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Occurrence and Molecular Characterization of Groundnut Ringspot Virus (GRSV) Infecting Groundnuts in Western Kenya

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Abstract: *Groundnut (Arachis hypogea) is an oilseed legume crop grown in Kenya on a small scale for income and nutritive value. However, its productivity remains low than its genetic potential of 1690 kg ha^{-1} attributed to biotic stressors. Viruses are among biotic stressors for low productivity globally. These include; Groundnut ringspot virus (GRSV), Tomato spotted wilt virus (TSWV),) among others. Groundnut ringspot virus had been reported in S.A, Ghana among others infecting groundnuts, peppers, watermelon, soybeans and many others. GRSV and TSWV have similar biological symptoms but differentiated serologically or by using molecular tests. Groundnut ringspot virus symptoms have been observed on groundnuts and other crops of economic importance in Kenya but no report had been documented on its occurrence or distribution. The objective was to Sequence nucleoprotein (N) genes of GRSV isolates obtained in a survey in western Kenya and compare with reference strains available in the GenBank. Plant GRSV Symptomatic samples were collected in a survey conducted in western Kenya and tested for GRSV using polyclonal antisera against GRSV. Samples that tested positive by DAS-ELISA was subjected to molecular tests. Total RNA was extracted from positive samples using CTAB and purified by DCC™-5 purification kit then using target primers GRSVnR (5'-GCGGTCTACAGTGTTGCACTT-3') and GRSVnF (5'TCTTGTGCATCATCCATTGT-3') used to amplify the nucleoproteins (N) genes, at 614-bp fragment of the nucleocapsid gene of GRSV corresponding to the part of the nucleocapsid (N) gene using RT-PCR tests. The PCR product sequenced. Sequence readings trimmed using Bio-edit software and phylogenetic analysis done in MEGA-X. Kenyan GRSV isolates clustered with Brazilian, USA, Argentinian, Ghanaian and South African isolates in GenBank. The study showed that GRSV occurs in surveyed counties of western Kenya, which is a big concern. Introgression of resistant genes into local groundnut varieties be done with urgency to come up with varieties resistant to GRSV.*

Keywords: *Nucleoprotein, RNA, GRSV, sequences, symptoms and molecular*

INTRODUCTION

1.1 Background of the study

Groundnuts (Arachis hypogaea L) is an oilseed legume crop of global importance grown by both smallholder and large commercial producers, for income (Kipkoech *et al.*, 2007) and nutritive value (Bajpai *et al*., 2017). World annual production is about 44 million tons (USDA, 2018) with China being the largest producer, followed by India, USA, Nigeria and Indonesia respectively (FAOSTAT, 2018). Groundnut is the 5th most widely grown crop in Sub-Saharan Africa behind maize, sorghum, millet and cassava (Rockstrom *et al*., 2003). Nigeria produces 30% of Africa's total yields, followed by Senegal and Sudan 8%, Ghana and Chad produce 5% total yield of Africa (Upadhyaya *et al*., 2006; Caliskan *et al*., 2008). In Kenya groundnuts are mainly grown in western Kenya and around Lake Victoria region (Ndisio, 2015). They are roasted or boiled and sold as snack in the streets and for manufacture of peanut butter in factories. Despite of its economic importance, yields of groundnuts in Kenya remains lower ;680kg ha^{-1} against its genetic potential of 1690 Kg ha^{-1} (FAO, 2015), due to unreliable rainfall, lack of high yielding varieties, pests and disease (Bucheyeki *et al*., 2008). Among diseases are viral diseases caused by; Tomato spotted wilt virus (TSWV), Groundnut bud necrosis virus (GBNV), Tobacco streak virus (TSV), Groundnut rosette assistor virus (GRAV), Groundnut rosette virus (GRV), Satellite RNA associated with GRV and/or GRAV, Peanut clump virus (PCV), Peanut stripe virus (PStV), Bean common mosaic virus (BCMV), Peanut mottle virus (PeMoV) and Cucumber mosaic virus (CMV) are of economic importance in groundnut production globally. Groundnut ringspot virus (GRSV) having similar biological symptoms to TSWV on infected host plant. Tomato spotted wilt virus also infects

