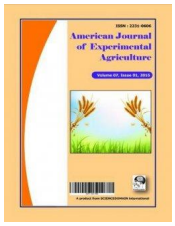

American Journal of Experimental Agriculture
11(1): 1-9, 2016, Article no.AJEA.21868
ISSN: 2231-0606

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Assessment of Sweet Potato Propagules for Re- infection by Various Viruses in East Kamagak Location – Homa Bay County-Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. Author RAO designed the study, wrote the protocol and wrote the first draft of the manuscript. Author WMT reviewed the experimental design and all drafts of the manuscript. Authors JOG and LK managed the analyses of the study. Author LK identified the plants. Authors RAO and WMT performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJEA/2016/21868
Editor(s):
(1) Yeamin Hossain, Department of Fisheries, University of Rajshahi, Bangladesh.
Reviewers:
(1) M. Angels Calvo Torras, Universidad Autonoma de Barcelona, Spain.
(2) Peng He, Guizhou University, China.
Complete Peer review History: http://sciencedomain.org/review-history/12665

Original Research Article

Received 6th September 2015
Accepted 3rd November 2015
Published 14th December 2015

ABSTRACT

Sweet potato, *Ipomea batatas* (L) Lam is an important subsistence food crop as well as cash crop in East Kamagak location and is also very popular in the major cities in the country including Nairobi. Sweet potato is easily managed with fewer field management practices compared to other root crops and it can similarly be stored for a prolonged length of time in the soil before harvesting. However sweet potato production is constrained by virus infection. At least 13 viruses are reported to infect sweet potato naturally of which most of them are insect transmitted. The study aimed at screening and selecting virus free germplasm. A survey was conducted in East Kamagak using questionnaire which aided in germplasm collection. Twelve genotypes were used for the study. The collected germplasm was virus indexed using visual scoring with severity of infection ranging from 1-9, serological and molecular detection. During the survey SPFMV and SPCSV were found to be

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common. Virus-free accessions were planted using Randomized Complete Block Design in three replicates. Harvesting was done 180 days post planting. The germplasm was again subjected to molecular detection of virus to ascertain whether the materials remained virus free and to detect new infections. All the germplasm tested positive for sweet potato feathery mottle virus (SPFMV) but negative for SPMMV, CMV, SPCSV and SPCFV an indication that SPFMV is a common virus in sweet potato and does not significantly affect sweet potato yield. Analysis of variance showed that Nyakowino, Nyawo, Zapallo and SPK004 total yield were significantly different at $p< 0.05$ with a range between 68.00-12.33.Sweet potato should be screened for viruses in commercial production.

Keywords: Germplasm; Ipomea batatas; indexing; virus free; serological; moleculer.

1. INTRODUCTION

Sweet potato, *Ipomea batatas* (L) Lam is an important subsistence food crop as well as cash crop in East kamagak location and is also very popular in the major cities in the country including Nairobi. Sweet potato also does well with little agronomical management practices and provides household food security because it stores well in the soil as a famine reserve crop [1].

The production of sweet potato is however constrained among others by virus infection, poor agronomic practices and lack of high yielding cultivars. At least 13 viruses are reported to infect sweet potato naturally [2]. Most of them are insect transmitted. In East Africa, symptoms of sweet potato virus disease were first reported in 1945 by Hansford [3]. However the presence of the viruses was not demonstrated until 1957 when Sheffield [4] associated two viruses with sweet potato plants having virus like symptoms. Virus A was later identified as Sweet potato feathery mottle virus (SPFMV) while virus B remained unclear. SPFMV has a worldwide distribution and is readily spread by aphids as per Cadena Hinojosa et al. [5]. Common sweet potato viruses in the country include Sweet Potato Mild Mottle Virus (SPMMV) and Sweet Potato Feathery Mottle Virus (SPFMV) (Ateka, et al. [6]), thus the germplasm collected needed virus indexing to ensure that all the germplasm collected were free of viruses as they may lower the yield and quality of sweet potato. The study aimed at screening and selecting virus free germplasm.

