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Evaluation of Four Varieties of Sweetpotato (*Ipomeoa Batatas* (L.) Lam) under Different Sources of Planting Material for Field Performance and Viral Load

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Abstract

Sweetpotato is an important crop for food security in many developing countries which is cultivated using vine cuttings. Studies have revealed that there are at least fifteen well characterized viruses known to infect sweetpotato of which 10 are economically important that contribute to yield reduction. Planting materials use by farmers are often infected by one or more of these viruses. The aim of this study was to evaluate three different sources of planting materials of different health status for their field performance and virus presence. The sources of planting materials were *in vitro* generated platelets, symptomless Field materials and Farmer's materials. Four sweetpotato varieties Apomuden, Bohye, Ligri and Dadanyuie were selected from each source of planting material. The trial was laid in a split plot design with the sources of planting material allocated to main plots and the varieties to sub-plots. The plantlets of the four varieties were planted at Botanga Irrigation Scheme in Northern region of Ghana. Viral symptom scores were taken twice, score 1 being the average from 4 - 7 weeks after planting (WAP) and score 2 being the average from 8 - 11 WAP. Nitrocellulose Membranes Enzyme Linked Immunosorbent Assay (NCM-ELISA) kits were employed for the detection of sweetpotato viruses on the field. The source of planting materials significantly influenced ($P < 0.05$) virus field visual observation. *In vitro* generated material showed the least symptoms of virus followed by Field materials. Apomuden and Bohye varieties recorded the highest virus score in the first and second virus symptom observational score respectively. NCM-ELISA revealed that the viruses SPFMV, SPMMV, SPMSV, SPCFV, SPCSV, and CMV were significantly present among the different sources of planting

materials. *In vitro*, Field and Farmer materials recorded NCM-ELISA score of 0.225, 1.075 and 1.500 respectively. Apomuden variety recorded the highest virus score in the assay. Vine and root yield was higher among the *in vitro* generated material. Farmers should use laboratory cleaned material however, in the absence of such material they should select field material showing no symptom of virus.

Keywords

In Vitro Plant Material, Sweetpotato, Virus, NCM-ELISA

1. Introduction

In tropical countries, sweetpotato is an important crop that helps to alleviate hunger [1]. It is cultivated in over 100 developing countries and ranks among the five most important food crops in over 50 of those countries including Ghana [2]. Sweetpotato is considered as active growing crop, with fast root formation and development which enhances greater survival rate of the seedlings leading to good yield [3]. It is an industrial raw material use in starch and pharmaceutical industry [4]. Yield in Ghana stands at 15 Mt/ha accounting for about 27% of potential yield [5]. The crop yield is significantly limited mostly by fungi, viruses, and bacteria that accumulate in planting material [6] [7] [8] [9]. Sweetpotato Feathery Mottle Virus (SPFMV) and other sweetpotato viral diseases cause serious yield losses [6] [7]. According to [10] SPFMV is the most destructive virus which causes infection across all over the world. Yield loss in the crop due to virus has been estimated to be about 15% - 48% in China, 34% - 97% in Egypt and 80% - 98% in East Africa [11] [12]. According to [8] virtually any sweetpotato growing from non-pathogen clean materials source will contain at least one virus Studies have demonstrated clear benefits in terms of higher yield and quality using pathogen-indexed planting material as compared to farmer's traditional non-tested material [13] [14] [15]. However, some studies have contradicted that, suggesting that virus-free planting materials yield the same or have no effect on storage roots and vines yields [16] [17]. A study has revealed that, SPFMV-infected plants produced better yield than an *in vitro* generated material [18].

Farmers in the study area continuously use their own stock for planting without replacing them. Others also use visual observation to discard apparently infected material. This study sought to assess the performance of *in vitro* indexed planting materials and other non-virus-indexed planting materials on growth and root yield of four known sweetpotato varieties in the Guinea savanna agro ecological zone in Ghana.

The specific objectives were to determine:

1) The virus load on different sources of planting materials.

2) The prevalent virus type in the planting material

3) How the health status of planting materials affects yield components of sweetpotato.

