

Full Length Research Paper

Survey of the incidence and distribution of groundnut rosette disease in major groundnut-producing regions of Western Kenya

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Groundnut rosette disease (GRD) is the most important viral disease of groundnuts in sub-Saharan Africa. In Kenya, GRD infection especially before flowering results in 100% loss in pod yield. Surveys were conducted in 2016 and 2017 to determine the incidence and distribution of GRD in five major groundnut growing Counties of western Kenya. A structured questionnaire was used to assess GRD incidence and severity and farmers' awareness about management of GRD. Reverse transcription (RT)-polymerase chain reaction (PCR) was used for the detection of GRD agents in collected symptomatic samples. Results revealed that GRD was prevalent in all the fields of the five counties. The highest mean disease incidence was in Busia County (35.7%) while the lowest incidence was in Siaya (23.1%). The most conspicuous symptoms observed in all the fields inspected were yellow/chlorotic rosette and green rosette. The highest mean disease severity was observed in farmers' fields in Busia (3.1) and Bungoma (3.0) Counties, while the lowest was observed in Siaya (2.8). RT-PCR detected GRD agents in all the symptomatic samples. This study demonstrated the widespread occurrence of GRD in major growing regions of western Kenya and warrants the implementation of effective virus disease control strategies.

Key words: *Arachis hypogaea* L., field survey, groundnut rosette disease, occurrence, severity.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an annual self-pollinated legume, widely grown in tropical and sub-tropical regions of the world. Asia and Africa account for 95% of global groundnut area where it is cultivated under rainfed conditions with low inputs by resource poor farmers. Groundnut is an important cash crop, an

affordable source of edible oil rich in omega-3 fatty acids, protein and vitamin E and its stover provides nutritious feed for livestock (Pandey et al., 2012; FAOSTAT, 2014). As a cash crop, groundnut has the potential for improving income and reducing poverty in the rural households (Diop et al., 2004). The crop can also be used to improve

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soil fertility by fixing atmospheric nitrogen (Andima et al., 2006). In Kenya, groundnut is a principal source of protein and major source of cash income for small-holder farmers. However, groundnut production in Kenya has continued to decline with farmers attaining less than 50% of the yield potential (FAOSTAT, 2014). A major constraint to achieving the yield potential of groundnuts in sub-Saharan Africa has been the presence of Groundnut rosette disease (GRD), fungal rust and Early Leaf Spot (ELS) diseases (Janila et al., 2013; Okello et al., 2013). Groundnut rosette disease is the most important disease of groundnut in Kenya (Okello et al., 2013; Janila et al., 2013).

GRD is the most destructive viral disease of groundnut, exclusively endemic to sub-Saharan Africa (SSA) and its off-shore islands (Deom et al., 2000; Waliyar et al., 2007). The disease was first described in Tanzania by Zimmerman in 1907 (Naidu et al., 1998) and since then epidemics have been reported in all groundnut growing regions of SSA and in Madagascar (Naidu et al., 1999). Groundnut rosette disease is caused by synergistic interactions of three viral agents, namely, *Groundnut rosette assistor virus* (GRAV), *Groundnut rosette virus* (GRV) and satellite RNA (satRNA) (Deom et al., 2000). All agents of GRD are persistently transmitted by aphids (*Aphis craccivora* Koch), may persist in the aphids for more than 10 days (Lynch, 1990), and there is no evidence of seed transmission to-date. Two distinct forms of GRD symptoms occur, chlorotic and green rosette (Waliyar et al., 2007) with variations due to diversity among the causal agents (sat-RNA variants), differences in genotype response, climatic conditions and mixed infections with other viruses (Naidu et al., 1999). Chlorotic rosette is the predominant form throughout sub-Saharan Africa, whereas green rosette has been reported in western and southern Africa regions (Naidu et al., 1998). Groundnut rosette can cause serious morphological disturbances to infected plants which take on bushy appearance and are stunted. Other symptoms include mottling, yellowing, leaf mosaic, and distortion of the shoots. GRD infection especially before flowering of the crop results in 100% loss in pod yield when susceptible varieties are used (Naidu et al., 1999; Waliyar et al., 2005; Okello et al., 2010).

