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Pathosystem, Epiphytology and Genomic Characterization of Groundnut Rosette Disease Pathogens

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Abstract: Synergism among the groundnut rosette disease (GRD) pathogens of *Groundnut rosette assistor virus* (GRAV, *Luteovirus*) and *Groundnut rosette virus* (GRV, *Umbravirus*) associated with a satellite-ribonucleic acid (sat-RNA), have declined groundnut (Peanut, *Arachis hypogaea* L.) production in Kenya. The polyphagous groundnut aphid (*Aphis craccivora* Koch; *Homoptera: Aphididae*) efficiently transmits GRD in sub-Saharan Africa. Inadequate information available on the pathosystem, epiphytology and genomic characterization of GRAV, GRV and sat-RNA pathogens in Kenya, have hampered control and management technologies due to their intimate complex etiology, the bottleneck which this study unravels. A survey of GRD was conducted in western Kenya among the four counties of Bungoma, Busia, Kisumu and Kisii during the short rains season of 2019. A total of 10 symptomatic leaf samples were selected from the collected samples and preserved until use. Total RNA was extracted from the symptomatic leaf samples using GeneJET Plant RNA Purification Mini Kit according to the manufacturers' protocol. RT-PCR detection of GRD pathogens was done using specific primers of GRAV, GRV and sat-RNA. DNA libraries were prepared and sequenced using the Sanger sequencing platform. Phylogenetic analyses and comparisons were performed using MEGA X software. The sequence quality were checked based on the peak of the electrophoregram and trimmed using CLC main work bench v20. The sequences were assembled with final consensus exported as FASTA file format and BLAST searched against NCBI database using BLASTn. The BLAST hit with nucleotide identity of at least 97% identity were considered, downloaded, uploaded to MEGA X and multiple alignment done with Gap Opening Penalty of 15 and Gap Extension Penalty of 5.5. Phylogenetic trees were constructed with best DNA/Protein model based on automatic Neighbor Joining Tree and Maximum Likelihood method of nucleotides substitution by Kimura 2 Parameter with Invariant Plus Gamma. The two GRAV isolates from Kenya (Ken_G10 and Ken_G2) clustered together in group II while the rest clustered in group I. The Kenyan novel GRAV isolates are more similar to each other than with any other sequences implying common ancestry than with the other African isolates. The Kenyan sat-RNA isolates formed two distinct groups with sub-groups within the clusters. Isolates Ken_G11 and Ken_G6 clustered together in group II while Ken_G10 and Ken_G7 clustered together in group I. Ken_G6 clustered with other Kenyan sat-RNA isolates implying a possible identity by descent (IBD), suggesting a possible impact of a genetic bottleneck whose cause should be investigated further to infer any conclusions.

Keywords: *Arachis hypogaea*, GRAV, GRV, sat-RNA, Genomics

1. Introduction

Leguminous groundnut (*Arachis hypogaea* L.) crop is an important food and oilseed plant in Kenya, grown in diverse environments between 40^0N and 40^0S in the world [1]. However, the farmers in western Kenya achieve less than 30-50% of the potential yield, with an average output of 600-700 kg/ha. The low yields are attributed to among other diseases, GRD that causes significant yield losses of upto 100% in

some individual farms. A survey in the groundnut growing seasons of 1997-1998 in western Kenya by Wangai *et al* (2001) showed that GRD incidence ranged between 24-40% [2]. A follow-up survey in 2016-2017 recorded a substantial increase of GRD incidence between 36-61% [3, 4]. Groundnut rosette disease is efficiently transmitted by the polyphagous groundnut aphid *Aphis craccivora* Koch in a persistent circulative manner, and inefficiently by *Aphis gosypii* Glover and *Myzus persicae* Sulzer [5] because the latter two vectors are not significant in the pathosystem ecology of GRD perpetuation. There is no evidence available for seed transmission of GRD pathogens [6]. The groundnut aphid maintains itself successfully throughout the dry and wet seasons because it is anholocyclic, parthenogenetic and ovoviviparous, reproducing almost everywhere throughout the year on some host crops with preference to groundnuts which are not drought stressed [7]. The GRD epiphytology is a complex involving intimate synergistic interaction between and among the aphid vector, GRAV, GRV and its sat-RNA, the host plant and environment [8].

