Research Application Summary

Occurrence and distribution of groundnut rosette disease in Western Kenya

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Abstract

Groundnut (*Arachis hypogaea* Linn) is an important legume in western Kenya, but yields are low and declining. Pests and diseases are ranked high among the yield reducing factors. Groundnut rosette disease (GRD) is the main disease and can cause upto 100% yield loss. Distinct chlorotic, green and mosaic rosette symptoms caused by synergism among groundnut rosette assistor luteovirus (GRAV), groundnut rosette umbravirus (GRV) and its satellite RNA (sat-RNA), make it a unique and fascinating virus disease, whose origin and perpetuation in nature still remains inconclusive in spite of substantial advance in knowledge since 1907 when it was first reported. Limited information is available on the occurrence and distribution of the disease in western Kenya. This study determined the distribution and tolerance/resistance of local germplasm to GRD pathogens. A survey of GRD was conducted in Bungoma and Kakamega counties in short and long rains of 2016-2017. Symptomatic leafy samples were collected and analyzed by molecular means. Data on incidence and severity was recorded and analyzed by Statistical Analysis Software (SAS) program version 9.3. Screenhouse experimental study was conducted at KALRO Kakamega. Five popular legume varieties and one solanaceous Physalis peruviana Linn plants at three leaf-stage in a 4x6 factorial design, were mechanically inoculated with GRD inoculum prepared from leaves of RT-PCR positive samples. The plants were monitored for symptom development in the screenhouse for 8 weeks. A total of 301 samples from 144 farms were collected. Rosette incidence was significantly higher in Bungoma (66.51%) followed closely by Kakamega (60.52%). All tested plants from the screenhouse developed symptoms typical of GRD. The fact that GRD occurs wherever groundnuts are grown in western Kenya, is of great concern and may be the reason for the observed low yields. Incorporation of GRD resistant genes in the local cultivars/varieties may be the only practical solution.

Key words: *Arachis hypogaea* Linn, biological characterization, pathogenicity, rosette disease, western Kenya

Résumé

L'arachide (*Arachis hypogaea* Linn) est une légumineuse importante dans l'Ouest du Kenya, mais les rendements sont faibles et en baisse. Les ravageurs et les maladies sont classés parmi les principaux facteurs de réduction du rendement. La maladie de la rosette de l'arachide (GRD) est la principale maladie et peut entraîner jusqu'à 100% de perte de rendement. Les symptômes distincts chlorotique, verte et mosaïque de la rosette causés par la synergie entre le lutéovirus assistant de la rosette d'arachide (GRAV), l'ombravirus de la rosette d'arachide (GRV) et son satellite ARN (sat-RNA), font d'elle une maladie virale unique et fascinante, dont l'origine et la perpétuation dans la nature demeurent non concluante malgré une avancée substantielle des connaissances depuis 1907, date où elle a été signalée pour la première fois. Des informations limitées sur la présence et la distribution de la maladie sont disponibles dans l'ouest du Kenya. Cette étude a déterminé la distribution et la tolérance/résistance du matériel génétique local aux agents pathogènes GRD. Une enquête sur le GRD a été menée dans les comtés de Bungoma et Kakamega lors des pluies courtes et longues de 2016-2017. Des échantillons foliaires symptomatiques ont été collectés et analysés par des moyens moléculaires. Les données sur l'incidence et la sévérité ont été enregistrées et analysées par le programme de logiciel d'analyse statistique (SAS) version 9.3. L'étude expérimentale en serres a été menée à KALRO Kakamega. Cinq variétés de légumineuses populaires et une plante solanacée Physalis peruviana Linn à trois stades foliaires dans un plan factoriel 4x6 ont été inoculées mécaniquement avec un inoculum GRD préparé à partir de feuilles d'échantillons positifs RT-PCR. Le développement des symptômes par les plantes a été surveillé dans les serres pendant 8 semaines. Un total de 301 échantillons provenant de 144 fermes a été collecté. L'incidence de la rosette était significativement plus élevée à Bungoma (66,51%), suivie de près par Kakamega (60,52%). Toutes les plantes testées des serres ont développé des symptômes typiques de GRD. Le fait que les GRD se produisent partout où les arachides sont cultivées dans l'Ouest du Kenya est très préoccupant et pourrait être la raison des faibles rendements observés. L'incorporation de gènes résistants aux GRD dans les cultivars/variétés locaux pourrait être la seule solution pratique

Mots clés: *Arachis hypogaea* Linn, caractérisation biologique, pathogénicité, maladie de la rosette, Ouest du Kenya