groundnuts and tomatoes, watermelon, pepper and soybean (Webster *et al*.,2011). Groundnut ringspot virus in Africa has been reported in South Africa and Ghana on groundnuts (Pappu *et al*., 2009) and soybean (Pietersen *et al*., 2002). In Ghana, GRSV reported co-infecting groundnuts with groundnut rosette disease (Appiah *et al*., 2016). Although GRSV has been documented occurring in South Africa but no record indicates its distribution, incidence and severity. In Ghana, reported viral infection rates to 69.5% (Appiah *et al*., 2016). This disease was also noted on cucumber (*Cucumis sativus* L.) in Brazil (Spadotti *et al*., 2014), coriander (*Coriandrum sativum* L.), eggplant, pepper, tomato and tomatillo in Florida, USA (Webster *et al.*, 2011). Chlorotic ringspots and leaf mosaic are common symptoms in groundnuts (Appiah *et al*., 2016). In peppers and tomatoes, chlorotic and necrotic spots on leaves, deformed leaves and fruits, necrosis of stems and terminal growing points are observed. Early infected tomato plant leaves roll inwards and develop a bronze cast followed by dark brown flecks. Fruits show uneven ripening, and raised bumps or ring patterns on the surface (Webster *et al*., 2011). GRSV symptoms on soybean are not described. This virus was reported for the first time on tomatoes in Florida and Brazil (Adkins *et al.*, 2010). GRSV induce same symptoms to TSWV transmitted by thrips causing necrotic spots and flecks on tomato stems and leaves, chlorotic ringspot on leaves and fruits, deformation of leaves, necrotic lesions on stems and petioles on tomatoes and bumps on fruits with uneven ripening of tomato fruits affecting their quality.

Methods and Materials

3.1 Survey for Occurrence and distribution of GRSV in western Kenya.

Extensive survey was conducted in major groundnut growing regions of western Kenya in short and long rain seasons covering four agro ecological zones: low midland zone 1 (LM1), low midland zone 2 (LM2), low midland zone 3 (LM3) and upper midland zone 1 (UM1). Two hundred and seventy six farms were visited randomly and A GPS device (Magellan Triton "Windows CE Core 5.0" X11-15302) used to determine the coordinates and altitude of the location. One to two sampling units measuring 10m x 10m were selected randomly on each farm depending on farm size, Typical visual symptoms of virus observed, recorded and leaf samples was collected in falcon tubes for DAS ELISA tests. Positive samples were then taken RT-PCR. leaf samples collected to determine virus occurrence. Data obtained (GRSV incidence, severity and altitude) was recorded and subjected to ANOVA

3.1.1 Double Antibody Sandwich ELISA (DAS ELISA)

Double antibody sandwich ELISA was done as described by Clark and Adams (1977). For detection of GRSV in groundnut leaf samples. Microtiters plates were coated with GRSV IgG diluted 1:1000 (v/v) in coating buffer and incubated for 4 hours at 37 ^{0}C. Sample extracts added and incubate at 4 ^{0}C. Extracts from GRSV commercial positive and negative standards were used as control experiment to check for negative and positive samples to GRSV, respectively. IgG- alkaline phosphate conjugates diluted 1:1000 (v/v) in conjugate buffer added and incubated for 2 h at 37 ^{0}C substrate. The positive samples were subjected to molecular tests for the virus.

3.1.2 TOTAL RNA Extraction

Total RNA from naturally infected groundnuts samples collected in western Kenya and serologically tested positive for the virus (DAS ELISA) were extracted using the CetylTrimethyl Ammonium Bromide (CTAB) method modified from (Lodhi *et al*., 1994). Leave samples were placed in falcon tubes and crushed completely. 1 ml of CTAB buffer was added to the crushed sample and allowed to settle for 10 minutes. Later on, the liquid was decanted off to a clean 1.5 micro centrifuge tube. The samples were then centrifuged at 12,000rpm for 5 minutes at room temperature (21ºc). 700µl of the supernatant was decanted off to a clean 1.5 micro centrifuge tube. Equal volume of chloroform isoamyl was added and mixed thoroughly. The mixture was centrifuged at 12,000rpm for 5 minutes at 4ºc. After the centrifugation, the supernatant was transferred to clean 1.5 micro centrifuge tube. 500µl of ice-cold isopropanol was added and mixed properly. After this the mixture was left at room temperature for approximately 10 minutes. The mixture was then centrifuged at 12,000rpm for 10 min at 4ºc. After the centrifugation, the liquid was eluted off while being keen to make sure the pellet is intact. The pellets later washed with 500µl of 70% ethanol. The sample was then centrifuged at 12,000rpm for 5 minutes. The supernatant was discarded. The pellet was air dried for approximately 30 minutes. Then air drying the pellet was suspended in 100µl of nuclease free water (NFW). 2/3 volume of Ammonium acetate 5M (67µl) and 2.5 volumes of ice-cold ethanol (250µl) was added to the mixture which then solution was stored at -80ºc for 2 hours. Later on, the samples centrifuged at 12,000rpm for 20 minutes. The liquid was eluted carefully while making sure the pellet is intact. 500 µl of 70% ethanol was added after the elution. The sample was centrifuged at 12,000rpm for 5 minutes and the ethanol was decanted off while ensuring that the pellet was intact. The 1.5 micro centrifuge tube was allowed to air dry for approximately 30 minutes. 100µl of Nuclease free water was used to suspend the nucleic acid. Quantification using the Nano drop was done to ascertain the quantity and quality of the nucleic acid. Later on, the sample was stored at -20ºc awaiting PCR reaction.

