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FIRST REPORT AND BIOLOGICAL CHARACTERIZATION OF COWPEA MILD MOTTLE VIRUS (CPMMV) INFECTING GROUNDNUTS IN WESTERN KENYA

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ABSTRACT

In western Kenya, groundnuts (*Arachis hypogaea* L.) is one of the major legumes where it is not only the principal source of protein but also a major source of small-holder cash income. However, groundnut production has continued to decline with farmers realizing less than 50% of the yield potential. Viral diseases play a great role in yield reduction. Cowpea mild mottle virus (CPMMV) has been reported to be one of the viruses infecting groundnuts but its status in western Kenya was not known. Therefore this study sought to establish whether the virus was already infecting groundnuts in this region. Two surveys were conducted in seven major groundnut growing Counties of western Kenya; Bungoma, Busia, Vihiga, Siaya, Migori, Nandi and Homabay, basing on various Agro-ecological zones (AEZs). Leafy samples were collected and tested by DAS-ELISA. Indicator plants were mechanically inoculated with CPMMV from positive samples and symptoms observed. The disease was found to be widespread in all the AEZs surveyed with high incidence at farm levels. Most farmers were found to use uncertified seed. ELISA tests confirmed the presence of CPMMV in the leafy samples collected from the farms. The virus induced varied symptoms on indicator plants tested. All the indicator plant used were major legumes grown in western Kenya and were found to be susceptible to CPMMV. This implies that CPMMV is a serious threat to legume production in western Kenya

Keywords: *Arachis hypogaea; incidence; legumes; severity; survey.*

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a self-pollinated, tropical annual legume which is fairly drought resistant and mainly cultivated in dry tropical areas. It has the advantage of generating residual nitrogen in the soil which benefits subsequent crops, especially when groundnut residues are incorporated into the soil during ploughing. In western Kenya, the crop is not only the principal source of protein but also a major source of small-holder cash income. However, groundnut production has continued to decline with farmers realizing less than 50% of the yield potential. This decline is attributed to a number of abiotic and biotic

factors among which viral diseases play a significant role. Groundnut rosette virus (GRV) is the most known virus among groundnut farmers since its symptoms are clear. CPMMV has been shown to cause necrotic lesions, chlorotic rings or line patterns followed by systemic leaf chlorosis, rolling and veinal necrosis (El-Hassan , 1997) which are quite confusing to the farmers. Cowpea mild mottle disease (CPMMD) caused by Cowpea mild mottle virus (CPMMV) was first reported on cowpea in Ghana (Brunt and Kenten, 1973). The disease has since been shown to have a very extensive geographical distribution and host range. The virus has been reported on cowpeas in eastern Uganda (Amayo , 2012) where it has been ranked second in terms of economic importance. This virus is mostly seed-borne (Naidu , 1998; Orawu , 2005; Amayo , 2012) meaning it can easily be spread when farmers use uncertified seed.

A survey in Sudan by El-Hassan , (1997) for CPMMV on groundnuts found that the virus was widespread in groundnut farms causing a very serious disease. The first report of CPMMV on groundnuts in Kenya was in 1976 mainly in the coastal regions (Bock , 1976). The last survey in Kenya was conducted by Bock and Wangai (1984) who noted that CPMMD was serious disease of groundnuts in eastern and coastal regions of Kenya. Since then, no work has been done to determine the status of this virus on groundnuts in other parts of the country and specifically western Kenya where groundnuts is a major legume. Therefore, this study sought to establish the status of this disease in western Kenya.

MATERIALS AND METHODS

Two diagnostic surveys (Long rains season; April-June and Short rains season; September-November, 2013) were carried out in seven major groundnut growing Counties of western Kenya in six agro ecological zones (AEZs; low midland zone 1 [LM1], low midland zone 2 [LM2], low midland zone 3 [LM3], upper midland zone 1 [UM1], low midland zone 4 [LM4] and low highlands 1 [LH1]) as follows: Busia (LM2, LM3), Bungoma (LM2), Homabay (LM2), Migori (LM2), Nandi (LH1, UM1), Siaya (LM3, LM4) and Vihiga (UM1). Disease incidence was calculated as the percentage of plants showing CPMMD symptoms to the total number of plants observed in the field. Disease severity was scored using a severity scale of 0 to 3.
Where; 0: No disease, 1: Mild, 2: Moderate, 3: Severe

Symptomatic leafy samples were collected and later analysed by DAS-ELISA using CPMMV specific antisera from DSMZ-Germany (Courtesy of Dr Stephan Winter). DAS-ELISA was done following the manufacturers instruction. Information on seed sources was obtained by interviewing the farmers.