2. Materials and Methods

Laboratory studies were done at the Biotechnological Laboratory at Crop Research Institute of CSIR, Fumusua-Kumasi from January to July 2017. The field study was conducted at Botanga Irrigation scheme, located within the Kumbungu District of Northern region of Ghana from September-December 2017. Botanga is located on latitude 009˚25'41"N, longitude 000˚58'42"W and altitude 183 m above sea level.

3. Experimental Design

The experimental design that was used to conduct the field trial was split plot where the source of planting materials represented the main plots and the varieties represented sub plots. The treatments were replicated three times. A sub-plot size of 4 × 5 m was used for planting 17 cuttings per row for 4 rows. The lengths of the cuttings were between 25 cm to 30 cm with four nodes each. Cutting were planted two nodes in the soil for rooting and two nodes outside for sprouting. Plantlets were spaced at 1 m between rows and 0.30 m within plants. The experiment was conducted during the dry season under irrigation in order to reduce cross infection by the virus insect vectors among the different sources of planting materials. Again, in order to minimize cross infection among different sources planting materials, a 10 m alleys were created in between main plots and Maize (Zea mays, L) were planted in the alleys.

4. Sources of Planting Material

Three sources of planting materials were used. The first planting material source was healthy tested vines (*in vitro* material) that were generated from the Biotechnology laboratory complex of the Crop Research Institute (CRI), Fumesua in Kumasi. Potted nodal cuttings were kept in heat therapy chamber to reduce the virus load, if any and this also enhanced sprouting of nodal cuttings to obtain partially clean meristems. The meristems were excised in the tissue culture laboratory with the help of stereomicroscope and then cultured on [19] media for about three months. Weaning and hardening processes were followed to obtained vigorous plantlets. The materials were further tested for virus by grafting them on *Ipomoea setosa* which is able to detect virus infected material grafted on it [20]. Healthy virus-free materials were further multiplied in glass house. In the second planting material source (Field Material), apparently healthy looking and symptomless materials were selected at the multiplication fields of International Potato Center located at Savanna Agricultural Research Institute experimental field, Nyankpala. Such materials were cleaned first before they were introduced to field five years earlier. Plants showing virus symptoms had been ro-

gued out leaving the healthy ones. Even though unhealthy plants were rogued some plants latently diseased could escape visual selection The third planting material source called farmer's material were materials that were taken from the open fields of a vine multiplier who had been given clean material five years earlier. These materials were sprouted from roots that have not been rogued of infected ones and were potentially infected. In each source of planting materials, four varieties, namely Apomuden, Ligri, Bohye and Dadanyuie were selected for the field experiment.

5. Virus Scores

Monitoring virus symptoms in the field was an important aspect of the trial to give details of infections which could affect the storage root yield. Field were inspected at two periods and severity viral symptoms score were undertaken using the scores in Table 1.

Infections in some varieties were difficult to detect as some virus were transient, mild, or may not appear at all on sweetpotato foliage. However, Nitrocellulose membrane-Enzyme Linked Immunosorbent Assay (NCM-ELISA) test was conducted to confirm the presence of viral symptoms and those viruses which were not induced on the foliage as well as virus that were mild and could not be detected by visual observation.

6. NCM-ELISA: Virus Detection Test

During the period of the experiment vine with leaves samples were taken from each plot at 8 WAP to evaluate virus load and virus type present in each source of planting materials and varieties. This was done by using an immuno-enzymatic virus reaction, NCM-ELISA, which involved the use of nitrocellulose membranes instead of the polystyrene micro titration plates as a support for the reagents used in the serological reaction.

Table 1. Severity viral symptoms scores (scale of 1 - 9) were used [21].