The effects of rosette disease can be devastating on groundnuts if not prevented (Waliyar et al., 2005; Okello et al., 2010). Successful development of effective GRD management strategies depends on a sound understanding of the distribution and incidence of the disease in different agro-ecologies and cropping systems. Although there have been reports of GRD in Kenya (Wangai et al., 1999; Wangai et al., 2001), there is no updated information on the distribution, incidence, and severity of the disease. The only survey on the occurrence of GRD (Wangai et al., 2001) was conducted several years ago and the evolving polyculture/cropping system in Kenya may have greatly impacted on the disease dynamics over the years. Therefore, in this study,

an extensive survey was conducted to determine the distribution, incidence, and severity of GRD in five major groundnut growing Counties of western Kenya. Farmers' awareness on the disease and management options were also investigated. This information is crucial in development of appropriate control interventions of the disease.

MATERIALS AND METHODS

Groundnut rosette virus disease (GRD) survey and sampling areas

A diagnostic field survey for GRD was conducted in major groundnut growing areas of Western Kenya from July 2015 to February 2016. The regions surveyed were the Counties of Homa Bay, Siaya, Busia, Bungoma, and Vihiga. Groundnut fields were selected at regular intervals of between 5-10 km along the major and feeder roads. The number of fields surveyed per county depended on the availability of groundnut farms at the time of survey. Farmers' groundnut fields were visited when the crop was between pod pegging and physiological maturity. A total of 76 symptomatic leaf samples were collected. Sampling was done on plants along diagonals and the number of samples collected per field depended on the variability of symptoms and field size. Coordinates were taken at each sampling site using a global positioning system (GPS) device (Magellan Triton 'Windows CE Core 5.0 X11-15302). During the surveys, a questionnaire was used to capture farmers' experience with GRD and to document current management practices being employed by farmers in addressing the problem. This was captured as a series of responses by asking farmers if they knew about GRD, if they recognized it on their farms, the frequency of the problem on their farms, rate of spread of GRD on their farms and how they described the symptoms of the disease. Data were also collected on respondents' efforts to control the disease. Farmers were shown colored photographs and also groundnut plants affected by GRD on their own farms to ensure they made the correct identification of the disease.

Disease assessment

Assessment of GRD incidence and severity was based on virus symptoms of plants in the field as described by Okello et al. (2014). Disease incidence was calculated by expressing the number of plants with virus symptoms as a percentage of the total number of plants in quadrants of each sampled field. The severity of GRD symptoms were assessed visually and recorded based on a scale of 1 to 5 where; 1 = no symptoms, 2 = mild symptoms on leaves, little distortion of leaf shape, apparent but negligible stunting, 3 = moderate symptoms on leaf, moderate distortion of leaf shape, moderate stunting, 4 = severe symptoms on leaf, severe leaf distortion with reduced size, plant partially stunted, 5 = very severe symptoms on leaf, severe leaf distortion, reduced size, plant severely stunted. Symptomatic leaf samples were collected and placed in 50 ml falcon tubes containing silica gel and kept at room temperature for subsequent molecular diagnosis.

Virus detection by RT-PCR

RNA extraction from groundnut leaves

Total RNA was extracted from groundnut leaf samples using a Plant

RNA MiniPrep (Zymo Research, Irvine, CA, USA) according to the manufacturers' instructions. The quantity and purity of the extracted RNA was assessed by determination of A_{260}/A_{280} and A_{260}/A_{230} absorbance ratio by NanoDrop spectrophotometer (Thermo Fisher, Wilmington MD). The integrity of the extracted RNA was verified in 0.8% agarose gel stained with ethidium bromide. The integrity of RNA was verified in 1.2% denaturing agarose gel stained with Gel Red (Biotium, Hayward, CA, USA), and visualized under UV transilluminator. Visual inspection of the gel images was used to confirm the presence of bands corresponding to the 28S and 18S ribosomal subunits, providing qualitative assessment of RNA integrity.

cDNA synthesis

First-strand cDNA was synthesized in a 20 μ l reaction containing approximately 5 μ g of total RNA using a RevertAid Premium First-Strand Synthesis Kit (Thermo Scientific, Waltham, MA, USA). Five μ l of RNA template was gently mixed with 4 μ l of 5x reaction mix, 0.5 mM dNTP, 0.5 μ l oligo dT₍₁₈₎ primers, 2 μ l enzyme mix, and made up to 20 μ l volume using nuclease free water. The mix was gently spun down for 30 s, then incubated in thermo cycler at 25°C for 10 min followed by 50°C for 60 min and terminated by heating at 85°C for 2 min.