Aphis craccivora commonly known as the cowpea aphid or groundnut aphid or black legume aphid, is the principal vector involved in the transmission of all the GRD pathogens in a persistent circulative manner. Studies have shown that all the GRD particles, whether they contain GRAV-RNA, GRV-RNA or sat-RNA, are acquired by the aphid vector from phloem sap in 4 hr and 8 hr acquisition access feeding for chlorotic and green rosette respectively [9]. The groundnut aphid does not always transmit all the GRD causal agents together [10]. During short inoculation feeding (test probe or stylet pathway phase), the vector probes groundnut leaves without reaching the phloem, hence transmitting only GRV and sat-RNA that multiply within the epidermal and mesophyll cells. Even if GRAV particles are deposited in the mesophyll cells, they cannot replicate because they can only replicate in the phloem cells [11]. The groundnut aphid can vector GRAV and GRV-sat-RNA when the stylets penetrate sieve elements (Salivation phase) of the phloem cells. When the inoculation feeding period is longer or the number of aphids per plant is increased, the success of transmitting all the three causal agents together is high. The aphid vector can fail to acquire or transmit GRV and its sat-RNA from diseased plants lacking GRAV. Such plants become sources of heavy GRD inoculum for volunteer groundnuts and related host plants. However, if such plants receive GRAV later due to A. *craccivora* feeding, the plants again serve as source of inoculum [12]. Reports of groundnut crop damage by GRD underscores the need for further pathosystem, epiphytology and genomic characterization studies to inform appropriate control and management strategies. This will prevent resistant and tolerant varieties from succumbing to high GRD inoculum pressure [13].

Groundnut rosette assistor virus (GRAV) belongs to the family *Luteoviridae* [1]. The GRAV virions are isometric shaped with 28nm diameter non-enveloped particles of polyhedral symmetry. It has a single stranded positive sense RNA with non-segmented genome of 6900 nt that encodes both structural and non-structural proteins [14]. It is suggested that GRAV encodes six open reading frames (ORFs) just like other *Luteoviruses*. The GRAV virions are composed of 24.5kDa single coat protein (CP) subunits. This virus is anti-genetically related to *Bean leaf roll virus (*BLRV*), Beet western yellows virus (*BWYV*), Chickpea Luteovirus (*CPLV*), Pea leaf roll virus (*PLRV*), Potato leaf roll virus-1 (*PLRV-1*) and Tobacco necrotic dwarf luteovirus (*TNDLV*) [15]. Replication of GRAV occurs autonomously in the cytoplasm of the phloem tissue. Vector transmission of GRAV is by *Aphis craccivora* in a persistent circulative manner. Mechanical artificial transmission by sap inoculation is possible experimentally but not through seed. The virus occurs wherever GRD has been reported and groundnuts crop is the only known natural host with a wider host range among *Leguminosae* and *Solanaceae* plants [4]. Infections by GRAV alone in groundnuts results to asymptomatic or transient mottling, and can cause substantial yield loss in susceptible cultivars/landraces [9].

Groundnut rosette virus (GRV) belongs to the genus *Umbravirus*. On isolation and characterization, Taliansky and Robinson (2003) found that the virus has no structural/coat protein gene and thus forms no conventional virus particles [16]. The GRV genome is non-segmented single-stranded linear molecule, positive sense RNA of 4019 nt that encodes four ORFs. Replication of GRV occurs in the cytoplasm of infected tissue autonomously [16]. The virus alone causes transient symptoms, but in association with a sat-RNA, typical clear GRD symptoms occur [9]. Encapsidation and vector transmission of GRV by *A. craccivora* (in a persistent mode) is dependent on GRAV [17]. Transmission of GRV is not possible through pollen, seed or contact between plants, however it is possible by mechanical sap inoculation and grafting [9]. The only natural host of GRV is groundnuts, but some experimental hosts in the *Chenopodiaceae* and *Solanaceae* families have been reported [9]. Only one strain (MC1) of GRV has been reported [18], and the virus is limited to sub-Saharan Africa (SSA) and its offshore islands of Madagascar [19].

