

Metagenomic analysis of viruses associated to maize lethal necrosis

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Acknowledgements

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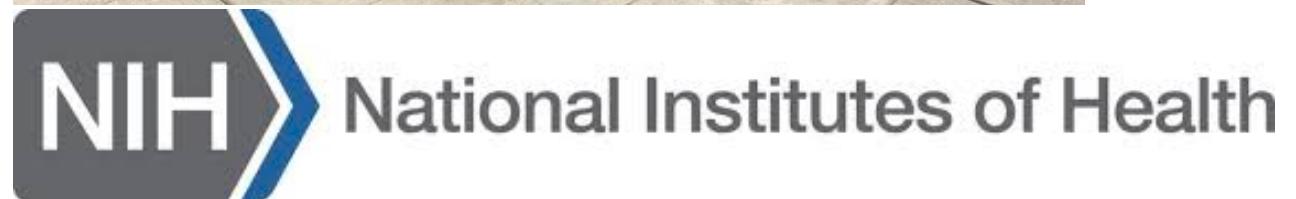
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**USDA Foreign
Agriculture Service**



SUMMARY

Maize lethal necrosis was first described in Kansas/Nebraska in 1978

Causal agents are

Maize chlorotic mottle virus

Sugarcane mosaic virus

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Nebraska



Kenya

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Maize lethal necrosis threatens food security in
sub-Saharan Africa

Diagnosis based on molecular approaches was erratic

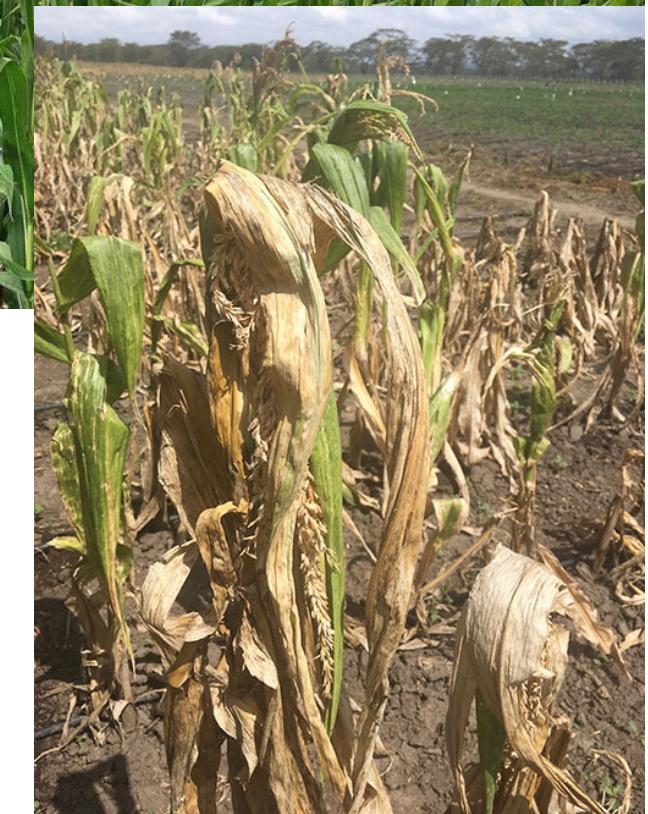
Antibodies and RT-PCR fail to detect

Sugarcane mosaic virus

Viruses causing Maize lethal necrosis?



Nebraska



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Viruses causing Maize lethal necrosis?

Metagenomic analysis (RNA sequencing)

A combination of two to eight viruses

At least three genetic variants of Sugarcane mosaic virus

Identification of genetic variation in Potyviruses



Nebraska



Kenya

Maize lethal necrosis is caused by a synergistic viral co-infection: Potyvirus + Maize chlorotic mottle virus



Maize chlorotic mottle virus

Sugarcane mosaic virus

Co-infection= **Maize lethal necrosis**

Erratic detection of Sugarcane mosaic virus

Sugarcane mosaic virus genome organization



ELISA test failed to detect SCMV
Anti-coat protein antibodies for Ohio isolate

RT-PCR is inconsistent
Primers to amplify the coat protein
Ohio isolate used as reference

RESEARCH

Open Access

Metagenomic analysis of viruses associated with maize lethal necrosis in Kenya



CrossMark

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Symptom variation in maize and other plants

Asymptomatic



Sorghum

Napier grass

Maize

Symptomatic



Maize

Maize

Maize

Model

Maize lethal necrosis is caused by novel viruses

Viruses associated to maize lethal necrosis are genetically different from known isolates

Prediction

Symptomatic plants are infected by viruses other than Maize chlorotic mottle virus and Sugarcane mosaic virus

Maize chlorotic mottle virus and Sugarcane mosaic virus from Kenya are genetically different to US isolates