Conventional reverse transcription PCR (RT-PCR)

The cDNA was amplified by PCR using corresponding 3' and 5' primers specific to coat protein sequence of *Groundnut rosette assistor virus* (GRAV) (Table 2) as described by Wangai et al. (2001). Two pairs of primers were used namely GRAV1 (HRP92: ATGAATACGGTCGTGGTTAGG / HRP93: TTTGGGGTTTTGGACTTGCC) and GRAV2 (HRP110: GGAGGGTCTGGCGAAACATT / HRP111: CCCTTGTAAGGAACCGGAAT), amplifying products of 547 and 890 base pairs, respectively.

The PCR amplifications were performed in a reaction of 20 μ l in Bioneer® premix, 2.0 μ l forward and reverse primers and 2.0 μ l cDNA. The PCR was performed in a MJ Mini™ Thermal Cycler (BIO-RAD, Singapore). The cycling profile was an initial denaturation at 94°C for 2 min followed by 36 cycles of 94°C for 45 s (denaturation), 53°C of 1 min (annealing) and 72°C for 1 min (extension) and a final extension of 5 min. The PCR products were electrophoresed on a 1% (w/v) agarose gel in 1X TAE buffer containing 0.5 μ g/ml of Gel Red and visualized in a UV transilluminator.

Data analysis

The data were analyzed through descriptive statistics (frequencies, percentages and mean values) for all continuous variables to generate summaries and tables. Data analysis was performed analyzed using the Statistical Package for Social Scientists (SPSS) version 16.0.

RESULTS

GRD symptoms and incidence

A total of 76 groundnut leaf samples were collected from farms in five counties of western Kenya. Groundnut rosette disease symptoms were observed in all the fields

surveyed in all the 5 Counties. Symptoms observed in the field were manifested mainly in the form of chlorotic rosette symptoms and stunting of severely affected plants (Figure 1). Other GRD symptoms observed ranged from green rosette, leaf curling, and bright mosaic. GRD was present in all the five counties surveyed. The disease incidence varied from one farm to another with the highest incidence of 60% recorded in some farms in Busia County. GRD incidences ranging from 23.1 to 35.7% were observed in the five counties. The mean disease incidence was highest in Busia (35.7%) while Siaya had the lowest (23.1%). Overall, the mean incidence for all the counties surveyed was 29.4% (Table 1).

GRD severity was assessed on infected plants using a scale range of 1 to 5. The scores of 3 to 4, which are characterized by chlorotic of leaves to a stunted growth, were more frequent than the scores of 2 which represent the initial symptoms manifested by mild chlorotic leaves with no stunting. Disease severity ranged from 2.86 to 3.14 across the five counties, with an overall mean of 2.96. The highest GRD severity (3.14) was scored in farmers' fields in Busia County while the lowest was in Siaya County with a score of 2.86 (Figure 2). The disease severity of 3 and 4 had the highest occurrence across the five counties while scale of 5 recorded the least occurrences at 9.9% (Table 2). There was no significant difference in viral disease severity between the counties ($p \geq 0.039$, $\alpha = 0.05$).

Conventional RT-PCR

Results of the RT-PCR tests conducted using primers specific to the coat protein sequence of GRAV showed that all the samples (100%) collected from the fields in all the five counties of western Kenya tested positive for GRD (Figure 3).

Distribution of GRD in western Kenya

Based on the survey and RT-PCR results, a distribution map of GRD occurring in western Kenya was drawn (Figure 4). GRD was found occurring in all the surveyed groundnut growing regions of western Kenya.