The sat-RNA is sub-viral RNAs of GRV and belongs to the sub-group-2 (small linear) satellite-RNAs. It is of size 895–903 nt, single-stranded, linear non-segmented RNA [20]. Its replication, encapsidation and movement within and between plants is entirely dependent on GRV. The sat-RNA plays a critical role as a helper virus dependent for transmission of GRV [21] and GRD symptom expression [14, 22, 23]. Ten variants of sat-RNA associated with GRV have been determined [20]. The different rosette symptoms of chlorotic, green and mosaic are caused by different variants of sat-RNA [14, 12]. The GRV sat-RNAs that cause chlorotic and green rosette symptoms in sub-Saharan Africa (SSA) are 895-903 nt long, and are at least 87% identical. The sat-RNA contains up to five ORFs in either positive or negative sense, but the role of any proteins expressed from these ORFs is unknown [20, 16]. Vector transmission of sat-RNA by aphids occur in the presence of GRAV and GRV. Artificial mechanical transmission occurs alongside GRV [9].

The synergistic etiology of GRD is a complex involving the three causal agents of *Groundnut rosette assistor virus* (GRAV), *Groundnut rosette umbravirus* (GRV) and a satellite-RNA (sat-RNA) of GRV [16]. These three pathogens synergistically depend on each other intricately, and they all have an important role in the pathosystem perpetuation and biological epiphytology of GRD. Groundnut rosette virus (GRV) needs assistor GRAV for encapsidation and transmission by *Aphis craccivora*, while presence of both GRV and GRAV helps in vector transmission of sat-RNA. However, the sat-RNA replication, encapsidation and movement within and between plants is entirely dependent on GRV. Therefore, the synergistic pathosystem of GRAV, GRV and sat-RNA with the epidemiological distribution of the three field rosette symptom types of chlorotic, green and mosaic occurring in western Kenya, have made GRD become three diseases in one, and has not been adequately documented to help understand its epiphytology in the region. This therefore necessitates the need to document the intricate pathosystem, epiphytology and genomic characteristics of GRD causal agents in western Kenya to facilitate designing of appropriate control/management strategies.

2. Materials and Methods

2.1. Field Survey

The extensive field survey to determine GRD pathosystem and epiphytology was conducted in all the major groundnut growing areas of the four counties of Bungoma, Busia, Kisumu and Kisii during the short rains season of 2019. A total of 10 samples were selected from the collected symptomatic leaf samples from farmers' fields, placed into falcon tubes containing RNAlater solution, and kept in a cool box until use. The systematic survey was conducted by walking through groundnut fields, and visually inspecting groundnut crops for symptomatic leaves. Depending on the farm size, quadrats of $10m^2$ were estimated with disease incidence and severity scored on the disease diagnostic score sheet for each quadrat through random sampling. The Geographical Information System for the latitude, longitude and altitude of the sampled farms was recorded using the GPS entrex venture HC GARMIN^{TM}.

2.2. Total RNA Extraction and Sanger Sequencing

Groundnut field samples with chlorotic, green and mosaic rosette symptoms were tested for GRAV, GRV and sat-RNA by two-step RT-PCR. Total RNA was extracted using the GeneJET Plant Purification Mini Kit (Thermo Scientific) according to the manufacturer's protocol with modifications on hybridization temperatures. The symptomatic leaf tissue was homogenized in liquid nitrogen and in lysis buffer provided in the kit. The GRAV, GRV and sat-RNA specific primers (Table 1) at 10 μM were used in transcription and amplification of the targeted coat protein (CP) gene for GRAV, and nucleotide bases for GRV and sat-RNA. The sense primers were also utilized in the Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) to transcribe and amplify genome fragments of interest for each causal agent. Amplification of the two-step RT-PCR products was done using both sense and antisense GRAV, GRV and sat-RNA specific primers (Table 1) under the following conditions; Denaturation at 94°C for 3 minutes followed by 30 cycles at 94°C for 1 minute, Hybridization at 60°C (for GRAV), 50°C (for GRV) and 60°C (for sat-RNA) for 1 minute, Elongation at 72°C for 1 minute and final extension at 72°C for 10 minutes. The resultant reaction mixture was stored at 4°C. The PCR products were loaded and visualized in 1% Agarose gel electrophoresis stained with Ethidium bromide (EtBr) in a X0.5 Tris Acetate EDTA buffer at 100V for 30 minutes to determine the DNA size by visualizing under UV light.

Table 1. Oligonucleotide Primers used in transcription and amplification of GRD pathogens.

