Saudi Journal of Biological Sciences (2016) 23, 237–242

King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Characterization of a *Sorghum mosaic virus* (SrMV) isolate in China

Yu Liang Zhang [a,b], Kayla K. Pennerman [c], Hongxing Wang [a], Guohua Yin [c,*]

[a] *Key Laboratory of Biology and Genetic Resources of Tropical Crops, Ministry of Agriculture, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou, Hainan 5711001, China*
[b] *Sugarcane Research Center, Chinese Academy of Tropical Agricultural Sciences, Haikou, Hainan 5711001, China*
[c] *Department of Plant Biology and Pathology, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901, United States*

Received 3 December 2014; revised 5 February 2015; accepted 7 February 2015
Available online 14 February 2015

KEYWORDS

SrMV;
Sugarcane mosaic disease;
Coat protein

Abstract *Sorghum mosaic virus* (SrMV), a causal agent of the destructive sugarcane mosaic disease, has a global presence. An isolate of SrMV infecting a commercially-grown sugarcane plant was recovered from the Hainan province of China. The virions were visualized by an electron microscope, and the coat proteins (CPs) were sequenced by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and tandem mass spectrometry. Discrepancies between the CP predicted and actual amino acid sequences were noted, which confounded the phylogenetic assignment of the isolate. The apparent variations may have physiological effects on the pathogenicity and virulence of SrMV.

1. Introduction

The main source of sugar production in China is from sugarcane (*Saccharum* spp.) grown in the southern part of the country, especially in the Yunnan and Guangxi provinces (Li et al., 2014; Lin et al., 2012; Luo et al., 2014). ROC22 is the most popular hybrid cultivar, covering more than 85% of the sugarcane planting area (Xu et al., 2008), and it is susceptible to one of the most common and destructive diseases of sugarcane, sugarcane mosaic disease (SMD). This disease occurs in sugarcane-growing countries worldwide and has significant economic impacts. Major yield losses caused by SMD have been reported as high as 21% in the United States (Grisham, 2000) and up to 42% in South Africa (Balarabe et al., 2014). In China, the incidence of mosaic disease ranges from 30% to 100% leading to a 3–50% decrease in cane yield and a 6–14% decrease in sucrose content; this corresponds to an annual loss of over US$30 million (Li et al., 2014). A characteristic symptom of SMD is the development of mosaic-like patterns of green and yellow shades along the leaves due to

* Corresponding author at: Rutgers University, Department of Plant Biology and Pathology, Foran Hall/Cook Campus, 59 Dudley Rd., New Brunswick, NJ 08901, United States. Tel.: +1 848 932 6235.
E-mail addresses: zhangyuliang@itbb.org.cn (Y.L. Zhang), k.pennerm@rutgers.edu (K.K. Pennerman), hxingw@163.com (H. Wang), guohuayin1997@gmail.com (G. Yin).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2015.02.013

chlorosis. Intensity of the mosaic pattern depends on the sugarcane cultivar, growth conditions, age of the leaf and viral strain (Grisham, 2000). Disease control is primarily through the use of resistant cultivars (Wu et al., 2012).

Known natural causal agents of SMD are members of the Potyviridae family: *Sugarcane mosaic virus* (SCMV) and *Sorghum mosaic virus* (SrMV) in the genus *Potyvirus*, and *Sugarcane streak mosaic virus* in *Poacevirus* (Grisham, 2000; Hall et al., 1998). These are transmitted via several aphid species in a non-persistent manner (Grisham, 2000). SrMV is the most common pathogen found in Chinese sugarcanes suffering from SMD (Li et al., 2014), though co-infections of SCMV and SrMV seem to increase virulence and yield loss compared to hosts infected with a single virus (Grisham, 2000; Xu et al., 2008).

