

Original Research Article

**ASSESSMENT OF SWEET POTATO PROPAGULES FOR INFECTION
BY VARIOUS VIRUSES IN EAST KAMAGAK LOCATION – HOMABAY
COUNTY.**

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 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. 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 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 common. Virus-free accessions were planted using Randomized Complete Block Design in three replicates. Harvesting was done 6 months after 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.

Key words: Germplasm, *Ipomea batatas*, Indexing, Virus free, Serological, Molecular

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 such as

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 (Mukasa et al, 2003).

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 (Moyer and Larsen, 1991). Most of them are insect transmitted. In East Africa, symptoms of sweet potato virus disease were first reported in 1945 by Hansford. However the presence of the viruses was not demonstrated until 1957 when Sheffield (1957) 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 (Cadena Hinojosa et al 1981). Common sweet potato viruses in the country include Sweet Potato Mild Mottle Virus (SPMMV) and Sweet Potato Feathery Mottle Virus (SPFMV) (Ateka, et al, 2004), 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.

Materials and methods

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 507km². 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 500mm and 1000mm and the short rain saeson which occurs between the months of August and November with rainfall ranging between 250mm and 700mm(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 98mg/kg respectively as per soil test results from KARI Njoro, 2011).

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.;2009). The other population details are summarized in the table 3.1

Table 3.1: Population distribution per location , Source MOPHS (2010)

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

Sampling Strategy

Multistage sampling procedure was used to select the 100 households who participated in 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 3.1) Questionnaires were used as instruments of data collection. A questionnaire was used because according Kothari (2004), 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.; 1995) 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.

3.3 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 (2010) 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

3.4 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; 2008).

- Leaf tissue (0.4 gm) was weighed and ground in 3ml of extraction buffer (composed of 20g CTAB 100mL 1M Tris-Hcl pH 8.0, 100mL 0.5M EDTA pH 8.0, 81.76g sodium chloride 10g sodium sulphite and 20g polyvinylpyrrolidone (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 1.5ml microfuge tubes and incubated at 65°C for 20 minutes in a water bath.
- Centrifugation was done at 13000 rpm for ten minutes before transferring 750µl of supernatant to a fresh tube.
- About 750µl 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 600µl) 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 500µL 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 4.2. and 4.3). This decision was based on earlier findings of (Karyeija et al, 2000; Mukasa et al, 2004) 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, 2003; Mukasa, et al.; 2003) to ascertain whether

146 the materials remained clean or they were affected by viruses and if so, what would be the
147 overall effect on the total yield.

148 **Results and discussion**

149 **4.1 Survey and germplasm collection**

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

152 Out of the 100 households interviewed 90 responded which translate to 90% reported to be
153 planting local landraces namely; Nyakowino, Abiro Nenywol, Rachar, Nyathi odiewo, Kuny
154 kibuonjo and Amina which they obtained from their own farms while 10% indicated that they
155 obtained their planting material from neighbor's farm. None of the farmers obtain their planting
156 materials from research stations (Table 4.1). This result is similar to finding from (Abidin.; 2004)
157 which pointed out that by the time of the official release, the two cultivars namely; SPK004 and
158 Ejumulla, they were spreading quickly through farmer-to-farmer exchange or purchase of
159 planting materials and promotions. (Tairo, et al 2008) also found out that sweet potato cultivation
160 is largely depended on locally available materials that are reserved in home gardens as source of
161 planting materials for the next season. Thus farmers keep on sharing landraces that are similar
162 but under different names due to poor record keeping.

163

164 Table 4.1: 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	90	10	None	75	25	72	28

potato			
Total	100	100	100

165

166 4.2 Serological and molecular detection of viruses

167 From table 4.2, Kuny Kibuonjo, Amina, Abiro nenywol and Nyakowino tested positive for
 168 Sweet Potato Feathery Mottle Virus (SPFMV) but tested negative for Sweet Potato Mild Mottle
 169 Virus (SPMMV), Cucumber Mosaic Virus (CMV), Sweet Potato Chlorotic Stunt Virus (SPCSV)
 170 and Sweet Potato Chlorotic Flake Virus (SPCFV), an indication that SPFMV is the most
 171 common virus in sweet potato and sometimes coupled with infection by both SPFMV and
 172 SPMMV. SPCSV is the most destructive sweet potato virus (Mukasa, at al 2004).