Score	Description of severity
1	Plants showing no symptoms
2	unclear virus symptoms
3	Clear virus symptoms > 5% of plants per plot
4	Clear virus symptoms at 6% to 15% of plants per plot
5	Clear virus symptoms at 16% to 33% of plant per plot
6	Clear virus symptoms at 34% to 66% of plants per plot
7	Clear virus symptoms at 67% to 99% of plants per plot
8	Clear virus symptoms at all plants per plot
9	Severe virus symptoms in all plants per plot

The steps below were used in NCM-ELISA virus detection under room conditions:

- Very minute amounts (30 µl) of the sample (plant sap) were blotted and dried.
- The portion that was not utilized by the samples were blocked with blocking solution.
- Specific antibodies (virus antibody 1) were used to react the virus particles.
- Then virus specific antibodies were detected by means of an appropriate substrate using the enzyme labeled antibodies (virus antibody 2).

6.1. Sample Selection for Virus Detection Test

Negative selection approach was used in the field to select those plants showing viral symptoms. Vines cuttings with leaves were collected from each plot into labelled envelopes. The envelopes were kept on ice in the field and then conveyed to the laboratory on the same day.

6.2. Sample Application to Nitrocellulose Membrane

The nitrocellulose membranes were cut into 10 pieces for the detection of 10 different viruses. The membranes were identified by writing the number coding each virus on the top. The membranes were pre-wet in Tris buffered saline (TBS) for at least 5 minutes prior to use. The dot blotting apparatus was connected to a vacuum pump. The pre-wet piece of Whitman's paper was placed over the dot blot manifold and pre-wet nitrocellulose membrane was also placed over the filter paper. A piece of parafilm was used to block the remaining area of the manifold not covered by the nitrocellulose membrane and carefully applied to a vacuum (230 mm of mercury) by turning the pump on. Using a clean tip for each sample, a 30 µl sample (plant sap) was pipetted into each well formed on the nitrocellulose membrane by the vacuum. The nitrocellulose membranes were removed from the gadget and were conveyed onto a well dry filter paper piece and it was allowed to dry for about 30 minutes.

6.3. Serological Test Process

The dry membranes of nitrocellulose were immersed in a blocking solution (TBS + 2% milk + 2% TRITON X-100). The first antibody 1 (virus specific antibody) and TBS plus 2% of milk was further added and gestated overnight at room conditions with gentle shaking (50 rpm). It was cleaned in Tween-Tris buffered saline (TTBS) for 3 minute by washing three times each with faster shaking (100 rpm). The second antibody, Goat anti-rabbit (GAR) was added in TBS plus 2% of milk and then incubated for one hour at room conditions with gentle shaking (50 rpm). Then the tissue nitro membranes were then washed again in TTBS (0.05%) four times for three minutes with very fast shaking (100 rpm). The tissue nitro membranes were incubated for about 30 minutes. In the case of SPCSV, tissue was incubated for 1.5 h in a substrate mixture (20 mg N, N-dimethylformamide, 1.2 ml) at room conditions with gentle shaking (50 rpm) for

colour formation process. The coloring process was stopped by disposing off the substrate mixture followed by dipping the nitrocellulose membranes in distilled water to stop the reaction completely. The nitrocellulose membrane tissues were rinsed in flowing tap water for three times for about 3 minutes each. The tissues were allowed to dry before the reactions data were recorded on the NCM-ELISA recording sheet using a scale of 0 - 5 [21] based on the intensity of the coloration comparing with positive controls. Where zero (0) represented negative reactions and 1 to 5 represented positive reactions, with one being the least. Positive reactions were those showing different shades of purplish colour.

6.4. Chlorophyll Content (SPAD Values)

Opti-Science Cc4-200 Chlorophometer SPAD readings were taken within the first month and were repeated two more times in the third and four months before harvesting and average was taken.

6.5. Root and Vine Yield

At harvest, total storage root yield and vine yield were determined.

6.6. Data Analysis

The Data collected were subjected to general Analysis of Variance (ANOVA) using Genstat statistical tool (4th Edition) and means were separated using least significant difference at the 5% probability level.