3.1.3 Detection of the virus using RT-PCR and primers

All set of reactions were carried out in a final volume of 50µl, which consisted of 25µl of 2X master mix, 10µm (1µl) GRSVnF (5'TCTTGTGCATCATCCATTGT-3') and, 10µm of GRSVnR (5'GCGGTCTACAGTGTTGCACTT-3') which amplify a 614-bp fragment of the nucleoprotein genes of GRSV (DeBreuil *et al*., 2007). Superscript™ III RT/Platinum™ 2 µl, 20µl of Nuclease free water and 1µl of the RNA template was prepared for the required number of reactions. The extracted RNA was denatured at 55 ºc for 30 minutes. The cycling conditions for RT-PCR were: one cycle of reverse transcription at 55 ºc for 30 minutes, one cycle of enzyme inactivation at 94 ºc for 2 minutes, 40 cycles of denaturation at 94 ºc for 15 seconds, 40 cycles denaturation at 94 ºc for 15 seconds, 40 cycles of annealing at 55 ºc for 20 seconds, 40 cycles of extension at 68 ºc for 1 minutes and one cycle of final extension at 68 ºc for 5 minutes. Nested PCR was done. The product was amplified with the Qubit 2.0 Fluorometer Kit amplification module (Thermo fisher Scientific Inc.). Agarose gel (1.5%) was used to confirm the PCR amplification success. The components were mixed gently to ensure all the components are at the bottom of the amplification tube. Then centrifuged briefly in a microcentrifuge.

3.5.2 Visualization of the PCR products

1.5g of agarose was weighed and then dissolved in 100ml of TBE buffer. The mixture was heated in a microwave for 2 minutes to facilitate dissolving of the agarose. It was allowed to cool and then 3µl of gel stain Invitrogen brand was added into the mixture and swirled. The mixture was poured into a casting tray with combs in place and left to solidify for 20 minutes to form a hard matrix. The combs were then removed and 5µl of each of the sample from the PCR machine was mixed with 3µl loading dye and loaded onto the wells formed by the combs.1kb DNA ladder was also loaded and the casting tray was then placed in gel tank containing TE buffer and connected to an electric power supply. The samples were run at100V for 1hour then observed in Azure™ Gel dock.

3.5.3 Sanger sequencing and phylogenetic analysis

The RT-PCR amplicons were directly sequenced in both directions. The Bio- edit software was used on sequence editing and generating the consensus sequences. BLAST analysis of the sequence was done to determine library sequences that resembled the query sequence. The resulting nucleotide sequences then aligned using the programs Electropherogram quality analysis and CLUSTAL W (Thompson *et al*., 1994) The nucleotide and deduced amino acid sequences of the open reading frame were compared with the corresponding sequences of other tospoviruses deposited in the GenBank. Phylogenetic analysis was done using MEGA-X software version 5.0 (Tamura *et al*., 2011) and a phylogenetic tree constructed using the neighbor-joining method with the Kimura 2-parameter model (Kimura, 1980)

RESULTS.

4.1 Distribution of GRSV in western Kenya.

Typical symptoms of groundnut ringspot virus in groundnuts were noted on groundnuts and other host plants bordering infected groundnuts in some farms surveyed in western Kenya. These include; chlorotic-ring spots, necrosis ring spot, leaf deformation and stunted growth as described by (Camelo *et al*., 2014). Other viral symptoms observed on groundnuts, include, leave mosaic, leaf chlorosis, stunted growth, reduced height of groundnut plant and leaf deformation. Disease symptoms also noted on alternative hosts bordering groundnut farms. In tomatoes plants displayed; inward cupping of leaves and leaves develop bronze cast and dark spots (Webster *et al*., 2011), necrotic spots and flecks, chlorotic areas on leaves, deformation on leaves, necrotic lesions on stems, and petioles on tomatoes affecting the quality of the fruit, as well (Adkins *et al*., 2015) (Figure1).