Farmers' knowledge and management strategies against GRD

Most of the 76 interviewed farmers (90%) were able to recognize plants infected with GRD in their fields, while the remaining 10% did not follow the disease symptoms. All of the interviewed farmers reported that the disease is systemic and, overtime, it spreads to all plants in the field. Ninety two percent of farmers reported that no



Figure 1. (a) GRD-infected plant showing chlorotic rosette; (b) GRD-infected plant showing stunted growth and; (c) and (d) healthy plants in the field.

Table 1. GRD incidence in five surveyed counties of western Kenya.

County	Sub-counties	No. of farms surveyed	GRD incidence (%)
Busia	Teso South, Butula, Nambale, Samia	27	35.7 ± 3.24 a*
Homa Bay	Rangwe, Ndhiwa, Rachuonyo	26	30.3 ± 2.23 b
Siaya	Gem	8	23.1 ± 3.16 c
Bungoma	Webuye, Sirisia	9	29.4 ± 1.87 b
Vihiga	Emuhaya	6	26.7 ± 1.77 b
Total/Mean		76	29.04

*Means in the same column followed by the same letter are not significantly different from one another ($p < 0.05$).

groundnuts are produced after infection. A small proportion of the farmers (7%) were not aware of the effect of GRD infection on groundnut production. Regarding the occurrence of the disease in the fields, 45.8 and 19% of the farmers attributed the cause of GRD to excess rainfall and presence of striga weeds, respectively. Other factors attributed to the disease by the farmers were soil fertility and drought stress. Eleven percent of the farmers said that the disease becomes more severe during dry season, although the disease is omnipresent during the whole year. However, ten percent of farmers had no idea as to what the causes GRD in the fields. Among the interviewed farmers, 65.1% reported to have used planting seeds from nearby (<10 km) local markets to establish the crop, 25.3% used their own seeds in the establishment of new fields, 6% obtained the seeds from the agricultural extension office, while 3.6% obtained seeds from their neighbours (home villages). In

terms of GRD management efforts, 44.6% of the farmers carried out rouging for disease control. The diseased plants were mainly removed two months after planting and during weeding. About 16.9% of the farmers interviewed sprayed the crop with pesticides for disease control. Thirty two percent of farmers were not aware about disease transmission and believed that the disease can be spread through the soil, while eight percent attributed new infections to the use of infected planting seeds.

DISCUSSION

Field surveys generate knowledge on the current status of GRD prevalence, incidence, and severity, which forms the basis of priority setting in the integrated disease management. Such knowledge is limited for GRD

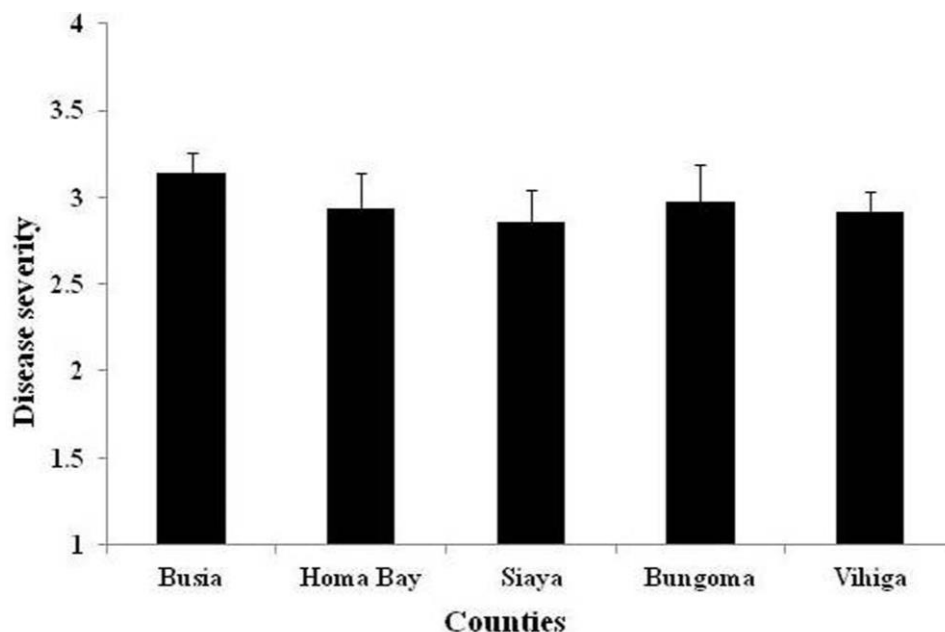


Figure 2. Mean GRD severity scores for the five surveyed Counties in western Kenya.