7. Results

Field virus severity score

Field observation for viral symptoms was done from week 4 - 7 after planting (First viral score) and then from week 8 - 11 after planting (Second viral score). The averages for the two periods were computed. The source of planting materials significantly influenced ($P = 0.018$) first viral score. The least virus severity score was realized in the *in vitro* generated material (**Figure 1**). Farmer's material recorded the greatest viral severity symptoms score followed by Field generated materials. The varieties did not show difference in viral symptom score in the first viral score ($P = 0.058$). However, it was observed that Ligri variety recorded lower viral symptom index score (**Figure 1**). Apomuden variety recorded relatively higher symptom index in Farmer and Field sources of planting material.

In the second viral symptoms score, it was observed that the trend was similar to the first viral score, there was general decreasing severity from Farmer's material to *in vitro* material of all the varieties (**Figure 2**). However, the severity was higher than the first viral score. The varieties had effect of viral symptom index ($P = 0.049$). Among the varieties Ligri showed the least viral symptoms scores in two source of planting materials except Farmer's material. Apomuden and Dadanyuie varieties showed the highest field viral score among Farmer's

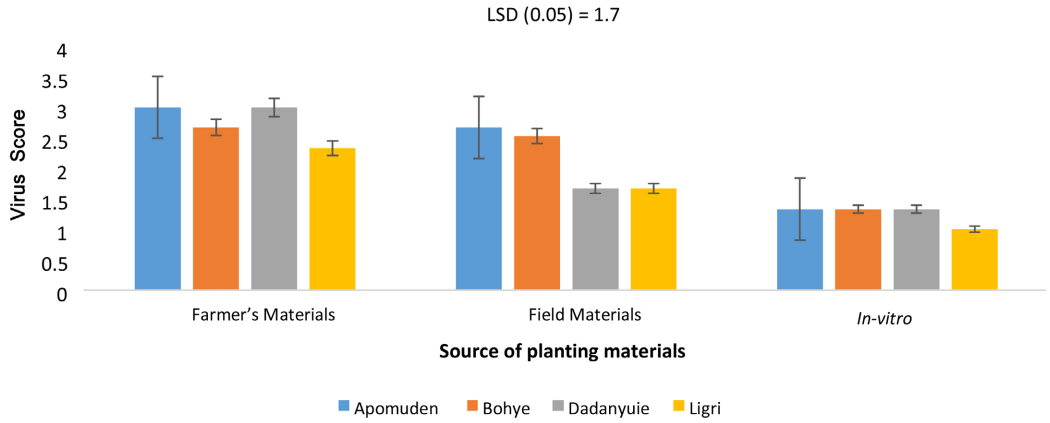

Figure 1. Effect of source of planting material and variety of sweetpotato on first field symptomatic virus score. The data represent the average virus scores from week 4 to 7. Error bars represent Standard Error of Means (SEM).

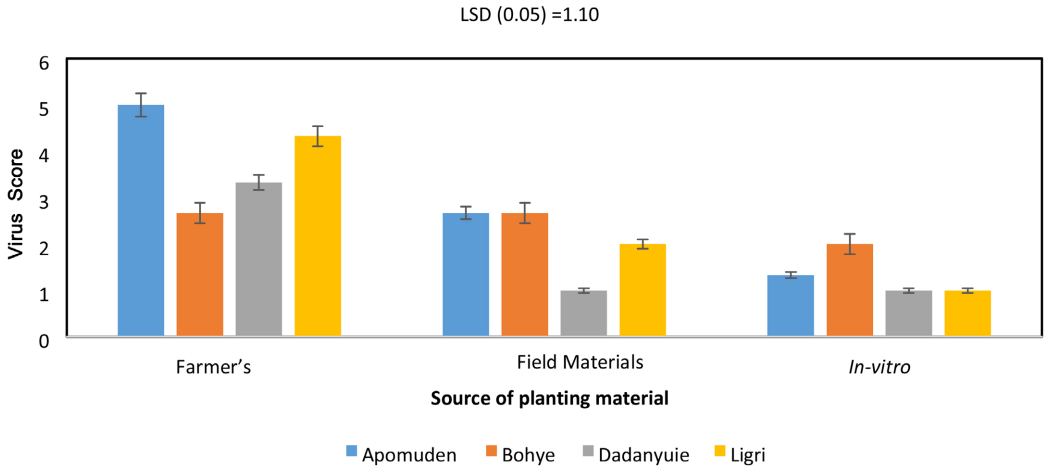

Figure 2. Effect of source of planting material and variety of sweetpotato on second field symptomatic virus score. The data represent average virus score from week 8 to 11. Error bars represent SEM.

materials and Bohye recorded the least. Among the Field materials, Apomuden showed the severest viral scores but was not significantly different from Bohye variety. In the *in vitro* source of planting material all the varieties recorded very low symptom index when compared to the other sources of planting materials.

