Enhancing Phytosanitary Systems for Healthy Plants, Safe & Sustainable Trade”

Title:

A distinct variant of Groundnut rosette virus associated satellite RNA (Sat-RNA) causing chlorotic rosette in western Kenya

Presented by:

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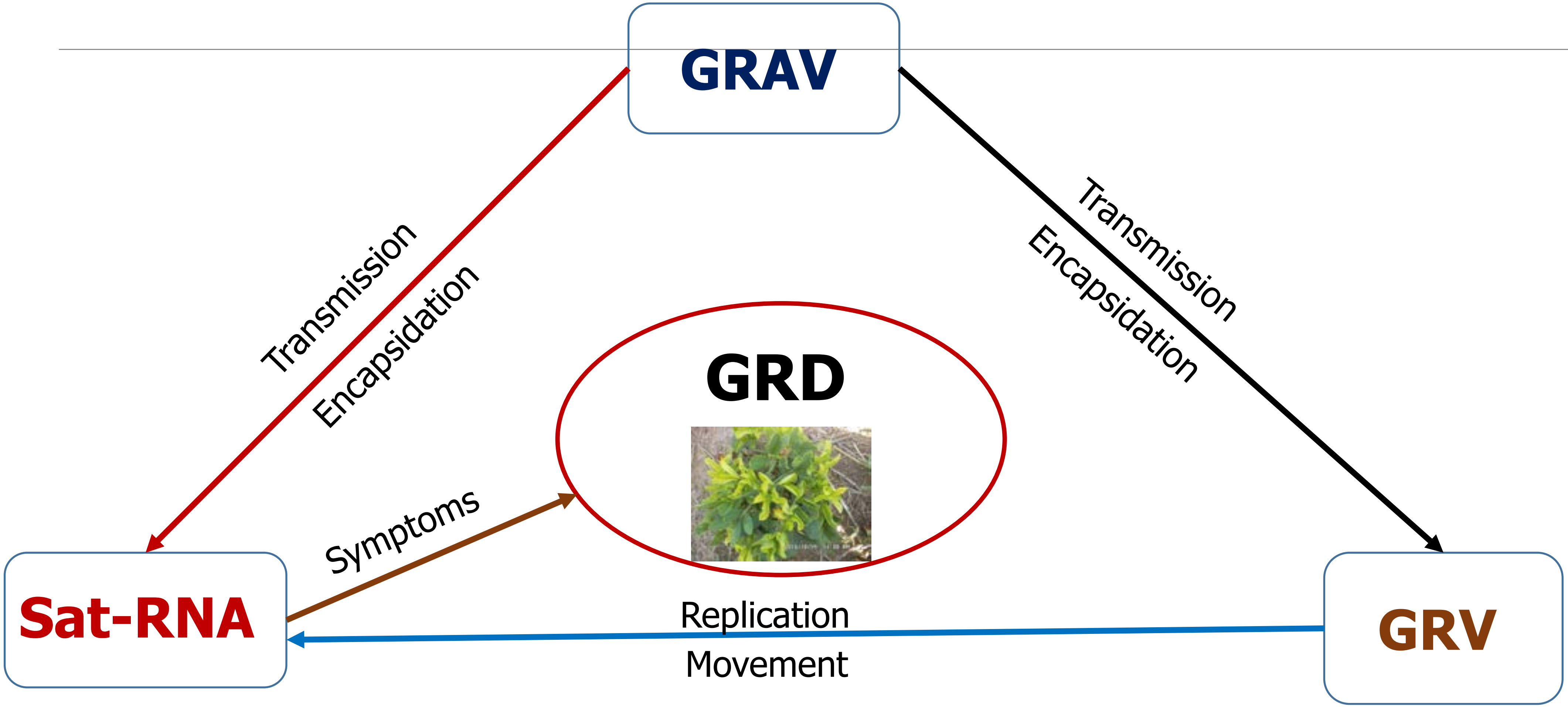
Introduction

- Groundnut (*Arachis hypogaea,* L.*),* is an annual oilseed and food legume crop
  - Grown in diverse environments > the semi-arid and sub-tropical regions countries.
- Poor yields of 500-800kg/ha, as opposed to the potential yield of >2.5t/ha
  - Attributed to poor agronomic practices, pests and diseases
- Viruses are responsible for the majority of the emerging diseases that threaten food production worldwide.
- Groundnut Rosette Disease (GRD) is by far the most endemic, destructive viral disease of groundnut in SSA.
  - Caused by two synergistic viruses; GRAV (*Luteovirus*) and GRV (*Umbravirus*) associated with a Sat-RNA.

