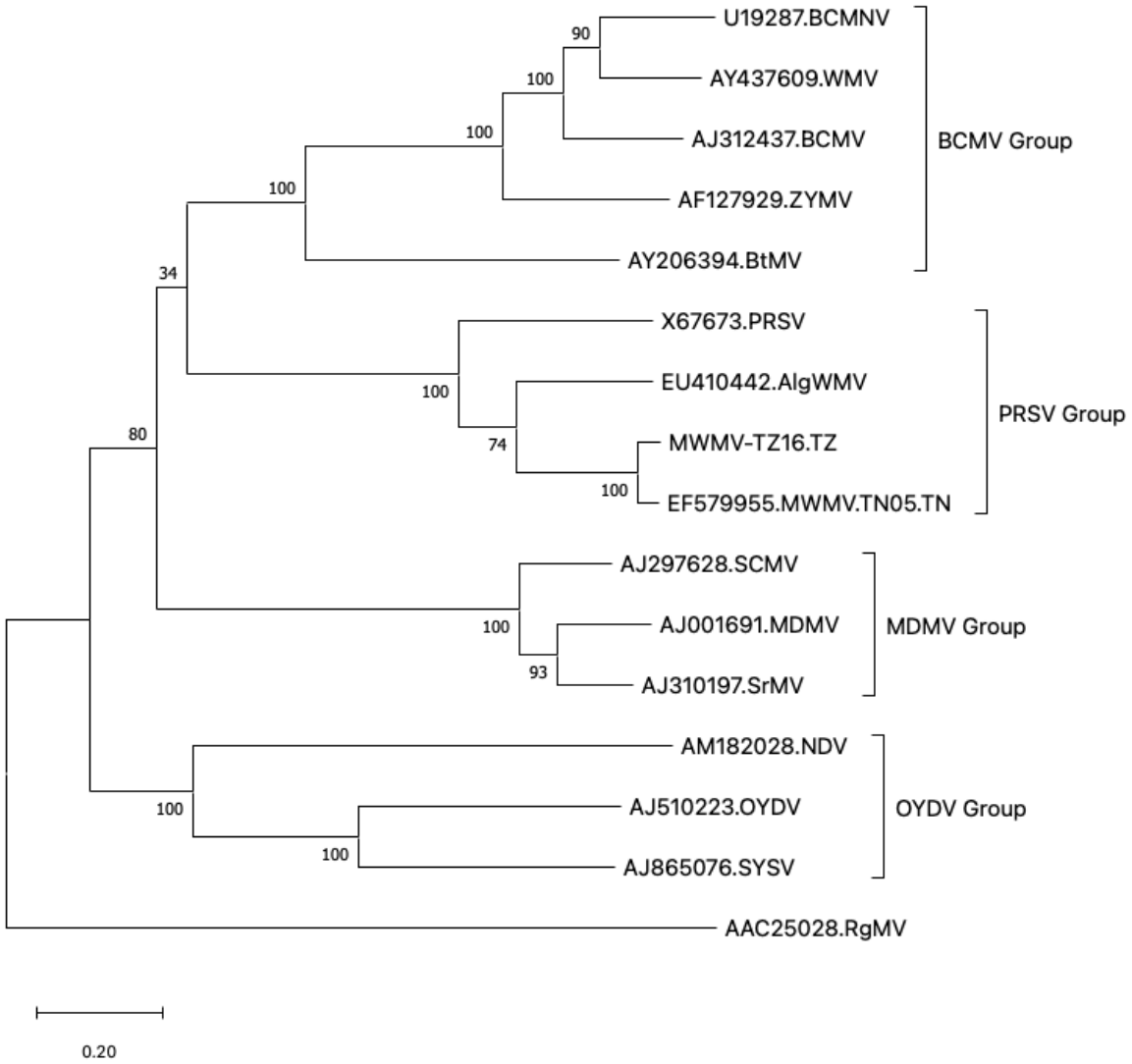
Table 2.1. Partial host range of an isolate of *Moroccan watermelon mosaic* virus (MWMV) from Tanzania (MWMV-TZ16)

Plant Species	Symptoms [a]	Symptom development in days post inoculation (dpi)[a]	Infectivity [b]
Cucurbits			
Cucurbita pepo cv. Sugar Pie	M, B, Vb, Cr	7	15/15 (100)
Cucurbita maxima cv. Big Max	M, B, Vb, Cr	10	15/15 (100)
Cucurbita moschata cv. Waltham Butternut	M, B, Vb, Cr	14	15/15 (100)
Cucurbita lanatus cv. Charleston Gray	M, B, Vb, Cr, S	21	15/15 (100)
Cucurbita lanatus cv. Crimson Sweet	M, B, Vb, Cr, S	28	15/15 (100)
Cucumis melo cv. Hales Best Jumbo	M, B, Vb, Cr	14	3/15 (20)
Cucumis melo cv. Sweet Delight	NS-ni	-	0/15 (0)
Cucumis sativus cv. Marketmore	NS-i	-	15/15 (100)
Non-cucurbits			
Solanum lycopersicum cv. Glamour	NS-ni	-	0/15 (0)
Nicotiana benthamiana	NS-ni	-	0/15 (0)
Nicotiana tabacum cv. Samsun	NS-ni	-	0/15 (0)
Phaseolus vulgaris cv. Topcrop	NS-ni	-	0/15 (0)
Chenopodium amaranthicolor	NS-ni	-	0/15 (0)
Chenopodium quinoa	NS-ni	-	0/15 (0)

[a] Symptom development was observed up to 30 d post inoculation (dpi). Abbreviations: M = mosaic/mottle; B = dark green blister; Vb = vein banding; Cr = crumpling; S = stunting; NS-i = no symptoms-infected; NS-ni = no symptoms-not infected.