NCM ELISA virus test

The ELISA result indicated that the sweetpotato sources of planting materials had significant viral load difference ($P < 0.05$) for six virus types. However, four virus types amongst the ten viruses tested for indicated negative reaction (Table 2). In the six virus types present in the planting materials *in vitro* source recorded the least viral load. SPCSV, SPMMV, SPMSV and CMV virus types were significantly higher in Farmer's material than in Field Material (Table 2). The six virus types were significantly ($P = 0.05$) present in the four varieties (Table 3). Apomuden variety had higher viral load as it recorded index of 2 or more. Sweetpotato chlorotic fleck virus (SPCFV) was the most prevalent across the

Table 2. The NCM ELISA virus test showing virus types and their load (0 - 5 scale) in each planting material source.

Virus type	Source of planting material				P-value	LSD
	Farmer.	Field	*In vitro*	Mean		
SPCSV	2.58	1.75	0.25	1.53	*<0.001*	0.6
SPFMV	2.83	2.5	0.5	1.94	*0.005*	0.9
SPCFV	1.92	1.58	0.5	1.33	*0.013*	0.7
SPLV	0	0	0	0	*0*	0
SPMMV	3.08	1.92	0.5	1.83	*0.003*	0.9
SPMSV	2.42	1.58	0.25	1.42	*<0.001*	0.5
SPVG	0	0	0	0	*0*	0
SPC-6V	0	0	0	0	*0*	0
SPCaLV	0	0	0	0	*0*	0
CMV	2.17	1.42	0.25	1.28	*<0.001*	0.4
Mean	1.50	1.08	0.22	0.97		

Table 3. The NCM ELISA virus test showing virus types and their load (0 - 5 scale) in each variety.

Virus type	Apomuden	Bohye	Dadanyuie	Ligri	Mean	P-value	LSD
SPCSV	2.22	1.11	1.22	1.56	1.53	<0.*001*	0.7
SPMMV	2.67	1.78	1.33	1.76	1.89	*0.007*	0.7
SPMSV	2.35	1.73	0.35	1.24	1.42	<0.*001*	0.5
SPCFV	2.89	2.11	1.11	1.67	1.95	*0.005*	0.7
SPLV	0	0	0	0	0	0	0
SPFMV	2.11	1.22	1.00	1.00	1.33	*0.003*	0.6
SPVG	0	0	0	0	0	0	0
SPC6V	0	0	0	0	0	0	0
SPCaLV	0	0	0	0	0	0	0
CMV	2.00	1.11	0.67	1.33	1.28	*<0.001*	0.5
Mean	1.42	0.91	0.57	0.86			

four varieties while cucumber mosaic virus (CMV) was the least across the varieties (Table 3).

Chlorophyll content (SPAD values)

The interaction between planting material source and varieties did not have significant effect on greenery of the crop (P = 0.298). The source of planting

materials significantly influenced ($P = 0.001$) chlorophyll content. The *in vitro* generated material recorded higher SPAD meter value, about 68% higher than Farmer's materials (Figure 3). There was no significant difference between *in vitro* and Field material in SPAD values. The varieties showed significant difference ($P = 0.005$) in greenness of the leaves. Dadanyuie looked greener than the other three varieties (Figure 4). Bohye and Ligri were not different in their SPAD values. Apomuden recorded the least SPAD value (Figure 4).