Interaction among the GRD agents



GRAV

Transmission
Encapsidation

Transmission
Encapsidation

GRD

Symptoms

Sat-RNA

Replication
Movement

GRV

Problem Statement

- Despite the importance of groundnuts, in western Kenya, farmers achieve less than 30-50% of the potential yield with an average yield of 600-700 kg/ha.
- Lack of sufficient research on the diversity of GRD causal agents:
  - Has resulted into increased viral load causing increased yield losses amongst groundnut farmers.
  - Information on GRD in western Kenya was not current since the last survey done was in 1998.
- Little understanding on the epidemiology of GRD and diversity of causal agents due to:
  - The complex etiology (potential permutations of GRD agents could lead to new disease patterns).

Justification

- In western Kenya, very severe and **highly variable GRD symptoms** were observed in groundnut farms (Mukoye *et al.*, 2018).
- The underlying cause possibly lies in the **genetic variability** in one or all of the GRD associated agents, mainly the **Sat-RNA** of GRV.
- The variants of the three GRD agents have potential permutations and therefore able to form **viable alternatives** that can adapt to diverse and changing "econiches".
- Over time and under high selection pressure, such "evolution" in the associated viruses can easily result into **new disease patterns**.

Objectives

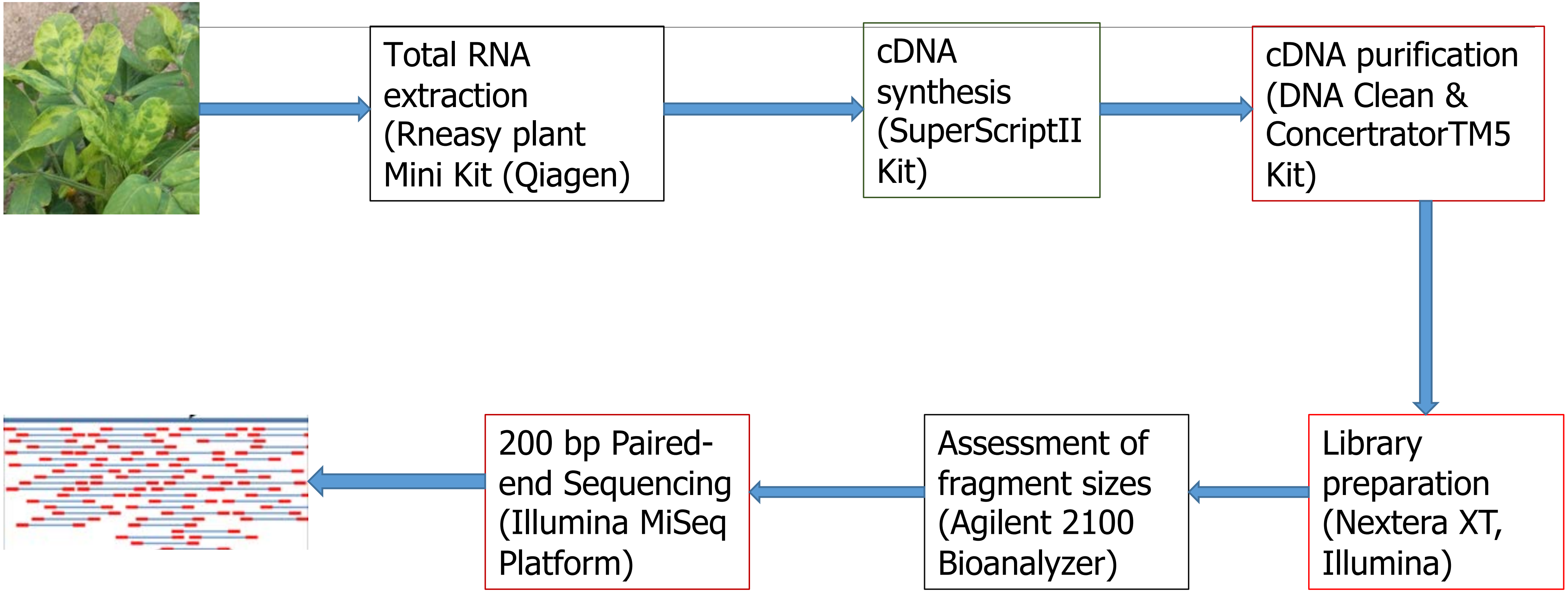
i. To determine the GRD symptom patterns in western Kenya.