The SCMV *Potyvirus* subgroup includes *Johnsongrass mosaic virus*, *Maize dwarf mosaic virus*, *Zea mosaic virus*, *Cocksfoot streak virus* and *Pennisetum mosaic virus* which infect other economically important Poaceous plants and crops (Seifers et al., 2000; Wang et al., 2010). A *Potyvirus* species has a single-stranded positive sense RNA genome which is polyadenylated and covalently linked to a viral protein. The genome is contained within a filamentous capsid consisting of about 2000 identical coat proteins (CPs) (Adams et al., 2005a; Urcuqui-Inchima et al., 2001). Most *Potyvirus* genomes are monopartite, around 10 kb in size and code for a single polyprotein which self-cleaves into ten mature proteins (Adams et al., 2005a).

We characterized a Chinese isolate of SrMV by its virulence, morphology and partial CP sequence. In doing so, we observed multiple discrepancies between the CP predicted and actual amino acid sequences, suggesting variations and modifications of the viral capsid.

2. Materials and methods

2.1. SrMV isolation, purification, biological identification and observation

SrMV isolate HN-lg-1 was detected in *Saccharum* hybrid cultivar ROC22 during a screen of sugarcane plants suffering from severe mosaic symptoms during the 2011 growing season in Hainan province of China (Zhang et al., unpublished). SrMV serological tests were performed as described elsewhere (Mohammadi et al., 2006). Fresh sugarcane leaves (600 g) from plants infected with SrMV HN-lg-1 were ground with extraction buffer (0.2 M sodium acetate, pH 5.0) containing 0.2% of β-mercaptoethanol to reduce oxidation. The purification procedure involved precipitation with polyethylene glycol, centrifugation with a 30% sucrose density gradient and isopycnic centrifugation with $CsCl_2$ as previously described (Hull, 2001). The purified SrMV particles were treated with 2% phosphotungstic acid solution for negative staining prior to observation under a JEM 2100 electron microscope (Johnson and Gregory, 1993).

Differential maize and sugarcane hosts were inoculated with isolate HN-lg-1 for viral species identification and maintenance of hosts infected with only SrMV for later SrMV CP purifications. Isolated HN-lg-1 in a buffer containing 1% K_2HPO_4, 0.1% Na_2SO_3 and small amounts of silicon carbide was added to sterile soil (sterilized at 120 °C, 0.11 MPa, 20 min) in which host seedlings were potted. Symptoms were observed and recorded every day. The control group was inoculated with sterile water.

2.2. RT-PCR

RNA was extracted from ground sugarcane leaf samples frozen in liquid nitrogen using the Plant RNA Kit (Omega Bio-Tek, USA) following the provided instructions. After treatment with DNase I (Takara, Japan), RT-PCR was performed to synthesize cDNA. Reverse transcriptase (M-MLV; TransGen, China), and random and oligo-dT primers were used following the manufacturer's instructions.

SrMV CP gene specific primers (Table 1) were used to detect the virus by PCR. These were constructed based on the SrMV complete genome (strain H, NCBI GenBank Accession No. U57358). Reaction mixtures consisted of 1 μl of cDNA (10–20 ng/μl), 0.5 μl or each primer (10 μM), 2 μl of 10× PCR buffer, 1 μl of 2.5 mM dNTPs, 0.25 units of Ex-Taq DNA polymerase (Takara, Japan) and RNase-free water to a final volume of 20 μl. The amplification reaction parameters were 94 °C for 3 min, 35 cycles at 94 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min, and 72 °C for a final 10 min. PCR products were subjected to gel electrophoresis on a 1% agarose gel at 80 V for 20 min and visualized with Goldview dye (Beijing SBS Genetech, China).

The RT-PCR products were purified using a Gel Extraction Kit (TianGen, China) and cloned into pMD19-T (Takara, Japan) by TA ligation following the manufacturer's instructions. The cloned vector was transformed into *Escherichia coli* DH5α as described elsewhere (Froger and Hall, 2007). Colonies containing the cloned vector were detected using the universal primers M13-47 and RV-M (Table 1) by colony PCR (protocol as described for amplification PCR). Vectors from three colonies were purified using the QIAGEN Plasmid Midi Kit (QIAGEN, Germany). These were separately sequenced using the automated ABI 3130xl Genetic Analyzer (Applied

Table 1 Primers used to detect and sequence SrMV CP.