Introduction

Groundnut (*Arachis hypogaea* L.) is a major food crop in western Kenya grown as oilseed, cash crop and animal feed. The crop is an annual/perennial plant that produces aerial flowers but fruiting below the soil level. Specific cultivar groups and genotypes are preferred for particular uses because of differences in flavour, oil content, size, shape and pest/disease resistance/ tolerance. The major groundnut producing countries include in Asia (China, India, Indonesia, Myanmar and Vietnam), Africa (Burkina Faso, Chad, Democratic Republic of Congo, Ghana, Kenya, Malawi, Mali, Mozambique, Nigeria, Senegal, South Africa, Sudan, Uganda and Zimbabwe), Unite States of America (USA) and South America (Argentina, Brazil and Mexico). Africa contributes about 24.4% of world production of groundnut and yields per hectare in Eastern and South Central Africa averages 1,604 kg/ha, which is consistently low

compared to the 3,393 kg/ha and 3,801 kg/ha recorded in China and the USA, respectively (Kidula *et al.*, 2010). Western Kenya farmers achieve less than 30-50% of the potential yield with an average output of 600-700 kg/ha, well below the expected yield of 3000-4000kg/ha obtained in on-station experimental fields (Mugisa *et al.*, 2016). Low yields are mainly attributed to poor agronomic practices, drought, low soil fertility, weeds, low quality seeds, numerous pests and diseases. Groundnut rosette disease (GRD) causes significant yield losses of 60-100% (Okello e*t al.*, 2017).

Rosette is a unique and fascinating virus disease whose origin and perpetuation in nature still remains inconclusive, in spite of substantial advance in knowledge since 1907 when it was first documented in Tanzania (Wangai *et al.*, 2001). Since then, GRD has been reported in several other sub-Saharan African (SSA) countries of Angola, Burkina Faso, Cote d'lvoire, Democratic Republic of Congo (DRC), Gambia, Ghana, Kenya, Madagascar, Malawi, Niger, Nigeria, Senegal, South Africa, Swaziland and Uganda (Mugisa *et al.*, 2016). In 1975, GRD affected 0.7 million ha of groundnut in northern Nigeria, and caused an estimated yield loss of 0.5 million tonnes, valued at US$ 5 million (Deom *et al.*, 2000). In 1995-1996, eastern Zambia lost 43,000 ha of groundnut to GRD viruses estimated at US$ 5 million. In 1994-1995, farmers in central Malawi abandoned the crop by 23%, following an unpredictable epidemic, whose annual loss was estimated at US$ 155 million (Appiah *et al.*, 2017). Key market class cultivars, including landraces have succumbed to GRD, resulting in yield reduction to as low as 800 kg/ha, compared with 3,000 kg/ha reported from on-station plots in Uganda (Okello *et al.*, 2017). Adoption of new varieties and specific cultivar genotypes is constrained by the low priority given due to lack of efficient seed production systems and pest/disease pressure tolerance/resistance.

Distinct field rosette symptoms of chlorotic, green and mosaic caused by synergism among groundnut rosette assistor luteovirus (GRAV), groundnut rosette umbravirus (GRV) and its satellite RNA (sat-RNA), makes it three diseases in one from the phenotypic symptom expression. The pathogens of GRD have not been detected elsewhere in the world except in SSA (Taliansky and Robinson, 2003). Despite the fact that groundnut is grown in western Kenya, and transmitted efficiently by the polyphagous groundnut aphid vector, *Aphis craccivora* Koch, and inefficiently by *Aphis gosypii* Glover and *Myzus persicae* Sulzer, all found in the region, limited information is available on the occurrence and distribution of rosette isolates. This study determined the occurrence, distribution and resistance of local groundnut germplasm to GRD. To date, there is no work on the host range studies of indicator plants for GRD causal agents through phenotypic screening and pathotyping biological characterization.

Materials and Methods

Field survey. Two disease diagnostic surveys to determine GRD virus occurrence and distribution were conducted in major groundnut growing areas of Bungoma and Kakamega counties of western Kenya. Symptomatic leafy samples were collected from farmers' fields and placed into falcon tubes containing RNAlater solution, and taken to the laboratory for molecular analysis. Groundnut fields were sampled during the short rains season

(September to November) of 2016 and long rains season (April to June) of 2017. Sampling was done in Bumula, Bungoma Central, Bungoma East, Bungoma South and Bungoma West in Bungoma County and Kakamega Central, Kakamega East, Kakamega North and Kakamega South in Kakamega County. Purposive sampling of groundnut farms was done by stopping at regular predetermined intervals along motorable roads that traverses each sampling area. The survey was conducted by walking through groundnut fields, and visually inspecting groundnut crops for symptomatic leaves. Depending on the farm size, quadrats were estimated, disease incidence and severity was scored on the disease diagnostic score sheet for each quadrat through random sampling. Disease incidence was calculated according to Reddy (1991), as the proportion of plants showing GRD symptoms. Disease severity was scored using a severity scale of 0 – 3, where: 0 = No disease, 1 = Mild, 2 = Moderate and 3 = Severe. Geographical position system (GPS) coordinates were recorded for each sampled field site. Data on incidence and severity was recorded and subjected to analysis of variance (ANOVA) using Statistical Analysis Software (SAS) program version 9.3. Pairwise comparison of means was done using the Least Significance Difference (LSD) at $P \leq 0.05$.

