International Journal of Academic and Applied Research (IJAAR)
ISSN: 2643-9603
Vol. 5 Issue 6, June - 2021, Pages: 13-25

Epidemiology And Occurrence Of Groundnut Ringspot Virus (Grsv) Infecting Groundnuts In Western Kenya

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ABSTRACT: *Groundnuts (Arachis hypogea) is an annual oilseed legume crop grown by small holder farmers in Kenya for its economic and nutritive value. However, its yields have declined upto 680 kg ha^{-1} than its genetic potential of 1690 kg ha^{-1} attributed to abiotic and biotic factors. Biotic factors are of economic importance which include Pests and diseases. Viruses are among biotic factors for yield reduction globally. These include; Tomato spotted wilt virus (TSWV) Groundnut bud necrosis virus (GBNV) among others. Groundnut ringspot virus (GRSV) has been reported in South Africa, Ghana, Brazil, USA and Argentina infecting groundnuts, tomatoes, watermelon, soy beans, peppers among others. GRSV and TSWV have similar biological symptoms thus can only be distinguished either serologically or by molecular tests. GRSV Symptoms have been noted on groundnuts and other hosts in Kenya but no documented report on its epidemiology, prevalence and distribution of GRSV in Kenya. The objective of the study was to determine prevalence and distribution of GRSV on groundnuts in western Kenya. Diagnostic survey was conducted in all agro ecological zones of western Kenya (LM1, LM2, LM3 and UM1) during short and long rain seasons. Disease incidence and severity observed, recorded and samples taken for serological and molecular tests. Leaf samples collected for serological analysis used polyclonal antisera against GRSV and TSW. GRSV occurs in surveyed regions with variant incidence; Chwele having the highest incidence (45.04 %) followed by Kimilili with incidence of 39.95 %. In some regions GRSV and TSWV co-infected groundnuts displaying same symptoms. Kapkateny region had the lowest incidence (17.75 %) with significant difference of (0.05). The study showed that GRSV occurs in surveyed counties of western Kenya with variant incidence and severity which is the first report in Kenya and third country in Africa to report on occurrence of GRSV which is a big concern. Introgression of resistant genes into local groundnut varieties to gain resistance to the virus be done with urgency. Additionally, farmers be advised to adopt integrated pest management to minimize thrips from transmitting virus from primary host.*

Keywords: GRSV, TSWV, Thrips, Incidence, Severity and prevalence

INTRODUCTION

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 (containing 48% edible oil and 25% crude proteins) (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, 2015). The crop is grown between 40° North and 40° South (Kumar *et al.*, 2007). 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). These viruses (GRSV and TSWV) belong to genera Tospovirus with same transmitting vectors. 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.

MATERIAL AND METHODS

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

Extensive diagnostic survey was conducted in 536 groundnut growing farms in three major groundnut growing counties (Bungoma, Busia and Kakamega) of western Kenya in short rain seasons of 2019 and long rain seasons of 2020. The survey covered 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). A total of 276 farms were visited in short rain seasons (September to December) of 2019 and 264 farms in long rains seasons (march to July) of 2020 covering four agro ecological zones. Eleven cluster regions were created based on agro ecological zones of western Kenya; Chebich (UM1), Kapkateny (UM1), Kimalewa (LM1), Kimilili (LM1), Chwele (UM1), Alupe (LM3), Chakol (LM3), Malaba (LM2), Mumias (LM2), Matungu (LM2) and Muhonje (LM1) Jaetzold *et al*., (2006). Simple random sampling design used to select farms to visit for data collection in each cluster. Total number of farms selected for study (sample size) in each cluster was based on number of farms for groundnuts in each cluster. One to two sampling units measuring 10m x 10m were selected randomly on each farm depending on farm size. Data obtained (GRSV incidence, severity and altitude) was recorded and Symptomatic leaf Samples taken for serological and molecular Analysis. A GPS device (Magellan Triton "Windows CE Core 5.0" X11-15302) was used to determine the coordinates and altitude of the location for survey to establish Agro ecological zones for study.

