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MOLECULAR CHARACTERISTICS OF *Tomato mosaic virus* INFECTING TOMATO IN UGANDA

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ABSTRACT

Viral diseases are part of the limiting factors to tomato (*Solanum lycopersicum* L.) cultivation worldwide, reducing both the quality and quantity of yield. *Tomato mosaic virus* (ToMV) is one of the damaging viruses of tomato. This paper describes molecular characteristics of the full length genome of ToMV isolated from tomato in Uganda (ToMV-Ug). The genomic, ribonucleic acid (RNA), of this isolate is 6383 nucleotides (nts) in length, encoding four open reading frames (ORFs). Based on the homology with other ToMV strains, the 5' proximal 130 kilo dalton (kDa) ORF and its read-through product (180 kDa) are expected to encode two proteins required for viral genome replication; while the 30 kDa middle ORF and the 17.5 kDa 3' proximal ORF are expected to encode the movement protein (MP) and coat protein (CP), respectively. The 5'- and 3'- untranslated regions (UTRs) are 71 and 201 nts, respectively. Comparison with previously published ToMV sequences showed that ToMV-Ug is 99% identical to ToMV strains from Africa (Egypt and Zimbabwe), as well as diverse locations such as China, Australia, Germany and Japan; suggesting high levels of sequence conservation within this virus. This is the first report detailing molecular analysis of a ToMV isolate from Uganda and the Eastern and Central Africa regions.

Key Words: Ribonucleic acid, *Solanum lycopersicum*, *Tobamovirus*

RÉSUMÉ

Les maladies virales font partie des facteurs limitant la production mondiale de la tomate (*Solanum lycopersicum* L.), réduisant à la fois la quantité et la qualité du rendement. Le virus de la mosaïque de la tomate (ToMV) est l'un des virus endommageant la tomate. Ce papier décrit les caractéristiques moléculaires de la longueur du génome de l'isolat ToMV de la tomate en Ouganda (ToMV-Ug). L'acide génomique, ribonucléique (ARN), de l'isolat a une longueur de 6383 nucléotides (nts), codant quatre cadres de lecture ouverts (ORFs). Sur la base de l'homologie avec les autres souches de ToMV, le proximal 5' de 130 kilo dalton (kDa) de l'ORF et sa lecture à travers le produit (180 kDa) sont espérés coder pour deux protéines nécessaires à la réplication du génome viral ; alors que les 30 kDa du ORF moyen et les 17,5 kDa du proximal 3' du ORF sont espérés coder pour le mouvement de la protéine (MP) et la protéine de l'enveloppe (CP), respectivement. Les régions non traduites du 5' et 3' (UTRs) sont de

71 et 201 nts, respectivement. La comparaison avec les séquences (ToMV) précédemment publiées a montré que ToMV-Ug est à 99% identique aux souches ToM de l'Afrique (Egypte et Zimbabwe), ainsi que diverses localités telles que la Chine, l'Australie, la Germanie et le Japon ; suggérant de hauts niveaux de séquence de conservation dans ce virus. Ceci est le premier rapport détaillant l'analyse moléculaire d'un isolat ToMV d'Ouganda et les régions Est et Centre de l'Afrique.

Mots Clés: Acide ribonucleic, *Solanum lycopersicum*, virus du Tobamo

INTRODUCTION

Tomato mosaic virus (ToMV) is among the most important viruses of tomato worldwide (Hanssen *et al.*, 2010). It is also the most resilient virus in terms of ability to survive outside plant cells and in dead tissues (Lanter *et al.*, 1982). The virus is a member of the *Tobamovirus* genus. It is mechanically transmitted, with a very wide host range including, more than 150 species of vegetables, flowers and weeds (Hanssen *et al.*, 1990; Ismaël *et al.*, 2012). The blend of its extreme survival ability and mechanical transmission makes its management a nightmare among most tomato growers worldwide. Management is even worse in the developing world where access to clean resistant tomato varieties is limited.

In Africa, literature on ToMV is scanty, with the first report of severe viral damage among East African vegetable growers being in the early 1990s (Brunt *et al.*, 1997). In Uganda, the occurrence of ToMV was first established in the late 1990s (Ssekyewa, 2006), then estimated to cause up to 100% yield losses. Recently, more than 60% incidence of ToMV was observed in field samples, with high levels of mixed virus infections (Arinaitwe, 2013). However, all these findings have been based on field symptom expression in Uganda (Goelet *et al.*, 1982; Klug, 1999; Arinaitwe, 2013) and antibody-based methods, which are at times reported as misleading (Ssekyewa, 2006). This is because of relatedness in symptom expression among many plant viruses and cross reactivity at serology level (López *et al.*, 2009).