173

174 Table 4.2: Results for NCM ELISA test of SPFM, SPMMV, CMV, SPCSV and
 175 SPCFV virus for sweet potato collected in East Kamagak

176

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

177

178 Key + indicates presence of virus, - indicates absence of virus

179 SPFMV- Sweet potato feathery mottle virus.SPMMV- Sweet potato mild mottle virus.CMV-
180 Cucumber mosaic virus.SPCSV- Sweet potato Chlorotic stunt virus.SPCFV- Sweet potato
181 Chlorotic flake virus..

182 From the table 4.3, Mugande A tested positive for SPMMV while Mugande B,C and D tested
183 positive for SPFMV. KSP20 A tested positive for sweet potato chlorotic stunt virus. SPK004 A,
184 B and C tested positive for sweet potato feathery mottle virus (SPFMV) but tested negative for
185 SPMMV, CMV, SPCSV and SPCFV. SPK013 A and B tested positive for SPCFV and SPK013
186 C tested positive for SPFMV and tested negative for SPMMV, CMV, SPCSV and SPCFV.
187 Zapallo A and B tested negative for SPFMV, SPMMV, CMV, SPCSV and SPCFV, Zapallo B
188 tested positive for SPFMV and Zapallo C tested positive for both SPFMV and SPCSV. Sweet
189 potato chlorotic stunt virus (SPCSV) is considered to be the most destructive viral disease
190 affecting sweet potato as the virus greatly reduces the crop yield and quality (Mukasa, et al
191 2004).

192

193 Table 4.3: NCM ELISA test results for SPFMV, SPMMV, CMV, SPCSV and
194 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	+	-	-	+	-

195

196 Viruses Identity

197 SPFMV- Sweet potato feathery mottle virus.SPMMV- Sweet potato mild mottle virus.CMV-

198 Cucumber mosaic virus.SPCSV- Sweet potato Chlorotic stunt virus.SPCFV- Sweet potato

199 Chlorotic flake virus.

200 The results in (Figure 4.1) reveals that SPMMV is not a serious sweet potato virus and maybe the

201 other samples did recover from the infestation by SPMMV and thus explaining the results

202 observed. This confirms earlier results by Mukasa et al (2004). Sample 9 (Rachar) and sample

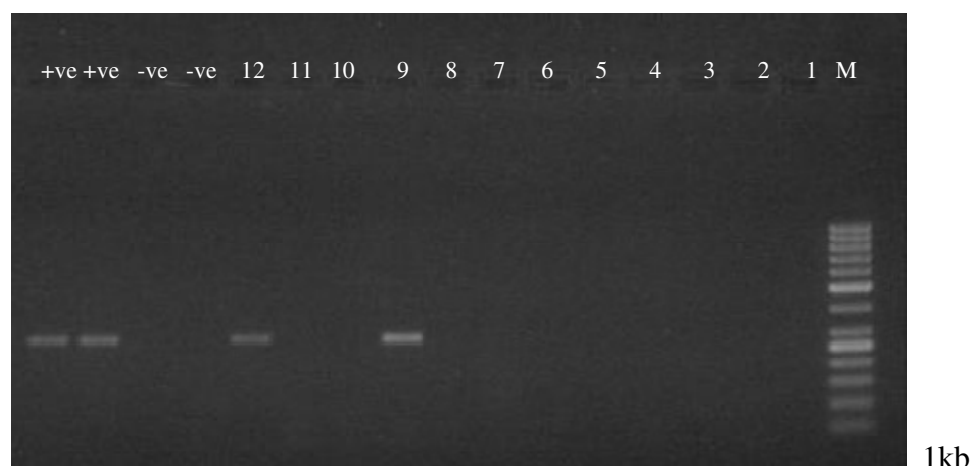
203 12 (KSP20) tested positive for SPMMV , one hundred and eighty days post planting. The other

204 samples, 1-SPK013, 2-Mugande, 3-Zapallo, 4-SPK004, 5-Nyawo, 6-Nyakowino, 7-Kuny

205 kibuonjo, 8-Nyathi, 10-Amina and 11-Abiro remained clean during the entire period of growth in

206 the field.