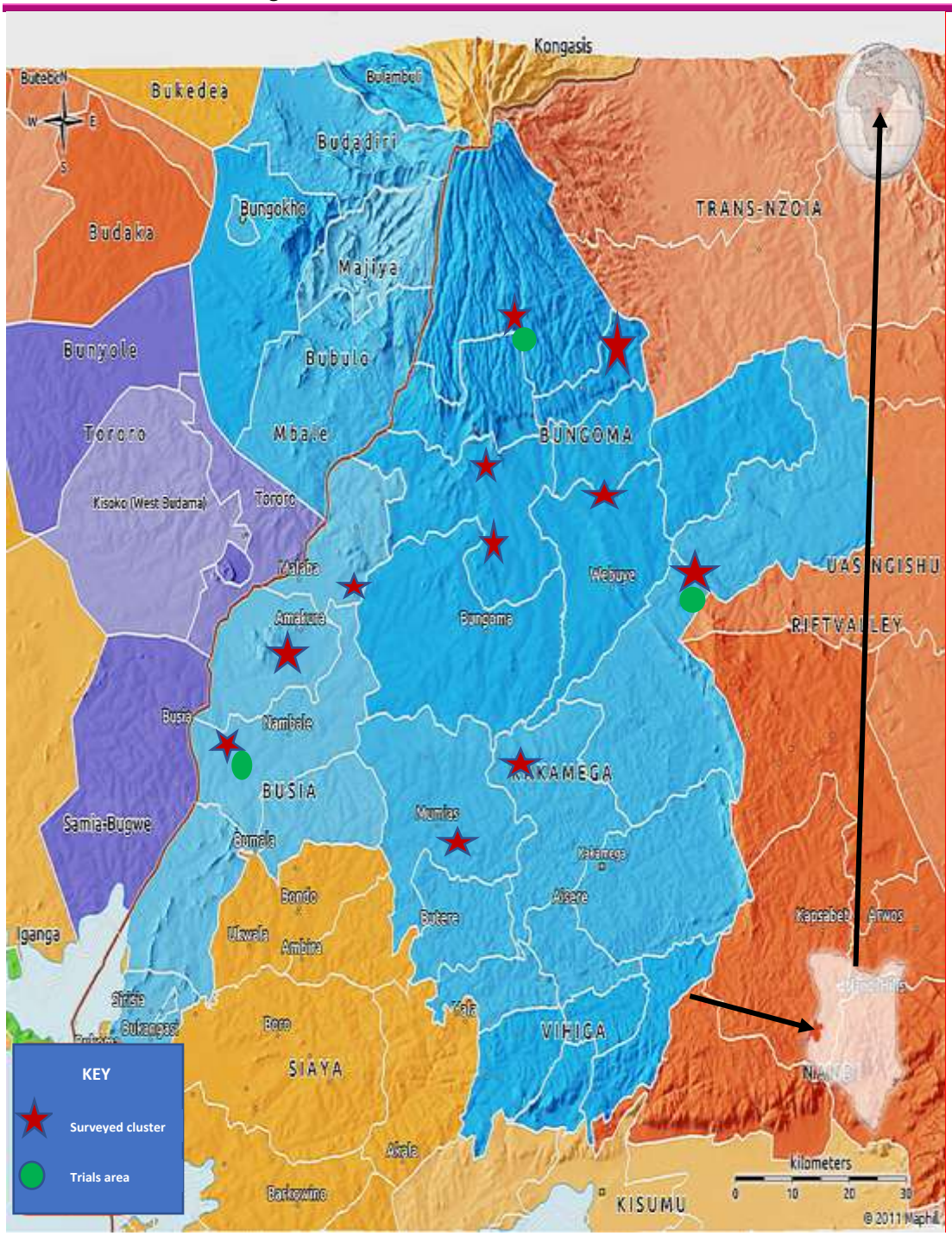

Figure 2: A map of western Kenya showing areas where Trials on effect of intercropping other legumes with groundnut varieties on incidences and severity of viral diseases on groundnuts and cluster areas for surveys on occurrence and distribution of groundnut ringspot virus.

3.1.1 Disease incidence and severity determination

Viral symptoms were recorded to determine the disease incidence and severity in each farm. Type of groundnut varieties grown and sources of seeds grown also recorded. Sampling points randomly selected on each farm. On each farm, incidence and severity were determined in an area of 10 m^2. A maximum of two sampling quadrats was sampled depending on farm size. Disease incidence calculated as

percentage of plants showing GRSV disease symptoms to the total number of plants observed in the field. The average incidence and severity of the sampling points per farm was use as the actual plot disease incidence and severity. The degree of disease (GRSV) incidences was assessed and analyzed according to (Nono-Womdim, 1996) as the proportion of diseased plants in an area.

Disease inci No. of plants infected x 100
Total number of plants observed

The presence and absence of viral disease on groundnuts varieties planted was scored using a rating scale basing on (Nono-Womdim, 1996) where low incidence=1-20%, moderate incidence= 21-49% and high incidence=50-100%. Disease severity was scored on a scale of 1-4, as described by Lyerly *et al*. (2002) and Nascimento *et al*. (2006), the scale used to evaluate the severity of symptoms of TSWV and adapted for GRSV since they belong in the same genus and have similar symptoms, where

1: plants without symptoms.
2: for leaves with symptoms of yellowing, mosaic, and/or chlorotic spots,
3: plants with symptoms of yellowing, mosaic or chlorotic spots, and height reduction,
4; for chlorotic plants and stunting symptoms.