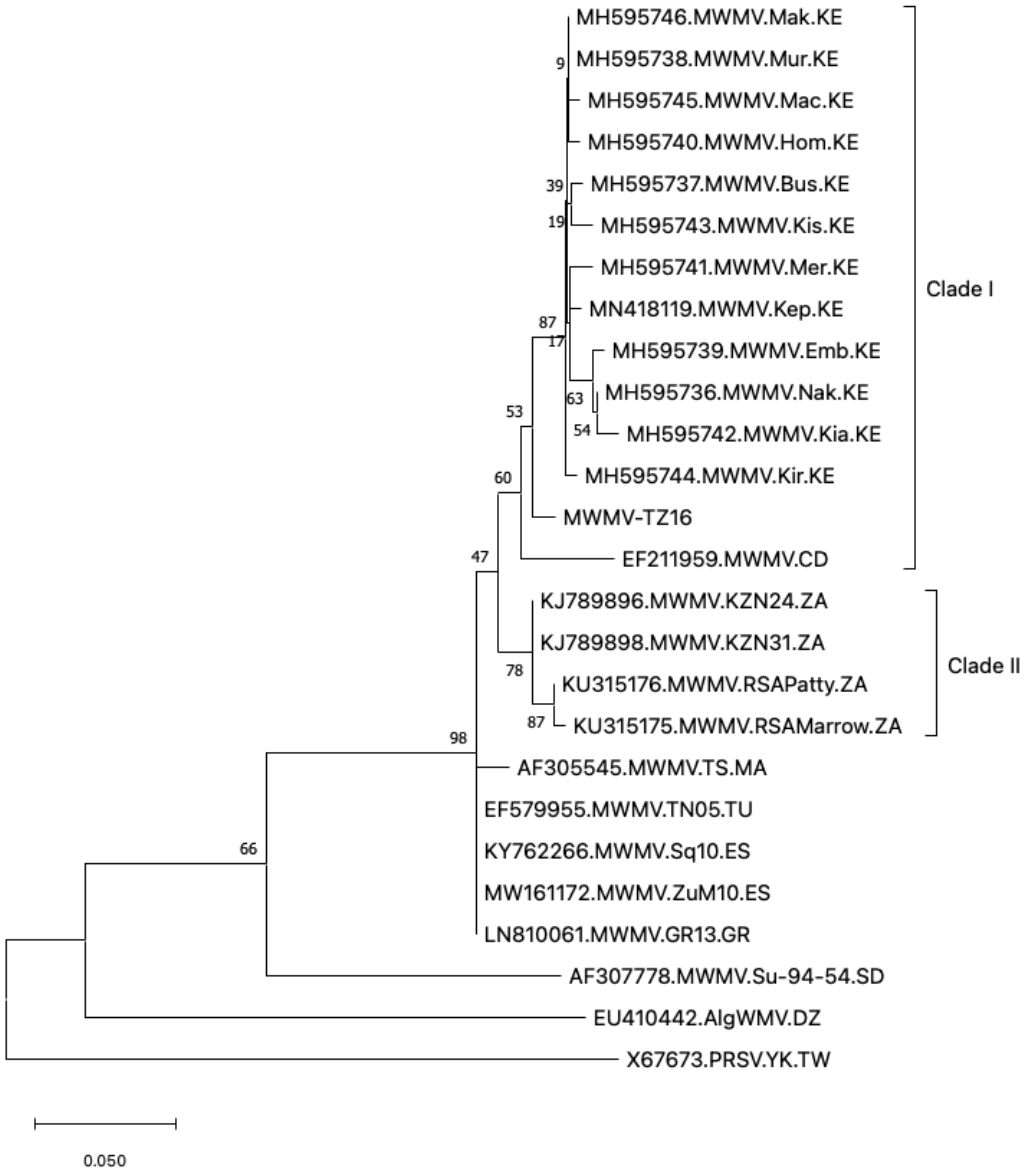
[b] Infectivity (number of infected plants/numbers inoculated with percentages in parentheses) was determined based on detection of viral RNA in newly emerging leaves by RT-PCR with MWMV-specific primers at 30 dpi.

Supplementary Figure 1



Phylogenetic tree generated based on an alignment of amino acid (aa) sequence of the full-length polyprotein of an isolate of *Moroccan watermelon mosaic virus* (MWMV) from Tanzania (MWMV-TZ16) and those of selected potyviruses representing major phylogenetic groups. The full-length polyprotein aa sequence of *Ryegrass mosaic virus* (RgMV) was used as the outgroup. The tree was constructed with MEGA X v.10.1.8 by the maximum-likelihood method, with the Jones-Taylor-Thomson (JTT) model.