Greenhouse experiment. Five popular legume varieties [beans (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*), groundnut (*Arachis hypogaea* L.), green gram (*Vigna radiata*), soybean (*Glycine max*)] and one Solanaceae golden berry (*Physalis peruviana* L.) plants were screened at the Kenya Agricultural, Livestock and Research Organization (KALRO), Kakamega. The experiment was arranged in a 4x6 factorial design with three replications in three plot sizes of 4 m by 2 m, with 60 cm spacing between the rows and 30 cm between the plants, and 1 m spacing between the replicated plots. The 30 cm diameter by 30cm height pots were filled with solarized soil and planted with 4 seeds each. The seeds were watered daily in the morning and evening throughout the growing period. At two leaf stage, the plants were thinned to two to ensure good crop stand. At three leaf stage, they were inoculated with GRD inoculum prepared from RT-PCR positive symptomatic leafy samples obtained from the field survey. The GRD inoculum was prepared by macerating the mixed distinct chlorotic, green and mosaic rosette symptomatic leaves in a mortar and pestle, in a chilled sterilized 0.01M cold phosphate buffer (K2HPO4+ KH2PO4), PH 7.0 containing 0.2% Sodium sulphite and 0.01M Mercaptoethanol (1:6 [w/v]) tissue: buffer. Inoculation at three leaf stage was mechanically done using the rub method on Carborundum dusted leaves. The plants were observed weekly for 8 weeks for phenotypic pathotyping symptom development. Seeds from less severely rosetted groundnut farms in the field with chlorotic, green and mosaic rosette symptoms were harvested, planted in screening caged pots and observed for 8 weeks. The screened symptomatic leafy samples were collected from each indicator plant based on visual symptoms in falcon tubes containing RNAlater solution in a cool box, and taken to the laboratory for molecular analysis. Asymptomatic leaves from screened rosetted seeds were also analyzed by molecular means.

Results and Discussion

Field survey GRD incidence and severity. A total of 301 symptomatic leafy samples from 144 farmers' fields were collected. Some individual farms recorded 100% chlorotic symptoms while others had 100% green rosette symptoms with 20-40% mosaic rosette symptom. Other farms had mixed symptoms across all the surveyed areas of 35-70% (Table 1).

Table 1. Visual mean Rosette incidence and severity score in Bungoma and Kakamega Counties

County	Season	N	Mean incidence	Mean severity
Bungoma	Long rain	45	30.89	1.49
	Short rain	47	66.51	2.21
Kakamega	Long rain	30	43.47	1.53
	Short rain	22	47.73	2.14
Overall	Long rain	75	35.92	1.51
	Short rain	69	60.52	2.19

Rosette was widespread in groundnut growing areas of western Kenya due to GRD and poor agronomic practices. This results concur with the earlier report by Wangai *et al*. (2001) who reported that GRD incidence in Kenya ranges from 24 to 40% in western Kenya. Rosette incidence in most farms of western Kenya recorded 30-100%. The disease has increased over time due to virulence and climate change causing complex mutations of GRD pathogens and etiology. This findings are also in agreement with Kidula *et al*. (2010) who estimated yield losses of 60-100% in the groundnut growing areas, and Okello *et al*. (2017) whose research indicated that mosaic rosette is of low incidence but of wide occurrence in east and southern Africa. Different variants of the naturally occurring isolates of sat-RNA nucleic particle are responsible for the distinct chlorotic and green forms of rosette symptoms (Deom *et al.,* 2000; Appiah *et al.,* 2017), while mosaic rosette is phenotypically induced by a mixture of both GRAV and GRV with its sat-RNA (Taliansky and Robinson, 2003). Although the sat-RNA is mainly responsible for GRD symptom diversity, groundnut genotypes possessing resistance to rosette disease are highly resistant but not immune to GRV and its sat-RNA, but are fully susceptible to GRAV which intensifies GRV sat-RNA induced symptoms elevating yield losses. Only limited field resistance is available for GRD cultivars, which have less than superior agronomic traits (Usman *et al.,* 2013).