These symptoms were used to score for disease severity in groundnuts and symptomatic leaf samples was collected in a cool box and falcon tubes for both serological (DAS-ELISA/ TAS ELISA) and molecular test for GRSV.

3.1.2 Enzymes- Linked Immunosorbent Assay (ELISA)

Detection of GRSV viral titre on leaf samples by serological techniques was based on the ability of the specific antibodies to react in the vitro with their antigens (virus particle), used polyclonal antibodies (IgG) for detection. Microtiter plants (Grainer microloan medium binding) was used and the volume for each reactant, kept at 100µl. between incubations, 3 intensive washing steps each lasting 3 min, carried out by repeated soaking of the plates in washing buffer for 4 minutes. The following buffers was used;

Coating buffer, pH 9.6 (per litre)

1.59 g Sodium carbonate (Na_2CO_3).

2.93 g Sodium bicarbonate ($NaHCO_3$)

0.20 g Sodium oxide (Na_2O)

dissolved in 900 ml H_2O

PBS (pH 7.4) phosphate buffer Saline

8.00 g Sodium chloride (NaCl)

0.20 g monobasic potassium phosphate (KH_2PO_4).

1.15 g Dibasic Sodium phosphate (Na_2HPO_4)

0.20 g potassium chloride (KCl)

0.20 g Sodium oxide (Na_2O)

Was dissolved in 900ml H_2O pH and adjusted from 7.4 to 11 with NaOH

PBS- Tween (PBST)

PBS+0.5 ml Tween 20 per litre

Sample extraction buffer (pH 7.4).

PBST+2% pvp (pvp- is polyvinyl pyrrolidone).

Conjugate buffer

PBST+2% pvp+0.2% egg albumin

Substrate buffer

97 ml diethanolamine

600 ml H_2O

0.20 g Sodium oxide (Na_2O)

Adjusted to pH 9.8 with HCl and make up to 1 litre with H_2O.

3.1.3 Double Antibody sandwich ELISA (DAS ELISA)

Double antibody sandwich ELISA done with no modification as per Clark and Adams (1977). For detection of GRSV in groundnuts 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 healthy groundnuts varieties and those of infected with known GRSV used as negative and positive controls, respectively. IgG- alkaline phosphate conjugates diluted 1:1000 (v/v) in conjugate buffer added and incubated for 2 h at 37 ^{0}C substrate.

3.1.4 Triple Antibody Sandwich ELISA (TAS ELISA)

The TAS ELISA was done as described by Were *et al*., (2013) Without modifications microlitre plates (96 wells) was coated with TSWV IgG diluted 1:1000 (v/v) in a coating buffer and incubated for 2 h at 37 °C. Blocking was done by adding 2% skimmed milk in PBST (200 µ1/well) and incubated for 30 min at 37 °C. Sap extracts sample was added and incubated at 4°C. Extracted from a healthy plant (groundnut) and those infected with TSWV were used as negative and positive controls, respectively. MAbs raised against TSWV was used in detecting antibodies at dilution of 1:100 (v/v) in conjugate buffer were used for detection. 100µ1 of each supernatant dilution was loaded onto microtitre plates and incubate for 2 h at 37°C. After washing the plates, an alkaline phosphate labeled phosphate as (99Rabbit- anti- mouse) diluted 1:1000 (v/v) in conjugate buffer was added and the plate incubated

for 45 min at 37 ºC. The substrate, P-Nitrophonyl phosphate diluted 1mg/ml in substrate buffer was added and incubate for 2 h at 37 ºC.

3.4.1 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 nucleocapsid gene 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.

RESULTS AND DISCUSSION

4.1 Occurrence and distribution of GRSV in western Kenya.

Typical symptoms of groundnut ringspot virus in groundnuts and tomato spotted wilt virus was noted on groundnut plants and other host plants bordering infected groundnuts in some farms surveyed in some agro ecological zones of western Kenya as shown in figure 3. These include; chlorotic-ring spots, necrosis ring spot, leaf deformation and stunted growth as described by (Camelo *et al*., 2014).

Figure 3: a) Groundnut plant serologically tested positive for GRSV and TSWV co-infecting the plant showing typical GRSV symptoms; chlorotic and necrotic ringspots with leaf deformation from Kimilili region in Bungoma county. b) Groundnut plant with no viral symptoms serologically tested negative for GRSV from Mumias from kakamega county of western Kenya.

Other viral symptoms observed on groundnuts, include, leave mosaic, leaf chlorosis, stunted growth, reduced height of groundnut plant and leaf deformation as shown in the figure 4. 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) additional symptoms include dark streaks on the main stem and wilting of the top portion of the plant. Necrotic lesions on stem and petiole epidermal tissues present. Fruit deformed with uneven ripening and raised bumps on the surface on tomato fruits (Webster et al.,2011).