Experiment

RNA sequencing

De-novo assembly

Alignment to plant virus database

Characterization / Identification of genetic

Experimental approach

Sample collection

16 maize growing
counties in Kenya



3 plant species:
maize, sorghum and
napier grass



Symptomatic and
asymptomatic plants

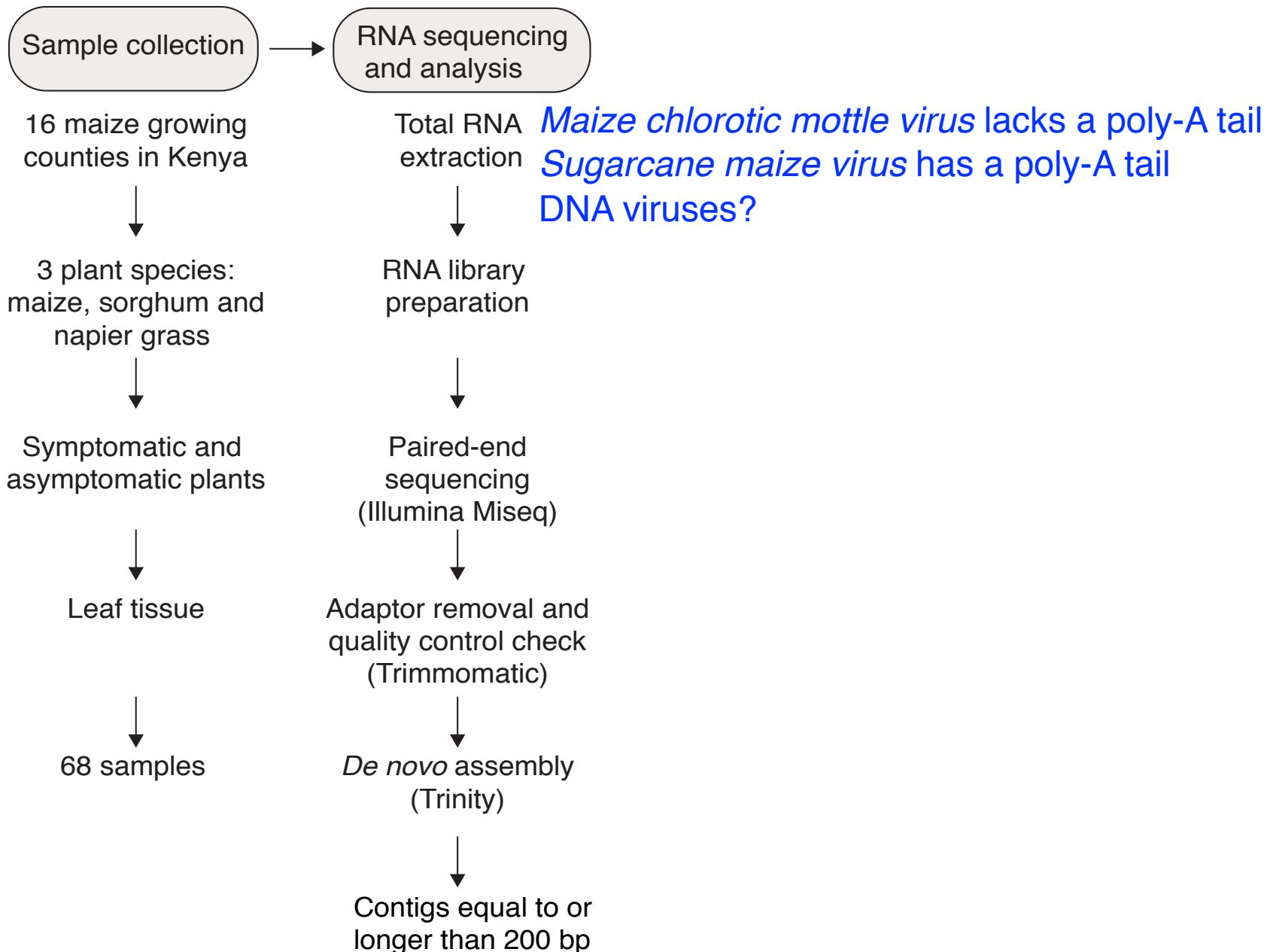


Leaf tissue

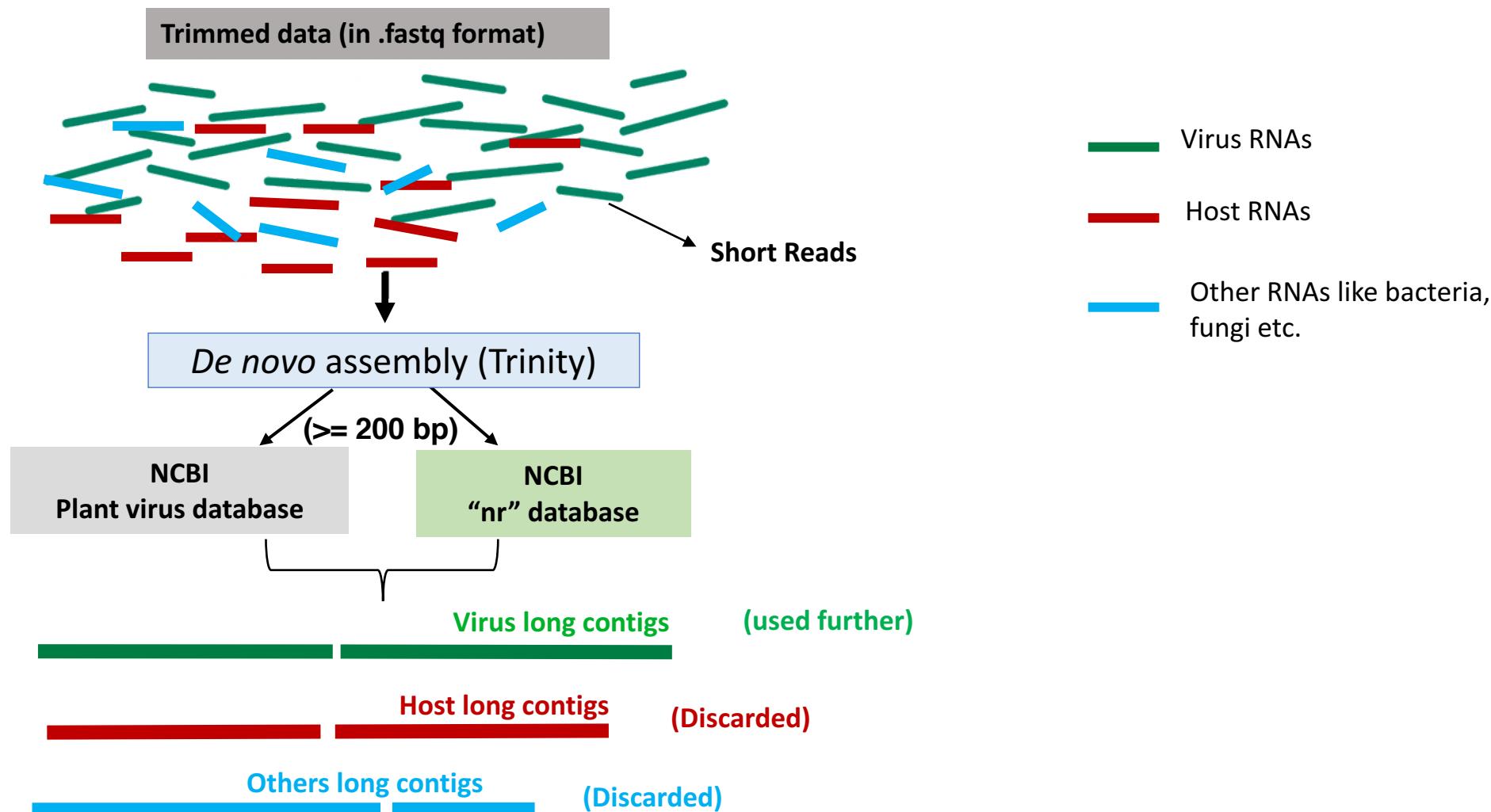


