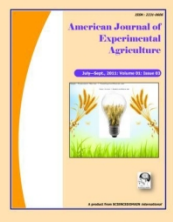

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Variability in Reactions of Groundnuts Varieties to Groundnut Rosette Virus Isolates from Uganda

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Authors' contributions

Both authors contributed equally. The second author collected and compiled the data while the first author analyzed the data and wrote the paper. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The study was conducted to assess the variability in reactions of groundnut varieties to groundnut rosette virus isolates from Uganda (Please correct the yellow highlighted sentence).
Study Design: The experimental design was randomized complete block design arranged as a split plot with three replications. The main plots were the groundnut varieties while the sub-plot were the groundnut rosette virus isolates collected from central, western and eastern Uganda.
Place and Duration of Study: The study was conducted at the screen house, Kyambogo University, Kampala during 2011 and 2012, respectively
Materials and Methods: Non-viliferous aphids obtained from Mukono zonal agricultural research and development institute (MUZARDI) fed on infected groundnut plants collected from central, western and eastern Uganda were used to infect 3 week old groundnut seedlings in a screen house.
Results: There were significant variation in reactions ($P<0.05$) among the groundnut varieties to groundnut rosette virus isolates. Similarly, significant variety x isolate interactions were observed for incidence, leaf area index and plant height.
Conclusion: This study has shown that there were significant variations in reactions of groundnut varieties to groundnut rosette virus isolates. In general, two groundnut rosette

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pathotypes exist in Uganda. Therefore, this is important in management of the disease.

Keywords: Groundnuts; isolates; reactions; rosette virus; pathotypes; variability.

1. INTRODUCTION

Groundnut rosette disease (GRD) is the most widespread and devastating disease of groundnuts in all the major groundnut growing regions in sub-Saharan Africa including Madagascar. According to Naidu et al [1], groundnut rosette disease manifests in two predominant forms namely, chlorotic and green rosette although, the variability in symptoms have been reported. However, chlorotic rosette is the most predominant symptoms in many sub-Saharan Africa countries whereas green rosette has limited geographic distribution reported only from west African countries, Uganda, northern Malawi and Angola. The green and chlorotic forms of rosette are caused by green and chlorotic variants of the satellite RNA. In contrast, the mosaic rosette is caused by mixed infection of chlorotic and mottle strains of GRV, is actually caused by mixed infection with chlorotic and mottle variants of the satellite RNA [2]. However, the mild symptoms consisting of faint mottling of leaves, chlorosis of the young leaves and veins generally remain dark green than the intervenial tissues while severe symptoms is characterised by curling of leaves in some cultivars and shortening of the internodes. Accordingly, the most severe and obvious symptoms occur when plants are infected when still young and growth of internodes is almost completely inhibited producing extremely dwarf plants of rosette habit which are normally chlorotic. The severity of groundnut rosette disease varies to a greater extent depending on the time of infection, plant density, cultivar of groundnuts and amount of inocula [3]. According to Naidu and Kimmins [4], groundnut rosette disease complex has significant effects on agronomic performance of groundnuts. In particular, groundnut rosette assistor virus (GRAV) affected plant height, leaf area index and yield in the absence of the groundnut rosette virus and satellite RNA.

Groundnut rosette disease is regarded as a polycyclic disease because each infected plant serves as a source of inoculum for initiating subsequent spread in the field [5]. According to Brook [6], winged aphids bring the virus into the crop but spacing affects rosette incidence because of the influence of ground cover on the landing response of the winged aphids. Although, vectored by aphids, GRD requires the existence of three agents namely, groundnut rosette virus (GRV) genus *Umbravirus* [7], satellite RNA (sat-RNA) [8] and groundnut rosette assistor virus (GRAV) genus *Luteovirus* [9] to produce visible symptoms. Individually, GRAV or GRV cause symptomless infection or transient mild mottle symptoms [10]. According to Murant and Kumar [2], groundnut rosette disease symptoms and different symptom forms are largely due to sat RNA and its variants. Yet, the expression of disease symptoms does not necessarily indicate the presence of aphid-transmissible groundnut rosette assistor virus GRAV in infected plants. Consequently, plants that show symptoms but lack GRAV play no role in the spread of the disease because the coat protein of GRAV is needed for encapsidation and transmission of GRV and sat-RNA [3]. Moreover, it is only the number of plants containing all three agents that play a crucial role in the secondary spread of the disease in a given field whereas the total number of plants showing disease symptoms irrespective of having GRAV influences yield [11]. Invariably, the nature and pattern of disease spread can be influenced by plant age, crop density, timing and efficiency of transmission by viruliferous aphid vectors reaching the crop as well as the virus isolates. In addition, the proximity to the source of primary inoculum, climatic factors, predators and

parasitoids of vector populations within the crop may also play a significant role in disease spread. Nonetheless, both types of groundnut rosette disease though sporadic and unpredictable cause significant yield losses amounting up to 100% [12].