Primers[a]	Target Genes	Sequences (5′ → 3′)	Reference (Accession No.)
SrMV-CP1	SrMV CP	GCAGGAGGCGGTACAGTAGATGCAG (8389–8413) GTGATGCTGCTGCACTCCAAGAAGG (9351–9375)	This work (based on U57358)
SrMV-CP2	SrMV CP	AGCAGCAAGTAAAGCACGAAAT (8634–8655) CAATAACGGGCTTGAGTGGAA (8999–9019)	This work (based on U57358)
M13-47	–	CGCCAGGGTTTTCCCAGTCACGAC	Universal primer
RV-M	–	GAGCGGATAACAATTTCACACAGG	Universal primer

[a] All primers have an annealing temperature around 50 °C except for the universal primers with an annealing temperature of 55 °C.

Biosystems, USA). Nucleotide sequences were analyzed using the DNAman software (5.2.9 Demo version, Lynnon BioSoft, Canada). The results were compared to CP sequences in NCBI GenBank to identify the virus species. The partial SrMV HN-lg-1 CP gene was uploaded to NCBI GenBank under the Accession No. KC179638.

2.3. SDS–PAGE, western immunoblotting and amino acid sequencing

SDS–PAGE and western immunoblotting were performed according to previously published protocols (Kollerová et al., 2008). Following electrophoresis, viral peptides were removed from the gel and subjected to matrix-assisted laser desorption/ionization tandem time-of-flight (MALDI-TOF/TOF) and tandem mass spectrometry (MS/MS) performed at the Beijing Genomics Institute, China. Three independent samples were sent for analysis. The results were analyzed using the Mascot database search tool (Matrix Science).

3. Results and discussion

3.1. SrMV isolation, purification, biological identification and observation

Sugarcane hybrid cultivar ROC22, infected with SrMV exhibited serious mosaic symptoms in the field, including dwarfing (Fig. 1A). ID-ELISA tests confirmed SrMV as a causal agent in 13 out of 20 field samples (Table 2). SrMV isolated from Sample No. HN-lg-1 was selected for further characterization. After $CsCl_2$ density-gradient centrifugation, a single light-scattering zone was observed in the upper middle part of the tube; its position coincided with a peak UV absorbance at 260 nm. The standard sedimentation constant was 160–175 S, and the buoyant density was 1.285–1.342 g/ml (Fig. 1B).

Table 2 ID-ELISA determination of virus isolate sample (A_{405}).

Test	OD_{405}	Results
Blank	0.084 ± 0.003	–
Control	0.113 ± 0.004	–
SrMV Sample	0.402 ± 0.021	+

Purified particles were inoculated in differential host maize and sugarcane cultivars. The maize leaves showed the systemic streak and mottled symptoms seven days post inoculation (Fig. 1D) while sugarcane showed atypical symptoms (data not shown) even after 30 days. Electron microscopic examinations of purified virions revealed a high concentration of long rod-shaped virus particles with sizes 800 ± 50 nm × 13 ± 2 nm (Fig. 1E). This was consistent with previous observations of SrMV and other members of *Potyvirus* (Edwardson et al., 1968; Hema et al., 1999; Lesemann et al., 1992; Pirone and Anazalone, 1966). SDS–PAGE and western immunoblotting indicated that the SrMV CP consisted of multiple copies of a peptide with a molecular weight of 35.68 kDa, which is close to the predicted size of about 36 kDa (Fig. 2).