Primers	Sequence (5' > 3')	Specific to	Source
sat-RNA F	ATGCAGATTGGTAGCCTTGG	sat-RNA	[24]
sat-RNA R	CTGTGTATGCGCCCATTAAG	sat-RNA	
GRAV F	GCAATGGACGAGCTAACAGG	GRAV-CP	[3]
GRAV R	ACTTGATGGTGAACCGGAAG	GRAV-CP	
GRV F	GCAAAATTTTTAGTCGGGGAAG	GRV ORF3 and ORF4	[24]
GRV R	GGTCTTATGTTCAGGCTGTCAA	GRV ORF3 and ORF4	

2.3. Bioinformatics Analysis

The sequence quality were checked based on the peak of the electrophoregram and trimmed using CLC main work bench v20 to remove the PCR primers. The sequences were assembled and any conflict was resolved from the consensus. The final consensus was then exported as FASTA file format. The sequences were BLAST searched against NCBI database using BLASTn to obtain closest match. The BLAST hit with nucleotide identity of at least 97% identity were considered and the sequences were downloaded from the NCBI database. The sequences were uploaded to MEGA X software and multiple alignment done with Gap Opening Penalty of 15 and Gap Extension Penalty of 5.5. Phylogenetic trees were constructed in MEGA X [25]. The best DNA/Protein model for phylogenetic tree was based on Automatic Neighbor Joining Tree and Maximum Likelihood method of nucleotides substitution. The best DNA model used for the phylogeny tree was Kimura 2 Parameter with Invariant Plus Gamma. The final tree was made with bootstrap value of 1000 replication.

3. Results

3.1. Field Symptom Diversity of GRD

The three GRD symptom types of chlorotic, green and mosaic rosette were observed in all the four surveyed counties (Figure 1). Chlorotic rosette was the most prevalent followed by green rosette and least was mosaic rosette (Figure 1).
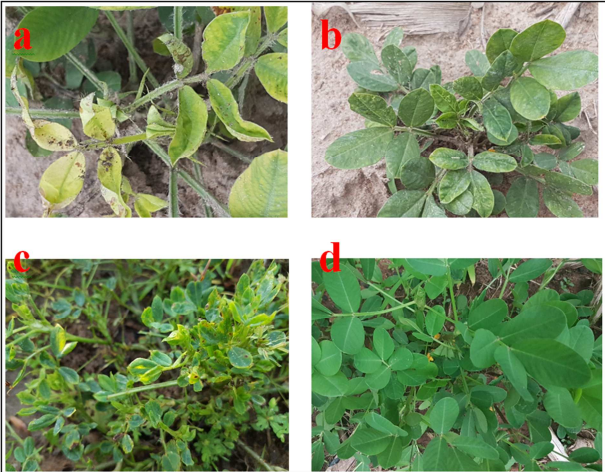

Figure 1. *GRD symptom types observed in the field.*

a: Chlorotic rosette symptom type; b: Green rosette symptom type; c: Mosaic rosette symptom type; d: Healthy groundnut crop.

3.2. RT-PCR Detection of GRAV, GRV and sat-RNA

Seven samples from the selected ten during the field survey were tested by two-step RT-PCR to detect GRAV, GRV and sat-RNA using their specific primers (Table 1). Five samples tested positive for GRAV, six samples tested positive for GRV and four samples tested positive for sat-RNA (Figures 2, 3 and 4).
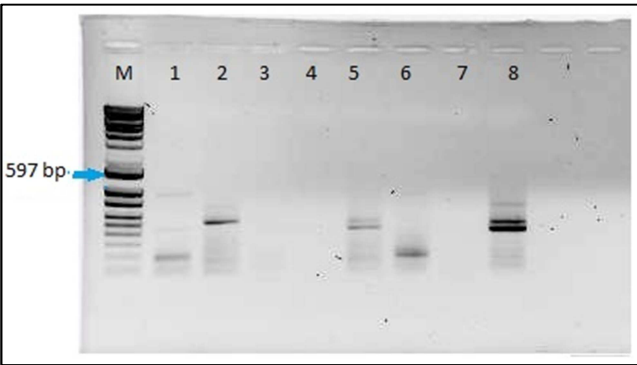

Figure 2. *Groundnut rosette assistor virus (GRAV).*

Expected band size was 597 bp. Lane M-1kb Ladder, Isolates in lanes 1-6 western Kenya, 7-Negative control (molecular grade water), 8-Positive control.