Vine yield

The interaction between planting material source and varieties did not have significant effect on vine yield ($P = 0.072$). There were significant differences ($P = 0.001$) in vine yield among the source of planting materials. Farmer material yielded the least and the yield of *in vitro* generated planting material was about twice that of the Farmer's material (Figure 5).

Significant differences existed among the varieties in vine yield ($P < 0.001$). Dadanyuie variety produced the highest vine yield (Figure 6). The other three varieties, Apomuden, Bohye and Ligri did not exhibit significant difference in vine yield (Figure 6).

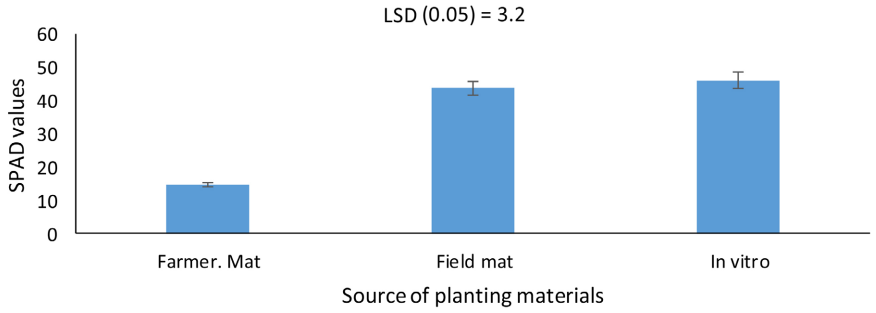

Figure 3. Effect of source of planting material on leaf chlorophyll content. Error bars represent SEM.

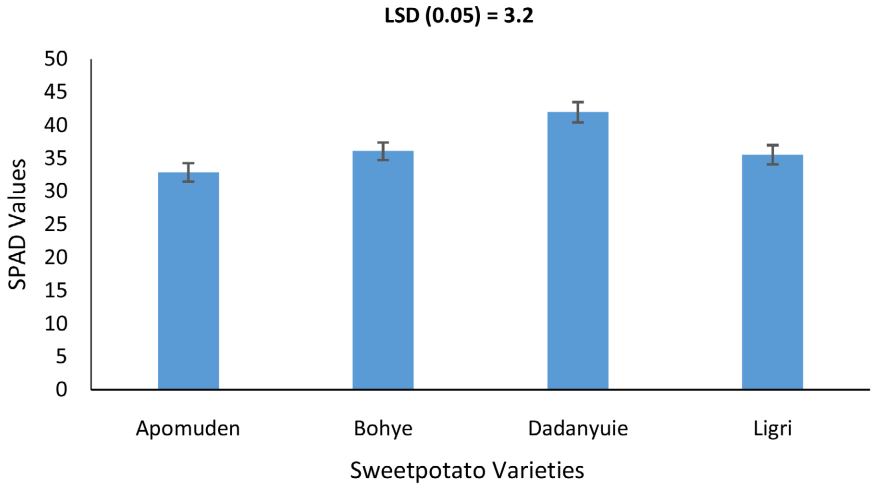

Figure 4. Effect of variety on sweetpotato leaf chlorophyll content; Bars represented standard error of means (SEM).

Root yield

The source of planting materials and variety interaction significantly influenced (P = 0.048) the root yield (Figure 7). The *in vitro* recorded the highest root yield (19.74 tons/ha) followed by Field generated planting materials (16.08 ton/ha). Farmer planting materials gave the least root yield (10.34 ton/ha) which was significantly lower than the two other sources of planting materials. Thus yield improvement of 47.6% and 18.5% were recorded by the *in vitro* over the Field and Farmer generated materials respectively.