3.2. SrMV coat protein nucleotide sequence

The 913 bp cDNA product from RT-PCR was found to have a nucleotide sequence nearly identical to other SrMV CP sequences, further confirming the species identification. *Potyvirus* species can be further differentiated into strains by their CP sequences (Shukla and Ward, 1988; Viswanathan

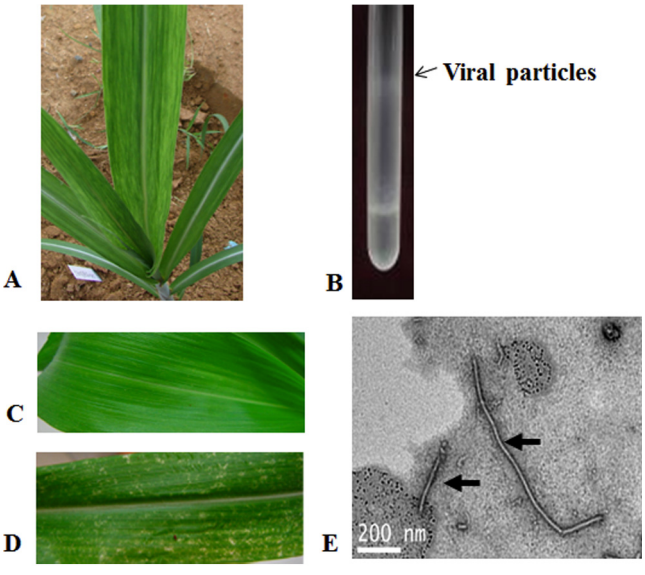

Figure 1 SrMV particles and mosaic symptoms in hosts. (A) Typical symptoms of SMD. (B) Virus particles from sugarcane hybrid cultivar ROC22 were purified by $CsCl_2$ density-gradient centrifugation. The arrow indicates the virus particle layer. SrMV was inoculated into (C) a control and (D) a differential maize host. Images depict symptoms seven days post inoculation. (E) Virus particles were observed under a JEM 2100 electron microscope. Arrows point to the virions, and the scale bar indicates 200 nm.

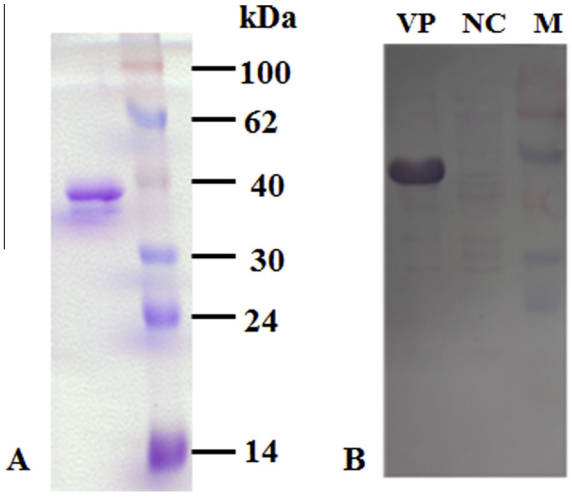

Figure 2 SDS–PAGE and western immunoblotting of SrMV. (A) The polyprotein of SrMV was separated by SDS–PAGE, suggesting that the molecular weight is ~36 kDa. (B) Western immunoblotting verified the presence of virus particles (VP). The negative control and protein marker are labeled as NC and M, respectively.