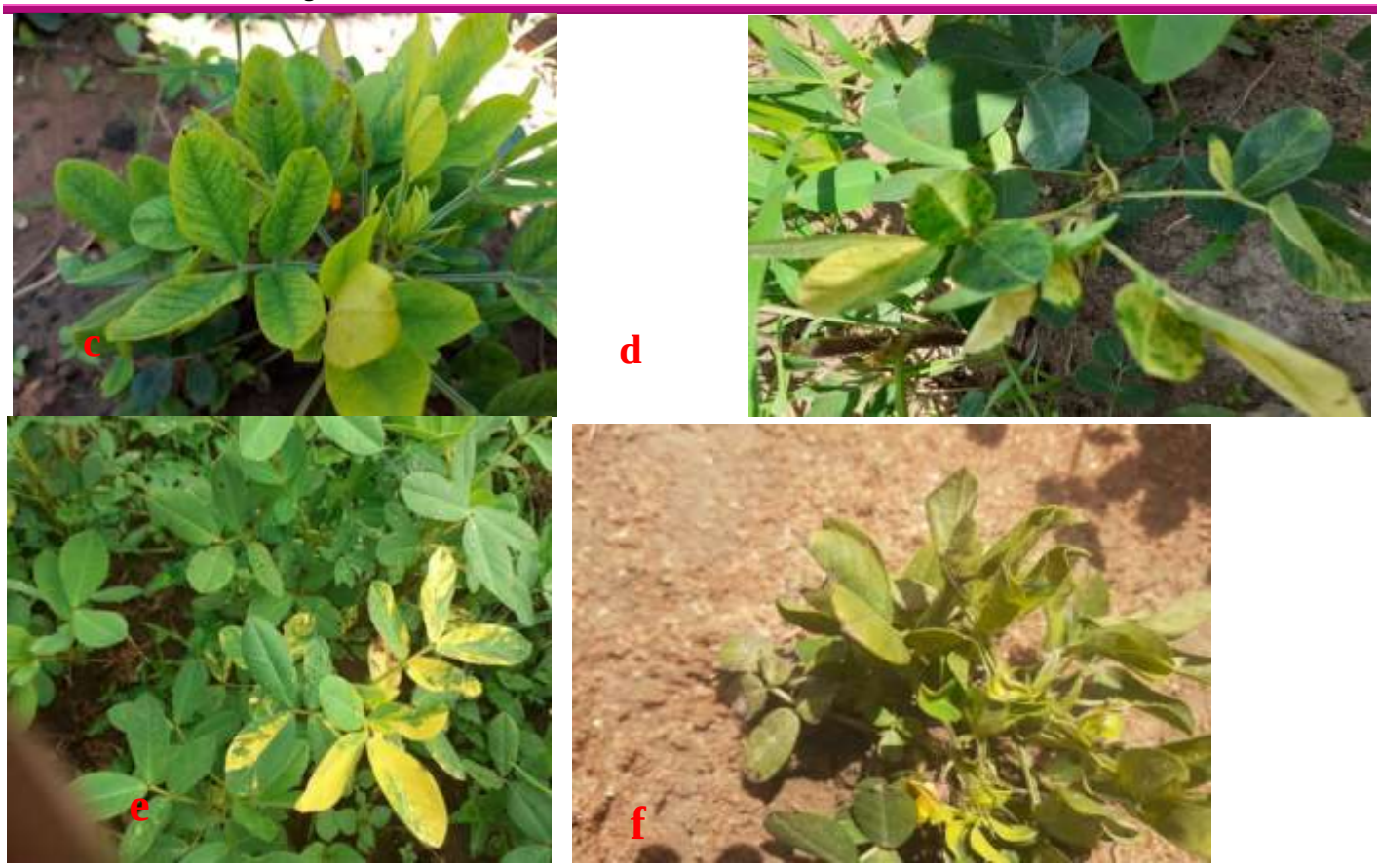

Figure 4. Groundnut plants obtained from western Kenya displaying symptoms of viral symptoms. c) groundnut plant from Aupe in Busia county with stunted growth, d) groundnut plant from Chebich in Bungoma county with leaf mosaic, e) groundnut plant from Muhonje in kakamega county with leaf chlorosis symptoms and f) groundnut plant from Chwele in Bungoma county with deformed leaves, necrosis and stunted growth.

4.1.1 Field symptoms and Sample analysis

Viral symptoms observed in all agro ecological regions surveyed with varied incidence. Chwele (LM1) had the highest disease mean incidence of (45.04 %), with maximum mean of (80 %). Chebich (UM1) was second with mean incidence of (41.19 %) followed by Kimilili (LM1) with viral incidence of (39.19 %). while kapkateny (LM1) region had lowest disease incidence (17.75 %) with maximum incidence of (60 %). Most of surveyed regions(clusters) of western Kenya showed moderate disease incidence (25.12 to 37.83 %). (Table 1). Post hoc ANOVA by SPSS, used to obtain least significant difference (L.S.D) to separate the means at p=0.05 showed positive correlation (r= 0.745; p<0.0001) between incidence and severity of GRSV.