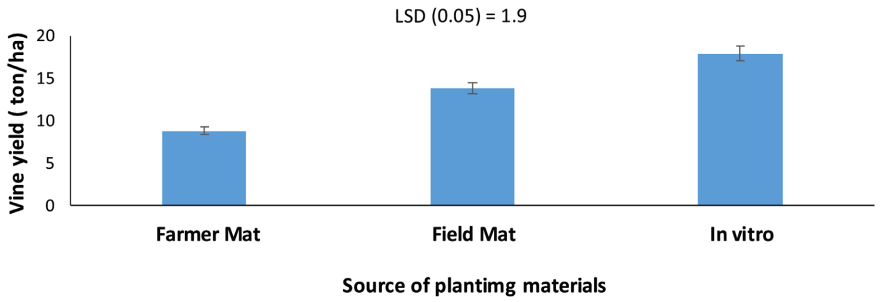

Figure 5. Effect of sweetpotato source of planting materials on vine yield. Error bars represent SEM.

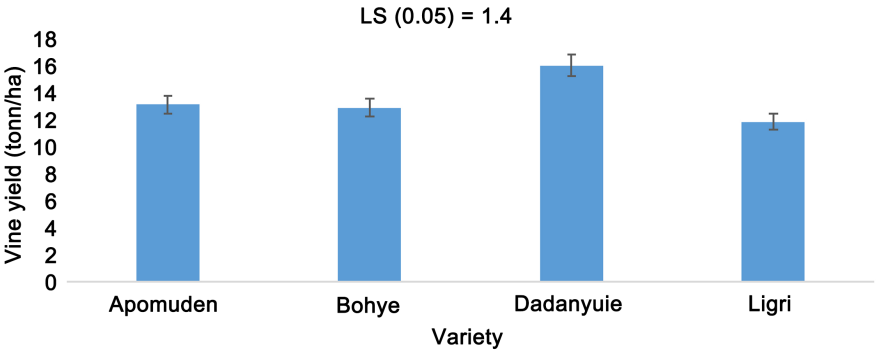

Figure 6. Effect of sweetpotato variety on vine yield of sweetpotato; Error bars represented SEM.

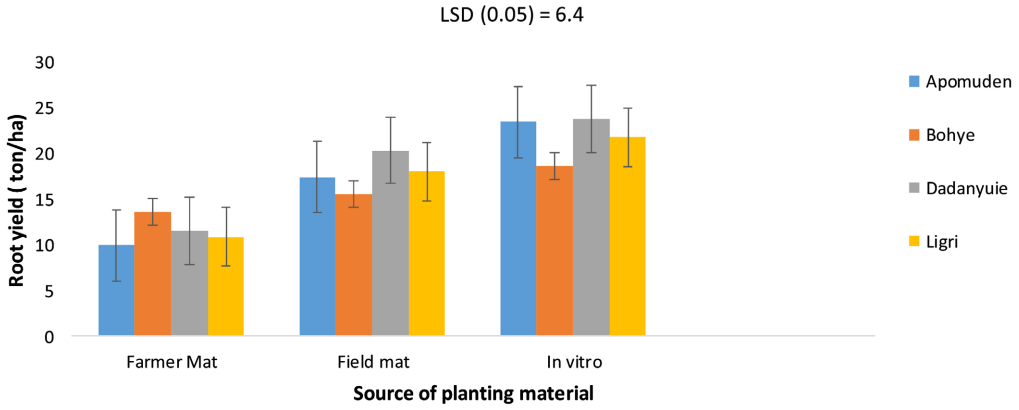

Figure 7. Interaction effect of source of planting materials and variety on total root yield. Error bars with represents SEM.

In the Farmer's material where virus infestation was higher Bohye variety recorded the highest root yield though not significantly different from the other varieties (**Figure 7**). In the less infected Field generated planting material and *in vitro*, Bohye recorded lesser yield than the other varieties though their differences were not significant. In those two sources of planting material, Field and *in vitro*, Apomuden *and* Dadanyuie varieties produced higher root yield though not significantly different from Ligri and Bohye (**Figure 7**).