Expected band size was 860 bp. Lane M-1kb Ladder, Isolates in lanes 1-6 western Kenya, 7-Negative control, 8-Positive control.
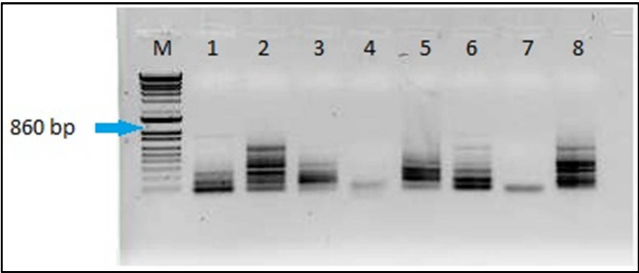

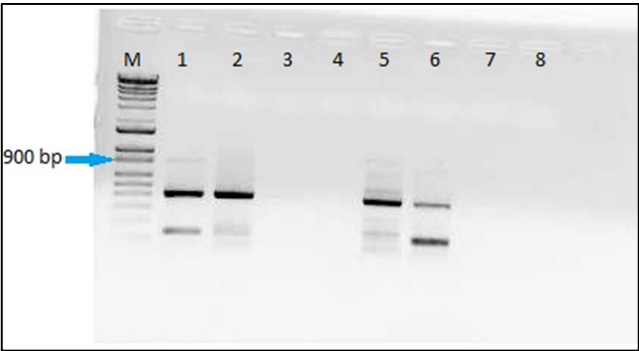***Figure 3.*** *Groundnut rosette virus (GRV).*

Figure 4. *Satellite-RNA (sat-RNA) associated with GRV.*

Expected band size was 900 bp. Lane M-1kb Ladder, Isolates in lanes 1-6 western Kenya, 7-Negative control, 8-Negative control.

3.3. Sequence Phylogenetics

The genomic characterization of GRD associated viruses was determined by analysis of the sequence reads obtained by Sanger Sequencing. The two GRAV coat protein (CP) gene sequences obtained were of good quality, hence assembled and compared with GRAV CP sequences available in the GenBank (Figure 5).
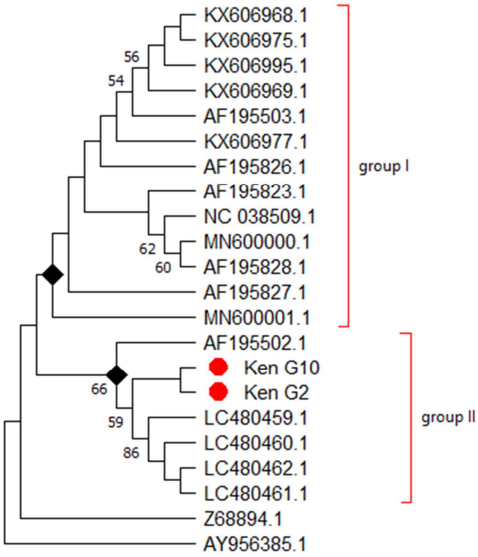

Figure 5. *Phylogenetic tree of GRAV CP and GenBank isolates.*

The evolutionary history was inferred by using Automatic Neighbor Joining Tree and Maximum Likelihood method of

nucleotides substitution. The best DNA model used for the phylogeny tree was Kimura 2 Parameter with Invariant Plus Gamma [26]. The final tree was made with bootstrap value of 1000 replication.

The two GRAV sequences were deposited in the GenBank with accession numbers LC576688 (Ken_G2) and LC576691 (Ken_G10).