Biological characterization of CPMMV

Common beans (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*), soyabean (*Glycine max*), green grams (*Vigna radiata*) and groundnuts (*Arachis hypogaea*), which are indicator plant for CPMMV, were planted in pots in greenhouse and mechanically inoculated with CPMMV obtained from positive samples. The inoculum was made by grinding the leaves in 0.01M potassium phosphate buffer (K_2HPO_4 +KH_2PO_4), pH 7.0, containing 0.2% sodium sulphite and 0.01M mercapto-ethanol (1:6 [w/v] tissue: buffer). The plants were observed weekly for symptom development for up to 5 weeks and leafy samples taken for ELISA tests.

RESULTS AND DISCUSSION

RESULTS

A total of 220 groundnut farms were sampled. Symptoms observed on groundnut farms included downward leaf rolling, chlorotic lesions, dwarfing, leaf deformation and mottling (Fig.1).

Figure 1. CPMMD symptoms on groundnuts

A- Downward leaf rolling and chlorotic lesions, B- Dwarfing, leaf deformation and mottling.

Disease incidence and severity

CPMMD was found to occur across all the AEZs with very high incidences at farm level. CPMMD incidence in LM3 was highest (33.81%) and significantly different to that of LM1 (19.52%) which was lowest (LSD=14.29; p=0.016). However the CPMMD incidence in LM3 was not significantly different from that of LM2, LM4 and UM1. The CPMMD incidence was highest during the SR but not significantly different from the incidence in LR. The mean severity scores for CPMMD on groundnuts were mild to moderate across the zones. There was a positive correlation between CPMMD incidence and severity (r=0.894; p<0.0001) indicating that severity increased with increase in disease incidence. Based on ELISA, LM1 had the highest incidence (8.50%) followed by LM2 (5.23%). Overall, the observed incidence was higher than that of ELISA (Table 1).

Table 1. CPMMD incidence and severity on groundnuts across the AEZs

AEZ	Season	N	Mean Incidence (%)		Mean severity
			Observed	ELISA	
LM1	LR	32	21.87	5.97	.88
	SR	39	17.59	10.45	.72
	Overall	71	19.52[b]	8.50	.79
LM2	LR	24	22.12	7.46	.92
	SR	36	35.69	3.50	1.28
	Overall	60	30.27[a]	5.23	1.13
LM3	LR	17	38.94	.00	1.12
	SR	41	31.68	3.50	1.24
	Overall	58	33.81[a]	1.96	1.21
LM4	LR	5	7.60	.00	.60
	SR	2	91.50	.00	3.00
	Overall	7	31.57[a]	.00	1.29
UM1	LR	9	15.56	.00	.33
	SR	15	24.00	.00	1.07
	Overall	24	20.83[a]	.00	.79
Overall	LR	87	23.80	13.43	.86
	SR	133	28.67	15.12	1.11

Means with same letter within a column are not significantly different at 0.05 level.

Seed sources

Most farmers (48.3%) obtained their seeds from local markets followed by the use of own seed (39.7%) while very few (1.9%) obtained seed from the agro-dealers (Fig. 2).

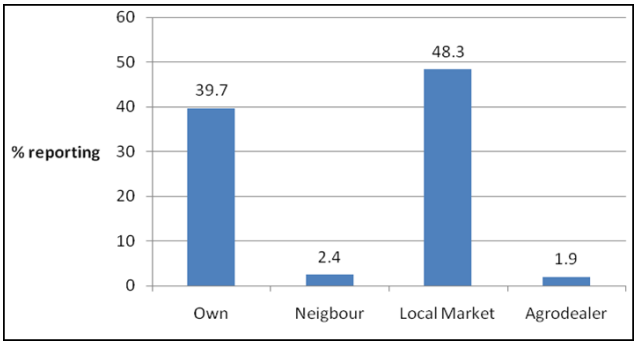

Figure 2. Percentages of farmers who obtained seeds from various sources

ELISA results

A total of 153 groundnut leafy samples were analysed. 22 samples were CPMMV positive and originated from farms in LM1, LM2 and LM3 (Table 2).

Table 2. DAS-ELISA results

Season	Virus	Total samples	ELISA +ve	Origin
LR	CPMMV	67	9	LM2 (5), LM1 (4)
SR	CPMMV	86	13	LM3 (3), LM1 (7), LM2 (3)

Symptoms induced by CPMMV on indicator plants

Varied symptoms were observed on the tested indicator plant upon inoculation with CPMMV. ELISA tests confirmed presence of CPMMV in all the legumes tested (Table 3).