2. MATERIALS AND METHODS

2.1 Study Site

The study was conducted in Rachuonyo District of Homabay County Kenya. The district has a population of 300,000 persons and an area of 507 km^2. The district lies at Latitude 0° 26' 24" (0.44°) south and longitude 34° 44' 20.4" (34.739°) east with average elevation of 1,378 meters (4,521 feet) above sea level (Rachuonyo district-Kenya mapcarta). The temperatures ranges between 14°C and 25°C. The district has two main rainy seasons; the long rains which start from late february and runs through June with rain fall ranging between 500 mm and 1000 mm and the short rain saeson which occurs beween the months of August and November with rainfall ranging between 250 mm and 700 mm (Rachuonyo district-Kenya mapcarta).

The main trading center is Oyugis and the study site that is Sino Sub-Location lies 5 km and Kachieng' Sub-Location 6 km southeast. Others are Kajiei Sub-Location 6 km north, Kasipul Location 7 km south and Karabondi Sub-Location 9 km north. The soil from Sino is of medium acidic at a pH of 5.8 while soil from Kachieng is extremely acidic at a pH of 4.5. Sino soil is rich in organic matter at 2.26% while Kachieng is 1.97%. Soil from both sites is adequate in phosphorus concentration at 88 and 98 mg/kg respectively as per soil test results from KARI Njoro, 2011).

2.2 Survey and Germplasm Collection

Rachuonyo District which comprises two divisions and seven locations was purposively selected as it is a major sweet potato growing area yet the divisions exhibit variations in some agro-ecological conditions for sweet potato production. The district has a total population of 300,000 persons (Central Bureau of Statistics; [7]) the other population details are summarized in the Table 1.

2.3 Sampling Strategy

Multistage sampling procedure was used to select the 100 households who participated in

Table 1. Population distribution per location, source MOPHS [8]

Location	Male	Female	Total population	Total households
Sino	32%	68%	7235	518
Kachieng	34%	66%	5071	482

the study. In the first stage, Rachounyo district was purposively selected and secondly, the two sites sampling. Fifty households in each location was selected using simple random sampling from a sampling frame of 1000 households of which 10% were selected (Table 1) Questionnaires were used as instruments of data collection. A questionnaire was used because according Kothari [9] it is free from bias of the interviewer, is appropriate in obtaining in-depth responses, is economical in terms of time and money and appropriate in analyzing the feelings, interests and motivations of the respondents. The respondents to the questionnaires were members of selected households in the two locations of Rachuonyo district, Homabay County.

The questionnaire had structured (closed ended) questions and unstructured (open ended) questions. According to Babbie [10] the closed ended questionnaire design, seems to be the best method available for collecting original data, to describe a population too large to be observed directly. This is because structured questions are easier to analyze since they are in an immediate usable form.

They are also easier to administer because each item is followed by alternative answer. They are economical to use in terms of time and money. In addition, it promotes detailed responses where the respondents are able to give reliable information. The questionnaire was divided into two sections. The first section consisted of background information which included gender, age, education status and employment status for demographic analysis. The second part consisted of information concerning sweet potato which included; Average size of land under sweet potato production, genotypes planted, preferred sweet potato character, source of planting materials, pests and diseases affecting sweet potato and the their control, average yield of sweet potato, modes of utilization and the source of market for their produce.

2.4 Virus Identification in the Field

Viral diseases were identified in the field using the procedure hereunder:

- The number of diseased plants per plot was counted.
- A visual score was used, especially for the virus infestation.
- The virus scoring scale of 1 – 9 adopted from Procedures for the evaluation and analysis of sweet potato trials' manual 2nd Version by CIP, IIAM, NaCRRI, CRI [11] was used in which;
- 1 refer to no symptoms (visual);
- 2 – unclear virus symptoms;
- 3-7 clear virus symptoms of increasing severity from 1-90%;
- 8- clear virus symptoms in nearly all plants 90-99%
- 9 – clear virus symptoms and clearly reduced growth in all plants.

The virus with their vector scores was recorded for each plot (treatment).