The four sat-RNA sequences obtained were of good quality, and were assembled then compared with those from the GenBank (Figure 6).
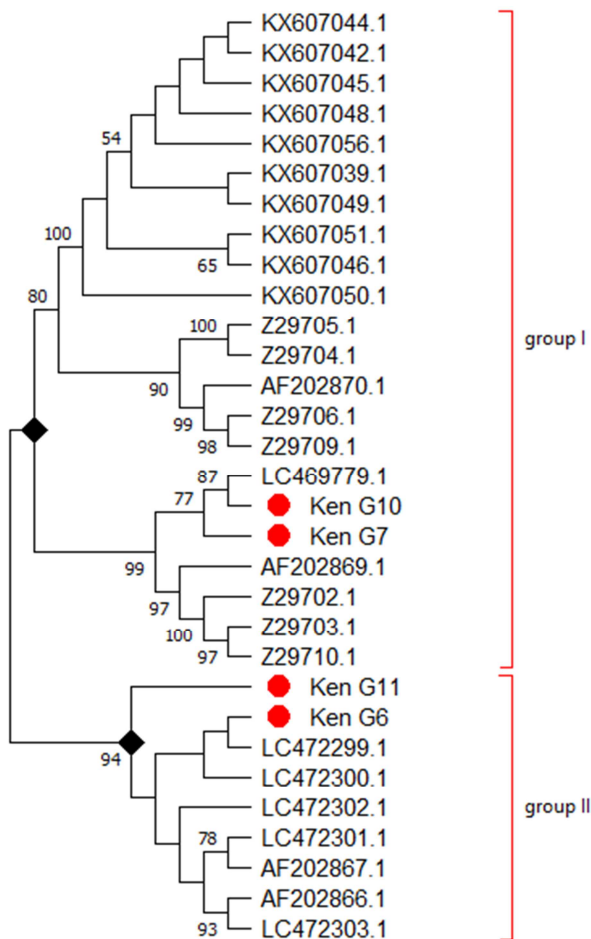

Figure 6. Phylogenetic tree of sat-RNA and GenBank isolates.

The evolutionary history was inferred by using Automatic Neighbor Joining Tree and Maximum Likelihood method of nucleotides substitution. The best DNA model used for the phylogeny tree was Kimura 2 Parameter with Invariant Plus Gamma [26]. The final tree was made with bootstrap value of 1000 replication.

The four Kenyan sat-RNA sequences were deposited in the GenBank with accession numbers LC576689 (Ken_G6), LC576690 (Ken_G7), LC576692 (Ken_G10) and LC576693 (Ken_G11).

4. Discussion

The GRD field survey symptom epiphytology was severely prevalent with incidences varying significantly. The sat-RNA variants occurrence in western Kenya caused the varied GRD symptom types observed in the field. Chlorotic rosette symptom type was the most prevalent followed by green rosette and then mosaic rosette. This supports the findings of Wangai *et al* (2001) and Mabele *et al* (2019) who reported chlorotic rosette to be the most significantly prevalent GRD symptom type in the region [2 and 3]. The high prevalence of chlorotic rosette could also be attributed to its higher transmission efficiency compared to green and mosaic rosette. This observation concurs with that of [27] who reported minimum acquisition feeding periods of 4 hrs and 8 hrs for chlorotic and green rosette respectively, and the median latent periods of 26.4 hrs and 38.4 hrs respectively for chlorotic and green rosette. The mosaic rosette symptom type minimum acquisition feeding periods have not been documented because it had not been previously reported, but was distributed in isolation in all the surveyed counties. This suggests that there is evolution of new variants of sat-RNA in western Kenya that might be causing these new symptoms. A total of 10 variants of sat-RNA have been reported to be associated with the various GRD symptoms [20, 21]. A mixture of either variants especially the chlorotic and green rosette or the mild ones, are likely to induce the mosaic symptom type [10]. It is therefore possible that some of these sat-RNA variants occur in western Kenya in mixed infections causing the mixed symptom types observed.

The two-step RT-PCR detected all the three GRD causal agents of GRAV, GRV and sat-RNA responsible for the disease pathosystem and epiphytology. On sequencing the PCR products, the genomic characteristics of GRAV and sat-RNA yielded good quality sequences while those of GRV were of poor quality. GRAV isolates from Kenya (Ken_G10 and Ken_G2) clustered together in group II while the other clustered in group I. All Kenyan isolates clustered in the same clade exhibiting closest identity, and grouped together with AF195502.1 (from Malawi and Nigeria) and LC480459.1, LC480460.1 and LC480461.1 (from Kenya). The two GRAV Kenyan isolates are more similar to each other than with any other sequences implying common ancestry than with the other African isolates. The sat-RNA isolates from Kenya formed two distinct groups with sub-groups within the clusters. Isolates Ken_G11 and Ken_G6 clustered together in group II while Ken_G10 and Ken_G7 clustered together in group I. Kenyan isolates in group II seems to be unique compared to other Kenyan sat-RNAs. It is reasonably long branched suggesting a possible impact of a genetic bottleneck whose cause should be investigated. Ken_G6 clusters with other Kenyan sat-RNA isolates implying a possible identity by descent (IBD). For group I sequences, Ken_G7 appears to be different from the other Kenyan isolates in this clade. Ken_G10 clusters closely with the other Kenyan isolates in uniqueness and closeness. Also worth noting is that the sample size of sat-RNA in this clade is so minimal to prefer any conclusion at this stage thus recommend for further research. The clustering of isolates from within geographical regions in distinct groups, indicates that isolates within each group are distinct and are evolving