Table 1. GRSV incidences in groundnut growing regions of western Kenya

County	Cluster	Mean Incidence	Max Incidence	Min Incidence	Std error
Bungoma	Chwele	45.04	80	0	2.43
Bungoma	Kapkateny	17.75	60	0	2.19
Bungoma	Kimalewa	28.26	75	0	1.92
Busia	Alupe	37.83	68	0	2.18
Busia	Chakol	25.12	56	0	3.02
Busia	Malaba	25.61	80	0	2.19
Kakamega	Mumias	27.36	80	0	3.23
Kakamega	Matungu	25.67	70	0	2.69
Kakamega	Muhonje	35.72	75	0	3.57
Bungoma	Kimilili	39.75	75	0	2.15
Bungoma	Chebich	41.19	76	0	2.67

4.1.2 Disease severity in surveyed regions of western Kenya

Disease severity occurred in all surveyed clusters with variations, Chwele had the highest disease severity (2.98) with maximum severity of (4) and minimum of (1), followed by Chebich with disease severity of (2.86) then Malaba with severity of (2.77). Kapkateny had lowest with disease severity of (2.22). Table 2 and figure 4.

Table 2. Disease severity in groundnuts growing regions in western Kenya

County	Cluster	Mean Severity	Max Severity	Min Severity	Std Error
Bungoma	Chwele	2.98	4	1	0.10
Bungoma	Kapkateny	2.22	4	1	0.11
Bungoma	Kimalewa	2.65	4	1	0.09
Busia	Alupe	2.65	4	1	0.13
Busia	Chakol	2.31	4	1	0.12
Busia	Malaba	2.77	4	1	0.12
Kakamega	Mumias	2.40	4	1	0.13
Kakamega	Kimilili	2.68	4	1	0.11
Kakamega	Muhonje	2.62	4	1	0.15
Bungoma	Matungu	2.76	4	1	0.14
Bungoma	Chebich	2.86	4	1	0.19