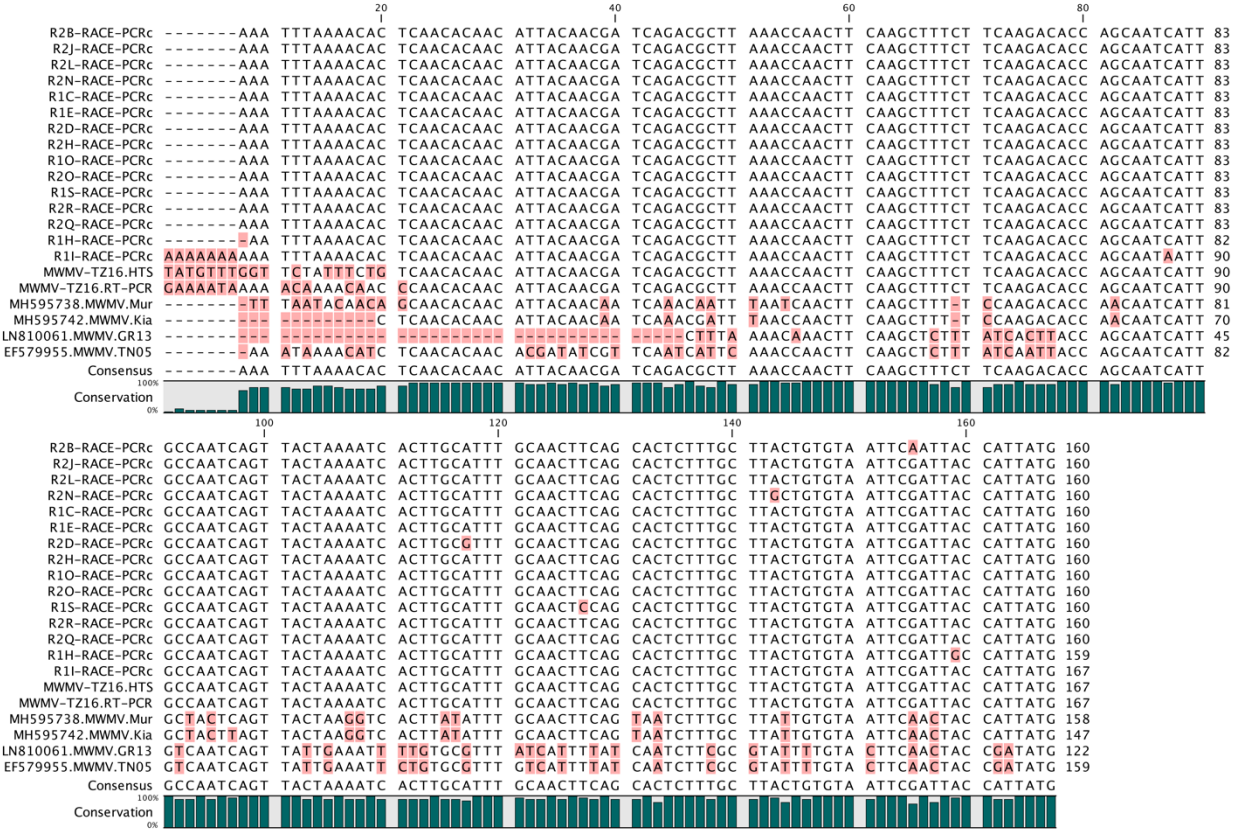
Supplementary Figure 2



Phylogenetic tree generated based on an alignment of the complete amino acid (aa) sequence of coat protein of an isolate of *Moroccan watermelon mosaic virus* (MWMV) from Tanzania (MWMV-TZ16) and those of other MWMV isolates, with those of *Papaya ringspot virus* (PRSV) and *Algerian watermelon mosaic virus* (AlgWMV) as the outgroup. The tree was constructed on MEGA X v.10.1.8 by the maximum-likelihood method, with the Jones-Taylor-Thomson (JTT) model. The country abbreviations are as follows: Kenya (KE), Tanzania (TZ), South Africa (ZA),

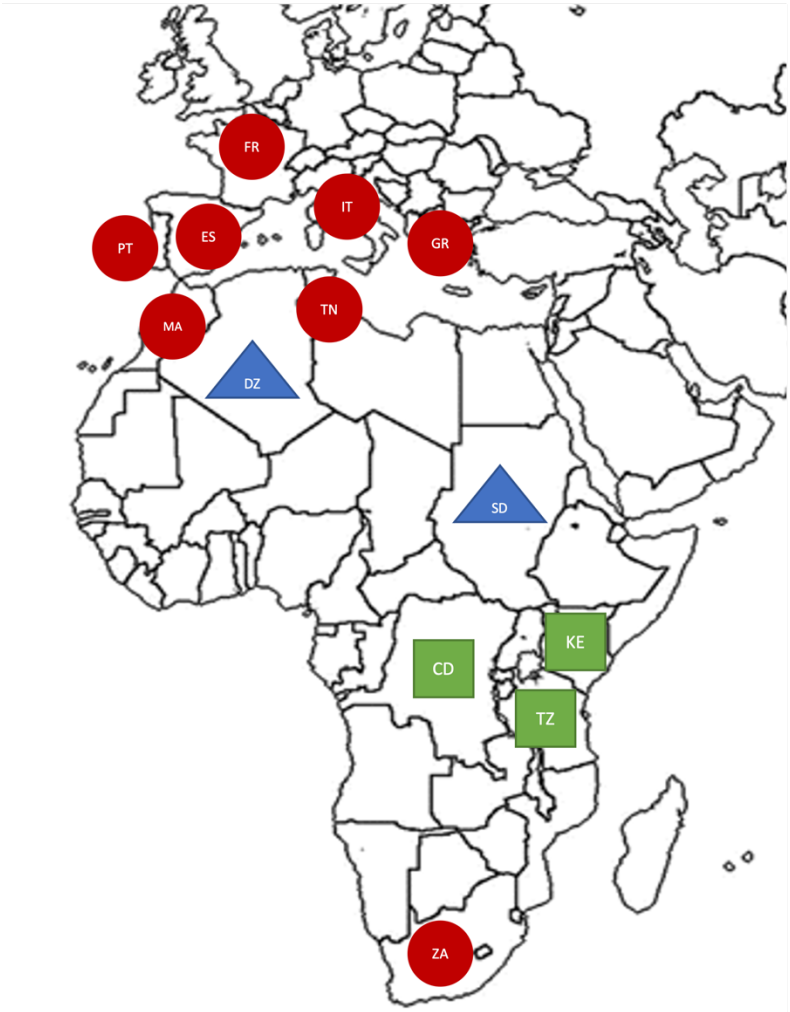
Tunisia (TU), Greece (GR), Spain (ES), Algeria (DZ), Taiwan (TW), Democratic Republic of the Congo (CD), and Morocco (MA).