López *et al.* (2009) indicated that successful management of many plant viruses

largely requires real-time detection and identification. To this effect, molecular-based detection and identification methods such as the polymerase chain reaction (PCR) and genome sequencing have been reported to be more informative in the crafting of durable management options for many viruses, including ToMV. Such techniques help in uncovering novel plant pathogens, and have been used in many crops (Deusdedith, 2011) and diagnostics for tomato infecting viruses (Chen *et al.*, 2011; López-Gresa *et al.*, 2012; Panno *et al.*, 2012; Andolfo *et al.*, 2014). Knowledge of the molecular composition of plant viruses is critical for development of diagnostics, and consequently facilitates management of such viruses.

This study was, therefore, executed to detect ToMV in major tomato growing districts of Uganda using PCR based techniques, and determine the identity and molecular characteristics of ToMV by sequencing its complete genome. This is the first ever complete genome of ToMV isolated from Uganda. Sequence knowledge generated in this study will, therefore, help in development of diagnostic tools for management of ToMV in Uganda.

MATERIALS AND METHODS

Source of virus isolates. Tomato leaf samples used in this study were collected from farmers' fields in eight major tomato growing districts (Fig. 1). The districts included Kasese (Zone VII), Mbarara (VIII-East / IX-West), Rukungiri and Ntungamo (IX) in western Uganda; Mpigi (VI) and Luwero (VIII) in central Uganda, Kamuli (V) and Mbale (X) in eastern Uganda. The climatic

conditions of these areas are those described in Ssekyewa (2006). A total of 69 fresh leaf samples, confirmed positive for ToMV or *Tobacco mosaic virus* (TMV) infections by enzyme linked immunosorbent assays (ELISA), were preserved by lyophilisation and with the RNA stable reagent (Biomatrix, Inc., San Diego, California, USA). They were then shipped and analysed at the Ohio Agricultural Research and Development Center (OARDC, Wooster, Ohio, USA). At OARDC, each lyophilised sample was ground in 2 ml of inoculation buffer (10 mM sodium phosphate buffer, 1% celite, and pH 7.0), and used to mechanically inoculate four young tomato plants of the "Peto" variety (Tiberini *et al.*, 2010). The inoculated plants were reared in a greenhouse, and systemically-infected foliar tissue harvested at 14 days post inoculation for total RNA isolation.

Isolation of total RNA. Total RNA was extracted from infected tomato leaves using the Trizol reagent (Invitrogen, Carlsbad, California, USA), following the manufacturer's instructions. Briefly, 0.3 g of leaf sample was ground to powder in liquid nitrogen using a mortar and a pestle, and homogenised in 1 ml Trizol reagent. The homogenate was then transferred to a 2 ml eppendorf tube and incubated at room temperature for 5 minutes (min), to permit complete dissociation of nucleoprotein complexes. Three hundred microliters (300 μ l) of cold chloroform was then added; followed by thorough mixing and the tube was then centrifuged at 12,000 rpm for 15 min at 4°C.

The aqueous phase that contained RNA was decanted into a sterile eppendorf tube and the RNA was precipitated with isopropanol. The RNA pellet was then washed with 1 ml 75% ethanol, and dissolved in 100 μ l RNase-free water. The concentration of extracted nucleic acid was determined using a Nano Drop ND-1000 photo spectrometer (Nano Drop Technologies Inc., Wilmington, Delaware, USA). The integrity of RNA was

also confirmed by agarose gel electrophoresis. Total RNA samples prepared in the Biotechnology Laboratory at the School of Agricultural Sciences, Makerere University, Uganda and preserved in RNastable® (Sigma Aldrich, Missouri, USA) was reconstituted by addition of 25 μ l of RNase-free water.

Amplification of viral RNA. Six pairs of primers were used to amplify ToMV complementary deoxyribonucleic acid (cDNA) with reverse transcriptase polymerase chain reaction (RT-PCR) (Table 1). Five of the six primer pairs were designed based on the sequences of a previous ToMV isolate (GenBank accession FN985165). The sixth pair of primers was derived from Letschert *et al.* (2002).