Due to its devastating and unpredictable effects, various methods have been employed to manage groundnut rosette disease including chemical spraying to reduce aphid vector populations, cultural practices to delay the onset and spread of vector and disease as well as breeding for virus and vector resistance. The use of cultural practices and insectidical sprays has been practice since the early days of groundnut growing in Uganda [13,14]. However, for a number of reasons, famers seldom use these practices. Therefore, host plant resistance remains the most economical and sustainable means of managing groundnut rosette disease. Resistance to groundnut rosette is in two forms namely, resistance to aphids vectors [15] and resistance to the virus [16] Although, some high-yielding, short, medium and long-duration genotypes with good levels of resistance to rosette disease and agronomically acceptable traits have been developed and made available to national programmes in different countries of sub-Saharan Africa, these always never last long and cannot be grown across environments due to the diversity of groundnut virus isolates which overcome the resistant genes [17]. However, the majority of these varieties are late maturity and therefore not suitable for production in areas with short rainy seasons. Therefore, the analysis of the genetic diversity structure and evolution of virus populations is critical for the development of efficient and stable control strategies. This is because the control strategies often fail due to the evolution of the resistant-breaking pathotypes/new pathogen population which overcomes resistance gene. Although, different approaches may be used to analyse the genetic diversity variation of plant viruses including biological properties such as the symptoms caused in different host plant species, host range or vector transmission properties, the choice of a given analytical technique depends on factors like the goal, sensitivity and cost of the analysis. According Nigam and Bock [18], genetic studies on Groundnut rosette virus disease suggest that resistance to this viral disease is complex, polygenic and governed partly by a pair of independent complementary recessive genes. However, in Uganda information on the variability of the reactions of groundnut varieties to groundnut rosette virus isolates is scanty and limited. Therefore, this study was conducted to assess the variability in reactions of groundnut varieties to groundnut rosette virus isolates from different parts of Uganda.

2. MATERIALS AND METHODS

The study was conducted in the screen house at Kyambogo University, 10km east of Kampala city during the period 2011 and 2012, respectively. Kyambogo University is located $0^{\circ}20N\ 32^{\circ}35E$, at an altitude of 1189 metres above sea level. Kyambogo lies in the equatorial climatic zone in the northern shores of Lake Victoria. The area is characterised by bimodal type of rainfall with a mean annual of 1000mm. The minimum and maximum annual temperatures range from 15º-18º and 27º- 30ºC, respectively. The predominant soil type in Kyambogo hill is ferric soils with loamy sandy soils [19]. The experimental design was a randomised complete block design (RCBD) arranged as a split plot with three replications. The groundnut varieties were the main plots and the virus isolates constituted the subplots**.** Four groundnuts varieties of varying resistance including Serenut 1R (susceptible), Serenut 3 (relatively resistant), Serenut 4T (highly resistant) and the landrace Egoromoit (very susceptible) were used for the study. The virus isolates used were obtained from Mukono (central), Serere (eastern) and Kyenjojo (western) Uganda. The groundnuts were planted on a 2 kg pots filled with soils obtained from Kyambogo university farm. The seeds were

watered once until after germination. The aphid colony used for inoculation was obtained from the groundnut fields in Mukono zonal agricultural research and development institute (MUZARDI). A single colony of *Aphis craccivora* was maintained on healthy groundnut plant in the screen house at Kyambogo University at 30ºC. The plants used for rearing the aphid colony were routinely monitored for freedom from groundnut rosette disease symptoms (Naidu et al., 1999). The aphid colony used for inoculation was given 24 hours acquisition period on rosette disease infected groundnut plants obtained from the different regions of Uganda. The viruliferous aphid colonies reared on groundnut plants infected with groundnut rosette virus isolates from different regions of the country were transferred and confined onto a- ten (10) day old groundnut seedlings per pot. The aphids were given a 72 hour virus inoculation access period (IAP). Aphids were then killed by spraying groundnut seedlings with Actellic 50EC (a.i 500g Pirimiphos methyl per litre) after 72 hrs. The inoculated groundnuts were maintained in insect free screen house.