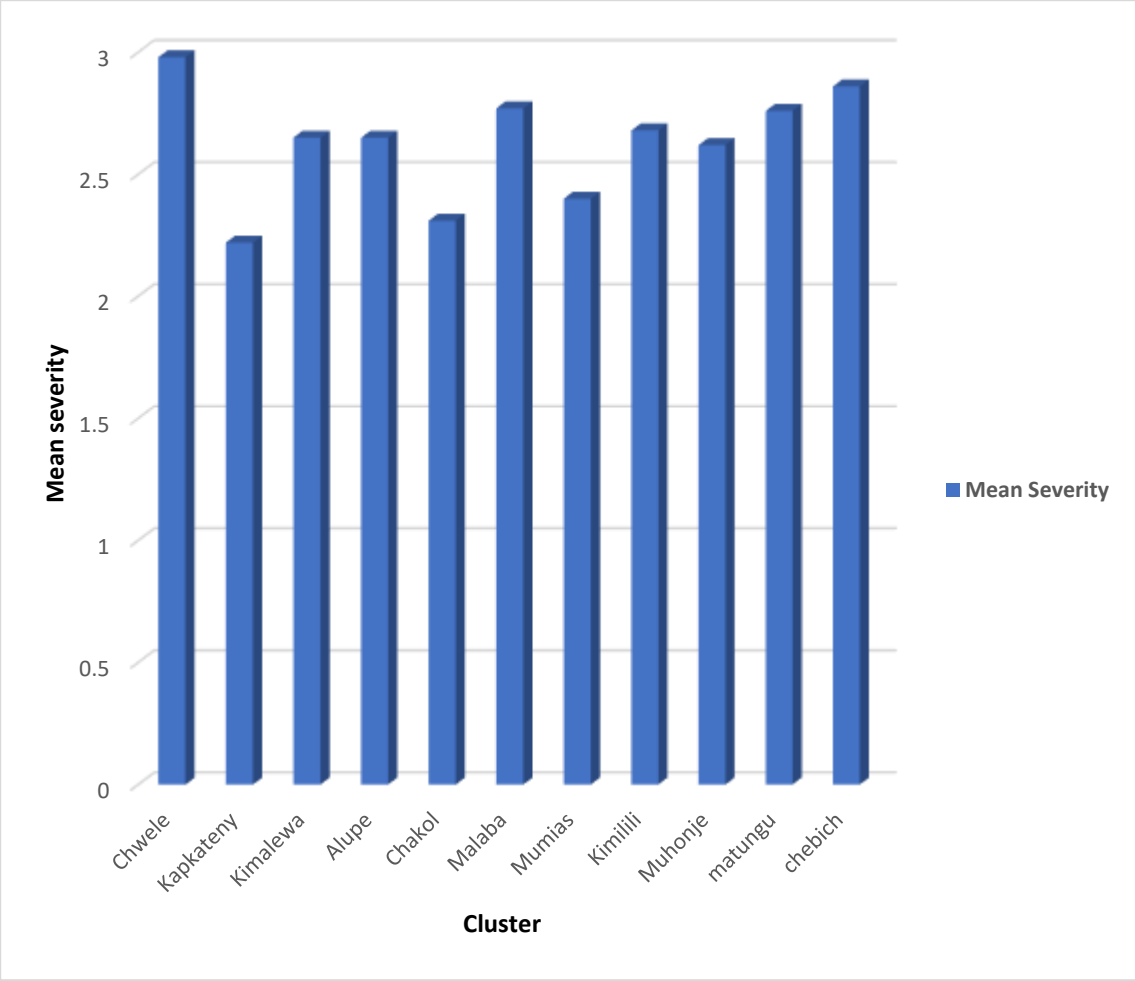

Figure 5: Graph of mean severity of viral disease on groundnuts in surveyed clusters in western Kenya**.**

4.1.3 Disease incidence and severity variations in rain seasons

Disease incidence and severity varied from long to short rains seasons in all clusters. Disease incidence in Chwele during short seasons was highest with (55.08%) to long rains season incidence of (35.00 %) followed by Chebich with short rains incidence of (50.38 %) to its long rains incidence of (32.00 %), then Kimilili with disease incidence of (48.50 %) during short rain seasons and (31.00 %) for long rain seasons respectively. Kapkateny had the lowest mean incidence during short rains season of (25.50 %) to Long rains season of (13 .00 %). Disease severity also varied from long rain seasons to short rain seasons in all clusters, like in disease incidence, Severity in short rain seasons, Chwele had the highest mean severity with 3.40 for short rain seasons and 2.46 for long rain seasons. Kapkateny had the lowest mean severity of 2.44 for short rain season and 2.00 for long rain seasons respectively (Table.3).

Table 3: GRSV incidence and severity for long and short rains seasons in groundnut growing regions in western Kenya

County	Clusters	Seasons	N	Incidence %	Severity
Bungoma	**Chebich**	Long Rains	18	**32.00**	2.72
		Short Rains	13	**50.38**	3.00
	Chwele	Long rains	31	**35.00**	2.56
		Short rains	37	**55.08**	3.40
	Kapkateny	Long rains	26	**13.00**	2.00
		Short rains	20	**25.50**	2.44
	Kimilili	Long rains	18	**31.00**	2.14
		Short rains	22	**48.50**	3.00
	Kimalewa	Long rains	30	**24.02**	2.30
		Short rains	41	**32.50**	3.00
Busia	**Alupe**	Long rains	17	**34.86**	2.60
		Short rains	20	**40.80**	2.70
	Chakol	Long rains	22	**22.00**	2.00
		Short rains	20	**28.24**	2.62
	Malaba	Long rains	15	**21.22**	2.44
		Short rains	19	**30.00**	3.00
Kakamega	**Matungu**	Long rains	19	**20.32**	2.52
		Short rains	26	**31.02**	3.00
	Muhonje	Long rains	15	**30.22**	2.50
		Short rains	14	**41.00**	2.74
	Mumias	Long rains	17	**22.30**	2.00
		Short rains	29	**32.42**	2.80