A two-step RT-PCR procedure was used to amplify the full length of ToMV. In the first step, cDNA was synthesized by mixing 5.75 ml DNase-treated total RNA; 2 ml dNTP mix (10 mM dATP, dCTP, dGTP, and dTTP); 1 μ l of 20 mM reverse primer, and 2.25 ml RNase-free water in a 0.5 ml tube. The tube was then incubated at 65 °C for 5 min, chilled on ice, before adding 2 ml of reverse transcriptase enzyme mix (Clontech, Palo Alto, California, USA) and 6 ml RNase-free water. The tube was then incubated at 25 °C for 10 min, 40 °C for 30 min, and 85 °C for 5 min. The synthesized cDNA was diluted 1:4 with ddH₂O and stored at -20 °C for future use.

In the second step, a PCR reaction consisting of 4 ml cDNA; 10 ml of 2x EconoTaq plus green master mix (Lucigen Corporation, Middleton, Wisconsin, USA); 2 ml of 10 mM of each primer (forward and reverse); 2 ml ddH₂O. The amplification conditions included an initial denaturation step at 94 °C for 2 min, followed by 40 cycles of 94 °C for 30 seconds (denaturation), 58 °C for 30 s (annealing), 72 °C for 2 min (extension); and a final extension at 72 °C for 6 min.

All PCR reactions were performed in a PTC-100 thermocycler (MJ Research Inc.,

TABLE 1. Primers used in amplification of ToMV isolated from tomato in Uganda

Primer name	Primer sequence (5' - 3')	Region	Size	Reference
ToMVspecR	CGG AAG GCC TAA ACC AAA AAG	Coat protein	672	Letschert <i>et al.</i> , 2002
Tob-Uni1F	ATTTAAGTGGASGGAAAAVCACT			
T7-ToMV-W-1F	GGG AAA TAA TAC GAC TCA CTA TAG TAT TTT TAC AAC AAT TAC	Methyltransferase	627	This study
ToMV-W-627R:	ACG TTT GGA AAG TAT CGT GAC AGA			
ToMV-W-633F	GCA TTC TCA AGA ATG TTA CAC GGG AA	Methyltransferase	1811	This study
ToMV-W-2444R:	CCA GTT ATC GCA CGT AAT AAT GCC AA			
ToMV-W-2326F:	GGC TAG TTA AAC CAT CCG CAA AGA AC	Replicase and movement protein	3434	This study
ToMV-W-5760R:	CAG CCC ATA CAG ATG ACA AAA ACA CA			
ToMV-6003F	GCA GAG TCC GAC AAC AGC TGA AA	Coat protein	450	This study
rolC-ToMV-W-6383R	GCA TAA AGG TCG AAT GGG CC			
ToMV-6174F	GCA TCT TAA ATG CAT AGG TGC TGA A	Coat Protein	380	This study

Waltham, Massachusetts, USA). Amplicons were analysed in 1.2% agarose gel in Tris-acetate-EDTA buffer. Gels were stained with ethidium bromide and DNA was visualised with UV light.

Cloning and sequencing. Amplicons were purified using a DNA Clean and Concentrator™-5 kit (Zymo, Irvine, California, USA), following the manufacturer's protocol and subsequently cloned into a pGEM-T Easy vector following the recommended protocol (Promega, Madison, Wisconsin, USA). The resulting plasmids were purified using a Zymo kit (Irvine, California, and USA) and 2 ml of each plasmid prep digested with EcoRI before sequencing. For direct PCR product sequencing, fragments were cleaned using a Zymo PCR clean-up kit and sequenced from both ends. Sequencing was done with the ABI 3100X1 sequencer at the Molecular and Cellular Imaging Center at OARDC in Wooster, USA. The resulting annotated full genome sequence was deposited in the GenBank under the accession number MG456601.