Using the visual scoring the plant materials which did not exhibit any virus symptoms were selected and subjected to serological virus test. The materials that tested both negative for visual virus scoring and serological test were put on yield trials alongside the released varieties obtained from KARI Njoro which tested negative for any viral infection

2.5 Serological and Molecular Detection of Viruses

Serological detection of virus was done in the laboratory using NCM-ELISA to confirm the field results on the presence or absence of the viruses in the landraces collected using the following protocol Nyaboga, et al. [12].

- Leaf tissue (0.4 gm) was weighed and ground in 3 ml of extraction buffer (composed of 20 g CTAB 100 mL 1 M Tris-Hcl pH 8.0, 100 mL 0.5 M EDTA pH 8.0, 81.76 g sodium chloride 10 g sodium sulphite and 20 g polyvinylpyrolidone (PVP-40) dissolved to make up one liter) in a mortar and pestle with aid of acid washed coarse carborundum.
- The slurry was then transferred to 1.5 ml microfuge tubes and incubated at 65°C for 20 minutes in a water bath.

- Centrifugation was done at 13000 rpm for ten minutes before transfering 750 µl of supernatant to a fresh tube.
- About 750 µl of chloroform: Isoamylalcohol (24:1) was added to the tubes. Tubes were shaken and centrifuged at 13000 rpm for ten minutes.
- The aqueous phase (about 600 µl) was transferred to a fresh tube and mixed with an equal volume of Chloroform: Isoamyl alcohol. This step was done twice.
- Aqueous phase (450 µl) was added to a fresh tube while avoiding the interphase.
- Nucleic acids was precipitated using ice cold Isopropanol before centrifuging at 6500 rpm for ten minutes.
- Isopropanol was decanted carefully leaving DNA pellet at the bottom of the tube. The pellet was washed with 500 µL of 70% ethanol before air-drying.
- The isolated total nucleic acids samples were then used for serological detection of the viruses and the remaining sample stored at -20°C was used for further molecular detection of viruses.

The materials collected from the field in East Kamagak location which tested positive for the various sweet potato virus such as sweet potato chlorotic stunt virus were discarded clean materials were put on yield trials. However since there was no clean Nyakowino sample for SPFMV, the samples were put on yield trials alongside the other varieties (Table 3. and 4). This decision was based on earlier findings of Karyeija et al. [13], Mukasa et al. [14] who found out that some sweetpotato cultivars recover from infection with SPFMV, SPMMV or co-infection with both viruses. At harvesting the same materials were subjected to molecular virus testing using PCR as described by IsHak et al. [15]; Mukasa, et al. [1] to ascertain whether the materials remained clean or they were affected by viruses and if so, what would be the overall effect on the total yield.

3. RESULTS AND DISCUSSION

3.1 Survey and Germplasm Collection

The survey conducted in East Kamagak location aided by the use a questionnaire yielded the following results;

Out of the 100 households interviewed 90 responded which translate to 90% reported to be planting local landraces namely; Nyakowino, Abiro Nenywol, Rachar, Nyathi odiewo, Kuny kibuonjo and Amina which they obtained from their own farms while 10% indicated that they obtained their planting material from neighbor's farm. None of the farmers obtain their planting materials from research stations (Table 1). This result is similar to finding from Abidin [13] which pointed out that by the time of the official release, the two cultivars namely; SPK004 and Ejumulla, they were spreading quickly through farmer–to-farmer exchange or purchase of planting materials and promotions. Tairo et al. [16] also found out that sweet potato cultivation is largely depended on locally available materials that are reserved in home gardens as source of planting materials for the next season. Thus farmers keep on sharing landraces that are similar but under different names due to poor record keeping.

3.2 Serological and Molecular Detection of Viruses

From Table 2, Kuny Kibuonjo, Amina, Abiro nenywol and Nyakowino tested positive for Sweet Potato Feathery Mottle Virus (SPFMV) but tested negative for Sweet Potato Mild Mottle Virus (SPMMV), Cucumber Mosaic Virus (CMV), Sweet Potato Chlorotic Stunt Virus (SPCSV) and Sweet Potato Chlorotic Flake Virus (SPCFV), an indication that SPFMV is the most common virus in sweet potato and sometimes coupled with infection by both SPFMV and SPMMV. SPCSV is the most destructive sweet potato virus as per Mukasa et al. [14].

