RUFORUM Working Document Series (ISSN 1607-9345), 2019, No. 18 (1): 232 - 238.
Available from http://repository.ruforum.org

Research Application Summary

Occurrence and distribution of viruses infecting groundnuts (*Arachis hypogaea* L) in Kenya

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Abstract

As well as providing food, animal feed and cash, groundnuts are important components of farming systems as they improve soil fertility. In Kenya, the current yields are well below potential and important constraints include the devastating impact of high levels of pests and diseases particularly viruses spread by insect vectors which cause approximately 80% of damage. About 31 viruses from 14 genera have been found infecting groundnuts naturally worldwide. Among these, Groundnut rosette assistor virus (GRAV), Groundnut rosette virus (GRV), satellite RNA associated with GRV and/or GRAV (rosette complex), Peanut mottle virus (PeMoV) and Cuncumber mosaic virus (CMV) are economically important in Sub-Saharan Africa. Recent diagnostic surveys in Kenya have not elucidated much about the viruses infecting the crop. Accordingly, this study identified virus diseases of groundnut in the main producing areas of Kenya. In October 2016 and May 2017, two diagnostic surveys were conducted in six major groundnut growing counties of Western Kenya. Leaf samples showing virus-like symptoms were collected and analysed either by serology and /or next generation sequencing (NGS). I n total, 103 groundnut farms were visited and 315 samples, collected. Of these, 261 were ELISA positive for one or more viruses tested. Fourteen viruses (CAB YV, CABMV, CMV, CPMMV, BYMV, GRSV, CCYV, BCMNV, BGMV, PeMoV, SPCFV, BCMV, CPMV and MCMV) were detected by ELISA while nine (GRAV, satellite RRA associated with GRV and/or GRAV, CPPV1, CPPV2, CpCSV, PBMYV, SPCFV, BCMNV and PeMoV) were detected by NGS. Most plants had mixed infections of more than one virus. The most common viruses detected by EL1SA were CABY V, PeMoV, CMV, CPMM V, BCMNV and BYMV. Of all the viruses detected in Kenyan groundnut fields only Cowpea aphid-borne mosaic virus (CABM V), CMV, PeMoV, and CPMMV are transmitted through groundnut seed. Therefore, fun her studies are needed to determine how In any more viruses are transmitted through this route.

Key words: *Arachis hypogaea*, groundnuts, Kenya, virus diseases

Résumé

As well as providing food, animal feed and cash, grotindnuts are important components of farming systems as they improve soi I t'ertility. I n Kenya, the current yields are well below potential and important constraints include the devastating inn pact of high levels of pests and diseases particulars viruses spread by insect vectors which cause approx. 80% of damage. About 31 viruses from 14 genera have been found infecting groundnuts naturally worldwide. Among these, Groundnut rosette

assistor virus (GRAV), Groundnut rosette virus (GRV), satellite RNA associated with GRV and/or GRAV (rosette complex), Peanut mottle virus (PeMoV) and Cucumber mosaic virus (CMV) are economically important in SSA. Recent diagnostic surveys in Kenya have not elucidated much the viruses infecting the crop. We identified virus diseases of groundnut in the main producing areas of Kenya. In October 2016 and May 2017, two diagnostic surveys were conducted in 6 major groundnut growing counties of Western Kenya. Leaf samples showing virus-like symptoms were collected and analysed either by serology and /or next generation sequencing (NGS). In total, 103 groundnut farms were visited and 315 samples, collected. Of these, 261, were ELlSA positive for one or more viruses tested. Fourteen viruses (CABYV, CAB MV, CMV, CPMM V, BYMV, GRSV, CCY V, BCMNV, BGMV, PeMoV, S PCF V, BCMV, CPMV and MC MV), were detected by ELISA while nine (GRAV, satellite RNA associated with GRV and/or GRAV, CPPV 1, CPPV2, CpCSV, PBMYV, SPCFV, BCMNV and PeMoV) were detected by NGS. Most plants had mixed infections of more than one virus. The most common viruses detected by ELISA were CAB YV, PeMoV, CMV, C PMM V, BC MNV and BYMV. Of all the viruses detected in Kenyan groundnut fields only Cowpea aphid-borne mosaic virus (CA BMV), CMV, PeMoV, and C PMMV are transmitted through groundnut seed, therefore, further studies needed to determine how many more virus are transmitted through this route.