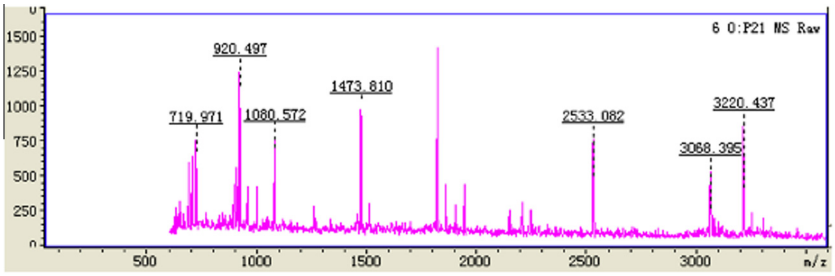

Figure 3 MS/MS sequencing results.

```
cDNA   GAGCAGCCGCAACAGATGCAACAGCACAAGCACAGCGTGATGCAGCAGCGAAAGCTCAACGAGACGCAGAAGCGGCAGAGAAGCAGAGACAAGATGCTGC  100
Pred.    A  A  A  T  D  A  T  A  Q  A  Q  R  D  A  A  A  K  A  Q  R  D  A  E  A  A  E  K  Q  R  Q  D  A  A  100
Ident.   A  A  A  A  E  A  T  A  Q  A  Q  R  D  A  A  A  K  V  Q  R  D  A  D  A  K  K  K  A  D  D  E  A  A
                    *  *

cDNA   AGCTAAGAAGAAAGCTGATGATGATGCGAAAGCTAAAGCTGACGCGGATGCCAAAGCAAAATCAGATGCTGACGCGAAAAAGAAAGCAGACGATGAAGCA  200
Pred.  A  K  K  K  A  D  D  D  A  K  A  K  A  D  A  D  A  K  A  K  S  D  A  D  A  K  K  K  A  D  D  E  A  200
Ident. E  K  Q  R  Q  D  A  A  G  K  K  K  A  D  D  D  A  K  A  K  A  D  A  D  A  K  A  K  S  D  A  D  A
             *  *  *     *  *  *

cDNA   GCAAGTAAAGCACAAAATCAAAAAGATAAGGATGTGGATGCCGGCACGTCCGGCACAGTGGCAGTGCCTAAACTCAAAGCAATGTCCAAGAAAATGAAGC  300
Pred.  A  S  K  A  Q  N  Q  K  D  K  D  V  D  A  G  T  S  G  T  V  A  V  P  K  L  K  A  M  S  K  K  M  K  L  300
Ident. A  N  K  V  Q  N  Q  K  D  K  D  V  D  V  G  T  S  G  T  V  A  V  P  K  L  K  A  M  S  K  K  M  K  L

cDNA   TACCACAAGCAAAAGGGAAAAACATTTTACACTTGGATTTTCTTTTAGGATATAAGCCACAACAACAAGACATTTCAAACACCAGAGCTACACGGGATGA  400
Pred.    P  Q  A  K  G  K  N  I  L  H  L  D  F  L  L  G  Y  K  P  Q  Q  Q  D  I  S  N  T  R  A  T  R  D  E  400
Ident.   P  Q  A  K  G  K  N  I  L  H  L  D  F  L  L  G  Y  K  P  Q  Q  Q  D  I  S  N  T  R  A  T  R  D  E

cDNA   GTTCGATAGGTGGTACGATGCATTGCAGAAAGAATATGAACTAGATGACACGCAGATGACAGTGGTCGCAAGCGGACTCATGGTTTGGGTCATAGAAAAC  500
Pred.  F  D  R  W  Y  D  A  L  Q  K  E  Y  E  L  D  D  T  Q  M  T  V  V  A  S  G  L  M  V  W  V  I  E  N   500
Ident. F  D  R  W  Y  E  A  L  Q  R  E  Y  E  L  D  D  T  Q  M  T  V  V  A  S  G  L  M  V  W  A  I  E  N

cDNA   GGATGCTCACCTAATATTAATGGTGTTTGGACAATGATGGATGGAGATGAGCAAAGGAAATTTCCACTCAAGCCCGTTATTGAGTATGCATCTCCAACAT  600
Pred.  G  C  S  P  N  I  N  G  V  W  T  M  M  D  G  D  E  Q  R  K  F  P  L  K  P  V  I  E  Y  A  S  P  T  F  600
Ident. G  C  S  P  N  I  N  G  V  W  T  M  M  D  G  D  E  Q  R  K  F  P  L  K  P  V  I  E  Y  A  S  P  T  F

cDNA   TTAGACAGATAATGCACCACTTTAGTGATGCAGCTGAAGCGTATATAGAGTATAGAAACTCGACAGAGCGTTACATGCCAAGATACGGACTTCAGCGAAA  700
Pred.    R  Q  I  M  H  H  F  S  D  A  A  E  A  Y  I  E  Y  R  N  S  T  E  R  Y  M  P  R  Y  G  L  Q  R  N  700
Ident.   R  Q  I  M  H  H  F  S  D  A  A  E  A  Y  I  E  Y  R  N  S  T  E  R  Y  M  P  R  Y  G  L
```

Notes: cDNA: cDNA sequence of SrMV CP gene from HN-lg-1 isolates.
Pred.: Amino acid sequence was predicted based on cDNA sequence.
Ident.: Amino acid sequence was identified via MADLI-TOF/TOF. Sequence was highlighted with red color indicated identified polypeptides. *: indicated the conformed unusual viral-preference codon.