68 samples

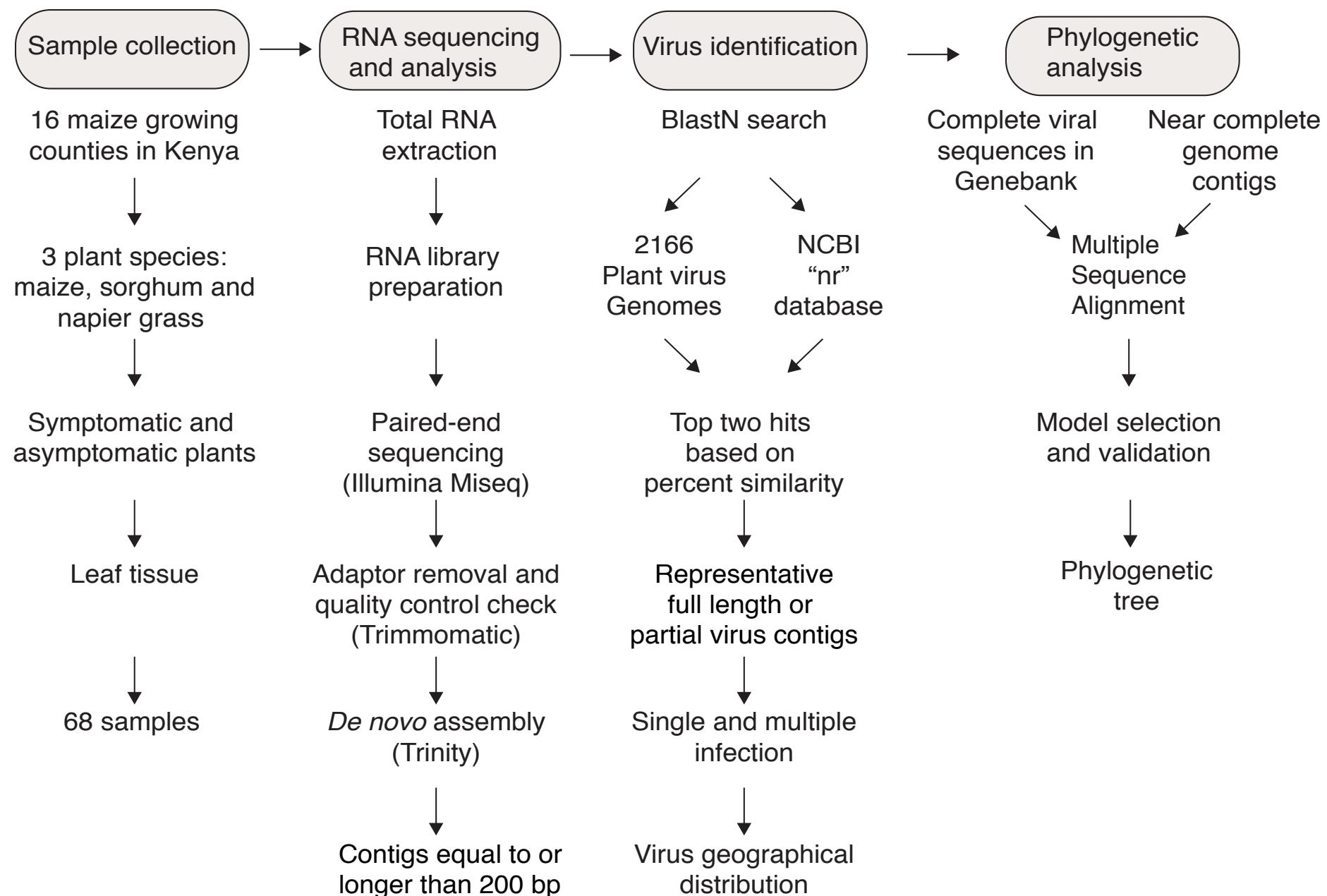
Experimental approach



De-novo assembly of RNA transcripts

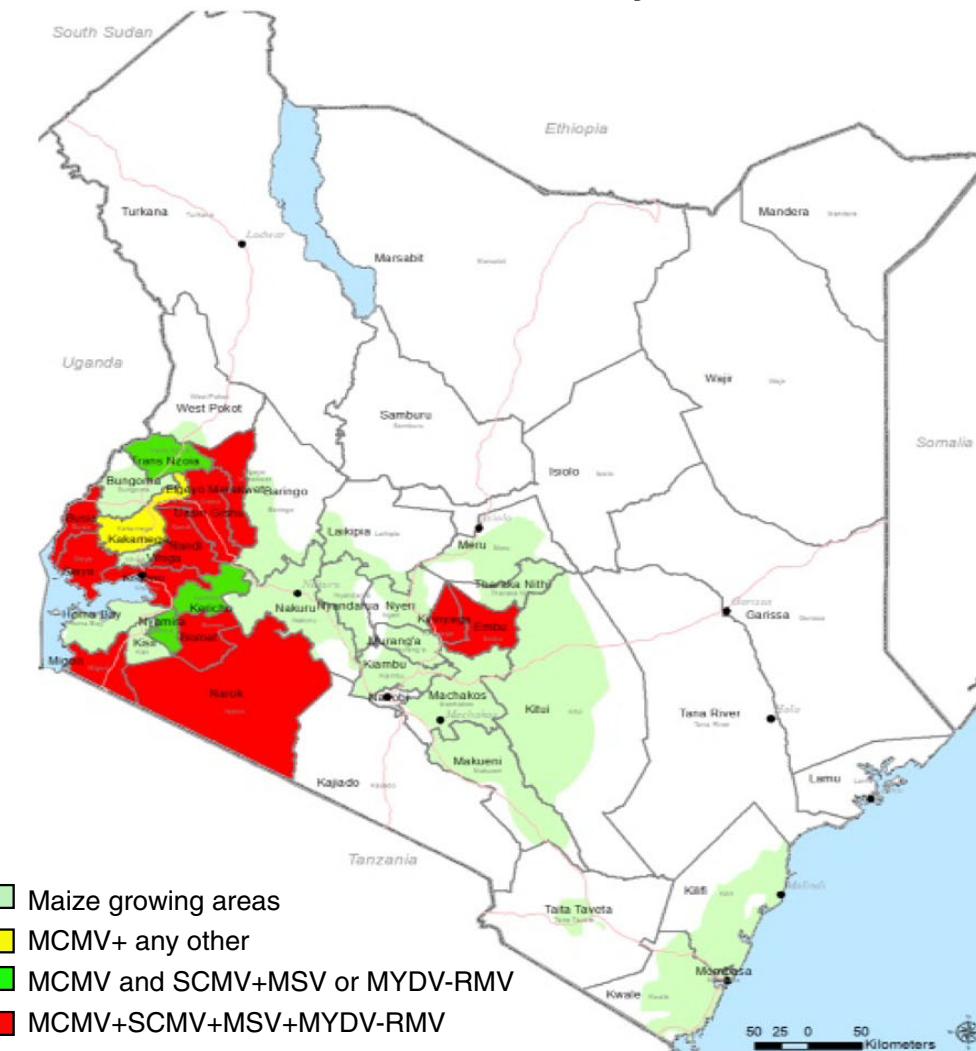


Experimental approach



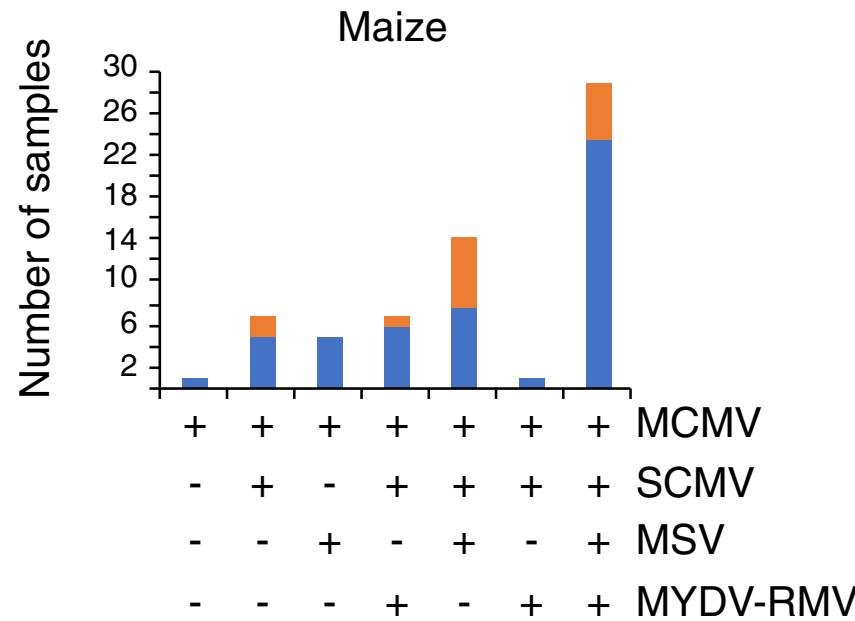
Geographic distribution of maize viruses in Kenya

b Distribution of maize viruses in Kenya



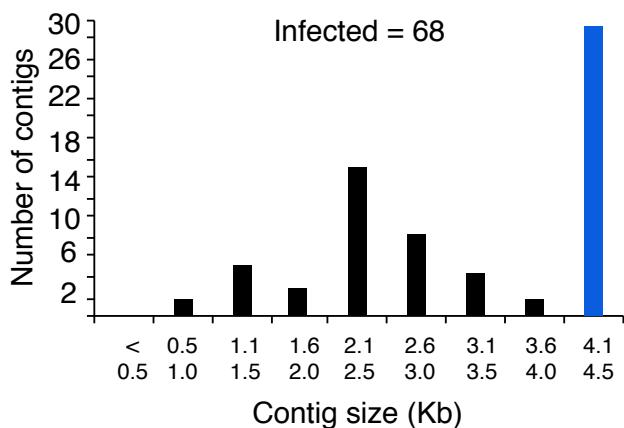
Abundance of maize-infecting viruses in Kenya