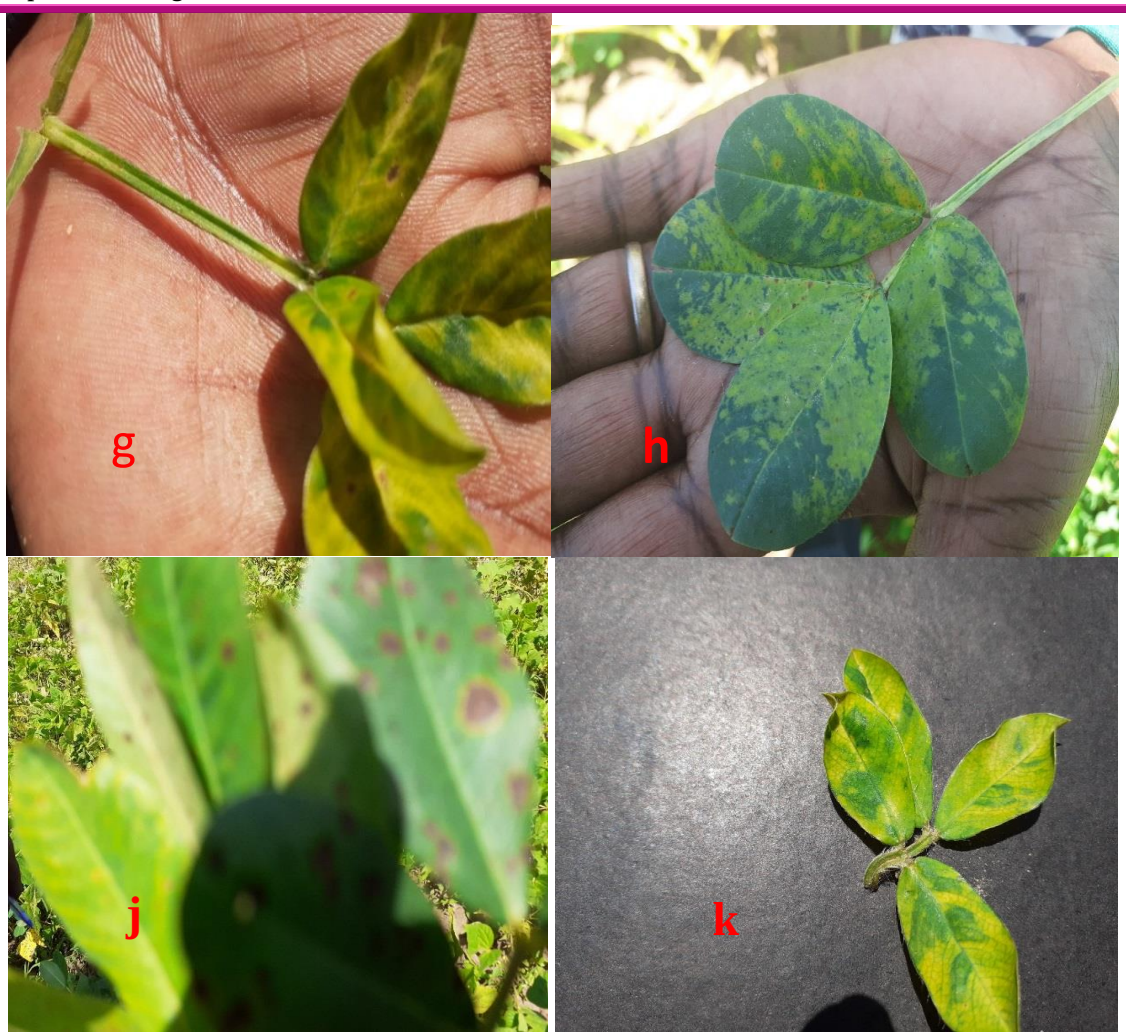

Figure 1: Groundnuts serologically testing positive for GRSV from western Kenya g) leaf from a Red Valencia groundnut from Alupe showing necrotic spots and chlorosis leaf veins. h) Leaf of Red Valencia groundnut from Chwele showing leaf mosaic symptoms. j) Leaf of ICGV129991 groundnut from Chwele showing necrotic ringspot on leaves and leaf mosaic. k) Leaf of ICGV129991 groundnut variety from Kimilili displaying upward leaf curling, leaf chlorosis. These are typical symptoms for GRSV

4.4 RT-PCR to detect GRSV

Six samples of groundnuts collected from symptomatic plants during survey in farms in a survey from Bungoma, Busia and Kakamega Counties of western Kenya, were tested by RT-PCR to detect GRSV in leaf samples using target GRSV primers. Total RNA eluted (Fig. 2).