207



208

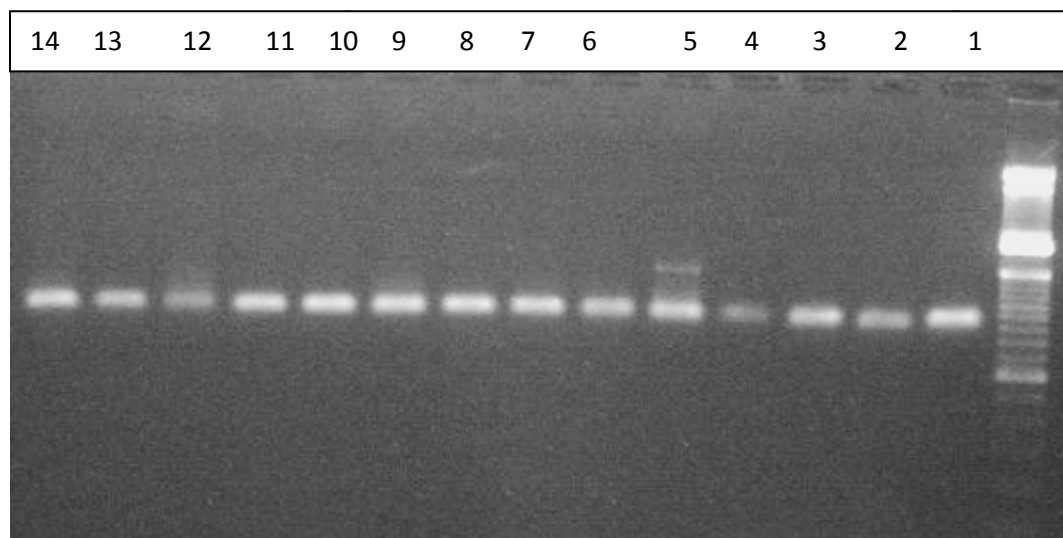
209 Fig 4.1: Molecular detection of sweet potato mild mottle virus

210 All the samples tested positive for Sweet potato feathery mottle virus (SPFMV) (Figure 4.2).

211 Sweet potato feathery mottle virus is the most common virus of sweet potato. This result is

212 similar to findings of (Carey et al 1999; Ateka et al, 2004). Similarly, the results confirms what

213 Karyeija et al, (2000) and Mukasa.; et al (2004) found out in the study of viruses and virus like
 214 diseases of sweet potato in Uganda, that some sweet potato cultivars recover from infection with
 215 SPFMV,SPMMV or co-infection with both viruses, as indicated by lack of symptoms and virus
 216 concentrations too low to be detectable in the newly developed leaves using serological test.
 217 For instance table 4.4 shows that Nyakowino which was put on yield trial despite testing positive
 218 for SPFMV did perform better than Nyawo, Zapallo and SPK004 which were put on trials upon
 219 confirmation by ELISA test that they were clean materials.



221 Figure 4.2: Molecular detection (using PCR) of sweet potato feathery mottle virus

222 1 Spk013 2 Mugande 3Zapallo 4 Spk004 5 Nyawo 6 Nyakowino 7 Kuny 8 Nyathi
 223 9 Rachar 10 Amina 11 Abiro 12 Ksp20 13 Zapallo 14 Nyakowino

226 Table 4.4: Variation in Vine internode diameter, leaf size diameter, average vine length, vine
 227 weight, small size storage root, marketable size storage root and total yield for varieties put on
 228 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	0.50 ^{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	2.03 ^{ef}	43.67 ^{bcd}	14.33 ^{ab}	44.17 ^d	58.67 ^{abc}
Nyakowino	0.46 ^d	1.53 ^e	9.35 ^e	3.10 ^b	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}

229

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

231 CHAPTER FIVE-Conclusion and Recommendation

232 5.1 Conclusion.

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

5.2 Recommendation

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

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