During the short and long rain seasons, disease incidence was significantly different (p<0.0001) with short rains season recording higher incidence than the long rains season. Bungoma County recorded 66.51 % mean incidence of rosette disease during the short rains season, higher than in the long rains season where it reduced to 30.89 %, registering a statistically significant difference of 0.633. Similarly, Kakamega County recorded statistically higher mean incidence in the short rains season of 47.73 % compared to 43.47 % in the long rains season. This phenomenon is attributed to the long rains being heavy and consistent after planting, washing off the vector aphids from the crop, reducing their build-up and contact hours for inoculum transmission, before much damage is done to the groundnut crop. In the short rains season, high incidence is attributed to massive build-up of the vector aphid colonies on the crop to very high densities, leading to further dispersal and secondary spread of the virus (Kidula *et al.,* 2010; Mugisa *et al.,* 2016). Between the two counties, Bungoma County had a higher mean incidence of GRD occurrence and symptom diversity distribution than Kakamega County with a standard error mean of 0.379 between the two growing seasons (Mabele *et al.,* 2018).

The mean severity in Bungoma County during the short rains season was statistically higher than in the long rains season with a significant standard error mean of 0.014. Kakamega County also recorded high severity index during the short rains season than the long rains season registering a statistically significant standard error mean of 0.066. The positive statistically significant severity of 0.633 between the two counties, is attributed to rosette disease and poor agronomic practices. Lower GRD incidence of 43.51% (Bungoma) and 32.68% (Kakamega) was recorded in the groundnut crops intercropped with maize and other tall cereals. This resulted into low inoculum of the virus transmitted by the vector A. craccivora reaching the groundnut crops. The maize and taller cereal crops provided a buffer zone that acted as a barrier and landing platform for the groundnut aphid. However, the severity was not statistically significant to those grown in open fields.

Screening legumes and *Physalis peruviana* Linn for resistance to GRD. Different levels of resistance/tolerance by legumes and Physalis peruviana Linn to GRD were evaluated in the screenhouse. Host range studies of screened GRD isolates through biological characterization from western Kenya, showed highly significant ($P \leq 0.05$) susceptibility of the germplasm to GRD pathogens. The screened germplasm expressed distinct pathotyping phenotypic field symptoms of stunted growth, shortened internodes, thickened stems, dwarfism with bushy appearance, dark green, yellowing with chlorosis lesions, mixed mosaic, reduced leaf area with twisted and distorted leaves curling downwards and upwards (Figure 1). Rosetted seeds were found not expressing either of the three GRD symptom types, confirming that rosette is not transmitted by seed on phenotypic screening through pathotyping biological characterization. However, molecular analysis is recommended to validate and support this findings. Rosette disease causes yield reduction possibly due to impairness of plant performance through limitation of photosynthetic production, thereby retarding growth and interruption of the supply of assimilates to pod and seed development. The RNA viruses exist as quasispecies in the infected plants and thus the population complexity of GRAV, GRV and sat-RNA in the field has the potential to be large with potential permutations among variants of the three causal agents (Taliansky and Robinson, 2003).This virus evolution and mutations results into new disease patterns and symptom diversity (Deom *et al*., 2000). Phenotypic symptom expression on all the screened germplasm samples infer that the western Kenya legumes and *P. peruviana* act as alternative hosts for GRD pathogens. The mean incidence and severity of GRD on the total screened legumes and P. peruviana in correlation to control isolates was statistically significant (p=0.05).

This study has shown that legumes and *Physalis peruviana* plants are susceptible and are potential alternate hosts to GRD pathogens. Okello *et al*. (2017) identified the leguminous weed vegetable, Oyado (*Cassia obtusifolia*) as a potential alternative host that tested positive for all the GRD agents through RT-PCR. Taliansky and Robinson (2003) reported a variant of the satellite RNA of groundnut rosette virus that induced brilliant yellow blotch mosaic symptoms in *Nicotiana benthamiana*. In all forms of rosette disease, early infection causes severe pod loss because rosetted plants may flower but few pods and seeds are produced (Kidula *et al*., 2010).

Figure1. From top left clockwise; screening groundnut rosette seeds, popular groundnut, common beans, cowpea, soybean and golden berry germplasm

Conclusion

This study has confirmed that GRD occurs wherever groundnuts are grown in western Kenya. This is of great concern and may be the reason for the observed low yields. Incorporation of GRD resistant genes in the local cultivars/varieties may be the only practical solution. Groundnuts should not be intercropped with other legumes and *Physalis peruvian*a Linn to significantly reduce GRD severity and inoculum in the field.

Acknowledgement

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