Table 2. GRD severity ranging from 1 to 5 scores (%) for the five surveyed counties in western Kenya.

Locations/sub-counties	County	Scores ranging from 1 to 5 (%)				
		1	2	3	4	5
Teso South, Butula, Nambale, Samia	Busia	12.6	16.8	28.3	28	14.3
Rangwe, Ndhiwa, Rachuonyo	Homa Bay	16.7	20.8	24.8	27.7	10
Gem	Siaya	16.3	21.3	28.1	28.1	6.2
Webuye, Sirisia	Bungoma	16.2	17.8	28.3	29.4	8.3
Emuhaya	Vihiga	15	23.3	27.5	23.3	10.9
Mean		15.4	20	27.4	27.3	9.9

pathosystem in Kenya. Although GRD is prevalent in Kenya, reports on its incidence and severity are scanty. This study confirms earlier reports on the occurrence of GRD in western Kenya (Wangai et al., 1999, 2001). The plants showing symptoms of GRD were easily identified due to the symptoms they exhibited. Although chlorotic rosette was predominant symptom type, isolated cases of green rosette, leaf curling, and mosaic symptoms were observed on groundnut plants. Similar symptoms were previously reported in Kenya, Uganda, and Nigeria (Wangai et al., 2001; A'Brook, 2007; Okello et al., 2014, 2017). However, green rosette symptom type is described as the most common symptom of GRD in West Africa (Naidu et al., 1998; Appiah et al., 2016).

The results reported in this study shows substantial incidence and severity of GRD during the survey carried out in the five-groundnut major growing counties of western Kenya. There were significant differences in disease incidence among the counties surveyed.

According to Hull (2002), variability in the pathological incidences of a viral disease is often attributed to several factors such as susceptibility and age of the host plant and environmental factors such as solar radiation and temperature. The variations in incidences observed in this study may be attributed to the fact that the surveyed counties are in different agro-ecological zones and as such the different environmental conditions prevailing may have influenced vector multiplication and spread. This result could also be due to intrinsic factors of the crops including susceptibility and agricultural practices employed within the different regions (Juarez et al., 2013). Studies by Edema et al. (1997) and Shoyinka et al. (1997) also indicated that weather conditions within seasons and cropping systems can affect distribution of viruses in different environments. The incidences were high in Busia, Siaya, and Homa Bay Counties compared to Bungoma and Vihiga. This confirms past reports that identified Homa Bay as one of the hotspots for GRD in