Sequence and phylogenetic analysis. A total of 34 other full genome sequences of tobamoviruses were downloaded from NCBI (<https://www.ncbi.nlm.nih.gov/>) and analysed together with the newly sequenced Ugandan isolate. Sequence alignment and percentage nucleotide identities were computed in Geneious Software v10.0.5 (Kearse *et al.*, 2012). Recombination events were detected using nine recombination detection programs within the RDP4 package (<http://darwin.uvigo.es/rdp/rdp.html>) namely: RDP, GENECONV, MaxChi, Chimaera, Bootscan, Siscan, PhylPor, LARD, and 3Seq (Martin *et al.*, 2015). Analyses were carried out using default settings (except sequences were set to linear) and the Bonferroni correction P-value cut-off of 0.05. Only breakpoints supported by at least three methods were considered putative (Posada, 2002). The sequences were aligned using ClustalW (Larkin *et al.*, 2007) in MEGA 7 (Kumar *et al.*, 2016) and edited

manually. The alignment was trimmed to give all sequences uniform length. The evolutionary history was inferred by using the Maximum Likelihood statistical method based on the best fitting nucleotide substitution model (General Time Reversible), (Nei and Kumar 2000). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach, and then selecting the topology with superior log likelihood value. All computational analyses were conducted in MEGA7 (Kumar *et al.*, 2016).

RESULTS

RT-PCR-based confirmation of ToMV identity. The presence of ToMV was confirmed by PCR in most of the tomato samples exhibiting typical symptoms of viral diseases, including mosaic and curly leaves, mottling of young leaves, leaf chlorosis, crinkling, flower abscission, necrosis, dieback, no fruit formation, yellow mosaic and bronzing, stunting, vein banding and clearing. The locations of the fields from which the samples were collected are denoted as red dots in Figure 1. ToMV presence in the samples was shown by the detection of a PCR fragment of expected size (672 bp) (Fig. 2). Positive samples were 74% (51/69) of the total field samples analysed. ToMV was detected in all districts surveyed, even in Mbale where no ToMV was detected with ELISA. Overall, Kasese had the highest incidence (92%), followed by Rukungiri (83%), Ntungamo (80%), Luwero (75%), Mbarara and Mpigi (73%), Mbale (67%) and Kamuli (33%). Generally, incidence of ToMV based on PCR was above 60% in most districts, except Kamuli district.

Sequence and phylogenetic analysis. By combining the sequences of five overlapping PCR fragments (Fig. 3), the full length sequence of ToMV-Ug was obtained (Fig. 4).

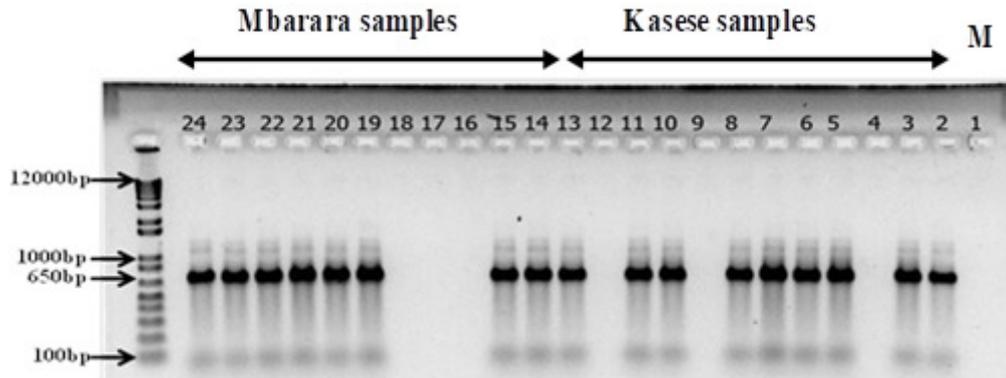


Figure 2. Gel electrophoresis. Image showing results of RT-PCR detection of ToMV in a selected subset of field samples. Lane 1 represents a sample isolated from a healthy plant serving as the negative control (M); Lanes 2-24 show the presence the ToMV-specific RT-PCR products in most reactions that used the field samples as templates.