C Frequency of mixed infections

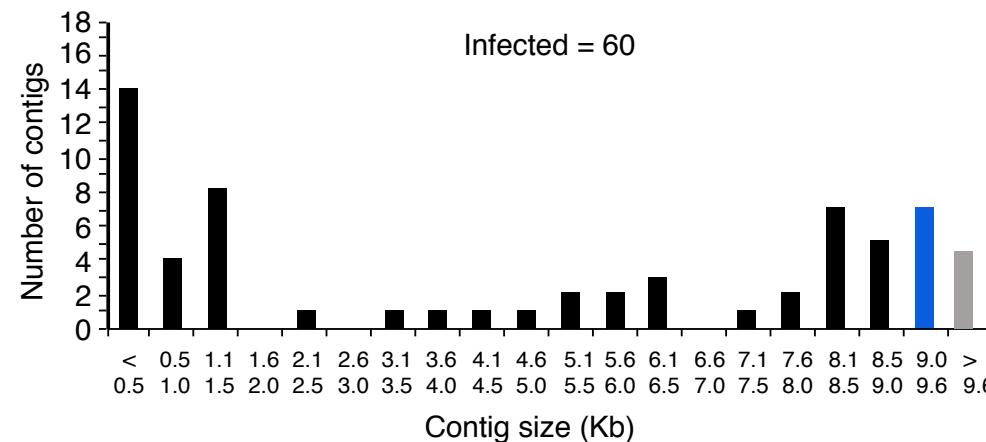


Size and frequency of *de novo* assembled virus contigs

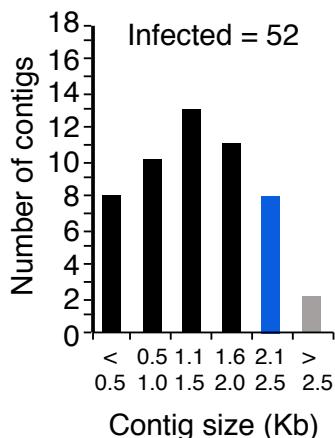
A Maize chlorotic mottle virus



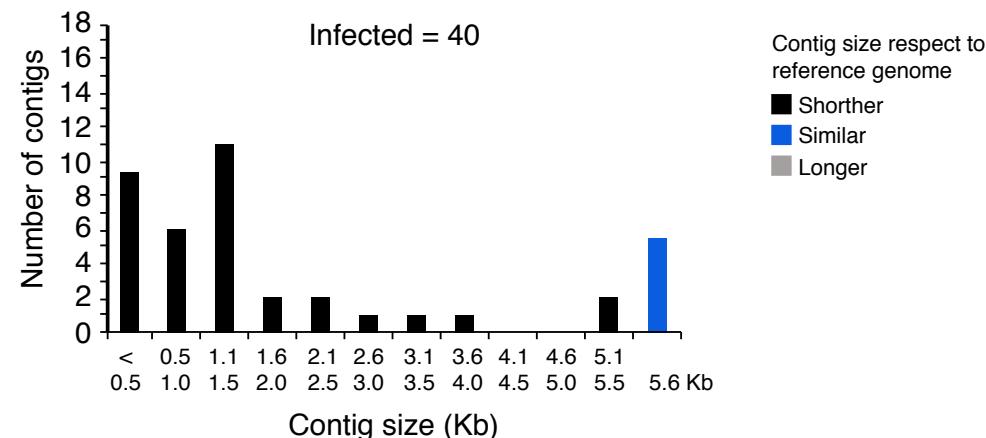
B Sugarcane mosaic virus



C Maize streak virus



D Maize yellow dwarf virus-RMV

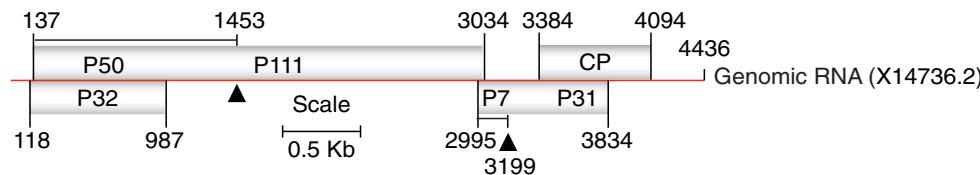


d Other viruses

| Virus | Reference Accession | Length (bp) | Contigs Number | Length (bp) | Similarity (%) | E-value | n | Sample number |
|------------------------------------|---------------------|-------------|----------------|-------------|----------------|----------|----|---|
| Hubei Poty-like virus 1* | NC_032912.1 | 9356 | 41 | 203 to 9323 | 75.2 to 87.3 | <3.6E-30 | 19 | 6,14,17,20,23,24,25, 27,30,32,34,35,40,41, 48,66,67,68,72 |
| Barley virus G isolate Gimje** | NC_029906.1 | 5620 | 26 | 242 to 5494 | 80.0 to 87.6 | <4.9E-65 | 11 | 18, 29, 30, 32, 33, 37, 40, 41, 42, 44, 45 |
| Scallion mosaic virus* | NC_003399.1 | 9324 | 9 | 260 to 961 | 71.6 to 90.0 | <1.8E-09 | 7 | 14, 20, 23, 28, 46, 47,68 |
| Jhonson grass mosaic virus* | NC_003606.1 | 9779 | 6 | 244 to 1630 | 75.0 to 84.0 | <4.4E-20 | 5 | 17, 18, 29, 30, 46 |
| Iranian johnsongrass mosaic virus* | NC_018833.1 | 9544 | 2 | 244 to 332 | 75.0 to 80.0 | <4.4E-25 | 2 | 26, 36 |

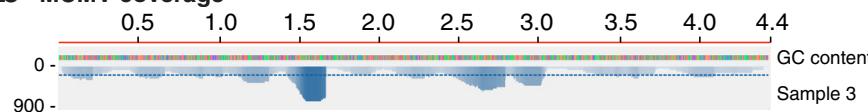
Alignment of *de novo*-assembled contigs to Maize chlorotic mottle virus