Figure 4 Predicted and actual amino acid sequence comparison of SrMV coat protein cDNA. Based on the cDNA sequence of the SrMV coat protein gene from HN-lg-1 clones, the predicted (Pred.) and actual (Ident.) amino acid sequences identified via MALDI-TOF/TOF were aligned. Portions highlighted in red were polypeptides verified by MS/MS. Asterisks (*) indicate a mismatch between the predicted and actual sequences identified by both MALDI-TOF/TOF and MS/MS.

et al., 2009). Typically, amino acid identities of different CP strains are 90–99% identical (Adams et al., 2005b; Shukla and Ward, 1988). A BLAST search of the CP predicted amino acid sequence of the HN-lg-1 isolate (Accession No. AGU12592) revealed that the isolate shared 100% identity with one other Chinese SrMV isolate from the Yunnan province (isolate YN11, Accession No. CAX36847). Up to 99% identity was achieved with several other SrMV isolates found in southern China (Wang et al., 2010), including those found by our group along with HN-lg-1. However, as noted in Section 3.3, the predicted and actual amino acid sequences of the HN-lg-1 isolate were considerably different, with only an 87% identity to each other. Thus, we performed a second BLAST search with the actual amino acid sequence. This resulted in a different list of SrMV CP sequences with 100% query coverage and identities ranging from 78% to 98% with one sequence scoring 100% identity (strain FJ10, Accession No. ABP68394). At this time, we are not able to definitively identify the strain in which HN-lg-1 belongs.

3.3. Discrepancies between SrMV coat protein predicted and actual amino acid sequences

The majority of the SrMV HN-lg-1 CP amino acid sequence was revealed by MALDI-TOF/TOF. A third of it was verified by MS/MS analysis (Fig. 3). A comparison of the CP predicted and actual amino acid sequences revealed multiple discrepancies between the two (Fig. 4). For residue identities determined by both MALDI-TOF/TOF and MS/MS, eight residues were not as expected. Furthermore, these mismatches

were not consistent. For example, predicted lysine residues were found to be glutamine, arginine or were as predicted. We do not know the reasons for these variations. However, we suspect that the apparent variations in the SrMV CP were mostly the result of post-translational modifications formed either *in vivo* or during sample processing.

We also considered codon bias to be a possible cause. Lower concentrations of certain tRNA molecules in the host may result in amino acid substitutions during translation. However, the apparent variations were consistent and only affected select amino acids, suggesting a more controlled mechanism such as post-translational modification. Additionally, there is a lack of evidence demonstrating that plant viral codon usage is adapted to that of the host; it instead seems to stem from mutational biases, possibly caused by viral genomic structure (Adams and Antoniw, 2004; Cardinale et al., 2013).

The physiological effects of these modifications and/or variations are not known. The CP plays many roles in cell-to-cell and systemic movement, encapsulation of the genome, association with aphid vectors and regulation of replication (Urcuqui-Inchima et al., 2001). Previous studies have shown that several key residues and regions of *Potyvirus* CPs are vital for proper functioning. When the N-terminal DAG motif or proximal residues are varied, aphid transmission of *Tobacco vein mottling virus* and *Tobacco etch virus* is no longer possible or significantly impaired (Atreya et al., 1991, 1995; López-Moya et al., 1999). Select mutations in the core of the *Plum pox virus* CP rendered the virus unable to move systemically throughout a host or assemble new virions (Varrelmann and Maiss, 2000). While we did not conduct any mutational studies, it is possible that the modified or varied residues play vital roles in SrMV pathogenicity and virulence. Moreover, we did not inspect the full CP. There may be other modifications or variations which have important implications. Future studies will have to further investigate the causes and effects of the discrepancies between the predicted and actual amino acid sequences.