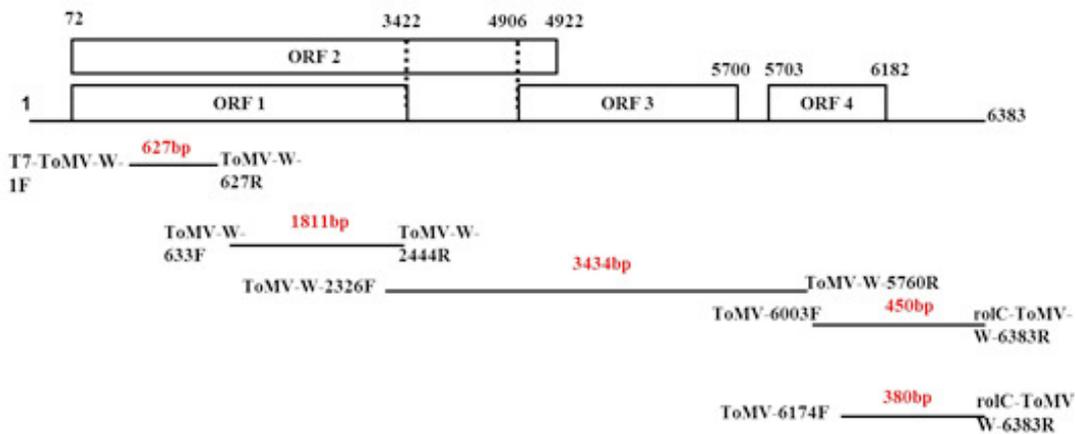


Figure 3. Methodological graphical representation showing how the full length of ToMV- Uganda was assembled.

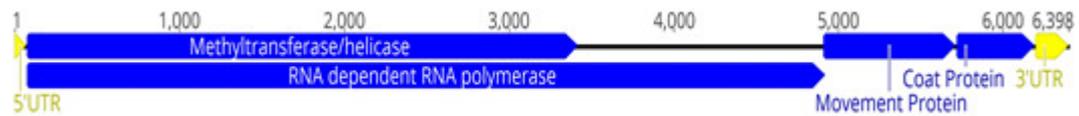


Figure 4. Genetic map showing composition of full length ToMV genome with respective annotated genes. The numbers represent nucleotide bases, while the blue coloured shows putative coding genes. The 5'UTR and 3'UTR are at the ends.

The sequence of T7-ToMV-W-1F used as a forward primer for amplifying the 5' terminal fragment, was derived from the sequences of other known ToMV isolates, which share a 19 nt highly conserved region, at their 5' termini. The 3' UTR sequence, which had only six conserved nucleotides (GGCCA) was obtained with two pairs of nested primers (ToMV-6003F and rolC-ToMV-W-6383R; ToMV-6174F and rolC-ToMV-W-6383R) that amplified two overlapping fragments (450 and 380 bp) respectively. These fragments were sequenced, and the derived sequences were assembled into the full length sequence.

The resultant complete sequence of ToMV-Uganda, after assembling different sequences of overlapping PCR products, was 6383 nt long with four ORFs. The first open reading frame (ORF-1) was 3351 nucleotides long, starting at nucleotide 72 and ending at nucleotide 3422; in effect encoding 1,116 amino acids (aa). The second ORF formed as a result of ORF-1 read-through (AUG) extended translation ended at the nucleotide position 4922. This ORF was 4851 nucleotides long, coding for 1616 aa. The third ORF (ORF-3) covered nucleotide bases 4906 to 5700, with a total of 795 nucleotides and coding for 364 aa. The last frame (ORF-4) was 480 nucleotides long, from nucleotide positions 5703 to 6182, encoding 159 aa. The 5' and 3' ends were 71 and 201 nucleotides long, respectively, with no known amino acids (Fig. 3).

In ORF-1, amino acid percentage identity ranged from 16% - 99%. Like in the 180kDa ORF, amino acid sequences for isolates from tomato were 99% identical while those from other crops ranged from 16% - 91%. In ORF-2, amino acid percentage identity to other tobamoviruses ranges from 64% - 99%. Amino acid sequences for species isolated from tomato were 99% identical while those from other crops such as broad beans, petunia, pepper and crucifers were between 64% and 91% (Table 2). In ORF-3, amino acid identity was in the range of 38% - 100%. Those compared from tomato had their identity range

between 76% and 100% while those from other crops ranged between 38% and 76%. Amino acid sequences for ToMV-Uganda were 100% identical to ToMV 11A from Fukushima, Japan, TMV-K and - L strains from Russia and China, respectively. In ORF-4, amino acid identity ranged between 48% and 100%. Comparison of ToMV-Ug with those isolated from tomato showed a similarity range of 96% - 100% - and 48% - 83% with isolates from other crops (petunia, broad bean, pepper, rib grass and crucifers) (Table 2). Generally, on the basis of comparison of ORFs 1 - 4, ToMV-Uganda was 100% identical to ToMV strains isolated from Australia, China, Japan and the TMV-L strain from China.