From the Table 3, Mugande A tested positive for SPMMV while Mugande B, C and D tested positive for SPFMV. KSP20 A tested positive for sweet potato chlorotic stunt virus. SPK004 A, B and C tested positive for sweet potato feathery mottle virus (SPFMV) but tested negative for SPMMV, CMV, SPCSV and SPCFV. SPK013 A and B tested positive for SPCFV and SPK013 C tested positive for SPFMV and tested negative for SPMMV, CMV, SPCSV and SPCFV. Zapallo A and B tested negative for SPFMV, SPMMV, CMV, SPCSV and SPCFV, Zapallo B tested positive for SPFMV and Zapallo C tested positive for both SPFMV and SPCSV. Sweet potato chlorotic stunt virus (SPCSV) is considered to be the most destructive viral disease affecting sweet potato as the virus greatly reduces the crop yield and quality [14].

Table 2. Source of planting material, pests and diseases of sweet potato

Attribute	Source of planting material			Pests		Diseases	
	Own farm	Neighbours farm	Research station	Weevil	Other pests	SPFMV	Other diseases
% of farmers growing sweet potato	90	10	None	75	25	72	28
Total	100			100		100	

Table 3. Results for NCM ELISA test of SPFM, SPMMV, CMV, SPCSV and SPCFV virus for sweet potato collected in East Kamagak

Sample ID	Variety	SPFMV	SPMMV	CMV	SPCSV	SPCFV
1	Kuny kibuonjo	+	-	-	-	-
2	Kuny Kibuonjo	-	-	-	-	-
3	Amina	+	-	-	-	-
4	Amina	-	-	-	-	-
5	Nyakowino	+	-	-	-	-
6	Nyakowino	+	-	-	-	-
7	Abiro Nenywol	+	-	-	-	-
8	Abiro Nenywol	+	-	-	-	-
9	Rachar	-	-	-	-	-
10	Rachar	-	-	-	-	-
11	Nyathi Odiewo	-	-	-	-	-
12	Nyathi Odiewo	-	-	-	-	-

Key + indicates presence of virus, - indicates absence of virus
SPFMV- Sweet potato feathery mottle virus.SPMMV- Sweet potato mild mottle virus.CMV- Cucumber mosaic virus.SPCSV- Sweet potato Chlorotic stunt virus.SPCFV- Sweet potato Chlorotic flake virus

Table 4. NCM ELISA test results for SPFMV, SPMMV, CMV, SPCSV and SPCFV viruses from materials sampled at KARI Njoro

Sample ID	Variety	SPFMV	SPMMV	CMV	SPCSV	SPCFV
1	Mugande A	-	+	-	-	-
2	Mugande B	+	-	-	-	-
3	Mugande C	+	-	-	-	-
4	Mugande D	+	-	-	-	-
5	Nyawo A	+	-	-	-	-
6	Nyawo B	-	-	-	-	-
8	Nyawo C	-	-	-	-	-
9	KSP 20 A	-	-	-	+	-
10	KSP 20 B	-	-	-	-	-
12	SPK004A	+	-	-	-	-
11	KSP 20 C	-	-	-	-	-
13	SPK 004 B	+	-	-	-	-
14	SPK 004 C	+	-	-	-	-
15	SPK 004 D	-	-	-	-	-
16	SPK 013 A	-	-	-	+	-
17	SPK 013 B	+	-	-	+	-
18	SPK 013 C	+	-	-	-	-
19	Zappalo A	-	-	-	-	-
20	Zappalo B	+	-	-	-	-
21	Zappalo C	+	-	-	+	-

Viruses Identity: SPFMV- Sweet potato feathery mottle virus. SPMMV- Sweet potato mild mottle virus. CMV- Cucumber mosaic virus. SPCSV- Sweet potato Chlorotic stunt virus. SPCFV- Sweet potato Chlorotic flake virus

The results in (Fig. 1) reveal that SPMMV is not a serious sweet potato virus and maybe the other samples did recover from the infestation by SPMMV and thus explaining the results observed. This confirms earlier results by Mukasa et al. [14]). Sample 9 (Rachar) and sample 12 (KSP20) tested positive for SPMMV, one hundred and eighty days post planting. The other samples, 1-SPK013, 2-Mugande, 3-Zapallo, 4-SPK004, 5-Nyawo, 6-Nyakowino, 7-Kuny kibuonjo, 8-Nyathi, 10-Amina and 11-Abiro remained clean during the entire period of growth in the field.