along discrete lineages unique to their regions of origin.

5. Conclusion

Groundnut rosette disease (GRD) is still the major disease of groundnuts in western Kenya, and is present whenever groundnuts are grown. Chlorotic rosette is the most prevalent symptom type on groundnuts followed by green rosette then mosaic rosette. The mosaic rosette symptom type is an emerging symptom evolving in groundnuts and could be due to dual infection by sat-RNA variants, or other agents inducing new disease encounter phenomenon. The genomic characteristics of the sat-RNA becomes more varied with wide geographical distance. The western Kenya sat-RNA variants of Ken_G6 and Ken_G11 were closely identical to LC472299.1 and AF202867.1 clustering in group II, while Ken_G7 and Ken_G10 were closely related to LC469779.1 and AF202869.1 clustering in group I. These new sat-RNA variants existing in western Kenya are probably contributing to the diverse symptoms expressed by GRD. The GRAV CP gene is less diverse even with wide geographical distance because its genome is highly conserved. All the two western Kenya GRAV isolates of Ken_G2 and Ken_G10 clustered together in group II showing close identity and unique to the other isolates.

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Conflict of Interest Statement

The authors declare that they have no competing interests.

References

[1] Deom, C. M., Naidu, R. A., Chiyembekeza, A. J., Ntare, B. R. & Subrahmanyam, P. (2000). Sequence diversity with the three agents of groundnut rosette disease. *Phytopathology.* 90: 214-219.

[2] Wangai, A. W., Pappu, S. S., Pappu, H. R., Okoko, N., Deom, C. M. & Naidu, R. A. (2001). Distribution and characteristics of groundnut rosette disease in Kenya. *Plant Disease.* 85 (5): 470-474.

[3] Mabele, A. S., Were, H. K., Ndong'a, M. F. O. & Mukoye, B. (2019). Distribution, molecular detection and host range of groundnut rosette assistor virus in western Kenya. *Journal of Plant Sciences. 7 (5): 100-105.*

[4] Mabele, A. S., Were, H. K., Ndong'a, M. F. O. & Mukoye, B. (2020). Occurrence and genetic diversity of groundnut rosette assistor virus in western Kenya. *Elsevier Crop Protection.* 139: 1-7.

[5] Todd, J. W., Culbreach, A. K. & Demski, J. W. (1993). Insect vectors of groundnut viruses. In: Reddy, D. V. R., McDonald, D. and Moss, J. P. (eds). Working together on groundnut virus diseases. *ICRISAT publication* 1994. pp 81.

[6] Anitha, S., Monyo, E. S. & Okori, P. (2014). Simultaneous detection of groundnut rosette assistor virus (GRAV), groundnut rosette virus (GRV) and satellite RNA (sat-RNA) in groundnuts using multiplex RT-PCR. *Archives of virology.* 159 (11): 3059-3062.

[7] Alegbejo, M. D. & Abo, M. E. (2002). Etiology, ecology, epidemiology and control of groundnut rosette disease in Africa. *Journal of Sustainable Agriculture.* 20 (2): 17-29.

[8] Naidu, R. A., Bottenburg, H., Subrahmanyam, P., Kimmins, F. M., Robinson, D. J. & Thresh, J. M. (1998). Epidemiology of groundnut rosette virus disease: current status and future research needs. *Annals of Applied Biology.* 132: 525–54.

[9] Waliyar, F., Kumar, P. L., Ntare, B. R., Monyo, E., Nigam, S. N., Reddy, A. S., Osiru, M. & Diallo, A. T. (2007). A Century of Research on Groundnut Rosette Disease and its Management. *Information Bulletin* no. 75. Patancheru 502 324, Andhra Pradesh, India. *International Crops Research Institute for the Semi-Arid Tropics,* pp. 40. ISBN: 978-92-9066-501-4.