ii. To determine the genetic diversity of GRV associated Sat-RNA viruses in western Kenya.

Materials and Methods

- Sampling was done in 6 Counties: Bungoma, Busia, Homabay, Kakamega, Siaya and Vihiga.
- The types of GRD symptoms observed were recorded.
- Leafy samples collected in RNA*later*® RNA Stabilization Solution and kept at 4^{o}C until further analysis.

Materials and Methods cont…



Total RNA extraction (Rneasy plant Mini Kit (Qiagen)

cDNA synthesis (SuperScriptII Kit)

cDNA purification (DNA Clean & ConcertratorTM5 Kit)

Library preparation (Nextera XT, Illumina)

Assessment of fragment sizes (Agilent 2100 Bioanalyzer)

200 bp Paired-end Sequencing (Illumina MiSeq Platform)

Materials and Methods cont…

Quality check (FastQC – v 0.11.5

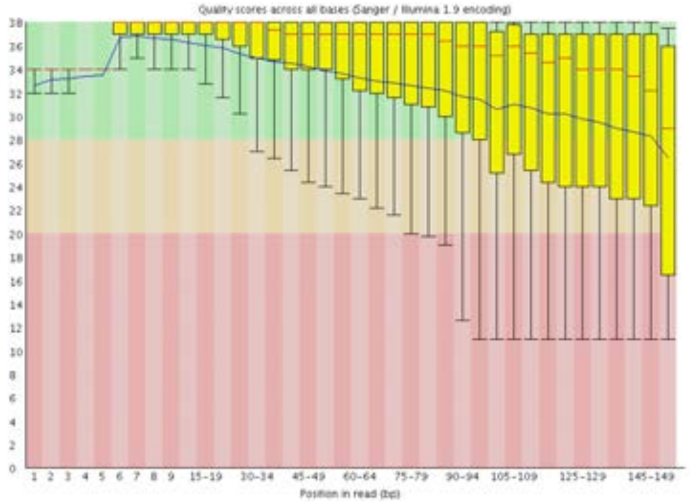
Trimming (Trimmomatic)

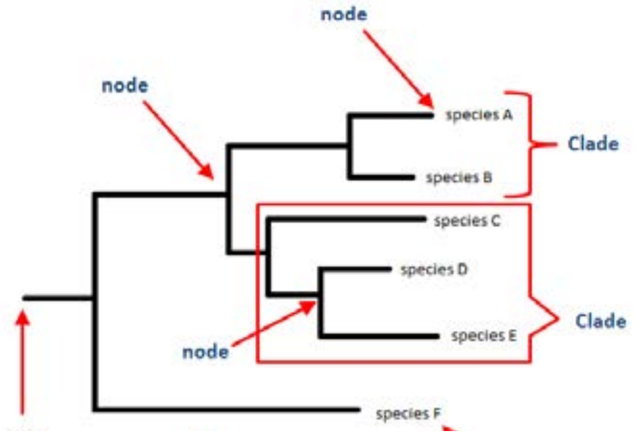
De novo assembly and alignment to viral genomes (CLC Genomics Workbench 10.1.2)

BLASTn

Phylogenetic analysis (MEGA X)