Plants were assessed for disease reactions at harvest using 0-9 scale where 0=no symptoms and 9=severely stunted and rosette plants as described by Kalule et al. [20] Prior to harvesting, data was taken on the leaf area index (LAI) and plant height. Leaf area indices were taken from individual plants in each replicate by measuring the length and width of one fully expanded leaf at the same position using a meter ruler [21]. Plant height was measured from each individual plant using the meter ruler. At harvest the number of pods per pot, fresh roots and shoots biomass was also recorded. The number of seeds per pod was counted after drying of the pods. All the data was compiled, entered in excel spread sheet and analysed using GENSTAT computer package. Where there was significance, means were separated using LSD at 5% probability level.

3. RESULTS AND DISCUSSIONS

3.1 Results

There was no significant variations ($P>0.05$) in reactions of groundnut varieties to virus isolates during the two trials. However, the groundnut rosette virus isolates behave significantly different ($P<0.001$) during both trials. In addition, significant differences ($P=0.001$) were observed in plant height, leaf area index and number of seeds for both the variety and virus isolates for the two trials. Similarly, significant variety x virus isolates interactions ($P=0.004$ and $P=0.056$) were observed during the first trial but not second trial. In fact, significant variety x virus isolates interactions ($P=0.001$) were observed for plant height during the two trials. However, significant variety x virus isolates interactions were observed for leaf area index only during the second trial ($P=0.001$) but not during the first trial ($P=0.112$). Similarly, no significant variety x virus isolates interactions were observed for the number of seeds during the two trials ($P=0.839$ and $P=0.059$), respectively (Tables 1 and 2). Groundnut rosette virus disease manifested as yellowing, mottling and mosaic of the leaves, stunting and distortion of the shoots. In addition, induced bushy stunted growth characterised by a diversity of symptoms were observed for all virus isolates. Indeed, during the first trial the most aggressive isolate was the one from western Uganda whereas during the second trials, the most aggressive isolates were those from eastern Uganda Table 1. Overall, all new symptoms appeared first on the top young leaves but later showed on older leaves. The effects of groundnut rosette disease on growth and yield of groundnut varieties is shown in Table 2. Egoromoit was the tallest variety compared to the other varieties during the two trials. Similarly, Egoromoit had the largest leaf area index compared to the other varieties during the two trials. Additionally, the highest number of seeds was recorded from

Egoromoit and Serenut 4T, respectively. Overall, all the isolates had different effects on growth and yield of groundnut varieties Table 3.

Table 1. Mean effects of groundnut rosette isolates on incidence and severity of 4 groundnut varieties grown at Kyambogo University, 2011/2012

Isolate	Incidence (%)	Severity
First trial Sep-Dec 2011		
Central	13.3	29.2
East	14.6	22.4
West	17.1	32.8
Water	0.0	0.00
Mean	25.0	25.00
LSD (0.05)	6.95	12.13
Second trial Feb-May 2012		
Central	1.33	0.43
East	2.28	0.95
West	1.08	0.41
Water	1.00	0.20
Mean	1.42	0.50
LSD (0.05)	0.34	0.24

Table 2. Mean effects of groundnut rosette disease on the plant height, leaf area index and number of seeds of four groundnut varieties grown at Kyambogo University, 2011/2012

Variety	Plant height (cm)	Leaf area index	Number of seeds
First trials Sep-Dec 2011			
Serenut 4T	14.56	4.79	2.92
Serenut 1R	12.37	3.90	2.25
Serenut 3	13.63	4.46	1.25
Egoromoit	29.59	6.43	6.25
Mean	17.54	4.90	3.17
LSD (0.05)	4.80	1.02	3.80
Second trial Feb-May 2012			
Serenut 4T	9.88	2.86	15.00
Serenut 1R	10.76	4.05	7.17
Serenut 3	8.58	2.97	9.50
Egoromoit	23.67	8.04	13.00
Mean	13.22	4.48	3.17
LSD (0.05)	2.93	0.77	3.80

Table 3. Mean effect of groundnut rosette isolate on plant height, leaf area index and number of seeds of four groundnut varieties grown at Kyambogo University, 2011/2012