All the samples tested positive for Sweet potato feathery mottle virus (SPFMV) (Fig. 2). Sweet potato feathery mottle virus is the most common virus of sweet potato. This result is similar to findings of Ateka et al. [6] Carey et al. [17]. Similarly, the results confirms what Karyeija et al. [18] and Mukasa et al. [14] found out in the study of viruses and virus like diseases of sweet potato in Uganda, that some sweet potato cultivars recover from infection with SPFMV, SPMMV or co-infection with both viruses, as indicated by lack of symptoms and virus concentrations too low to be detectable in the newly developed leaves using serological test.

For instance Table 4 shows that Nyakowino which was put on yield trial despite testing positive for SPFMV did perform better than Nyawo, Zapallo and SPK004 which were put on trials upon confirmation by ELISA test that they were clean materials.

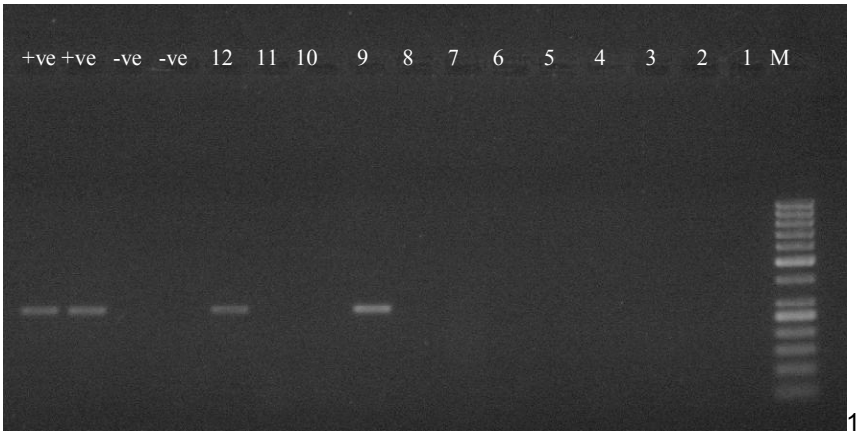

Fig. 1. Molecular detection of sweet potato mild mottle virus

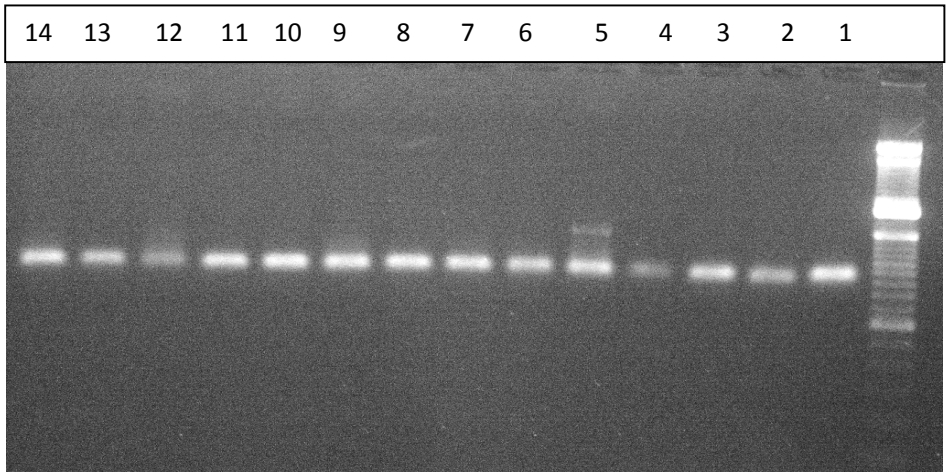

Fig. 2. Molecular detection (using PCR) of sweet potato feathery mottle virus
1 Spk013 2 Mugande 3Zapallo 4 Spk004 5 Nyawo 6 Nyakowino 7 Kuny 8 Nyathiodiewo 9 Rachar 10 Amina 11 Abiro 12 Ksp20 13 Zapallo 14 Nyakowino