[10] Naidu, R. A., Robinson, D. J. & Kimmins, F. M. (1998a). Detection of each of the causal agents of grountnut rosette disease in plants and vector aphids by RT-PCR. *Journal of Virology Methods.* 76: 9-18.

[11] Naidu, R. A., Kimmins, F., Holt, J., Robinson, D. J., Deom, C. M. & Subrahmanyam, P. (1999b). Spatiotemporal separation of groundnut rosette disease agents. *Phytopathology.* 89: 934-941.

[12] Olorunju, P. E., Ntare, B. R., Pande, S. & Reddy, S. V. (2001). Additional sources of resistance to groundnut rosette disease in groundnut germplasm and breeding lines. *Annals of Applied Biology.* 159: 259-268.

[13] Appiah, A. S., Offei, S. K., Tegg, R. S., & Wilson, C. R. (2016). Varietal response to groundnut rosette disease and the first report of groundnut ringspot virus in Ghana. *Plant Disease.* 100 (5): 946-952.

[14] Murant, A. F. & Kumar, I. K. (1990). Different variants of the satellite RNA of groundnut rosette virus are responsible for the chlorotic and green forms of groundnut rosette disease. *Annals of Applied Biology.* 117: 85-92.

[15] Scott, K. P., Farmer, M. J., Robinson, D. J., Torrance, L. & Murant, A. F. (1996). Comparison of the coat protein of groundnut rosette assistor virus with those of other luteoviruses. *Annals of Applied Biology.* 128: 77-83.

[16] Taliansky, M. E. & Robinson, D. J. (2003). Molecular Biology of umbraviruses*: Phantom warriors. Journal of General Virology.* 84: 1951-1960.

[17] Robinson, D. J., Ryabov, E. V., Raj, S. K., Roberts, I. M. & Taliansky, M. E. (1999). Satellite RNA is essential for encapsidation of groundnut rosette *umbravirus* RNA by groundnut rosette assistor luteovirus coat protein. *Virology*. 254: 104-114.

[18] Taliansky, M. E., Robinson, D. J. & Murant, A. F. (1996). Complete nucleotide sequence and organisation of the RNA genome of groundnut rosette umbravirus. *Journal of General Virology*. 77: 2335-2345.

[19] Kayondo, S. I., Rubaihayo, P. R., Ntare, B. R., Gibson, P. I., Edema, R., Ozimati, A. & Okello, D. K. (2014). Genetics of resistance to groundnut rosette virus disease*: African Crop Science Journal.* 22 (1): 21-29.

[20] Blok, V. C., Ziegler, A., Robinson, D. J. & Murant, A. F. (1994). Sequences of 10 variants of the satellite-like-RNA-3 of groundnut rosette virus. *Virology*. 202: 25-32.

[21] Taliansky, M. E. & Robinson, D. J. (1997). Trans-acting untranslated elements of groundnut rosette virus satelitte RNA are involved in symptom production. *Journal of General Virology*. 78: 1277-1285.

[22] Taliansky, M. E., Robinson, D. J. & Murant, A. F. (2000). Groundnut rosette disease virus complex: Biology and Molecular Biology. *Advances in virus research.* 55: 357-400.

[23] Mukoye, B. & Mabele, A. S. (2019). Genetic diversity of Groundnut rosette disease causal agents towards its management: A review. *International Journal of Genetics and Genomics.* 7 (1): 12-17.

[24] Mukoye, B., Mabele, A. S., Ndong'a M. F. O., Mangeni, B. C. & Were, H. K. (2019). Distribution of groundnut rosette disease and sequence diversity of groundnut rosette virus associated satellite RNA (Sat-RNA) in Western Kenya. *International Journal of Genetics and Molecular Biology.* 12 (1): 22-29.

[25] Kumar, S., Stecher, G., Li M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35: 1547-1549.

[26] Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution. 16: 111–120.

[27] Misari, S. M., Abraham J. M., Demski J. W., Ansa O. A., Kuhn C. W, Casper R. and Breyel E. (1988). Aphid transmission of the viruses causing chlorotic and green rosette diseases of peanut in Nigeria. *Plant Disease*. 72: 250-253.