Variety	Plant height (cm)	Leaf area index	Number of seeds
First trials Sep-Dec 2011			
Central	9.63	3.37	0.67
East	14.61	3.82	0.92
West	13.43	3.79	1.58
Water	32.48	8.60	9.50
Mean	17.54	4.90	3.17
LSD (0.05)	4.03	1.18	3.16
Second trial Feb-May 2012			
Central	11.59	4.50	8.17
East	9.16	2.78	3.58
West	14.51	4.85	6.58
Water	17.62	5.78	8.25
Mean	13.22	4.48	6.65
LSD (0.05)	1.58	0.49	2.62

3.2 Discussion

This study assessed the variability in reactions of groundnut varieties to groundnut rosette virus isolates from different parts of Uganda. Groundnut varieties reacted differently to the groundnut rosette virus isolates. In fact, the improved varieties behave similarly to groundnut rosette disease compared to the landrace. In other words the improved varieties succumbed to different levels of symptoms of groundnut rosette compared to the landrace which consistently recorded higher plant height, leaf area index and number of seeds. This is because the improved varieties were bred for different sources of resistance, for example, Serenut 1R and Serenut 3R were bred and released against the rosette disease whereas Serenut 4T was released as aphid vector resistant varieties. Accordingly, this means that each of these varieties can only be grown and managed under different conditions [13;14]. Therefore, where the disease pressure is high, improved varieties can only be grown under continuous spraying against the vector or grown where the disease pressure is relatively low without spraying. However, insecticidal spraying may not be very feasible under the smallholder farming systems because of the cost of the insecticide and also the unpredictability of weather conditions which relies on early planting and close spacing. Groundnut rosette isolates induced a variety of symptoms on the groundnuts including bushy stunted growth characterised by veinal chlorosis, greenish mosaic leaves, and small rosetted, twisted and curled leaves among others. Characteristically, both types of groundnut rosette pathotypes were observed during both trials [1]. These results probably suggest differences in cultivar resistance to groundnut rosette disease. This is in line with the earlier findings which indicated that improved groundnut varieties such as Serenut 3R have been developed and incorporated with the genes for virus resistance as opposed to Egoromoit which is a local landrace. Although, this may imply that host plant resistance is the most economical and sustainable way of managing groundnut rosette disease, sources of resistance may be lacking in most of the newly improved varieties compared to the local landrace Egoromoit. Accordingly, the evolution of more aggressive and virulent isolates always overcomes the existing sources of resistance. Therefore, the continuous search for sources of resistance is always the major preoccupation of the groundnut breeders to

identify genotypes which can resist the new strains of the rosette pathogens and other constraints limiting groundnut production as well as genotypes with improved agronomic traits [16]. However, this study has confirmed the varying level of resistance among the improved varieties. For example, Serenut1R was the most susceptible among the improved varieties to groundnut rosette disease.

The effect of groundnut rosette disease on the growth and yield of groundnut was observed on plant height, leaf area indices and number of seeds per plant. In fact, groundnut rosette disease may express as a significant reduction in plant height due to shortened internodes, low leaf area indices or poor pod setting with no seeds per pods and subsequently zero yield per unit area. Besides, its sporadic and unpredictable nature, groundnut rosette disease is known to cause a significant yield loss amounting up to 100% [12]. Indeed, groundnut rosette disease has become a discouraging factor to many of the farmers growing groundnuts in sub-Saharan Africa. Therefore, the implication of this study is that it is imperative to sensitize the stakeholders on the means of recognition, spread and control in order to facilitate the effective management of the disease. Consequently, this will promote increased groundnut production and reduced yield losses attributed to the disease attack. Basing on this study findings, groundnut rosette disease severity depends on the variety of groundnuts grown by the farmers and other factors. Therefore, it is imperative for the farmers to adopt improved groundnut varieties to obtain higher yields as opposed to the yield from local land race such as Egoromoit that are susceptible to disease.

4. CONCLUSION

Groundnut varieties reacted different to the groundnut rosette virus isolates. In fact, groundnut rosette disease induced a variety of symptoms on the groundnuts including bushy stunted growth appearances characterised by veinal chlorosis, greenish mosaic leaves, and small rosetted, twisted and curled leaves among other symptoms. Overall, groundnut rosette disease expressed reduced plant height, low leaf area indices and poor seeds yield per plant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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