a MCMV genome and contig alignment

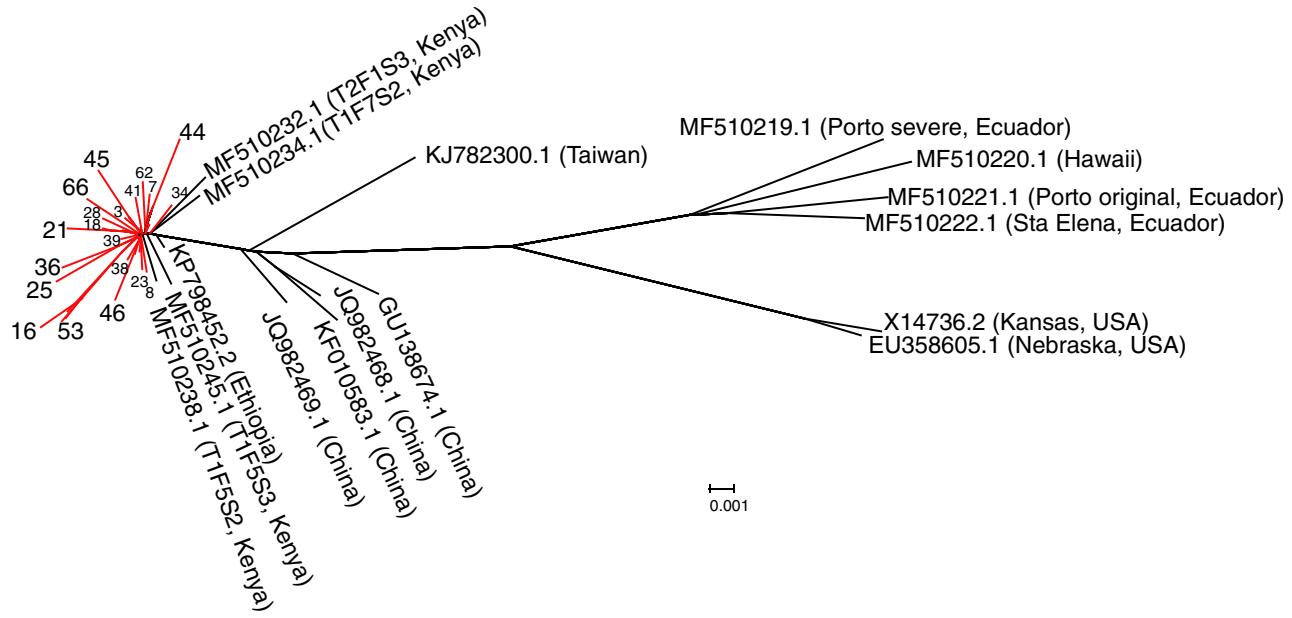


| | Alignment size (Kb) | Contig size (Kb) | Similarity (%) | Sample (symptoms) | County | Contig polarity |
|--|---------------------|------------------|----------------|-------------------|-----------|-----------------|
| | 4.4 | 4.4 | 96.7 | 3 (S) | Bomet | — (+) |
| | 3.0 | 3.0 | 96.9 | 4 (S) | Bomet | — (-) |
| | 2.0 | 2.0 | 96.5 | 5 (S) | Bomet | — (+) |
| | 3.6 | 3.7 | 96.9 | 6 (S) | Bomet | — (-) |
| | 4.4 | 4.5 | 96.7 | 7 (A) | Kirinyaga | — (+) |
| | 4.4 | 4.7 | 96.7 | * 8 (A) | Embu | — (-) |
| | 1.5 | 1.5 | 96.2 | 9 (A) | Kirinyaga | — (+) |
| | 2.2 | 2.2 | 96.4 | 11 (S) | Transzoia | — (-) |
| | 3.6 | 3.7 | 96.5 | 12 (S) | Transzoia | — (-) |
| | 4.2 | 4.2 | 96.6 | 13 (S) | Bomet | — (+) |
| | 4.4 | 4.4 | 96.7 | 14 (A) | Transzoia | — (-) |
| | 4.4 | 4.4 | 96.7 | 15 (A) | Busia | — (+) |
| | 4.4 | 4.4 | 96.6 | 16 (S) | Busia | — (-) |
| | 2.1 | 2.1 | 96.7 | 17 (A) | Busia | — (+) |
| | 4.3 | 4.3 | 96.6 | 18 (S) | Busia | — (-) |
| | 3.4 | 3.4 | 96.5 | 20 (A) | Busia | — (+) |
| | 4.3 | 4.5 | 96.6 | 21 (S) | Bomet | — (-) |
| | 2.8 | 2.8 | 96.6 | 22 (S) | Narok | — (+) |
| | 4.4 | 4.4 | 96.7 | 23 (S) | Bomet | — (-) |
| | 4.4 | 4.5 | 96.7 | 24 (S) | Narok | — (+) |
| | 4.4 | 4.5 | 96.6 | 25 (S) | Nandi | — (-) |
| | 3.0 | 3.0 | 96.6 | 26 (S) | Siaya | — (+) |
| | 2.0 | 2.0 | 96.5 | 27 (S) | Bomet | — (-) |
| | 4.3 | 4.3 | 96.6 | 28 (A) | Busia | — (+) |
| | 4.4 | 4.4 | 96.6 | 29 (S) | Embu | — (-) |
| | 4.4 | 4.4 | 96.7 | 30 (S) | Migori | — (+) |