Comparison of the full length of the ToMV-Ug isolate with other isolates in the GenBank revealed 99% amino acid similarity with ToMV isolates from Germany, China, Japan and Australia, and the TMV-L strain from China (Table 2). Only 9% identity was realised with tobamoviruses that infect crucifers. Phylogenetic analyses placed the newly sequenced ToMV-Uganda in close relationship with ToMV isolates from China, Japan and Australia. Furthermore, the monophyletic clade of ToMV with 99% bootstrap support shared closer relationship with *Tomato mild mottle virus* another tobamovirus (Fig. 5).

DISCUSSION

Detection of *Tomato mosaic virus* in Uganda. The polymerase chain reaction was thus used in this study to confirm the identity of and characterise ToMV infecting tomato in Uganda. Much higher incidences of the virus were observed with PCR, even in districts where ELISA results were negative. This could be attributed to latent infections or low titres that sometimes occur with viral disease infection (Biosca *et al.*, 2006). In addition, previous reports show that commercially available serology-based kits for most economically important pathogens are not suitable for analysing latent infections as they usually have relatively low sensitivity and do

TABLE 2. Percentage identity of ToMV-Uganda with other sequenced tobamoviruses

Accession no.	Virus name	Source	Amino acid identity (%)					
			Host	180kDa	130kDa	30kDa	17.5kDa	Full length
AF332868	<i>Tomato mosaic virus</i> isolate Queensland	Australia	Tomato	99	99	99	100	99
GQ280794	<i>Tomato mosaic virus</i> strain N5	China	Tomato	99	99	99	96	99
AJ132845	<i>Tomato mosaic virus</i>	China	Tomato	99	99	99	100	99
AJ417701	<i>Tomato mosaic virus</i> Camellia strain	China	Tomato	99	98	99	98	98
AB083196	<i>Tomato mosaic virus</i> L11A-Fukushima	Japan	Tomato	99	99	100	100	99
DQ873692	<i>Tomato mosaic virus</i> strain 1-2	Germany	Tomato	99	99	99	98	99
FN985165	<i>Tomato mosaic virus</i> - China	China	Tomato	99	99	99	100	99
AJ011933	<i>Tobacco mosaic virus</i> strain IM	China	Broad bean	91	90	76	83	85
Z92909	<i>Tobacco mosaic virus</i> K strain	Russia	Tomato	99	99	100	96	93
AF155507	<i>Tobacco mosaic virus</i>	China	Tomato	98	98	91	96	85
EF392659	<i>Tobacco mosaic virus</i> pet-TW	Taiwan	Petunia	91	91	80	83	85
AF165190	<i>Tobacco mosaic virus</i>	China	Tomato	91	91	76	83	49
X02144	<i>Tobacco mosaic virus</i> L strain	China	Tomato	99	99	100	100	99
D63809	<i>Tobacco mosaic virus</i>	Japan	Rakkyo	90	90	76	81	84
DQ355023	<i>Bell pepper mottle tobamovirus</i>	S. Korea	Pepper	87	88	69	81	35
AB276030	<i>Pepper mild mottle virus</i> strain L4BV	Japan	<i>Capsicum</i>	76	18	63	71	19
DQ223770	<i>Ribgrass mosaic virus</i> isolate Impatiens	Germany	Impatiens	76	16	37	48	19
AY318866	Crucifer tobamovirus	Britain	Crucifers	64	16	38	48	9