Supplementary Figure 3



An alignment of 5'-end sequence of an isolate of *Moroccan watermelon mosaic virus* from Tanzania (MWMV-TZ16) generated from RACE-PCR fragments, HTS sequence data, the sequence from a cloned RT-PCR fragment and compared with the 5'-end sequences from full-length sequences of MWMV isolates from the East/Central Africa and North Africa/Southern Europe.

Supplementary Figure 4



Geographic distributions of *Moroccan watermelon mosaic virus* (MWMV) isolates according to major clades/lineages. The isolates of Clade I are shown in the red circles (●), isolates of Clade II with the green squares (■), and closely related species, *Algerian watermelon mosaic virus* (AlgWMV) and *Sudan watermelon mosaic virus* (SuWMV) with blue triangles (▲). The country abbreviations are as follows: Kenya (KE), Tanzania (TZ), South Africa (ZA), Tunisia (TU), Greece (GR), Spain (ES), Portugal (PT), Italy (IT), France (FR), Democratic Republic of the Congo (CD), Sudan (SD), Algeria (DZ) and Morocco (MA).

Supplementary Table 1. Sequences of the oligonucleotide primers used in this study

Primers	Nucleotide sequence[a]	Target region	Fragment size (kb)	Reference
CIFor	5'-GGIVVIGTIGGIWSIGGIAARTCIAC-3'	Potyvirus CI gene		Ha et al. 2008
CIRev	5'-ACICCRTTYTCDATDATRTTIGTIGC-3'	Potyvirus CI gene	0.7	Ha et al. 2008
F4P1 CPF	5'- GCAAGAAGGATAAGGACAAAGACG-3'	F4P1 CP Region		This study
N1T	5'-GACCACGCGTATCGATGTCGAC$(T)_{17}$V-3'	General 3'-end	1.0	Ha et al. 2008
MWMV P3-For	5'- GCCAGTGATGCCAGACATTTT-3'	MWMV P3 gene		This study
MWMV P3-Rev	5'- TCCCGCAAACTTAGCTCTTC-3'	MWMV P3 gene	0.5	This study
MWMV P1F3	5'-CAGAATGCCATGAAGAGG-3'	P1 gene		This study
MWMV HCM	5'-CTCTTGGCATGATATTGGCGC-3'	MWMV HC-Pro gene	1.1	This study
MWMV GSP-R2	5'- GCCCTTCCCTTCTTATCC-3'	MWMV P1 for RACE	-	This study
Abridge Anchor	5'-GCC CAC GCG TCG ACT AGT ACG GGI IGG GII			
Primer (AAP)	GGG IIG-3'	Poly-C tail	-	Gibco-BRL
M13(-21)	5' GTAAAACGACGGCCAGT 3'	pGEM vector	-	Invitrogen
M13R	5' CAGGAAACAGCTATGAC 3'	pGEM vector	-	Invitrogen

[a]In the primer sequences, I = inosine; Y = C/T; R =G/A; W=A/T; V=A/C/G; S=C/G and D=A/G/T

Supplementary Table 2. Sequences of *Moroccan watermelon mosaic virus* (MWMV) isolates obtained from NCBI and identities of coat protein, 3'-UTR and 5'-UTR sequences with those of a divergent strain of MWMV from Tanzania (MWMV-TZ16)