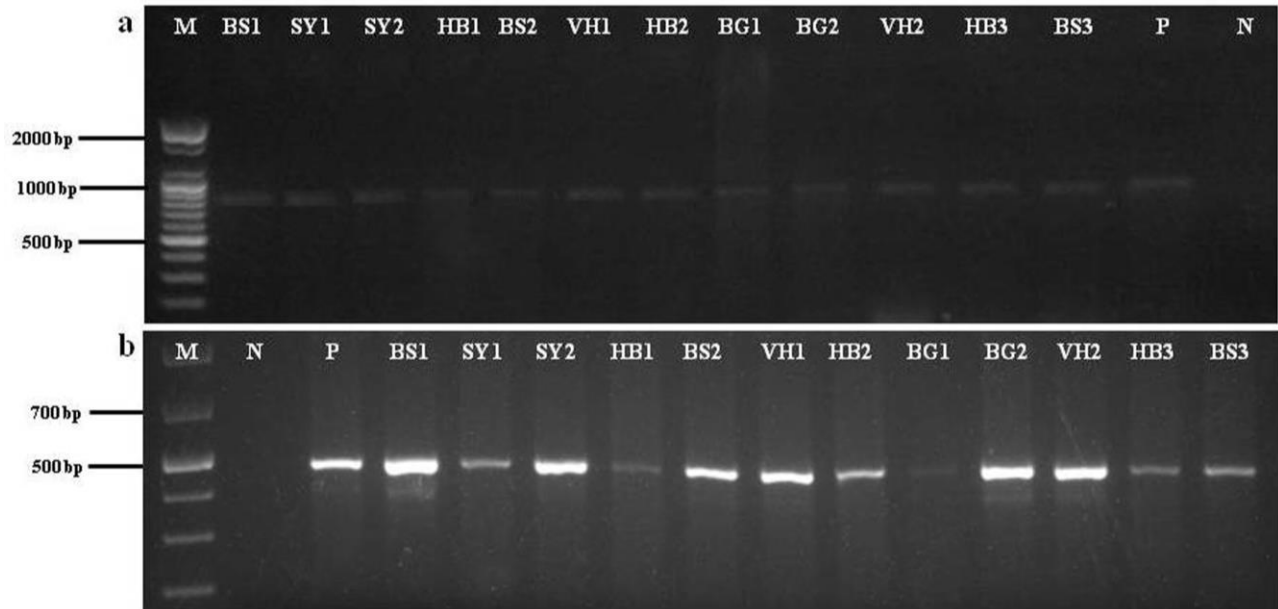


Figure 3. Gel electrophoresis of amplified RT-PCR products using (a) *Groundnut rosette assistor virus* (GRAV2) and; (b) *Groundnut rosette assistor virus* (GRAV1) primers. Lanes P = positive control; N = negative control (healthy plant); BS, SY, HB, BG and VH represent samples from Busia, Siaya, Homa Bay, Bungoma and Vihiga Counties, respectively.

Kenya (Wangai et al., 2001). The variations in incidences in this study can be due to the fact that the low virus incidences in Bungoma and Vihiga may suggest presence of low virus inoculum source to cause high incidence in groundnut fields.

A striking feature of this study was that there was no farm with 0% GRD incidence, an indication that all groundnut varieties in the surveyed area were susceptible to the viruses and that all farms were infected. RT-PCR detected GRAV in all GRD symptomatic plant leaves collected from all the surveyed counties in western Kenya. These results demonstrate a wide distribution of the disease in all groundnut growing regions. This confirms previous reports by Waliyar et al. (2007) that GRD is an important disease of groundnut across all groundnut growing regions in Sub-Saharan Africa.

Although most of the farmers could recognize the disease, they associated the symptoms to abiotic stresses such as drought, too much rain and poor soil fertility. None of the groundnut farmers associated the disease with viruses and aphid vector. Although most farmers observed that the susceptibility of the disease varied among cultivars, they continued growing susceptible cultivars because of their superior agronomic traits such as high yield. The farmers' perception and lack of knowledge on the disease could be some of the factors that might be contributing to the high prevalence of GRD in major groundnut growing regions in western Kenya. The study demonstrated that despite the several strategies used by farmers for the management of GRD, including rouging, cultural practices, and chemical sprays

(Naidu et al., 1999), the disease continues to be a major constraint to groundnut production in Kenya.

The study revealed that groundnut rosette disease is prevalent and widely distributed in all major groundnut growing counties in western Kenya and therefore control interventions are urgently required. The results of this survey reflect the need for creation of awareness to enhance farmers' knowledge on GRD in Western Kenya. Emphasis should be on accurate disease identification, sources of seeds for planting and feasible management practices such as rouging and use of resistant varieties. Farmers use their own uncertified seeds or those from neighbors most of which are from susceptible landraces and serve as source of virus. Therefore, for effective control of GRD, farmers need to be sensitized on the benefits of using seeds of improved groundnut varieties. Further work on molecular characterization of GRD agents identified in this study will facilitate the understanding of diversity of viruses infecting groundnut in western Kenya. There is a need to promote production and dissemination of seeds of improved varieties to reduce virus disease incidence in major groundnut growing regions of Kenya. The determination of the areas with high insect vectors and virus disease incidences indicates where resistance breeding and other control strategies are urgently needed. The study has demonstrated the need for breeding to develop improved varieties that have high levels of resistance to GRD. Host plant resistance will be the best long-term solution to the problem of virus diseases, as they will reduce yield losses even if farmers lack knowledge about the spread