Tomato mosaic virus in Uganda

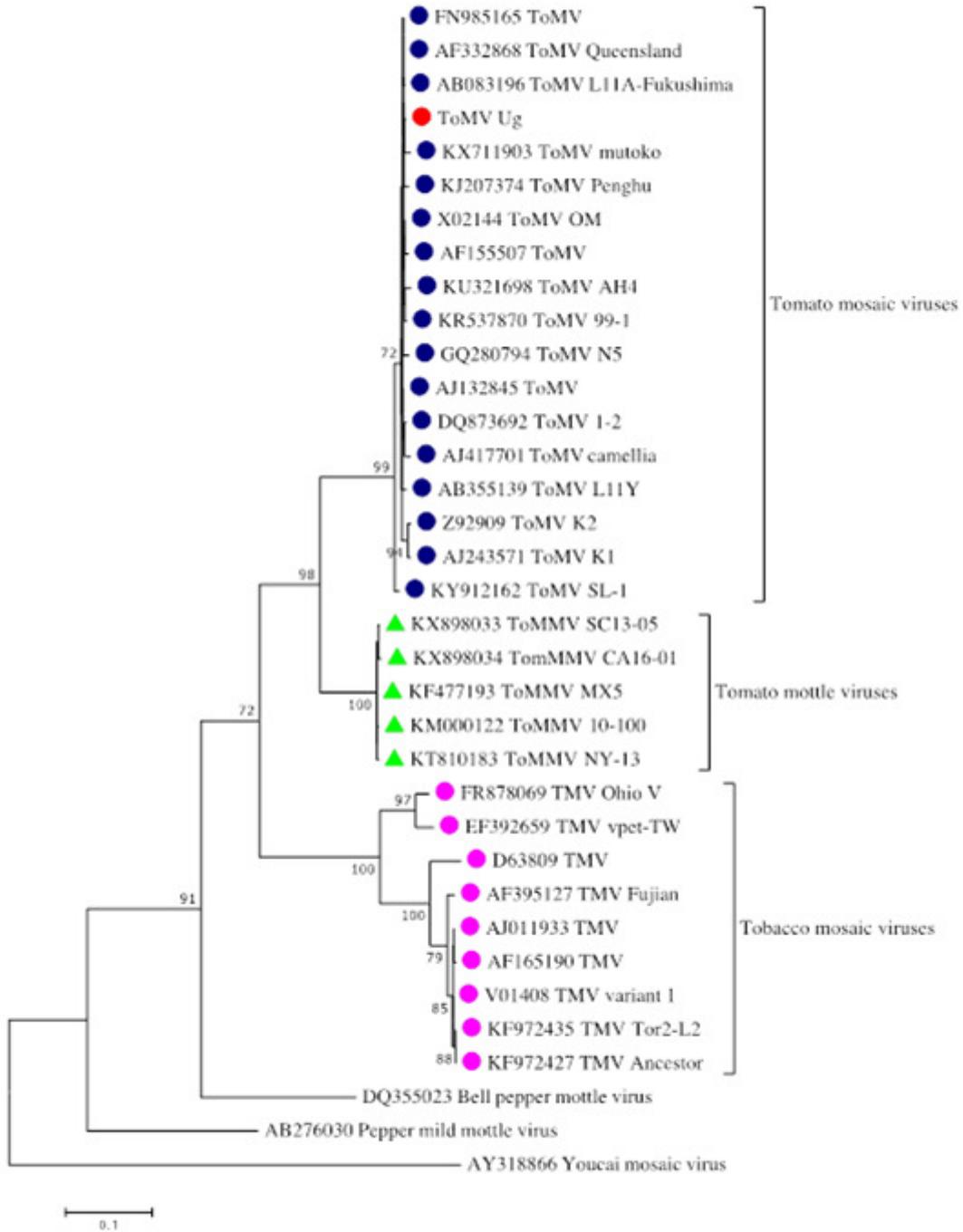


Figure 5. A maximum likelihood dendrogram showing phylogenetic relationship between ToMV-Ug with other representative ToMV isolates as well positioning of ToMV with other tobamoviruses. Only bootstrap values higher than 70% are shown. The scale is in substitutions/site.

not detect low titres of the target pathogen in asymptomatic tissues (López *et al.*, 2009). Successful PCR amplification of ToMV-Uganda with the TobUni1/ToMVspec (Letschert *et al.*, 2002) suggests possible genomic similarities with the China strain whose coat protein gene was used in the primer design. However, differentiation of the genus *Tobamovirus* based on the coat protein gene has been reported as misleading since ToMV uses all its four proteins in infection (Hanssen *et al.*, 2010). Nevertheless, reproduction of a similar result with Letschert *et al.*, (Letschert *et al.*, 2002) indicated that the primer pair (TobUni1/TobUni1/ToMVspec) can be reliably used in subsequent rapid diagnosis of ToMV in Uganda. After all, most biological and serological assays used in identification of tobamovirus species are reportedly time consuming and laborious and at times lead to ambiguous results (Letschert *et al.*, 2002). In summary, considering the high prevalence of ToMV, even in mixed infections (Sseyekwa, 2006), this primer could play a pivotal role in future epidemiological studies on tomato viruses in Uganda.