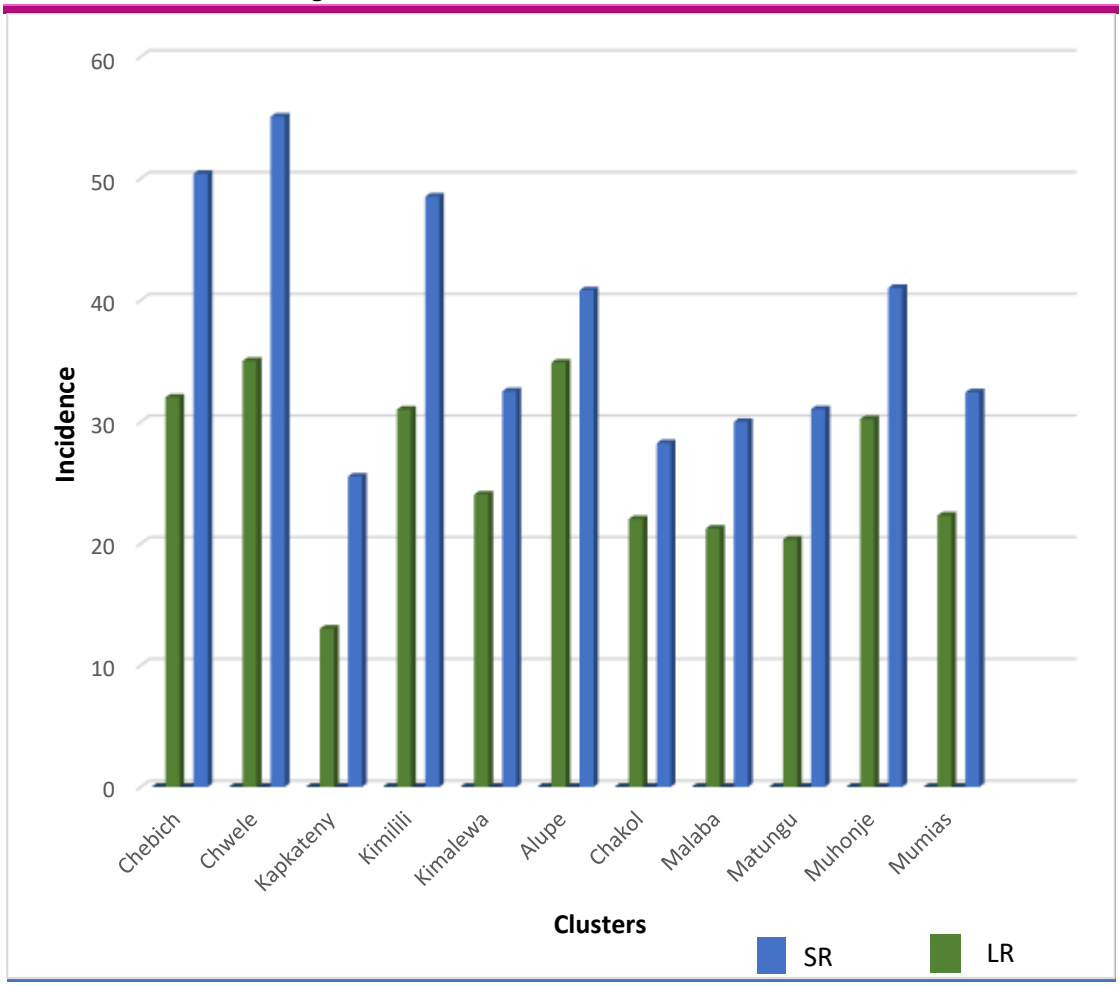

Figure 6: Graph for visual variation in viral incidence in surveyed regions during Long and short rains seasons of western Kenya

4.1.4 Serological Tests

Symptomatic leaf samples collected from a survey and subjected for serological tests, some tested positive for GRSV, TSWV or both. Samples from chwele had the highest number (6) testing positive for GRSV followed by samples from Kimalewa and Mutungu with two testing positives for the virus each. However, some samples from Chebich and Muhonje tested positive for TSWV. It was noted that a Sample from Kimilili tested positive both for GRSV and TSWV. (Table .4)

Table 4. ELISA for GRSV and TSWV on groundnut samples collected from W. Kenya

County	Cluster	Samples (N)	GRSV	TSWV	AEZs
Bungoma	**Chwele**	**12**	**6(+)**	**_**	**LM1**
Bungoma	**Kapkateny**	**13**	**_**	**1(+)**	**UM1**
Bungoma	**Kimalewa**	**11**	**2(+)**	**_**	**LM1**
Busia	**Alupe**	**19**	**1(+)**	**_**	**LM2**
Busia	**Chakol**	**10**	**_**	**_**	**LM3**
Busia	**Malaba**	**6**	**_**	**_**	**LM4**
Kakamega	**Mumias**	**10**	**_**	**_**	**LM2**
Bungoma	**Kimilili**	**9**	**1(+)**	**1(+)**	**UM1**
Kakamega	**Muhonje**	**7**	**_**	**_**	**LM1**
Bungoma	**Matungu**	**11**	**2(+)**	**_**	**LM3**
Bungoma	**Chebich**	**10**	**_**	**4 (+)**	**UM1**

4.1.4 Effect of GRSV on groundnut yield production

Infected groundnuts that displayed GRSV symptoms; leave chlorosis, leaf mosaic, stunted growth, reduced stem height, necrotic leaf spot that tested positive for the virus, failed totally to bear nuts or if were available then was of poor quality. Those groundnuts with no disease symptoms produced nuts with good quality. (Figure 7).

Figure 7: Groundnuts plants obtained from Chwele region in a survey showing the effect of GRSV on crop yields; G) groundnuts with Infected with GRSV having failed to bear nuts, H) Health groundnuts having nuts.