Isolate Code	Location	Collection Year	Full length availability	Identity (%)			
				5'-UTR NT	CP AA	CP NT	3'-UTR NT
MH595740.MWMV.Hom.KE	Kenya	2017	Yes	86.5	97.5	92.3	91.9
MH595736.MWMV.Nak.KE	Kenya	2017	Yes	86.5	96.4	92.1	92.4
MH595741.MWMV.Mer.KE	Kenya	2017	Yes	86.5	96.4	92.2	92.4
MH595742.MWMV.Kia.KE	Kenya	2017	Yes	85.7	95.7	92.0	91.9
MH595738.MWMV.Mur.KE	Kenya	2017	Yes	84.8	97.8	92.8	91.9
MH595739.MWMV.Emb.KE	Kenya	2017	Yes	84.8	96.1	92.6	91.3
MH595744.MWMV.Kir.KE	Kenya	2017	Yes	84.0	96.8	92.3	90.7
MN418119.MWMV.Kep.KE	Kenya	2018	Yes	84.0	96.8	91.6	90.7
MH595743.MWMV.Kis.KE	Kenya	2017	Yes	83.1	96.8	92.3	91.9
MH595746.MWMV.Mak.KE	Kenya	2017	Yes	82.3	97.8	92.3	91.3
MH595745.MWMV.Mac.KE	Kenya	2017	Yes	81.5	97.1	92.5	91.9
MH595737.MWMV.Bus.KE	Kenya	2017	Yes	80.6	97.5	92.2	92.4
KU315176.MWMV.RSAPatty.ZA	South Africa	2012	Yes	72.2	95.7	85.8	86.7
KU315175.MWMV.RSAMarrow.ZA	South Africa	2012	Yes	72.2	95.4	85.4	86.7
KJ789896.MWMV.KZN24.ZA	South Africa	2011	No	N/A	95.4	85.6	86.7
KJ789898.MWMV.KZN31.ZA	South Africa	2011	No	N/A	95.4	85.3	86.7
EF579955.MWMV.TN05.TN	Tunisia	2005	Yes	71.4	94.7	85.1	89.0
KY762266.MWMV.Sq10.ES	Spain	2010	Yes	69.7	95.4	85.4	88.4
MW161172.MWMV.ZuM10.ES	Spain	2010	Yes	69.7	95.4	85.4	88.4
LN810061.MWMV.GR13.GR	Greece	2014	Yes	69.7	95.0	85.3	89.0
AF305545.MWMV.TS.MA	Morocco	1974	No	N/A	94.0	85.6	90.1
EF211959.MWMV.CD	DRC	2006	No	N/A	92.9	86.4	87.8
AF307778.MWMV.Su-94-54.SD	Sudan	1994	No	N/A	74.6	70.1	70.1

Supplementary Table 3. Nucleotide (nt) and amino acid (aa) identities for the full-length polyprotein and each functional mature protein encoded by the genome of an isolate of *Moroccan watermelon mosaic virus* (MWMV) from Tanzania (MWMV-TZ16) and with those of closely related potyviruses

		Identity Matrix (%)																							
		ORF		P1		HC Pro		P3		6K1		CI		6K2		VPg		Nia		Nib		CP		PIPO	
Species of *Potyvirus*	Years	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa
EF579955.MWMV.TN05	2005	79.9	91.0	70.8	68.6	80.8	96.4	80.5	87.3	83.9	96.1	81.8	96.0	74.2	80.7	80.5	88.4	79.4	94.9	79.8	94.9	85.1	94.7	90.5	76.2
EU410442.ALgWMV	2008	66.0	71.6	49.7	37.6	64.2	71.1	57.3	47.2	76.2	80.7	67.8	74.4	63.1	59.6	66.9	72.6	66.0	72.3	71.5	81.2	69.8	77.5	60.0	36.9
X67673.PRSV	1994	68.1	65.9	32.4	29.7	65.4	66.5	51.2	38.4	67.9	65.3	65.9	72.1	54.6	42.1	67.1	68.9	61.6	64.4	68.4	74.4	67.0	72.8	53.3	32.6
AJ312437.BCMV	2002	53.7	49.8	31.0	15.5	51.9	44.5	43.0	25.5	52.5	40.3	56.4	55.5	48.2	21.0	57.6	53.1	54.0	49.1	58.3	55.8	60.5	61.0	43.8	28.2
AF127929.ZYMV	2001	53.3	49.9	30.8	17.8	52.4	46.3	42.7	24.7	51.9	40.3	55.8	55.1	39.7	17.5	56.3	52.6	51.2	49.1	60.6	57.5	59.0	60.5	43.8	30.4
AF127929.ZYMV	2001	53.3	49.9	30.8	17.8	52.4	46.3	42.7	24.7	51.9	40.3	55.8	55.1	39.7	17.5	56.3	52.6	51.2	49.1	60.6	57.5	59.0	60.5	43.8	30.4
AY437609.WMV	2004	53.2	50.3	35.6	15.5	51.1	46.2	44.2	25.2	57.6	44.2	55.9	55.9	50.3	28.0	54.5	52.6	53.7	47.1	58.7	56.3	59.9	62.7	43.3	23.9
AY206394.BtMV	2004	52.9	49.6	33.6	16.2	52.2	44.7	37.9	22.8	58.3	36.5	57.4	57.4	48.2	35.0	58.3	53.6	53.2	45.3	60.0	59.6	57.1	55.9	41.1	19.5
AJ001691.MDMV	1998	52.6	48.2	34.3	14.5	51.6	42.2	44.2	25.8	50.6	37.3	56.6	55.4	46.0	33.3	55.3	48.9	47.1	37.8	59.2	57.3	62.2	63.9	47.2	23.9
AJ297628.SCMV	2002	52.6	48.3	32.9	18.5	51.5	42.9	44.8	24.0	52.5	35.8	56.9	54.8	48.9	28.0	53.7	50.0	48.9	41.1	59.3	58.7	63.0	66.5	50.0	21.7
U19287.BCMNV	1995	52.4	49.5	32.8	14.5	51.9	46.0	41.0	22.9	60.2	50.0	57.0	56.2	48.9	26.3	53.1	48.9	51.0	46.3	57.5	56.2	59.4	61.8	44.4	30.4
AJ310197.SrMV	2002	51.8	47.8	34.0	14.0	50.7	42.0	44.1	24.6	49.3	35.8	57.0	56.2	48.2	33.3	51.6	45.2	47.9	38.6	58.4	59.1	64.3	65.2	47.7	26.0
AM182028.NDV	2007	49.7	43.4	31.4	17.8	49.1	42.2	38.3	18.9	53.8	44.2	54.7	50.0	45.3	24.5	49.3	39.7	49.6	38.2	57.2	57.1	56.3	55.5	32.0	17.3
AJ510223.OYDV	2000	48.7	43.4	30.7	15.5	49.5	43.7	37.6	20.1	50.6	42.3	53.8	49.0	45.3	28.0	55.8	41.3	50.2	42.7	60.0	57.9	64.3	63.9	37.5	21.7
AJ865076.SYSV	2005	48.1	43.6	30.0	16.0	49.5	42.6	39.1	19.0	51.9	42.3	54.3	49.5	46.8	24.5	47.5	35.4	50.3	40.3	60.3	61.2	62.2	64.8	38.5	10.8