Key words: *Arachis hypogaea*, groundnuts, Kenya, virus diseases

Introduction

Groundnut (*Arachis hypogaea* L) is an important oil, food and cash legume crop in countries of sub Saharan Africa (SSA). In Kenya, it is the second most important legume grown mostly by small scale farmers for food and income and it contributes more to farmers' incomes than maize or bean crops (Farmlink, 2018). As well as providing food and animal feed, groundnuts are important components of farming systems as they improve soil fertility. The current yields (>1 ton/ha) in Kenya, are well below the potential yield (FAOSTAT, 2016). This may be attributed to a combination of factors such as high levels of pests and diseases, poor access to improved seeds, unreliable rains, traditional small-scale farming with little mechanization, increased and/or continued cultivation on marginal land, poor agronomic practices and limited extension services. These in addition to the devastating impact of high levels of pests and diseases particularly viruses, make the crop unprofitable for farmers (Abang 2013). The situation is aggravated by the fact that the climate favours virus vector insects and there is a high incidence of aphids, whiteflies and leafhoppers which spread many virus diseases on groundnuts and other vegetable crops. Virus diseases account for 80% of groundnut damage (Radhakrishnan *et al*., 2016).

Unlike fungi and bacterial infections, farmers do not recognize virus symptoms, but viruses can reduce yields up to 100%. In previous published surveys, only groundnut rosette disease (caused by a complex of viruses) has been reported in Kenyan crops but groundnuts are reported to be affected by > 31 viruses from 1 4 genera worldwide (Sreenivasulu 2008). Nine of them belong to genus Potyvirus, six to Tospovirus, two each to Cucumovirus, Pecluvirtis, Soymovirus and Umbravirus, and one each to Begomovirus, Bromovirns, Carlavirus, Ilarvirus, Luteovirus, Potexvirus, Rhabdovirus and Tymovirus (Sreenivasulu *et al*., 2008). Most of these (19) were first isolated from groundnut and the rest, from other hosts but they commonly occur on groundnut. Diseases in groundnut caused by Tomato spotted wilt virus (TSWV), Groundnut bud necrosis virus (GBNV), Tobacco streak virus

(TSV), Groundnut rosette assister virus (GRAV), Groundnut rosette virus (GRV), satellite RNA associated with GRV and/or GRAV, Peanut clump virus (PCV), Bean common mosaic virus (BCMV), Peanut mottle virus (PeMoV) and Cucumber mosaic virus (CMV) have been reported as the most economically important causing serious yield losses regionally or globally (Sreenivasulu, 2008). Previous studies have reported that many plants are often co-infected by different plant viruses and viroids (Coetzee *et al*., 2010; Jo *et al*. 2015; Taiwo *et al*. , 2007; Wylie *et al*. , 2012). Groundnut crops affected often have plants with virus-like symptoms (plants can be severely stunted or killed) and the yields from such plants are low.

Despite the economic importance of groundnuts in Kenya, limited information is available about viral disease incidence, distribution and molecular diversity. Such information is needed as the first step toward the design of future effective disease management strategies. This study identified virus diseases of groundnut in the main producing areas of Kenya.

Materials and methods

In October 2016 and May 201 7, two diagnostic surveys were conducted in six major groundnut growing counties (Kakamega, Bungorna, Busia, Siaya, Nandi and Homabay) of Western Kenya. In each field, 3-5 leaf samples were randomly collected from groundnut plants showing virus-like symptoms (green mosaic, leaf distortion, downward curling, mottling, chlorotic areas, necrotic spots, local lesions, stunting or a combination of these) as well as one sample from asymptomatic plant, and kept at 4°C until fun her analysis either by Enyme linked immunosorbent Assay (ELISA) or next generation sequencing (NGS).