DISCUSSION AND CONCLUSION

The survey revealed that GRSV or TSWV or both occur in all agro ecological zones of groundnut growing regions of western Kenya. Groundnut ringspot virus is distributed in all regions surveyed in Bungoma, Busia and Kakamega Counties of western Kenya with variant incidence and severity. The symptoms observed on infected groundnuts were Stunted growth, leave chlorosis, necrotic and chlorotic ringspot on leaves, yellow leaf mosaic and leave deformation which are typical symptoms of GRSV and TSWV (Pappu *et al*., 2009). Serological tests using DAS- ELISA and TAS-ELISA confirmed the occurrence of GRSV and TSWV in regions of western Kenya with variant incidence and severity (Adkins *et al*.,2002). Chwele region (UM1) had the highest incidence (45.04 %) with mean severity (2.98), followed by Chebich (UM1) with incidence (41.19 %) and severity (2.86), then Kimilili (LM1) had incidence (39.19 %) with mean severity (2.68) respectively. Alupe (LM2) of Busia county had incidence of 37.83 % with severity 2.65, Muhonje (LM1) in Kakamega County had an incidence of 35.72 % with severity of 2.62. In Kimilili region it was noted that GRSV and TSWV were co infecting groundnuts. These indicates the viruses

(GRSV and TSWV) co-exist in some agro ecological zones of groundnuts growing regions of western Kenya. Chebich exhibited GRSV symptoms but samples serologically tested positive for TSWV. This imply that TSWV exhibit same biological symptoms with GRSV thus can only be distinguished serologically (Adkins *et al*.,2002). Disease incidence and severity had positive direction of linear relationship ($r=0.559$, $p<0.05$) with moderate magnitude of association. implying that severity increased dependently with viral incidence (Chuang *et al*.,1987). This is a characteristic of non- seed borne viruses where GRSV and TSWV belong (Gallitelli, 2000). This reveals that the source of infection in most farms was from other host plants transmitted by thrips (Cardoso *et al*.,2003). Therefore, disease symptom development occurred progressively with time and disease became more severe at later stage of growth thus serving as source of inoculum in the field for transmission (Hanssen *et al.*,2011). Therefore, this leads to late infection of plants thus moderate viral incidence and reduced built up of viral titre which lead to moderate severity of the disease (Takahashi *et al*.,2019). The difference in incidence and severity in all agro ecological zones surveyed could imply that the GRSV and TSWV inoculum level differ due to differences in geographical and climatic conditions (Andre *et al*.,2018) or the virus detected serologically could be having a wide host range imply that the virus could exist in areas where groundnut is not grown (Fermin *et al*.,2018). Most of its alternative hosts are the commonly cultivated crops and wild weeds thus act as sources of inoculum posing great threat not only to groundnuts but also other crops of economic importance (Webster et al.,2011).

Disease incidence and severity was high during short rain seasons compared to long rains season. The population intensity of thrips (vectors) may had reduced in number during long rain seasons, due to heavy rains which may have washed them out of the host plant (groundnut) thus lowering their activities in transmission of GRSV and TSWV to host plants (Agneroh *et al*.,2012). In short rains season, a biotic factor was conducive for vectors to multiply and carry on their activities effectively thus transmission of GRSV was high which resulted into high disease incidence than long rains (Kone *et al*.,2017). which might have resulted in thrips hibernating due to heavy and long rain periods thus transmission being lowered thus lowered disease pressure on the plant as a result showed low disease incidence and severity (Saroj *et al*., 2019). Disease incidence and severity increased with time of plant growth this is an indication that GRSV and TSWV are neither seed nor soil born disease but transmitted by vectors (Gutierrez *et al*.,2013). GRSV is transmitted by a number of Thrips (Frankliniella occidentalis, F. schultzei, F. gemina, F. fusca, F. intonsa, T. palmi, T. subnudula and T. tabaci) (Adkins *et al.*,2015). Therefore, the absence and variation in occurrence and distribution of GRSV may have been due to population intensity of Thrips in those regions or insects present in such regions are not vectors of GRSV. thus, lowed the rate of transmission (Karavina *et al*.,2017).

Conclusion

Groundnut ringspot virus (GRSV) and Tomato spotted wilt virus (TSWV) occurs in western Kenya exhibiting same symptomatic characteristics (Boari *et al*.,2002) on groundnut varieties grown in Kenya.

Recommendations

This study Revealed the occurrence and distribution of GRSV in Bungoma, Busia and Kakamega Counties of western Kenya infecting groundnuts and other alternative hosts. There is need for urgent measures to manage GRSV in western Kenya through planting resistant variety to GRSV or introgression of resistant genes into other groundnut varieties (Culbreath et al.,2003) which are more productive but susceptible to GRSV.

Acknowledgement

Our appreciation goes to Masinde Muliro University of Sciences and Technology for provision of materials and facilities for serological tests of samples and KEPHIS Nairobi for provision of manpower, materials and facilities for both Molecular and serological tests.

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