Removal of host sequences





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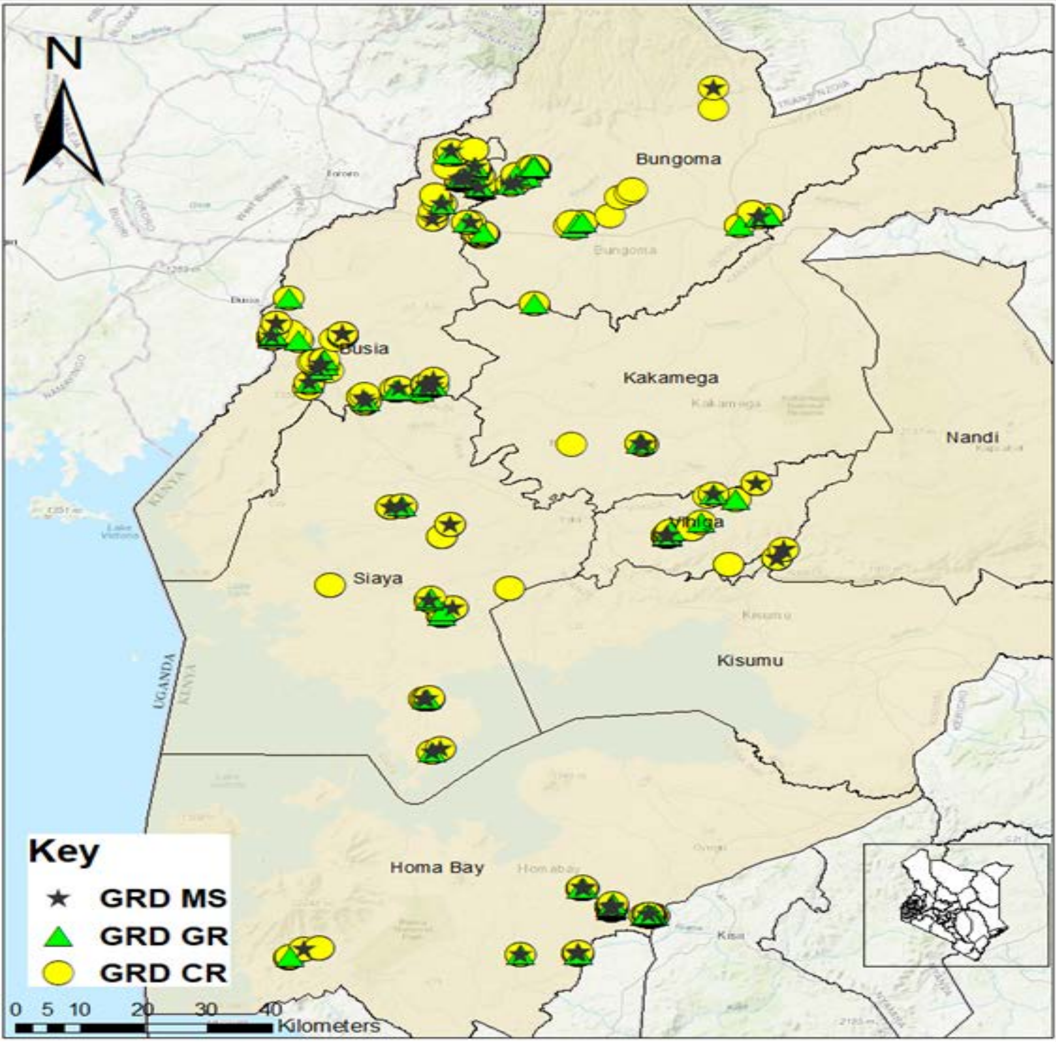
Results