Table 3. Reaction of legumes and indicator plants to CPMMV

Legume	No. inoculated	Local reaction	Systemic reaction	No. symptomatic	ELISA +ve
Common beans	10	cl	vc, lm, ld,lt, st	6	2
Groundnuts	10	nl	ld,dc, vc	8	4
Cowpea	8	cl	st, cb, vc	7	5
Green grams	8	nl	vc	4	2
Soyabean	6	nl	m	5	3

Key: cb-chlorotic blotches, cl-chlorosis, dc-downward leaf curling, lm-leaf mottling, ld-leaf deformation, nl-necrotic lesions, vc-vein chlorosis, st-stunting, m-mosaic. The observed symptoms on some indicator plants inoculated with CPMMV were as shown in Fig. 3.

Figure 3. Symptoms observed on various legumes inoculated with CPMMV

From top left clockwise: Leaf mottling, deformation and stunting on common bean, veinal chlorosis on cowpea, mosaic on soyabean and chlorotic lesions on groundnuts

DISCUSSION

This is the first report of CPMMV in western Kenya. Earlier report of the disease in Kenya was in eastern coastal regions of Kenya by Bock and Wangai (1984). The symptoms that were observed on groundnuts; downward leaf rolling, chlorotic lesions, dwarfing, leaf deformation and mottling, are the same as those observed by El-Hassan , (1997) in his study. Further analysis of the leafy samples with CPMMV specific antisera confirmed the presence of CPMMV. This therefore confirms that the symptoms observed were as a result of CPMMV and thus the virus is infecting groundnuts in western Kenya.

The disease was observed across all AEZs with high incidences at farm levels. This may be attributed to the rampant use of uncertified seed which could be viral contaminated. In this study it was found that most farmers used seeds from the local markets or recycled their own seeds from one planting season to another. This is very risky as CPMMV has been known to be one of the viruses that are highly seed-borne (Naidu , 1998; Orawu , 2005; Amayo , 2012). Therefore the crops becomes infected at early stages of growth which bring about more damage preventing the plants from reaching their full potential (Booker , 2005; Taiwo , 2007) and thus the high incidence and severity that was observed in the farms. Disease incidence correlated positively with severity. This could still be attributed to early infection of the plants due to the seed-borne nature of the virus which results to

more infected plants and viral titre built-up as the plant matures. Thus high incidence and severity. The wide distribution of CPMMD observed in this study is a clear indication of the impact of CPMMV on the yield of groundnuts in Kenya.

All indicator plants tested were found to be susceptible to CPMMV and symptoms induced were varied. The indicator plants used in this study are the major legumes cultivated in western Kenya. This implies that CPMMV infects all major food legumes. There have been many reports of CPMMV on other legumes where it has been reported to cause yield loses. The virus has been reported on groundnuts in Sudan inducing downward leaf curling and chlorosis (El-Hassan , 1997) and in eastern coastal regions of Kenya (Bock , 1976). The virus has recently been reported on soyabean in Brazil (Zanardo , 2013) and in India (Yadav , 2013) where it caused leaf mosaic and stem necrosis. Most of the symptoms observed on the indicator plants (chlorotic blotches, chlorosis, downward leaf curling, leaf mottling, leaf deformation and necrotic lesions) in the greenhouse tests were similar to those observed in different parts of the world (El-Hassan , 1997; Yadav , 2013; Zanardo , 2013). The other varied symptoms (veinal chlorosis on groundnuts and mosaic on soyabean) could imply that the viral strain (s) in Kenya is different due to differences in geographical and climatic conditions or the virus detected serologically could just be closely related to CPMMV. Efforts to amplify the CPMMV isolate from western Kenya using specific Carlavirus pair of primer failed. A similar scenario was observed by Amayo , (2012) where he could not amplify the CPMMV isolates from eastern Uganda using the similar pair of primers. This supports the fact that the CPMMV in western Kenya could be related to the one in eastern Uganda and different from those in other parts of the world. Most of CPMMV alternative hosts are the commonly cultivated legumes and thus acts as sources of inoculum posing great threat not only to groundnuts but also other legumes of economic importance.

In conclusion, CPMMV is highly infecting groundnuts in western Kenya and is expected to increase as a result of use of uncertified seeds by local farmers and the susceptibility of other legume species to the virus.

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