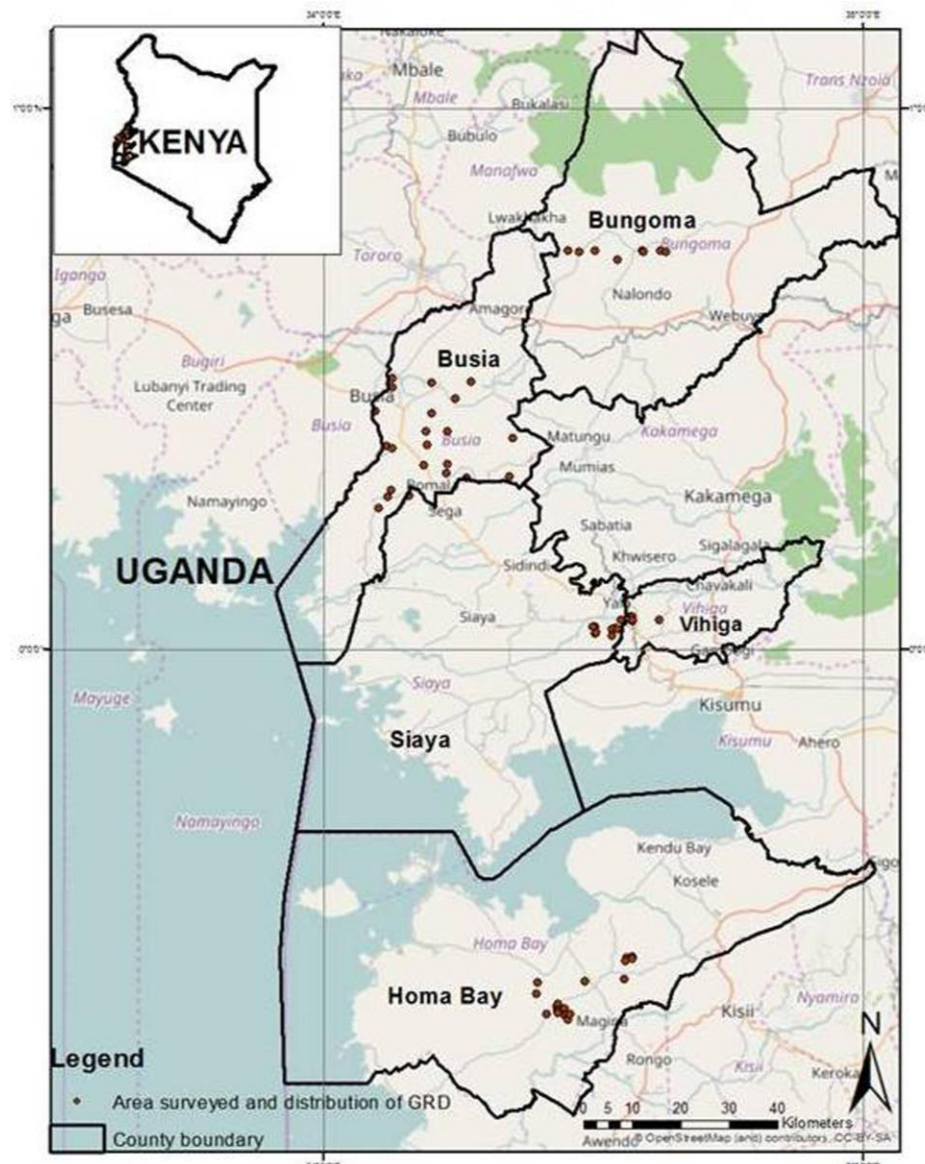


Figure 4. Map of western Kenya showing surveyed counties and distribution of GRD.

of plant virus diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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