Supplementary Table 4. Nucleotide (nt) and amino acid (aa) identities for the full-length open reading frame (ORF) and individual functional mature proteins of an isolate of *Moroccan watermelon mosaic virus* (MWMV) from Tanzania (MWMV-TZ16) and those of other isolates of MWMV

		Identity Matrix (%)																							
		ORF		P1		HC Pro		P3		6K1		CI		6K2		VPg		Nia		Nib		CP		PIPO	
MWMV Isolate	Year	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa
MH595746.MWMV.Mak.Ken	2017	87.7	94.6	83.9	81.4	89.4	96.7	90.7	96.2	91.6	98.0	91.8	98.2	87.1	91.2	81.7	92.6	80.8	93.7	84.3	95.3	92.3	97.8	97.7	93.2
MH595745.MWMV.Mac.Ken	2017	87.7	94.6	83.7	82.8	89.6	97.3	90.9	96.5	91.6	98.0	91.3	97.6	85.9	89.4	82.8	93.6	80.7	92.4	84.2	95.3	92.5	97.1	97.2	93.2
MH595744.MWMV.Kir.Ken	2017	87.7	94.6	83.6	82.6	89.4	96.9	91.1	96.8	90.3	98.0	91.7	97.6	87.7	91.2	82.9	93.1	80.4	92.8	84.2	95.7	92.3	96.8	97.2	91.5
MH595743.MWMV.Kis.Ken	2017	87.8	94.6	83.4	80.8	89.7	97.3	91.0	96.5	91.0	98.0	91.6	98.2	85.9	91.2	82.4	93.1	81.5	93.3	84.6	95.7	92.3	96.8	97.7	93.2
MH595742.MWMV.Kia.Ken	2017	87.9	94.6	83.6	81.4	89.6	97.1	91.6	96.8	91.6	98.0	91.7	97.9	86.5	91.2	83.1	94.2	81.4	92.8	84.5	95.9	92.0	95.7	97.7	93.2
MH595741.MWMV.Mer.Ken	2017	87.7	94.5	83.5	82.3	89.3	96.7	91.0	96.8	91.6	98.0	91.7	97.6	86.5	89.4	82.9	94.2	81.1	93.7	84.0	95.1	92.2	96.4	96.6	91.5
MH595740.MWMV.Hom.Ken	2017	87.6	94.8	83.5	82.8	89.0	96.9	91.1	96.5	91.6	98.0	91.3	98.2	85.3	89.4	82.8	93.6	81.0	92.8	84.0	95.7	92.3	97.5	96.6	89.8
MH595739.MWMV.Emb.Ken	2017	87.8	94.6	84.1	82.8	89.2	96.7	91.1	96.8	91.0	98.0	91.6	97.7	84.2	89.4	82.6	93.6	81.5	93.3	84.3	95.9	92.6	96.1	97.7	93.2
MH595738.MWMV.Mur.Ken	2017	87.9	94.8	84.0	83.1	89.7	97.1	90.9	96.8	91.6	98.0	91.8	97.6	87.1	89.4	83.5	94.7	80.8	92.8	84.3	95.5	92.8	97.8	97.7	93.2
MH595737.MWMV.Bus.Ken	2017	87.7	94.6	83.1	82.3	89.4	97.1	90.9	95.9	91.0	98.0	91.9	98.1	85.3	89.4	83.1	94.2	80.8	92.8	84.2	95.3	92.2	97.5	97.7	93.2
MH595736.MWMV.Nak.Ken	2017	87.8	94.7	83.9	81.1	89.2	97.5	91.3	96.8	91.0	98.0	91.6	98.1	87.1	89.4	82.8	94.2	81.0	92.8	84.6	95.9	92.1	96.4	97.2	91.5
MN418119.MWMV.Kep.Ken	2018	87.7	94.5	83.3	81.1	89.4	97.3	91.0	96.5	91.6	98.0	91.5	97.6	85.9	89.4	82.6	93.6	81.3	93.7	84.7	95.5	91.6	96.8	97.2	91.5
KU315176.MWMV.RSAP.SOA	2013	80.2	91.3	70.2	67.5	80.2	96.0	81.2	89.0	83.3	96.1	81.8	96.5	80.1	84.2	78.7	88.4	79.0	94.9	81.8	95.5	85.8	95.7	93.8	81.3
KU315175.MWMV.RSAM.SOA	2013	80.1	91.4	70.2	67.8	80.1	95.8	81.3	89.3	83.3	96.1	81.8	96.3	79.5	84.2	79.1	89.4	78.9	94.9	81.8	95.7	85.4	95.4	93.8	81.3
EF579955.MWMV.TN05.Tun	2008	79.9	91.0	70.8	68.6	80.8	96.4	80.5	87.3	83.9	96.1	81.8	96.0	74.2	80.7	80.5	88.4	79.4	94.9	79.8	94.9	85.1	94.7	90.5	76.2
LN810061.MWMV.GR13.Gre	2015	80.2	91.1	70.7	67.8	80.8	96.4	80.6	87.8	83.9	96.1	82.2	96.5	74.8	80.7	80.7	87.8	79.6	95.3	80.6	95.5	85.3	95.0	91.1	77.9
MW161172.MWMV.ZuM10.SPA	2020	79.8	91.0	69.6	67.2	80.8	96.2	80.8	87.6	82.6	96.1	81.7	96.2	75.4	82.4	80.0	88.4	79.4	95.3	79.9	95.3	85.4	95.4	91.1	77.9
KY762266.MWMV.Sq10.SPA	2010	79.8	90.9	69.5	67.2	80.8	96.2	80.7	87.3	83.3	96.1	81.7	96.2	75.4	82.4	80.0	88.4	79.3	94.9	79.9	95.1	85.4	95.4	91.1	77.9