GRD symptom patterns



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GRD MS - Mosaic

▲

GRD GR – Green rosette

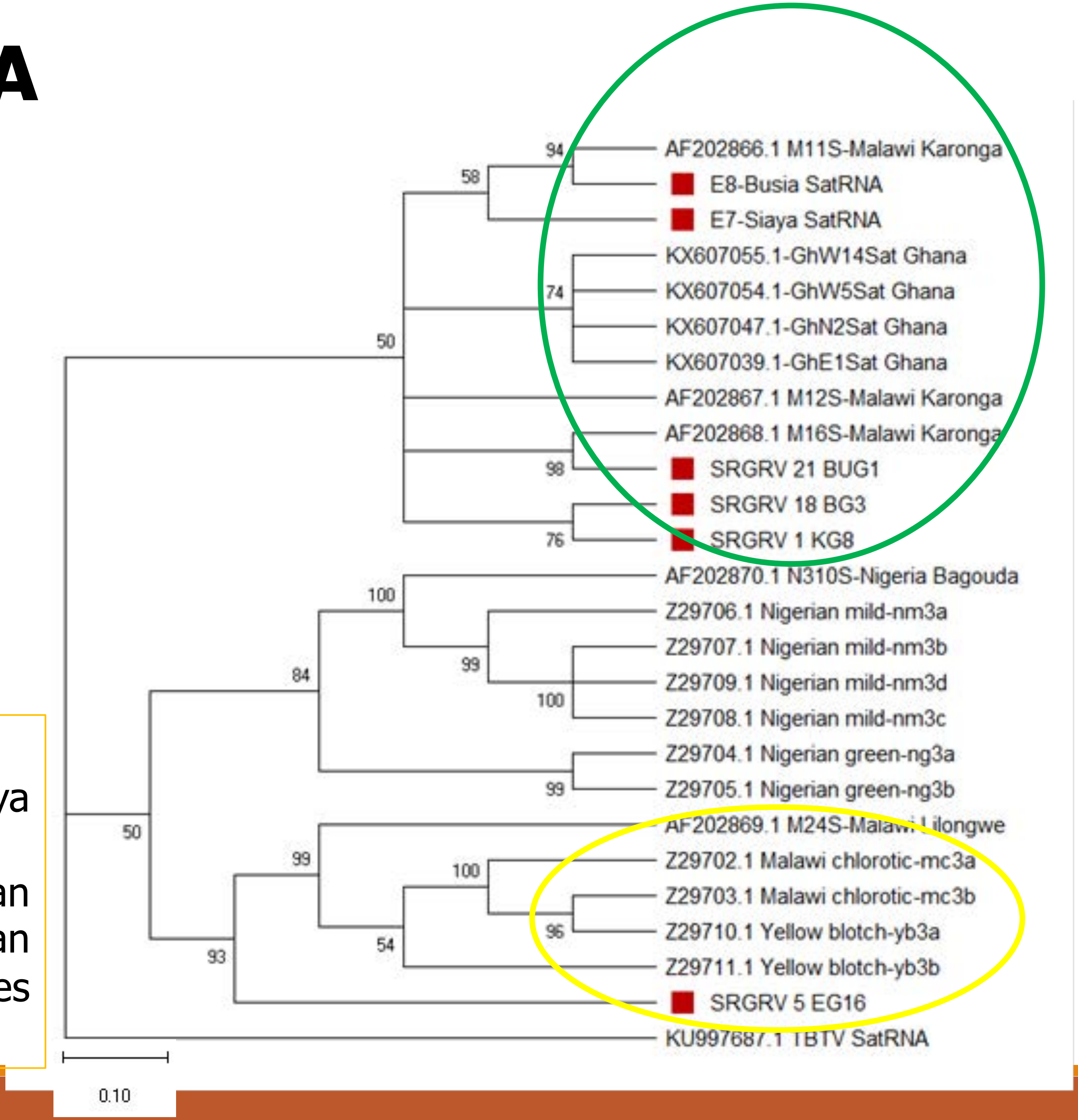
●

GRD CR – Chlorotic rosette

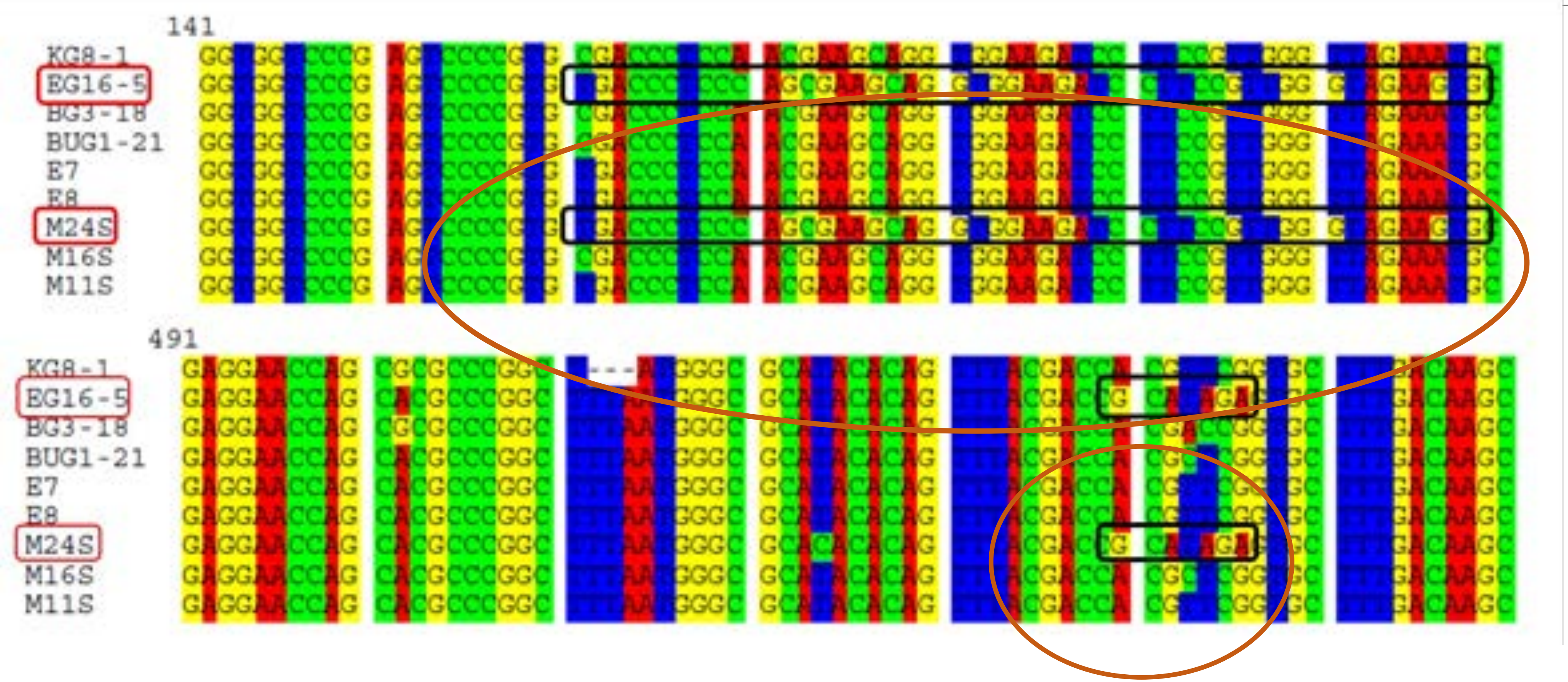
Diversity of GRV-SatRNA

Sample ID	Sat-RNA ID	Sequence length (nt)
EG16	SRGRV_5-EG16	901
E7	E7-Siaya SatRNA	896
E8	E8-Busia SatRNA	897
BUG1	SRGRV_21-BUG1	901
KG8	SRGRV_1-KG8	898
BG3	SRGRV_18-BG3	901

- Two distinct clusters formed by Kenyan isolates.
- Sequence identities: 88-100% of the western Kenya isolates and those from Malawi, Nigeria and Ghana.
- Very close identities: 92-100% between the Kenyan isolates and those from Malawi, followed by Nigerian isolates (90-93%) and least with Ghanaian isolates (86-89%).



Regions of divergence between the green rosette and chlorotic rosette Sat-RNA isolates



Conclusion

- GRD is still the major viral disease of groundnuts in western Kenya.
- Genetic diversity of the Sat-RNA is more varied with wide geographical distance.
- New variants of Sat-RNA exists in western Kenya that are contributing to the diverse symptoms expressed by GRD.

Recommendations

- There is need for research in breeding for resistance to GRD.
- Need for more sequencing of the GRD agents to develop strain specific diagnostic tools.
- There is need for a reliable seed production and certification for groundnuts in western Kenya.

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Acknowledgements

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