Serological analysis . Commercial ELISA kits with specific antibodies against Cucurbit aphid-borne yet lows virus (CABYV), Cowpea aphid-borne mosaic virus (CABMV), Cucumber mosaic virus (CMV), Cowpea mild mottle virus (CPMMV), Bean yellows mosaic virus (BYMV), Groundnut ringspot virus (GRSV), Cucurbit chlorotic yellows virus (CCYV), Bean common mosaic necrosis virus (BCMNV), Bean golden mosaic virus (BG MV), Peanut mottle virus (PeMoV), Sweet potato chlorotic heck virus (SPCFV), Bean common mosaic virus (BCMV), Cowpea mosaic virus (CPMV), Tomato yellow leaf curl virus (YLCV) Pea nut stunt virus (PSV), Sugarcane mosaic virus (SCMV) and Maize chlorotic mosaic virus (MCMV) together with their positive/negative controls and reagents were purchased from DSMZ, Germany. For the detection of the viruses BYMV, CPM MV, BCMN V, BYMV, CABMV, CMV, MCMV, SPCFV, CCYV, GRSV, PSV, CPMV, PeMoV, SCMV, TYLCV and C PMMV, Double Antibody Sandwich (DAS)-ELISA was conducted essentially as described previously (Were *et al*., 2013) following manufacturer's instructions. For each of the viruses, Microtiter plates were coated with the respective IgG diluted I:1000 (v/v) in coating buffer and incubated for 2h at 37°C. To block, 2% skimmed milk in PBST (200ql/well) were added and incubated for 30 min at 37°C. Sap extracts prepared by grinding leaf tissues of virus-infected plants I : 10 (w/v) in sample extraction buffer (PBST + 2% PVP) were added and incubated overnight at 4°C. Extracts from manufacturers for each virus's negative and positive control were used as negative and positive controls, respectively. lgG alkaline phosphatase conjugate, diluted 1:1000 (v/v) in conjugate buffer, was added and incubated for 2b at 37°C. The substrate, p-Nitrophenyl phosphate diluted at 1 mg/ml in substrate buffer (DEA*H_2O *NaN_3) was added and incubated for 1 h at 37°C or until there was colour change. Quantitative measurements of the p-nitrophenol substrate conversion resulting in yellow color were made by determining the absorbance at 405 nm (A 405) in a Biotek® model spectrophotometer (Labsystems Co., Finland). Twice the mean absorbance readings of healthy controls were used as the positive thresholds.

To detect BC MV, CABYV and BGMV, Triple Antibody Sandwich (TA S) EU SA was conducted as described previously (Were *et al*., 2013) and following manufacturer's instructions. For each virus,

microtitre plates (96 wells) were coated with respective IgG diluted 1 : 1000 (v/v) in a coating buffer and incubated for 2h at 37°C. Blocking was done as above. Sap extracts prepared as described above were added and incubated overnight at 4°C. Hundred µl/well of MAbs raised against BCMV and diluted I :100 (v/v) in conjugate buffer added and incubated for 2 h at 37°C were used for detection. As explained above, extracts from the manufacturer's negative and positive control were used as negative and positive controls, respectively. Alkaline phosphatase labelled Rabbit-anti-mouse RaM-AP, (DSMZ, Germany) diluted I :1000 v/v in conjugate buffer was added and the plates incubated for 45 min at 37°C. Substrate addition, incubation and absorbance readings were done as described for DAS ELISA above.

Six samples that were ELISA positive for CPPMV, SPECFV, PeMoV, BCMNV, CABYV and one that was negative but severely stunted with small green leaves, were subjected to next generation sequencing. Total RNAs from infected the samples was extracted using the Qiagen RNeasy Plant Mini Kit (Qiagen, Germany) and used for double stranded cDNA synthesis using the SuperScript II (Thermo Fisher Scientific, Waltham, USA) kit. The cDNA was column-purified with the DNA C lean & ConcentratorTM-5 — DNA kit (Zymo Research, Irvine, USA) and quantified with the Qubit 2.0 Fluorometer (Thermo Fisher Scientific). The sample was then processed with the transposon-based chemistry library preparation kit (Nextera XT, Illumina) following manufacturer's instructions. The fragment size structure of the DNA library was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa C lara, USA). The indexed denatured DNA library was sequenced (200-bp paired-end sequencing) on the Illumina MiSeq platform (Illumina). Trimmed reads (Haas *et al*., 20 13) were used for de novo assembly and contigs aligned to the viral genomes database (ftp://ftp.ncbi.nih.gov/genomes/Viruses/all.fna.tar.gz/, downloaded on October 20 19) using CLC Genomics Workbench 10.1.2. The assembled contigs were subjected to a BLA STn search against the GenBank database (Altschul *et al*., 1990). Complete and partial respective viral sequences used for comparison and phylogenetic analyses were retrieved from GenBank (http://www.ncbi. nlm. nih.gov/). Phylogenetic analyses and comparisons were performed using the MEGA v.7 (Kumar *et al*., 2016) and DnaSP v.5 (Librado and Rozas, 2009) programs.