Chapter III

Future Directions

Survey of *Moroccan watermelon mosaic virus* (MWMV) Distribution and Genetic Diversity

In Chapter II, we established that MWMV is an emerging disease that threatens the production of cucurbits in Africa and the Mediterranean Basin. Furthermore, the MWMV isolates characterized to date in Africa and the Mediterranean Basin have a great degree of genetic diversity, some of which is associated with geographic distribution, i.e., isolates of the North and South Africa/Southern Europe and Central/East African clades (Fig. 2B). However, the diversity and genetic structure of the MWMV isolates in the Central/East African clade have remained less clear. The analysis of the sequence of MWMV-TZ16 further support a Central/East African lineage and provided new insights into strain diversity in this lineage. The MWMV-TZ16 sequence was also used to develop primer pairs for specific detection of these viruses. Finally, our work also raised many questions about the MWMV strain distribution in Africa.

The number of currently available full-length sequences of MWMV isolates is limited. Partial sequences, usually of the 3'-end of the genome [1,4], have been determined and used in phylogenetic studies of other studies [5]. Moreover, some of these isolates are particularly interesting in terms of strain diversity, geography and population structure [1,12]. Thus, a comprehensive survey of MWMV distribution in African and Mediterranean countries is needed, including more extensive characterization of diverse MWMV isolates.

Further biological characterization of *Moroccan watermelon mosaic virus* (MWMV) isolates

We also conducted a partial host range study with MWMV-TZ16. Based on this study, this isolate was restricted to cucurbits, and more pathogenic to squash and pumpkin. Moreover, MWMV-TZ16 induced the diagnostic chlorotic mottle and dark green blister in all infected

cucurbits, but did not induce necrosis or shoestring symptoms. This experiment also revealed biological differences between MWMV-TZ16 and isolates of the North and South Africa/Southern European lineage, the latter also infecting non-cucurbit hosts, e.g., inducing local lesions in *Chenopodiaceae* and systemic infection in *N. benthamiana*, and necrosis and shoestring symptoms in some cucurbits [5]. These biological properties may be useful in identifying MWMV isolates of these and other lineages, and should be determined for MWMV isolates chosen for full genome sequencing.

PRSV is a cucurbit- and papaya-infecting potyvirus, with cucurbit-infecting (PRSV-W) and papaya-infecting (PRSV-P) isolates or biotypes [6]. The association of MWMV with severe virus-like symptoms in papaya, makes it important to complete the Koch's Postulate for these diseases and to assess the host range of MWMV isolates from papaya [2,3]. The genetic diversity of papaya and cucurbit isolates of MWMV could be compared to identify regions of the genome that involved in host adaptation. Mapping of the genetic factors influencing host adaptation would involve genome exchanges with infectious clones of cucurbit- and papaya-infecting strains of MWMV.

Mixed infections of MWMV with other potyviruses, e.g. WMV and ZYMV, were previously reported [7,12]. More recently, mixed infection of MWMV with carlaviruses, e.g., *Cowpea mild mottle virus* (CpMMV) and *Cucumber vein-clearing virus* (CuVCV), was also reported [8]. The synergistic or antagonistic interactions between MWMV and each of these co-infecting viruses during infection of cucurbit plants should be investigated to better understand the impact to the biology of these viruses and symptom development.

Seed Transmission of *Moroccan watermelon mosaic virus* (MWMV)

Some members of the genus *Potyvirus* are known to be seed-transmitted, e.g., BCMV, LMV, SMV and ZYMV [9-11]. Seed transmission often plays an important role in the global distribution of plant viruses [9,11], though some whitefly-transmitted begomoviruses and criniviruses are notable exceptions [13]. Although there is no evidence of seed transmission of MWMV [14], this was based on small numbers of seeds. Thus, a more thorough investigation of seed transmission is warranted, and with different cucurbit crops. For example, although ZYMV is seed-transmitted in *C. pepo*, this occurs at low frequency and latent infections may be involved [15,16].

Phylogenetic analyses showed that MWMV isolates from South Africa were most closely related to those from Morocco, Tunisia and Southern Europe, despite the great geographic distance between South Africa and these other countries. This suggests long-distance movement may have been involved, e.g., with seed or transplants. Therefore, potential mechanism for long-distance movement of MWMV should be investigated. This could lead to determination of management strategies to prevent the long-distance movement of the virus.