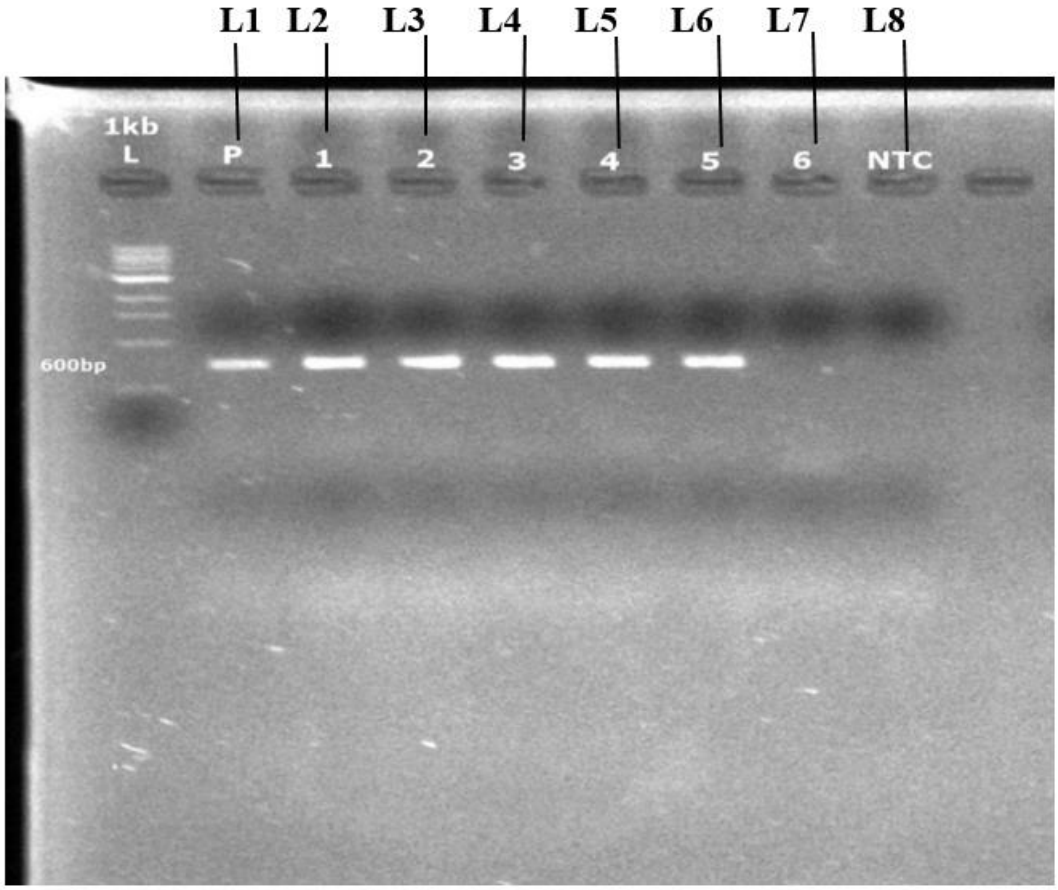

Figure 2: Gel electrophoresis of RT-PCR amplified RNA using primers specific for GRSV for symptomatic samples collected in a survey conducted in western Kenya. Expected band size was 600 bp. Lane L- 1 kb ladder, L1- positive control, L2- GRSV-KE1 groundnut sample from Chwele with stunted growth with necrotic leaf spot, L3- GRSV-KE2 groundnut sample from Alupe with leaf mosaic, L4- GRSV-KE3 groundnut sample from Chwele L5- GRSV-KE4 groundnut sample from Matungu L6- GRSV-KE5 groundnut sample from Kimalewa with leaf mosaic and necrotic leafspots L7- GRSV-KE6 groundnut sample from Chwele with chlorotic leafspots L8- Negative control

4.4.5 Comparison of Kenyan isolate nucleoproteins with those in GenBank

The six Kenyan GRSV isolates nucleoprotein(N) gene sequences (600 bp) was compared with GRSV isolates nucleoproteins (N)/nucleocapsid proteins gene sequences available in GenBank from other countries. The comparison revealed that Kenyan isolates had 96.13 to 99.98 % identity with GRSV isolates available in GenBank. Kenyan Isolates; GRSV-KE1 with accession number (LC616779), GRSV-KE2 (LC616780), GRSV-KE3 (LC616781), GRSV-KE4 (LC616782), GRSV-KE5 (LC616783) and GRSV-KE6 (LC616784) had very close percentage identity with isolates from different alternative of various counties. Brazilian soybean Isolate LEM (MH686229.1) had closest identity of 99.93% followed by USA infecting insect isolate +GRSV (HQ634665.1) had identity of 99.82 %. Brazilian Pisum sativum isolate (ER1) (KY778230.1) had identity of 99.30 %. Tomato isolate (SA-05) of accession number (MH742958.1) from South Africa had identity of 99.28 %. Groundnut isolates from South Africa (SA-05) (Accession number S54327.1) and Ghana (GRSV-N-Gh) of accession number KT345728.1 had identity of 97.56 and 98.02 % respectively with Kenyan isolates. Other host plants with close identity with Kenyan isolates; watermelon isolate (GRSV leaves) (MN364668.1) from Brazil had identity of 96.89 %, Solanum americanum isolate (11.102) of accession number KM007024 from USA had identity of 96.13 % and Glycine max isolate (S30) of accession number MG029625 from Brazil had identity of 96.62 %. In general, all western Kenya isolates exhibited close identity and grouped together with some isolates, from Argentina, Brazil, Ghana, USA, South Africa of all tested host range. table 1

Table 1. Comparison of Kenyan GRSV isolates nucleoproteins with other isolates from other countries in GenBank.