Results and discussion

A total of 103 groundnut farms were visited and 315 samples collected, 138 in the long rain and 177 in the short rain seasons, respectively. visually, mean virus incidence was higher in the short rain season (50.2%) than in the long rain season (35.6 o/o). In the short rain season, Kakamega county had the highest mean virus incidence (47.6) followed by Bungoma (47.4%), Homabay (44.3%), Busia (41.1 %) while Siaya had the lowest (31.6%). In the long rain season, Bungoma county had the highest mean virus incidence (52.5%) followed by Busia (49%) and Kakamega had the lowest. Three farms in Bungoma county had an incidence of up to 100% while a farm in Kakamega had the least viral incidence of 20%. The average temperature and rainfall during June were 22.5 °C and 525 min in the western areas and 16.5 °C and 225 mm in eastern areas (Min. Env. and Forestry. 2019). This shows that it is generally warmer in the short rain season than in the long rain season. Warm climates favour multiplication and survival of insect vector, which may help explain the high incidence in the short rain season. Similar results were observed by Mukoye *et al*. (2014).

From the samples collected, 256 (81%), were EL ISA positive for one or more viruses tested, implying that 19% of the samples where either infected by viruses whose antisera we did not have or that symptoms were due to other causes as reported by Sherwood and Melouk (1995). These findings concur with earlier observations (Were *et al*., 2013; Mukoye *et al*., 2014; Mangeni *et al*., 2014) when working with potato, cowpea and beans, respectively. The findings also indicate that viruses are the major cause of the low yields observed in groundnut crops and are a major constraint to groundnut production in Kenya. Using ELISA, 14 viruses, i.e., Cucurbit aphid-borne yellows virus (CABYV),

Cowpea aphid- borne mosaic virus (CABMV), Cucumber mosaic virus (CMV), Cowpea mild mottle virus (CPMMV), Bean yellows mosaic virus (BYMV), Groundnut ringspot virus (GRSV), Cucurbit chlorotic yellows virus (CCYV), Bean common mosaic necrosis virus (BCMN V), Bean golden mosaic virus (BGMV), Pea nut mottle virus (PeMoV), Sweet potato chlorotic fleck virus (SPCFV), Bean common mosaic virus (BCMV), Cowpea mosaic virus (CPMV) and Maize chlorotic mosaic virus (MCMV), were detected by ELISA in groundnut samples. This is the first extensive survey and detection of a large number of viruses in groundnut fields in Kenya. The most abundant viruses were CABYV (24%), PeMoV (18%), CMV and CPMMV each (17%), followed closely by BCMN V and BYMV (each with 16%) while the least abundant virus was BGMV (3%). It is interesting to note that MCMV, a virus that in combination with Sugarcane mosaic virus (SCMV) causes maize lethal necrosis virus, a devastating disease of maize crops in Kenya (Wangai e/ al., 2012b), was detected in groundnuts. This is the first report of this virus to be found infecting a legume and this may be due to intercropping of maize with legumes and the subsequent infestation of the fields by polyphagous aphids.