b MCMV coverage

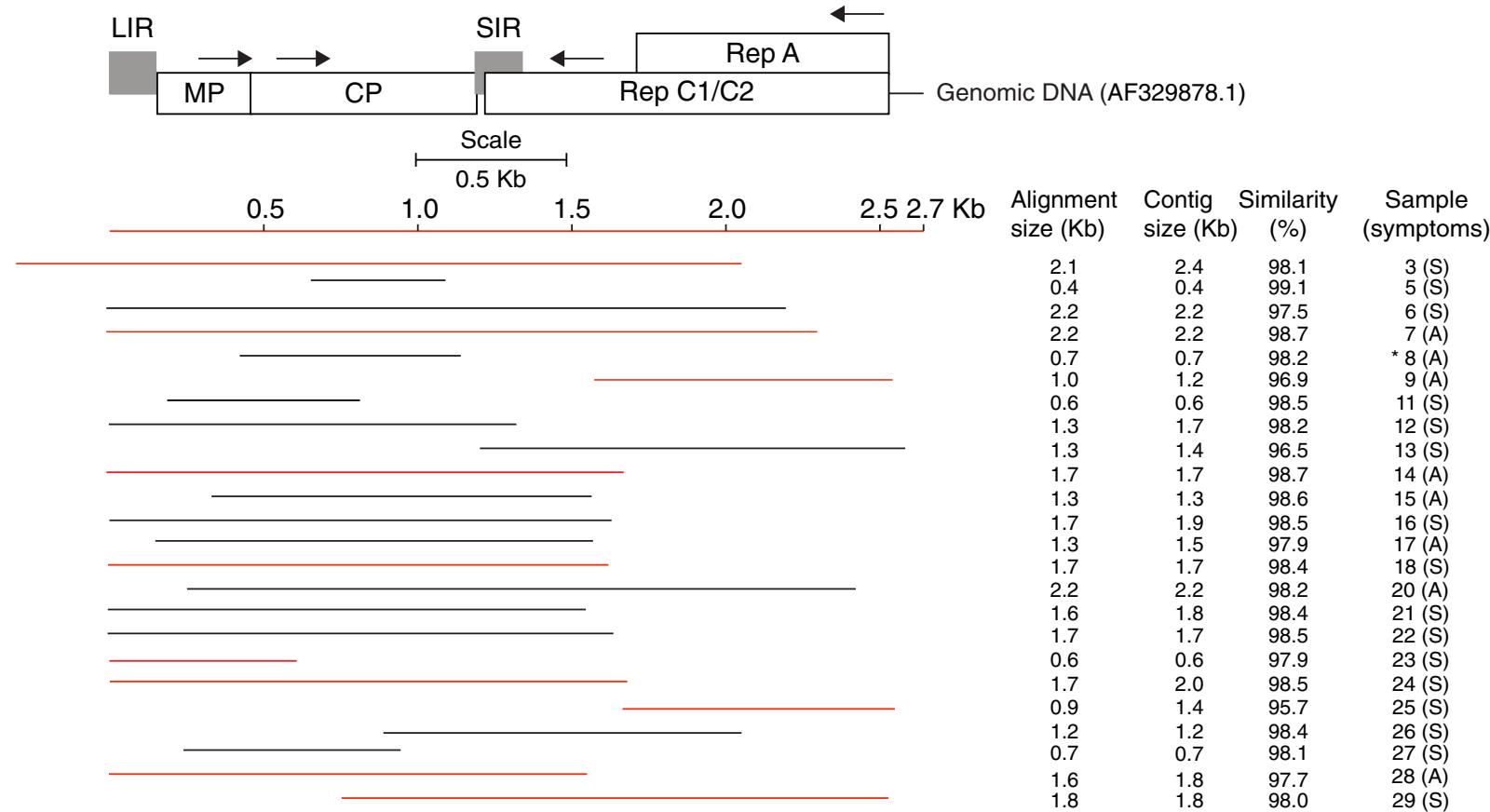


Maize chlorotic mottle virus exhibits low genetic variation

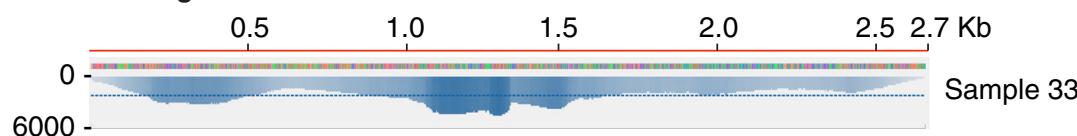


Alignment of *de novo*-assembled contigs to Maize streak virus

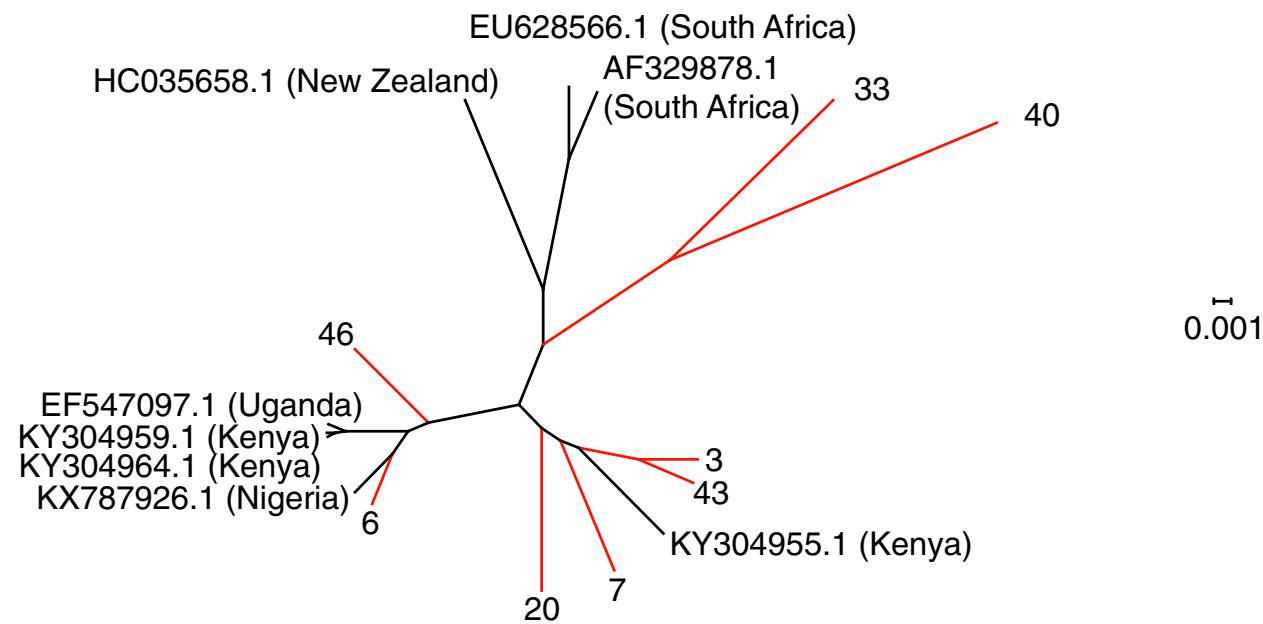
a MSV genome and contig alignment



b MSV coverage

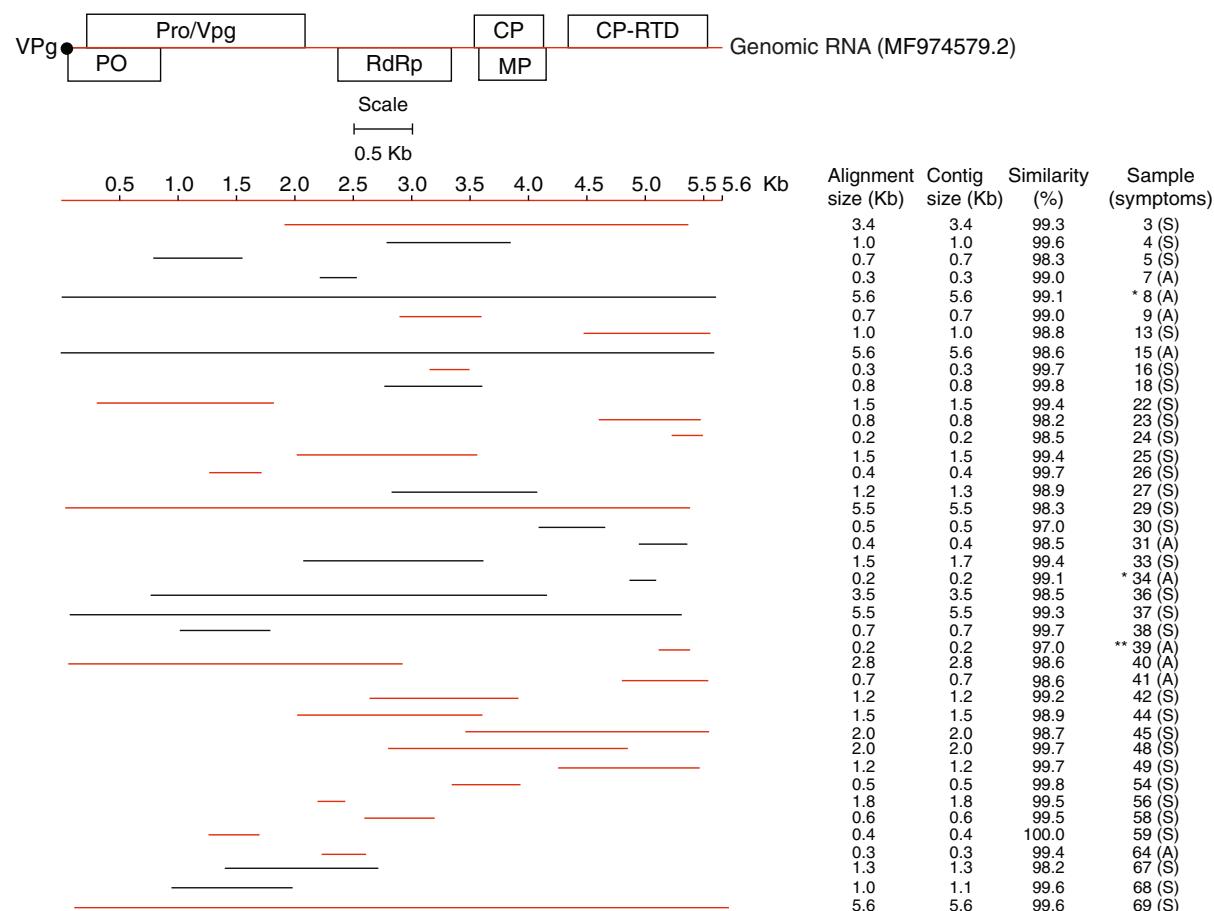


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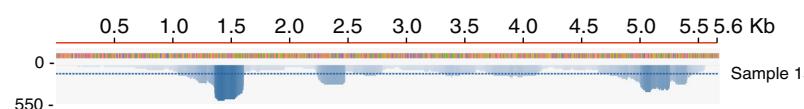