Description	Scientific name	Host plant	Country	Query cover %	E Value	Per Ident %	Acc Les	Accession number
Groundnut ringspot virus isolate	GRSV	Tomato	S. Africa	98	0.0	99.28	3038	**MH742958.1**
Groundnut ringspot virus isolate	GRSV	Peanut	Argentina	90	0.0	96.38	777	**MT423636.1**
Groundnut ringspot virus isolate	GRSV	Peanut	Argentina	90	0.0	96.38	777	**MT423645.1**
Groundnut ringspot virus isolate	GRSV	Peanut	Argentina	90	0.0	97.40	777	**MT423626.1**
Groundnut ringspot virus isolate	GRSV	Watermelon	Brazil	98	0.0	96.89	3074	**MN364668.1**
Groundnut ringspot virus isolate	GRSV	Peanut	Brazil	98	0.0	97.04	3069	**KY400110.1**
Groundnut ringspot virus isolate	GRSV	Solanum Americanum	USA	76	0.0	96.13	542	**KM007024.1**
Groundnut ringspot virus isolate	GRSV	Soybean	Brazil	97	0.0	99.93	3040	**MH686229.1**
Groundnut ringspot virus isolate	GRSV	Peanut	Brazil	76	0.0	96.93	522	**KF511798.1**
Groundnut ringspot virus isolate	GRSV	Pisum sativum	Brazil	78	0.0	99.30	557	**KY778230.1**
Groundnut ringspot orthotospovirus isolate	GRSTV	Glycine max	Brazil	81	0.0	96.62	562	**MG029625.1**
Groundnut ringspot virus isolate	GRSV	Thrips	Brazil	99	0.0	97.04	3074	**MG797643.1**
Groundnut ringspot virus isolate	GRSV	Soybean	S. Africa	98	0.0	98.67	857	**AF487516.1**

Groundnut ringspot virus isolate	GRSV	Soybean	S. Africa	98	0.0	98.37	857	**AF487517.1**
Groundnut ringspot virus isolate	GRSV	Peanut	Ghana	88	0.0	98.02	768	**KT345728.1**
Groundnut ringspot virus isolate	GRSV	Groundnut	S. Africa	98	0.0	97.56	928	**S54327.1**
Groundnut ringspot virus isolate	GRSV	Groundnut	Kenya		0.0	99.99	6041	**LC616779** ★
Groundnut ringspot virus isolate	GRSV	Groundnut	Kenya		0.0	99.98	6041	**LC616781** ★
Groundnut ringspot virus isolate	GRSV	Groundnut	Kenya		0.0	99.98	6041	**LC616782** ★
Groundnut ringspot virus isolate	GRSV	Groundnut	Kenya		0.0	99.9	6041	**LC616780** ★
Groundnut ringspot virus isolate	GRSV	Infecting insect	USA	83	0.0	99.82	569	**HQ634665.1**
Groundnut ringspot virus isolate	GRSV	Groundnut	Kenya		0.0	100	6041	**LC616784** ★
Groundnut ringspot virus isolate	GRSV	Groundnut	Kenya		0.0	100	6041	**LC616783** ★

Table1: showing accession number of GRSV isolates nucleoproteins in GenBank from different countries and sources with identity percentage to Kenyan isolates. Kenyan isolates accession numbers are flagged with a star.

4.5 Phylogenetic analysis of Kenyan isolates

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei., 1993). Groundnut rosette assistor virus (LC480463.1) was used as an out-group that gave a better rooting stability than the other possible Tospoviruses; Tomato chlorotic spot virus (TCSV), Irish yellow spot virus (IYSV), Tomato spotted wilt virus (TSWV), Impatians necrotic spot virus (INSV). Phylogenetic tree constructed in MEGA X for evolutionary comparison revealed the clustering of the six Kenyan isolates forming two groups with other isolates available in GenBank. Kenyan isolates have a common ancestral origin with isolates from South Africa, Ghana, Brazil, Argentina and USA. GRSV-KE5 (LC616783) had most recent evolutionary origin with USA isolate (HQ634665) having closest clustering with 99.82 % identity. Kenyan isolate GRSV-KE6 (LC616784) and GRSV-KE2 (LC616780) each had 97.56 % identity SA (S54327.1). The comparison reveals that Kenyan isolates GRSV-KE6, GRSV-KE2 and South African isolate SA (S54327.1) are monophyletic (have same recent evolutionary origin on phylogenetic tree). Kenyan groundnut isolates GRSV-KE4 (LC616782), GRSV-KE3 (LC616781) and GRSV-KE1 (LC616779) showed monophyletic relationship with soybeans isolates (AF487516.1), (AF487517.1) and groundnut isolates SA (S543227.1) of south Africa and Peanut isolate (KT345728.1) from Ghana with identity of 98.67%, 98.37%, 97.56% and 98.02% respectively. Kenyan

isolates had paraphyletic relationship with Brazilian isolates; peanut isolate (KF511778.1), Pisum sative isolate (KY778232.1), Glycine max isolate (MG029625.1) and Thrips isolate (MG797643.1) with identity of 96.93%, 99.31%, 96.62% and 97.04 respectively. Although the Kenyan isolates had common ancestral origin with some isolates available in GenBank, formed divergent cluster with the following isolates; tomato isolate (MH742958.1) of South African with identity 99.28%, Brazilian watermelon isolate (MN364668.1) with identity of 96.89 %, USA Solanum americanum isolate (KM007024) with identity of 96.13 %. and Brazilian soybeans isolate (MH686229.1) with identity of 99.93 %. Some isolates of different species available in GenBank also exhibited divergent from Argentinian peanut isolates; MT423636.1, MT423642.1, MT423645, all having identity of 96.38 and MT423626.1 with identity of 97.40 %. In general, all western Kenya isolates exhibited closest identity and grouped together with some South African, Brazilian, Ghanaian isolates. figure 16 and table11