Basing on ELISA results, CABYV, PeMoV, CMV and CPMMV were the most abundant viruses, an indication that the toiir viruses were well established throughout the main groundnut growing areas in Kenya, possibly due to substantial seed transmission and availability of insect vectors. This finding agrees with that of earlier workers (de Breuil *et al*., 2008), who found PeMoV very widespread in Argentina. Despite having antisera, three viruses, SCMV, Tomato yellow leaf curl virus (YLCV) and Pea nut stunt virus (PSV) were not detected in groundnuts samples from the surveyed areas. This suggests that the three viruses may not be infecting groundnuts in Kenya or that the strains available could not be detected by the antisera used in this study. Most plants had mixed infection of up to four viruses in any combination (Fig. 1) and only about 2% of the samples had single infections. This may be due to the fact that most of these viruses are transmitted by aphids, whiteflies, thrips, hoppers and beetles, which were observed in large numbers in the fields during the stir vey.

Using next generation sequencing, nine viruses, i.e., Groundnut assistor virus (GRAV), satellite RNA associated with GRV and/or GRAV, Cowpea polero virus 1 (CPPV1), Cowpea polero virus 2 (CPPV2), Chickpea chlorotic dwarft cirus (CpCDV), Phasey Bean Mild Yellows Virus (PBMYV), SPCFV, BCMNV and PeMoV) were detected. Interestingly, only three viruses out of the nine had been detected by ELISA. This may be attributed to the low titre of the viruses in question or that we did not have antisera for the viruses and it may also be that NGS was more sensitive than ELISA. All in all, this study has found more viruses in groundnuts in Kenyan than hitherto known. It is becoming increasingly clear that intercropping may lead to evolution of viruses that infect a cross-section of plants that are normally intercropped.

The NGS results revealed the PeMoV genome of isolate KAL RO2-23 to be 970 1 nucleotides long. The overall genomic organization of PeMoV is thus similar to that of other potyviruses (Ivanov *et al*. , 20 14). The KALRO2-23 isolate sequence was 95 - 99% identical to the other six PeMoV complete genomes available in Genebank. From phylogenetic analysis, it was observed that a high diversity in the PeMo V species existed between the strains of the old world and those of the new world. Based on the analysis of complete genomes (n=7), the average nucleotide divergence between PeMoV isolates had a small variation (1 - 5%), despite their different geographical origins and isolation hosts. These comparisons indicate that the viral isolate (KALRO2-23) belong to the PeMo V species and are the causal agent of green mosaic, chlorotic and necrotic spots observed on groundnuts in Kenya.

Below each of them are the viruses detected in the respective plant by E: ELISA and N: NGS The viruses CABMV, CMV and PeMoV are seed transmitted (Adams and Kuhn, 1977; de Breuil *et al*., 2008), therefore, planting clean seed would help manage them in the field if other natural hosts are absent. The common practice by many small holder farmers of planting more than one legume in

one field helps spread viruses to healthy legumes thereby acting as source of inoculum. However, for those viruses that are not seed transmitted such as groundnut rosette disease complex, several methods including use of pesticides to reduce vector populations, cropping practices to delay onset and spread of both vector and disease, and cultural practices (reviewed in Naidu *et al*. , 1999) have been investigated for management of the disease but with only limited success. The question remains, could there be other natural hosts that habour the viruses when the crop is out of the field or is it the practice of intercropping system that has to be changed to manage the viruses? The answer to this question remains an enigma and needs to be investigated.

E: BCMV, BGMV, BYMV, CABMV
N: CPPV2, GRAV, SRGRV

E: BCMV, BYMV, CABMV, CPMMV
N: CpCSV, SRGRV

Figure 1. Groundnut plants displaying different symptoms as seen in the field

Conclusions

Groundnut fields in Kenya are highly infested with viral diseases and all groundnut varieties were susceptible to one or more viruses. This suggests that groundnut plants in Kenya may be having more viruses than the 20 fotind in this study. Breeding groundnut varieties for resisting iniltiple viruses is therefore needed to contain the virus menace in the country.

Acknowledgments

This work was funded by The royal society of the UK and next generation sequencing was done at the BecA ILRI hub. The write rip was done in Prof. Suziiki Nobnhiro's lab, Okayama University, Japan under funding from JSPS.This paper is a contribution lo the Fifteenth RUFORUM A nniial General Meeting held 2-6 Decein ber 2019 in Cape Coast, Ghana.

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