In the present study, we developed two primer pairs for RT-PCR detection of MWMV, one predicted to allow detection of all MWMV isolates/strain and the other to allow specific detection of isolates from East Africa, e.g. MWMV-TZ16. It will be of interest to use these tools for detection of MWMV in comprehensive field surveys to better understand the worldwide distribution and prevalence of MWMV strains. Furthermore, these surveys should not be limited to cucurbits, as some MWMV isolates appear to infect and cause severe disease symptoms in papaya [2,3]. It would be important to identify other hosts in the field, with or without symptoms.

References

1. Lecoq H, Dafalla G, Desbiez C, Wipf-Scheibel C, Delécolle B, Lanina T, Ullah Z, Grumet R (2001) Biological and molecular characterization of Moroccan watermelon mosaic virus and a potyvirus isolate from Eastern Sudan. Plant disease 85:547-552. https://doiorg/101094/PDIS2001855547
2. Read DA, Muoma J, Thompson GD (2020) Metaviromic analysis reveals coinfection of papaya in western Kenya with a unique strain of Moroccan watermelon mosaic virus and a novel member of the family Alphaflexiviridae. Arch Virol 165:1231-1234. https://doi.org/10.1007/s00705-020-04582-x
3. Mumo NN, Mamati GE, Ateka EM, Rimberia FK, Asudi GO, Boykin LM, Machuka EM, Njuguna JN, Pelle R, Stomeo F (2020) Metagenomic analysis of plant viruses associated with papaya ringspot disease in *Carica papaya* L in Kenya. Front Microbiol 11:205. https://doi.org/10.3389/fmicb.2020.00205
4. Lecoq H, Justafré I, Wipf-Scheibel C, Desbiez C (2008) Moroccan watermelon mosaic virus newly reported on zucchini squash in France. Plant Pathology 57:766. https://doi.org/10.1111/j.1365-3059.2008.01848.x
5. Yakoubi S, Desbiez C, Fakhfakh H, Wipf-Scheibel C, Marrakchi M, Lecoq H (2008) Biological characterization and complete nucleotide sequence of a Tunisian isolate of Moroccan watermelon mosaic virus. Arch Virol 153:117-125. https://doi.org/10.1007/s00705-007-1074-2
6. Lecoq H, Desbiez C (2012) Viruses of cucurbit crops in the Mediterranean region: an ever-changing picture. Adv Virus Res 84 67–126 https://doiorg/101016/B978-0-12-394314-900003-8

7. Yakoubi S, Desbiez C, Fakhfakh H, Wipf-Scheibel C, Marrakchi M, Lecoq H (2008) Biological characterization and complete nucleotide sequence of a Tunisian isolate of Moroccan watermelon mosaic virus. Arch Virol 153:117-125. https://doi.org/10.1007/s00705-007-1074-2
8. Menzel W, Abang MM, Winter S (2011) Characterization of cucumber vein-clearing virus a whitefly (Bemisia tabaci G)-transmitted carlavirus. Arch Virol 156:2309-2311. https://doi.org/10.1007/s00705-011-1124-7
9. Simmons HE, Munkvold GP (2014) Seed transmission in the Potyviridae. In: Gullino ML, Munkvold (Ed) Global perspectives on the health of seeds and plant propagation material. Springer, Dordrecht, pp 3-15
10. Hajimorad MR, Domier LL, Tolin SA, Whitham SA, Saghai Maroof MA (2018) Soybean mosaic virus: a successful potyvirus with a wide distribution but restricted natural host range. Mol Plant Pathol 19:1563-1579. https://doi.org/10.1111/mpp.12644
11. Baldodiya GM, Baruah G, Sen P, Nath PD, Borah BK (2020) Host-Parasite Interaction During Development of Major Seed-Transmitted Viral Diseases. In: Kumar R and Gupta A(Ed) Seed-Borne Diseases of Agricultural Crops: Detection Diagnosis & Management. Springer, Singapore, pp 265-289
12. Ibaba JD, Laing MD, Gubba A (2016) Genome sequence analysis of two South African isolates of Moroccan watermelon mosaic virus infecting cucurbits. Virus genes 52:896-899. https://doi.org/10.1007/s11262-016-1372-4
13. Gilbertson RL, Batuman O, Webster CG, Adkins S. (2015) Role of the Insect Supervectors Bemisia tabaci and Frankliniella occidentalis in the Emergence and Global

Spread of Plant Viruses. *Annu Rev Virol* 2:67-93. doi:10.1146/annurev-virology-031413-085410

14. Roggero P, Gotta P, Stravato VM, Dellavalle G, Ciuffo M (1999) Further spread of Moroccan watermelon mosaic potyvirus in Italy in 1998. J Plant Pathol 81:149
15. Simmons HE, Holmes EC, Gildow FE, Bothe-Goralczyk MA, Stephenson AG (2011) Experimental verification of seed transmission of Zucchini yellow mosaic virus. Plant Disease, 95(6), pp.751-754. https:// doi:10.1094 / PDIS-11-10-0843
16. Simmons HE, Dunham JP, Zinn KE, Munkvold GP, Holmes EC, Stephenson AG (2013) Zucchini yellow mosaic virus (ZYMV, Potyvirus): vertical transmission, seed infection and cryptic infections. Virus Res. 176:259-264. https//doi:10.1016/j.virusres.2013.06.016