4.5 Phylogenetic tree of Kenyan isolates

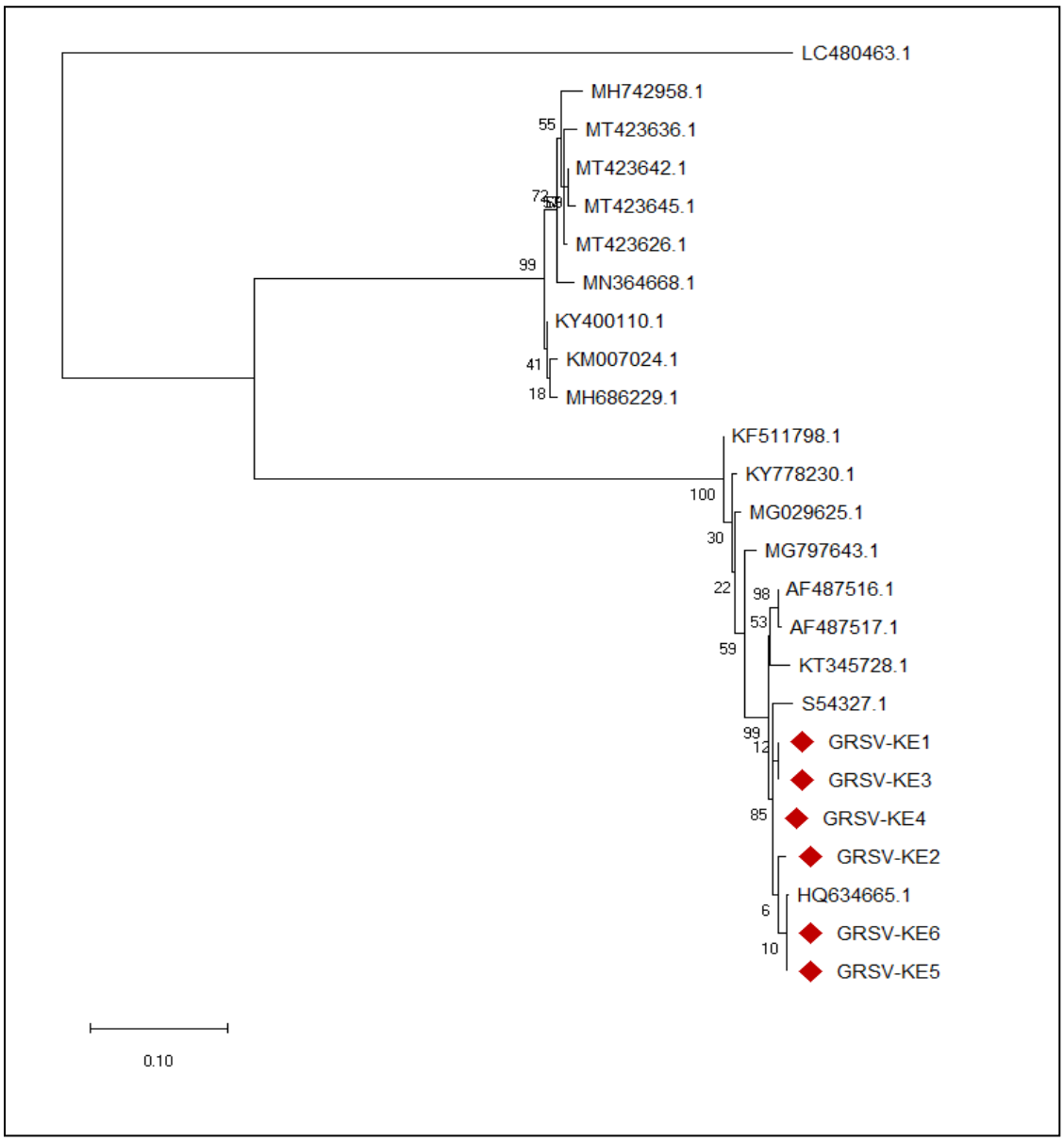

Figure 3. Phylogenetic analysis of six GRSV Kenyan isolates (flagged red) and some GRSV isolates from GeneBank. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [Tamura and Nei, 1993]. The percentage of trees in which the associated taxa clustered together is shown next to the branches (1000 replications). Evolutionary analyses were conducted in MEGA X [Kumar et al., 2018]. LC480463.1 (Groundnut rosette assistor virus) was used as an outgroup