Author contributions

G. Yin designed the experiments. Y.L. Zhang and H. Wang performed the experiments. All authors co-prepared the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

The authors are grateful to Professor Y.W. Wei (Crop Genetic Improvement and Biotechnology Lab, Guangxi Academy of Agricultural Sciences, Guangxi, China) for technical assistance and SrMV strain identification. Funding for this work was supplied by the National Nonprofit Institute Research Grant of CATAS-ITBB, China (ITBB110303), and the Special Fund for the Industrial Technology System Construction of Modern Agriculture (No. nycytx-24). The funding sources had no role in experimental design, data collection and interpretation, decision to publish or preparation of the manuscript.

References

Adams, M.J., Antoniw, J.F., 2004. Codon usage bias amongst plant viruses. Arch. Virol. 149, 113–135.

Adams, M.J., Antoniw, J.F., Beaudoin, F., 2005a. Overview and analysis of the polyprotein cleavage sites in the family Potyviridae. Mol. Plant Pathol. 6, 471–487.

Adams, M.J., Antoniw, J.F., Fauquet, C.M., 2005b. Molecular criteria for genus and species discrimination within the family Potyviridae. Arch. Virol. 150, 459–479.

Atreya, P.L., Atreya, C.D., Pirone, T.P., 1991. Amino acid substitutions in the coat protein result in loss of insect transmissibility of a plant virus. Proc. Natl. Acad. Sci. U.S.A. 88, 7887–7891.

Atreya, P.L., Lopez-Moya, J.J., Chu, M., Atreya, C.D., Pirone, T.P., 1995. Mutational analysis of the coat protein N-terminal amino acids involved in *Potyvirus* transmission by aphids. J. Gen. Virol. 76, 265–270.

Balarabe, D.D., Adama, Y., UU, K.A., Aisha, Z.M., 2014. Identification of virus isolates inducing mosaic of sugarcane in Makarfi Local Government Area of Kaduna State, Nigeria. Afr. J. Biotechnol. 13, 1351–1357.

Cardinale, D.J., DeRosa, K., Duffy, S., 2013. Base composition and translational selection are insufficient to explain codon usage bias in plant viruses. Viruses 5, 162–181.

Edwardson, J.R., Purcifull, D.E., Christie, R.G., 1968. Structure of cytoplasmic inclusions in plants infected with rod-shaped viruses. Virology 34, 250–263.

Froger, A., Hall, J.E., 2007. Transformation of plasmid DNA into *E. coli* using the heat shock method. J. Vis. Exp. 253.

Grisham, M.P., 2000. Mosaic. In: Rott, P., Bailey, R.A., Comstock, J.C., Croft, B.J., Saumtally, A.S. (Eds.), A Guide to Sugarcane Diseases. CIRAD: ISSCT, Montpellier, France, pp. 249–254.

Hall, J.S., Adams, B., Parsons, T.J., French, R., Lane, L.C., Jensen, S.G., 1998. Molecular cloning, sequencing, and phylogenetic relationships of a new *Potyvirus*: *Sugarcane streak mosaic virus*, and a reevaluation of the classification of the Potyviridae. Mol. Phylogenet. Evol. 10, 323–332.

Hema, M., Joseph, J., Gopinath, K., Sreenivasulu, P., Savithri, H.S., 1999. Molecular characterization and interviral relationships of a flexuous filamentous virus causing mosaic disease of sugarcane (*Saccharum officinarum* L.) in India. Arch. Virol. 144, 479–490.

Hull, R., 2001. Methods for assay, detection and diagnosis. In: Matthews' Plant Virology. Academic Press, New York, pp. 627–674.

Johnson, R.P., Gregory, D.W., 1993. Viruses accumulate spontaneously near droplet surfaces: a method to concentrate viruses for electron microscopy. J. Microsc. 171, 125–136.