Alignment of *de novo*-assembled contigs to Maize yellow dwarf virus

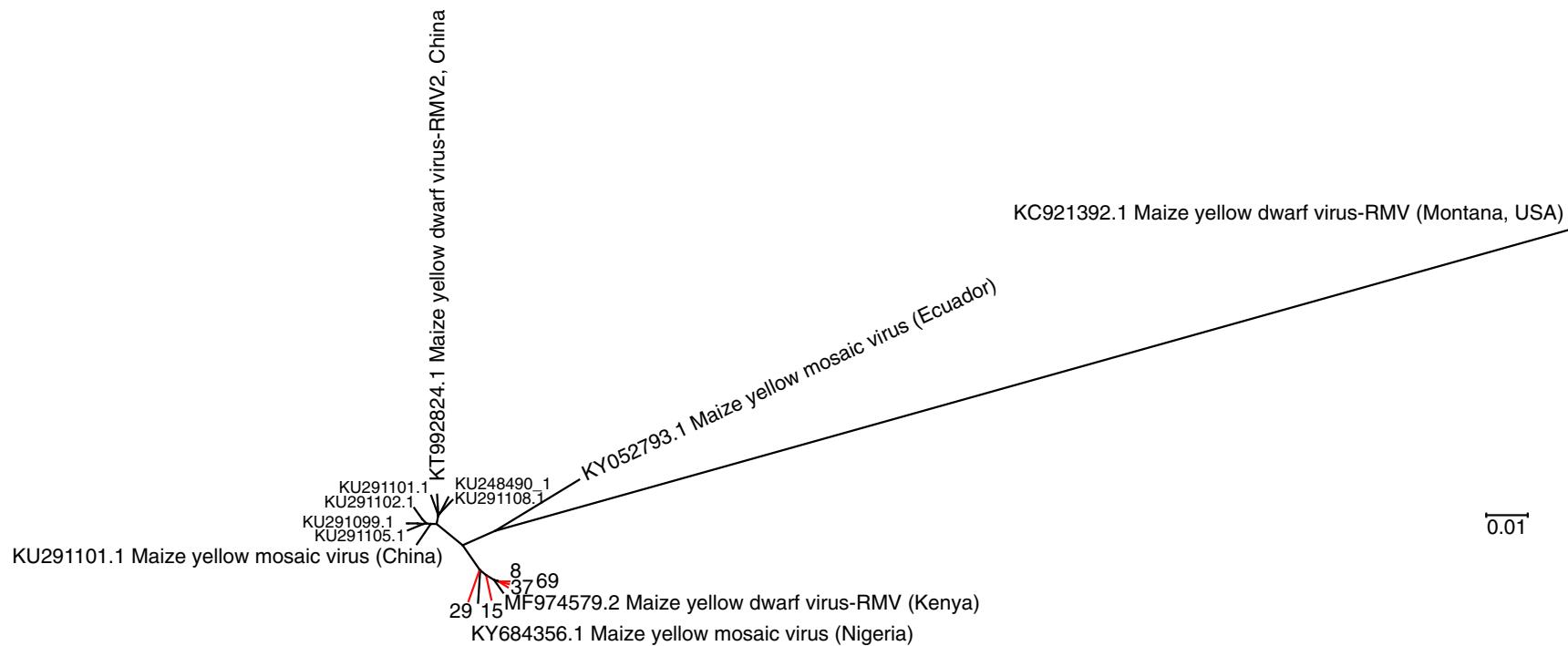
a MYDV-RMV genome and contig alignment



b MYDV-RMV coverage

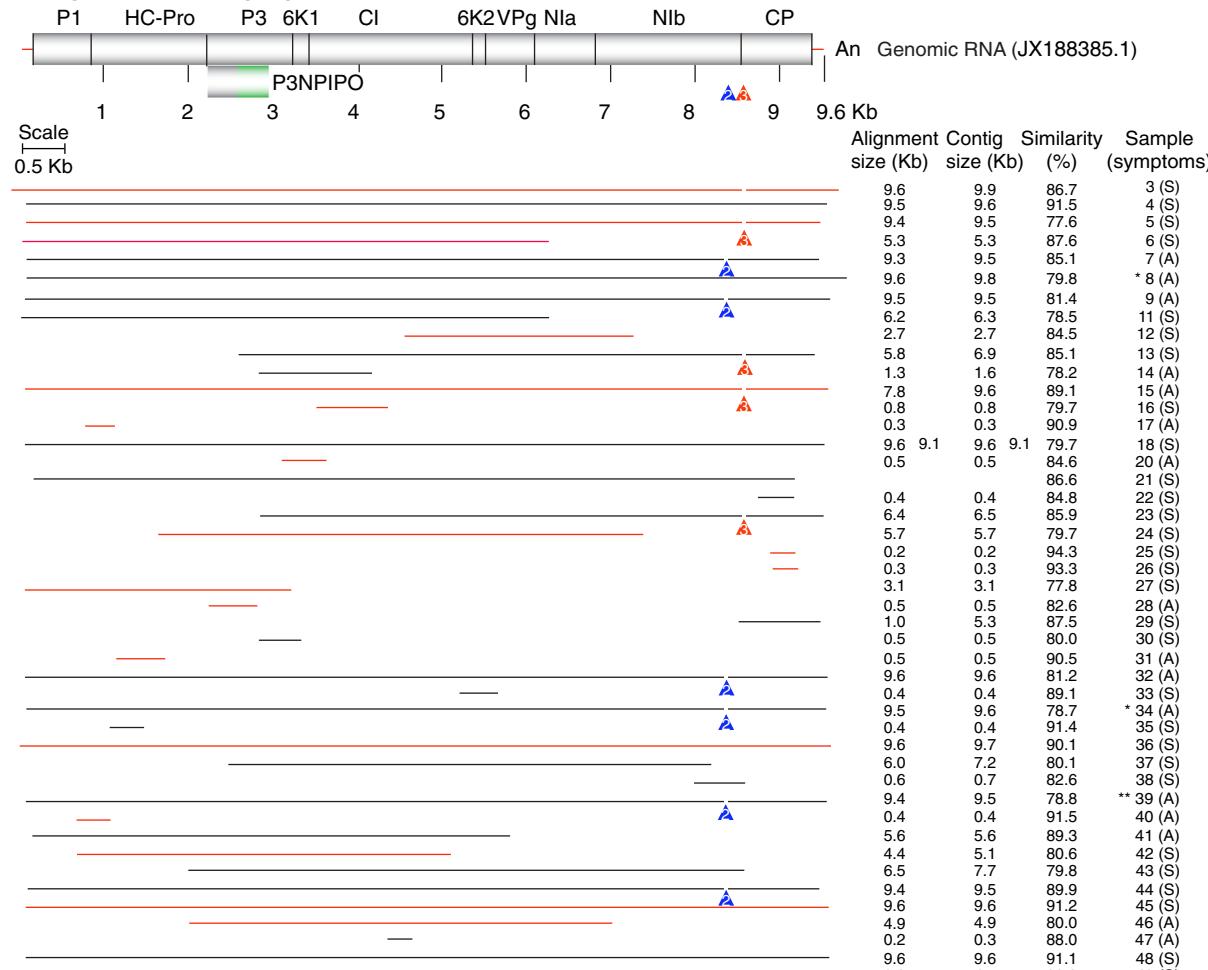


Maize yellow dwarf virus exhibits low genetic variation

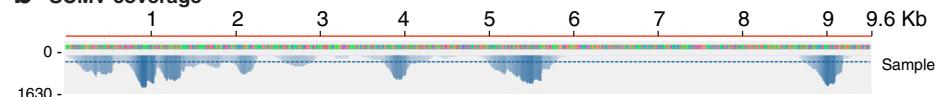


Alignment of *de novo*-assembled contigs to Sugarcane mosaic virus

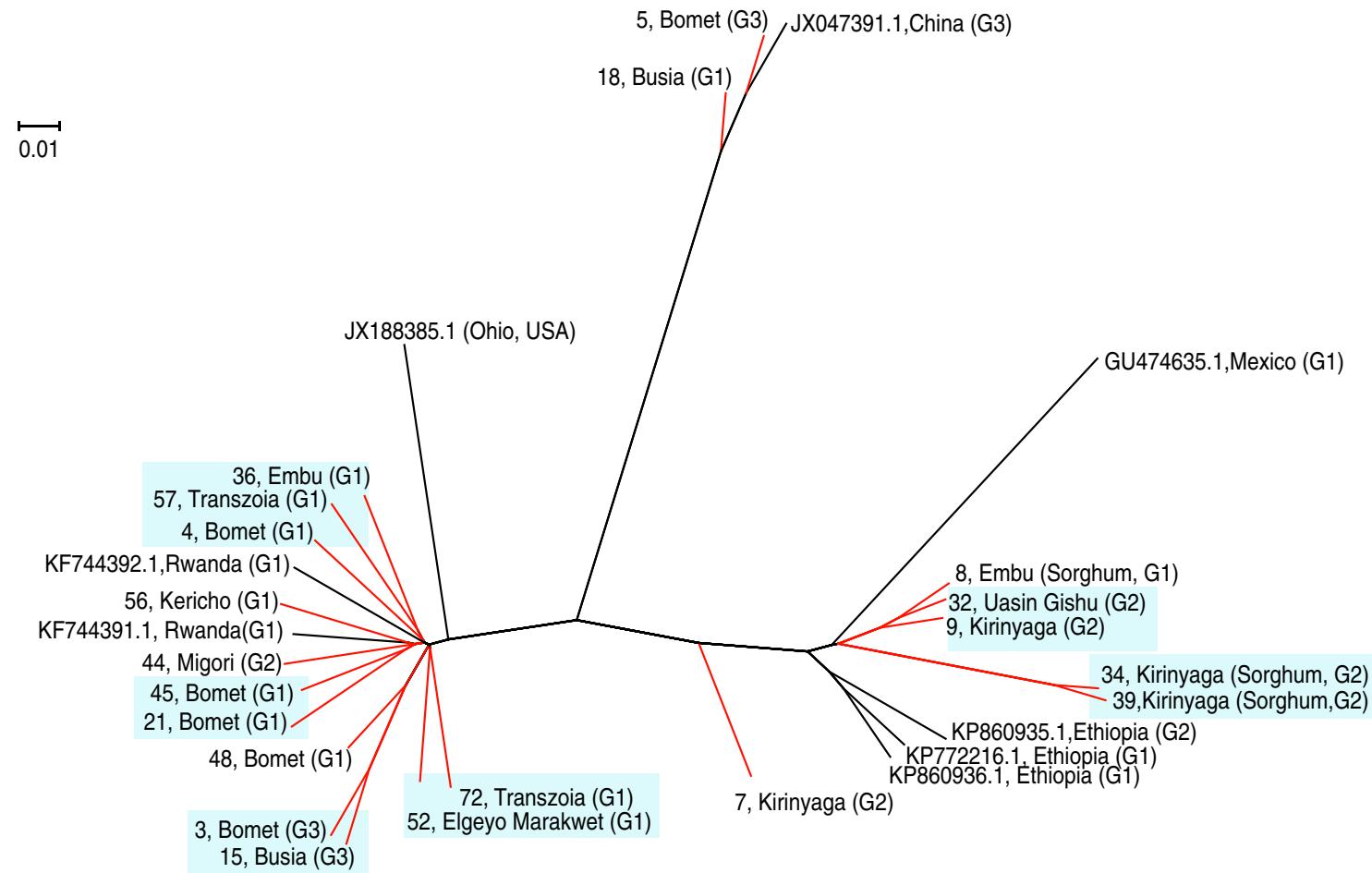
a SCMV genome and contig alignment



b SCMV coverage

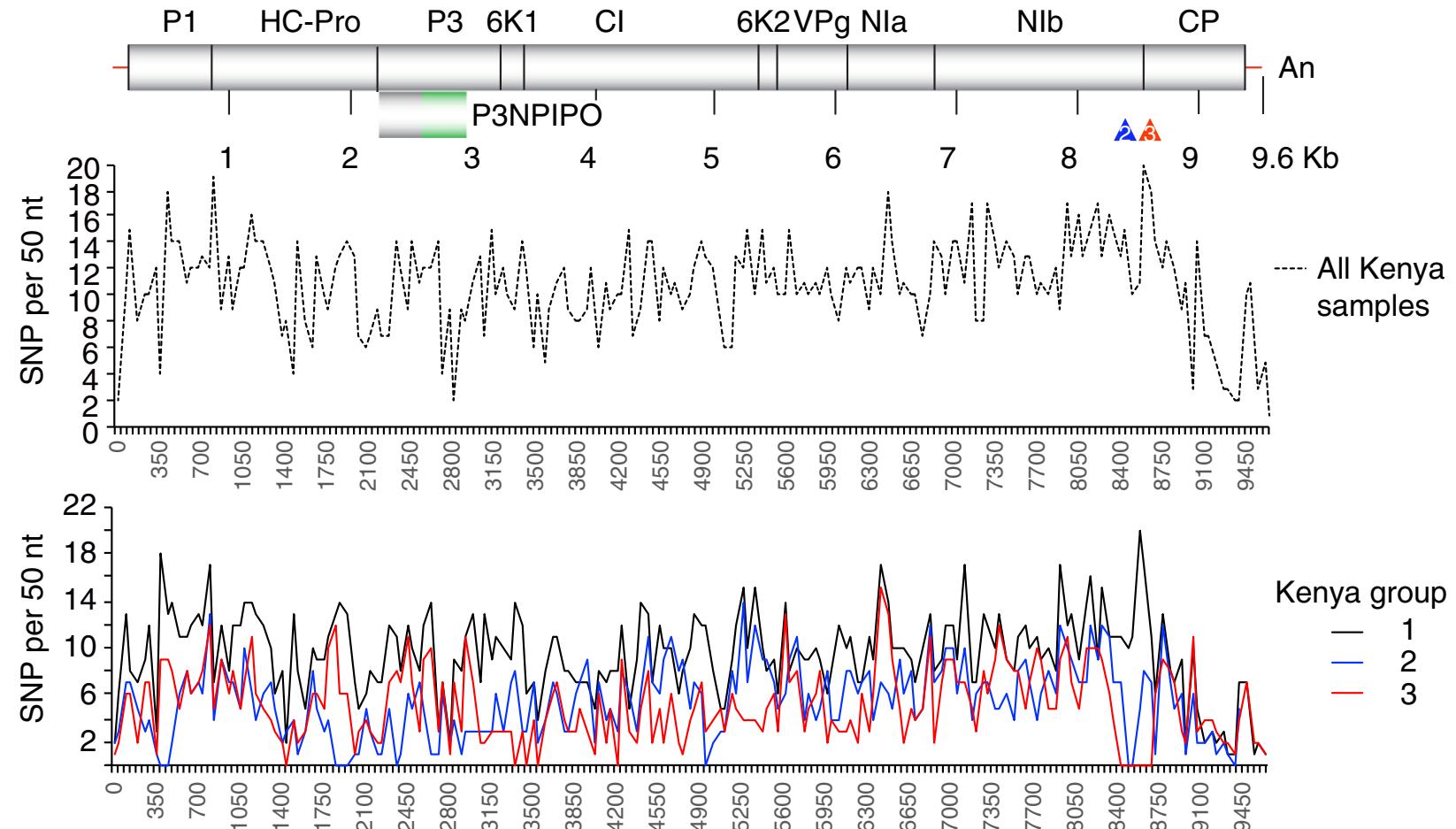


Sugarcane mosaic virus is genetically diverse



Region between the Nib and CP showed hyper-variability

a SCMV single nucleotide polymorphism



Partial alignment of contigs to reference genome

Alignment of amino acid sequence to reference genome

b SCMV partial polyprotein sequence alignment

Summary

Widely distributed viruses

Maize chlorotic mottle virus
Sugarcane mosaic virus
Maize yellow dwarf virus
Maize streak virus

Other viruses

Potyviruses

Hubei Poty-like virus 1
Scallion mosaic virus and
Jhonson Grass Mosaic Virus
Iranian Jhonson Grass Mosaic Virus

Poleroviruses

Barley virus G

Three genetically distinct strains of Sugarcane mosaic virus were detected