The Kenyan GRSV isolates nucleoproteins (N) shared 96.13-99.98 % identity with those available in the GenBank. This implies that there is close identity among GRSV nucleoproteins from western Kenya with other GRSV gene isolates available in GenBank which confirms that they are not new viruses (King *et al*., 2012). The six GRSV isolate nucleoproteins (N) of western Kenya (GRSV-KE1, GRSV-KE2, GRSV-KE3, GRSV-KE4, GRSV-KE5 and GRSV-KE6) clustered together with each other on phylogenetic tree with identity up to 100 %. This implies that they have same recent evolutionary origin and from same geographical region thus share same transmitting vectors and alternative host for the virus (Erkenbrack *et al*.,2019). This finding concurs with Wangai *et al*., (2001) and Appiah *et al.,* (2017) who observed closer identity between sequences from the same geographical region as compared to those from separate geographical regions. The six Kenyan GRSV isolates nucleoproteins had 96.13 to 99.82 % identity with isolates of different species available in the GenBank; soybeans, watermelon, tomato, Solanum americanum, Pisum sativum, Thrips and infecting insects of South Africa, Ghana, Brazil, USA and Argentina. This implying that the genetic sequence of Kenyan GRSV isolates of groundnuts was same with genetic sequence of GRSV from other plant species (Kweon *et al*.,2020). This is an indication that GRSV RNA is more stable to mutation from one alternative host to the next thus very few strains of GRSV occurs in hosts (Peris *et al*.,2010). although this virus is picked by thrips does not undergo gene alteration along the transmission process (Sharp *et al*.,2011). Kenyan GRSV isolates; GRSV-KE4 (LC616782), GRSV-KE2 (LC616780), GRSV-KE3 (LC616781) and GRSV-KE1 (LC616779) exhibited closest evolutionary origin with groundnut isolates of South Africa (S54327.1) with identity of 97.56 %, Ghanaian's groundnut isolate (KT345728.1) with 98.02 % identity and Brazilian groundnut isolate (KF511798.1) with identity of 96.93 %. It is worth noting that the Kenyan isolates had highest in identity 99. 82 % with USA isolates of infecting insect (HQ634665.1) having same closest evolutionary origin with GRSV-KE6 (LC616784) and GRSV-KE5 (LC616783). This implies that the Kenyan isolates had recent evolutionary origin with those from South Africa, Ghana and Brazilian isolates, that is why they clustered together with closest identity (Harkins *et al*.,2017). Kenyan nucleoproteins of GRSV-KE6 (LC616784) and GRSV-KE5 (LC616783) had same evolutionary node on phylogenetic tree and clustered together with infecting insect isolate (HQ634665.1) from USA with identity of 99.83 %, may imply that the infecting insects of GRSV in USA belong in the same clade with vectors transmitting GRSV into groundnuts in western Kenya (Jehle *et al*., 2006).

Kenyan GRSV isolates had close identity 96.13 – 99.93 % with Solanum americanum isolate (KM007024) of USA, peanut isolate (MT423642.1) of Argentina, peanut isolate (MT423626.1) of Argentina, watermelon isolate (MN364668.1) of Brazil, peanut isolate (KY400110.1) of Brazil, tomato isolate (MH42958.1) of South Africa and soybean isolate (MH686229.1) of Brazil but did not cluster together. This implies that these isolates had same ancestral origin but due to wide variation in geographical region resulted into differences in environmental conditions causing variations in evolution of GRSV (Appiah *et al.,* 2017). In general, all GRSV nucleoprotein gene sequences in this study and those in GenBank shared 96.13-100% nucleotide identity. This implies that GRSV nucleoprotein gene is highly conserved across the wide geographical region globally (Zheng *et al*.,2005). Basing on this characteristic of fitness of GRSV gene to mutation can for development of pathogen resistant cultivars of groundnuts through genetic engineering that can be used globally (Deom *et al*., 2000; Appiah *et al*., 2017).

Conclusion

Nucleoproteins of GRSV Kenyan isolates had closest identity with those in the GenBank despite of their varied wide geographical distance. The western Kenya GRSV groundnut isolates were closely identical with those of Ghana, South African, Brazilian, USA and Argentina. Kenyan groundnut isolates had closest identity with isolates of other species; Soybeans, from south Africa and Argentina, Solanum americanum of USA, Watermelon isolate of Brazil, Pisum sativum isolate of Brazil, Glycine max of Brazil, Thrips of Brazil, infecting insect of USA and Tomato isolate of S.A, apart from peanut isolates available in GenBank.

Recommendations

This study Revealed that Nucleoproteins (N) genes sequences of Kenya isolates infecting groundnuts have close identity with those nucleoproteins and nucleocapsid sequences available in the GenBank, therefore to manage the transmission of GRSV within and between nations, there is need for introgression of resistant gene into groundnut varieties that are productive but susceptible to the virus to improve on their resistance (Culbreath et al.,2003). KEPHIS to reinforce importation regulation and rules on contaminated farm inputs to control vectors being imported into the Country (Elena *et al*.,2014).

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