Nucleotide identity and genome structure of ToMV-Uganda isolate. The complete nucleotide sequence of ToMV-Uganda was also determined. Sequence analysis clearly confirmed ToMV-Ug as a tobamovirus with four major ORFs in the genomic RNA (gRNA). The sequence had a -TAG- stop codon at position 3422 that marked the end of the methyltransferase protein, which was subsequently suppressed to form the RNA dependent RNA polymerase protein. At position 3423 - 3428, it had a -CAATTA- motif that is normally recognised by the GUA anti-codon, which leads to the intermittent production of the 180-kDa protein (Fillmer *et al.*, 2015). The 30kDa movement protein and the 17.5K coat protein were separated by -AT- at positions 5700 - 5703. All these characteristics are common among tobamoviruses (King *et al.*, 2012; Ismael *et al.*, 2012; Fillmer *et al.*, 2015).

The ToMV-Uganda sequence was also 6.3 kilo bases (kb) in length and fell within the 6.3 to 6.6 kb range for most tobamovirus genomes (Chen *et al.*, 2011; Panno *et al.*, 2012; Fillmer *et al.*, 2015; Sui *et al.*, 2017). Moreover, the Ugandan strain of ToMV was positive-sense and single-stranded, which concurred with previous descriptions of the genomic structure for ToMV strains from USA and China (Goellet *et al.*, 1982; Klug, 1999).

Evolutionary phylogenetic relationship of ToMV-Uganda with other known strains.

Phylogenetic comparisons of full genome sequences showed closest relationship of ToMV-Uganda to strains from China (accessions AJ132845, FN985165; 99%), Japan (accession AB083196; 99%) and Germany (accession DQ873692; 99%); while distant relation occurred with the Australian isolate (AF332868; 84%). Aguilar *et al.* (1996) suggested that tobamoviruses, which are 95% or higher in nucleotide identity, should be considered strains of the same virus while those that are 80-95% identical would be closely related viruses. Tobamoviruses that are 60-79% identical would then be considered different virus species. The Ugandan isolate of ToMV can, therefore, be considered a strain closely related to strains of ToMV from Japan, China, Australia and Germany (Fig. 5). This finding could provide insights on studies on migration of the virus species worldwide.

Coat protein amino acid comparison alone revealed 100% homology of ToMV-Ug with the China strains (accessions FN985165 and X02144), Japan strains (AB083196) and Queensland strain (AF332868). This shows that the CP amino acid is highly conserved implying that resistance sources found in one of those countries can serve across all the other regions with similar homology. This is key in disease management and surveillance as resistance sources found in one of those countries can serve across all the other regions with similar homology. In the past, most comparisons were based on coat protein identity (Hanssen *et al.*, 2010). While this may

be merely speculative, close resemblance of ToMV-Uganda with these isolates in the CP region might suggest a likelihood of the Ugandan strain having evolved from those in the Asian, European and Australian continents. If proven, this finding would have implications for future disease surveillance. It should be noted that climatic conditions such as temperature, relative humidity, rainfall and wind speed are considered as the most important factors that favor development and spread of plant diseases in general (Jones, 2016). It is, thus, necessary to put measures of control to avoid any disease introductions through unwarranted sharing of crops from one region to another.

The ToMV-Uganda isolate exhibited low genomic variability and its nucleotide substitutions were highly randomised. For instance, the 180kDa protein had the highest substitutions while the 17.5kDa CP had the lowest. According to Pagán *et al.*, (2010), the rate of evolution among tobamoviruses is generally low at 8×10^{-5} to 1.3×10^{-3} nucleotide substitution/site/year. This is so because most tobamoviruses tend to conserve most of their nucleotides for successful infection (Pfleger and Zeyen, 2008). Randomisation of nucleotide substitution could, thus, be due to functional or geographical adaptation. A comprehensive study, comparing different isolates from different agro ecological zones, is necessary to provide more insight into the structure of ToMV populations infecting tomato and other crops in Uganda.

CONCLUSION

This is the first study on full genome molecular characterisation of a ToMV isolate from Uganda as well as the Eastern and Central Africa regions. The isolate was notably, 98 to 100% similar to ToMV strains from China, Japan and Germany implying it is a strain of the same virus species occurring in Asia and Europe. The study has documented low genomic variability and consequently

nucleotide substitutions. This finding has an implication for stability of the pathogen if a new race emerges. Low variability at genomic level would lead to less resistance. This, therefore, means that other sources of resistances need to be deployed through introducing new variability (crossing, importing new sources of resistances from other areas). The information generated herein will help in further diagnostics and disease management. Further analysis of the evolutionary relationships of *Tomato mosaic virus* strains occurring within and outside Africa will add knowledge on viral migration and evolutionary dispersal of the species.

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