Table 5. Variation in vine internode diameter, leaf size diameter, average vine length, vine weight, small size storage root, marketable size storage root and total yield for varieties put on yield trials a cross site

Genotype	Vine internode diameter	Leaf size diameter	Petiole length	Average vine length	Vine weight	Small size storage root	Marketable size storage root	Total yield
Ksp20	1.00^{a}	7.33^{b}	9.85^{d}	2.73^{c}	23.83^{e}	12.50^{abc}	36.50^{d}	49.00^{c}
Rachar	0.58^{b}	7.03^{bc}	10.03^{cd}	2.15^{de}	38.50^{d}	9.67^{abcd}	44.83^{d}	54.50^{c}
Mugande	0.51^{c}	6.70^{c}	10.85^{b}	1.90^{f}	39.00^{cd}	16.33^{a}	62.17^{abc}	78.50^{a}
Zapallo	050^{cd}	1.48^{e}	4.92^{g}	0.95^{g}	9.17^{f}	1.17^{e}	11.17^{e}	12.33^{d}
Spk013	0.50^{cd}	9.08^{a}	10.83^{b}	2.32^{d}	47.00^{abcd}	2.67^{e}	64.50^{ab}	67.17^{abc}
Nyathi Odiewo	0.50^{cd}	7.20^{bc}	11.43^{a}	4.05^{a}	55.17^{a}	8.83^{bcd}	73.50^{a}	82.33^{a}
Kuny Kibuonjo	0.48^{cd}	4.63^{d}	9.78^{d}	2.03ef	43.67^{bcd}	14.33^{ab}	44.17^{d}	58.67^{abc}
Nyakowino	0.46^{d}	1.53^{e}	9.35e	3.10b	48.33^{abc}	15.83^{ab}	52.17^{bcd}	68.00^{ab}
Spk004	0.40^{e}	0.15^{f}	8.53^{f}	2.08^{ef}	46.50^{abcd}	11.17^{abc}	47.83^{cd}	59.00^{bc}
Amina	0.38^{e}	7.02^{bc}	10.02^{cd}	3.22^{b}	45.00^{bcd}	6.83^{cde}	43.00^{d}	49.33^{bc}
Nyawo	0.30^{f}	4.82^{d}	10.25^{c}	2.02^{ef}	39.50^{cd}	11.83^{abc}	52.67^{bcd}	64.50^{abc}
Abiro Nenywol	0.23^{g}	1.55^{e}	10.03^{cd}	3.27^{b}	51.50^{ab}	6.83^{cde}	50.50^{bcd}	57.33^{bc}

Means with the same letter(s) within the column are not significantly different at p< 0.05

3.3 The Effect of Virus on Sweet Potato Yield

In Table 5, among other parameters that were scored in the field, Nyakowino, a local landrace which was put on yield trials along-side other varieties, did perform well despite testing positive for Sweet potato Feathery Mottle virus (SPFMV).

4. CONCLUSION AND RECOMMENDATION

4.1 Conclusion

All the germplasm tested positive for SPFMV an indication that, SPFMV is the most common virus occurring in sweet potato however it has little effect on the yield since Nyakowino which was put on yield trials despite testing positive for SPFMV did perform well. One landrace Rachar and one released variety KSP20 tested positive for SPMMV while the rest of the germplasm remained clean, an indication that SPMMV is not a common virus of sweet potato like SPFMV.

4.2 Recommendation

Sweet potato germplasm should be screened for viruses in commercial production.

DISCLAIMER

This manuscript was presented in the conference available link is- "http://www.ku.ac.ke/schools/agriculture/index.php/89-faculty?start=10" date 1st to 5th December 2014, Kenyatta University, Nairobi, Kenya.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/12665