Kollerová, E., Glasa, M., Šubr, Z.W., 2008. Western blotting analysis of the *Plum pox virus* capsid protein. J. Plant Pathol. 90, 19–22.

Lesemann, D.-E., Shukla, D.D., Tosic, M., Huth, W., 1992. Differentiation of the four viruses of *Sugarcane mosaic virus* subgroup based on cytopathology. Arch. Virol. Suppl. 5, 353–361.

Li, W.-F., Shen, K., Huang, Y.-K., Wang, X.-Y., Zhang, R.-Y., Shan, H.-L., Yin, J., Luo, Z.-M., 2014. Evaluation of resistance to *Sorghum mosaic virus* (SrMV) in 49 new elite sugarcane varieties/clones in China. Crop Prot. 60, 62–65.

Lin, L., Li, Z., Hu, C., Zhang, X., Chang, S., Yang, L., Li, Y., An, Q., 2012. Plant growth-promoting nitrogen-fixing enterobacteria are in association with sugarcane plants growing in Guangxi, China. Microbes Environ. 27, 391–398.

López-Moya, J.J., Wang, R.Y., Pirone, T.P., 1999. Context of the coat protein DAG motif affects *Potyvirus* transmissibility by aphids. J. Gen. Virol. 80, 3281–3288.

Luo, J., Pan, Y.B., Xu, L., Zhang, H., Yuan, Z., Deng, Z., Chen, R., Que, Y., 2014. Cultivar evaluation and essential test locations identification for sugarcane breeding in China. Sci. World J. 2014, 302753.

Mohammadi, M.R., Koohi-Habibi, M., Mosahebi, G., Hajieghrari, B., 2006. Identification of prevalent *Potyvirus* on maize and johnsongrass in corn fields of Tehran province of Iran and a study on some of its properties. Commun. Agric. Appl. Biol. Sci. 71, 1311–1319.

Pirone, T.P., Anazalone, L., 1966. Purification and electron microscopy of *Sugarcane mosaic virus*. Phytopathol. Notes 56, 371–372.

Seifers, D.L., Salomon, R., Marie-Jeanne, V., Alliot, B., Signoret, P., Haber, S., Loboda, A., Ens, W., She, Y.M., Standing, K.G., 2000. Characterization of a novel *Potyvirus* isolated from maize in Israel. Phytopathology 90, 505–513.

Shukla, D.D., Ward, C.W., 1988. Amino acid sequence homology of coat proteins as a basis for identification and classification of the *Potyvirus* group. J. Gen. Virol. 69, 2703–2710.

Urcuqui-Inchima, S., Haenni, A.L., Bernardi, F., 2001. *Potyvirus* proteins: a wealth of functions. Virus Res. 74, 157–175.

Varrelmann, M., Maiss, E., 2000. Mutations in the coat protein gene of *Plum pox virus* suppress particle assembly, heterologous encapsidation and complementation in transgenic plants of *Nicotiana benthamiana*. J. Gen. Virol. 81, 567–576.

Viswanathan, R., Karuppaiah, R., Balamuralikrishnan, M., 2009. Identification of new variants of SCMV causing sugarcane mosaic in India and assessing their genetic diversity in relation to SCMV type strains. Virus Genes 39, 375–386.

Wang, J.-G., Zheng, H.-Y., Chen, H.-R., Adams, M.J., Chen, J.-P., 2010. Molecular diversities of *Sugarcane mosaic virus* and *Sorghum mosaic virus* isolates from Yunnan province, China. J. Phytopathol. 158, 427–432.

Wu, L., Zu, X., Wang, S., Chen, Y., 2012. *Sugarcane mosaic virus* – long history but still a threat to industry. Crop Prot. 42, 74–78.

Xu, D.-L., Park, J.-W., Mirkov, T.E., Zhou, G.-H., 2008. Viruses causing mosaic disease in sugarcane and their genetic diversity in southern China. Arch. Virol. 153, 1031–1039.