8. Discussion

The virus symptoms among the source of planting materials and varieties varied in severity in the field. Among these virus symptoms observed were chlorotic spot bordered by purple pigment, vein discoloration, and leaves curls, slightly orange leaves, yellowing of upper and middle leaves, yellow veins, and stunted growth. In the first phase of viral symptoms score (First viral score) it was observed that, the Farmer's materials showed more viral symptoms compared to Field generated materials and *in vitro* generated materials showed very minimal or negligible virus symptom. The second viral score which was taken between eighth and eleventh week after planting showed similar result but severity increased across the three sources of planting materials as well as the varieties. This could be attributed to the fact that the viral symptoms are endogenous and develop with time. The *in vitro* generated planting material showed less of these symptoms when compared with the other two sources. These symptoms observed agree with earlier reports on potato viruses [20] [22]. The Field material had less severity score than the Farmer's material. Removal of infected material from the Field material by farmers lead to less infection with Sweetpotato virus and this practice is believed to control Sweetpotato virus spread [22].

The NCM-ELISA result also revealed variations in viral load of important sweetpotato viruses on the different sources of planting materials and sweetpotato varieties. Sweetpotato viruses with economic importance that have been reported on Farmers' fields include SPFMV, SPCSV, SPMMV, SPCFV and CMV and the most widely spread in the major sweetpotato production areas are SPCSV, SPFMV and SPMMV [23] [24] [25]. Virus serology test confirmed the field viral symptoms score that were observed were actually due to virus. The Farmer materials that have been on the field for about five years without virus re-cleaning recorded higher viral load than Field materials that were subjectively selected based on the absence of virus symptoms. The Field materials were also found to be highly infected when compared with *in vitro* generated planting materials. The Farmer's material having higher viral load is due to non-cleaning and rogueing of infected materials from the stock leading to accumulation of viral complex. [10] has reported that all sweetpotatoes grown from non-virus-tested source of planting materials revealed the presence of one or more viruses in them.

The SPAD meter reading is an index of chlorophyll content which relates to

greenness. In this experiment when cleaned *in vitro* generated planting materials were compared with the other two planting materials, chlorophyll content was higher than Farmer and Field planting materials. This variation in sources of planting materials on chlorophyll content could be attributed to variation of viral load which consequently influenced photosynthetic ability. Sweetpotato virus disease (SPVD), characterized by small, distorted leaves have considerable effects on cell metabolism such as photosynthesis, respiration, and transpiration [26]. Symptom induction is primarily by the perturbation of the cell metabolism and damage to cell organelles such as chloroplasts [26] [27] [28]. Loss of chlorophyll from plants can be due to virus infection [29]. Several strains of cucumber mosaic virus (CMV) are known to induce chlorosis [30].

Varietal variation among sweetpotato with respect to chlorophyll content was observed among the four varieties. Though there were difference in chlorophyll content among the varieties only Dadanyuei stood out in greenery. Apomuden variety recorded higher titer load of the virus in the serology test and this manifested in lower greenery of the leaves when compared with Dadanyuie which posted very low viral load. Though the varieties have inherent ability to manufacture chlorophyll more than others the contributory effect of viral complex interfering with photosynthetic apparatus cannot be ignored [28] [29].

The results revealed that vine yield was influenced by the degree of infection by the sweetpotato viruses. *In vitro* generated material produced more vines than the two sources that posted higher viral load. [31] did not find any vine yield difference between apparently clean materials raised in the open field (Field material) and materials cleaned and kept in netted chamber (in our case equivalent to *in vitro* material). Vine yield of 8.9 to 9.6 ton/ha recorded for Open field and Netted chamber in [31] was similar to what we obtained under Farmer material plot but far below the vine yield obtained under Field and *in vitro* plots (**Figure 5**) [31]. Growing the same four varieties [31] did not find difference in their vine yield however in our study, Dadanyuie variety that had lower titer value of the viruses studied produced more vines. Apomuden that had higher viral load produced lower vine yield than Dadanyuie. This strengthens the belief that there is correlation between viral infection and yield attributes reduction.

Root yield obtained from *in vitro* planting material was higher than the other two sources. The viral load was low in the *in vitro* planting material and that might have contributed to its significant higher root yield. Virus free plantlets derived from these seed stocks have been reported to increase root yield in sweetpotato [32] [33] [34]. It is believed that "apparently" healthy planting material is as effective as pathogen-tested planting material [31]. In our study, root yield improvement of about 47% was obtained in the *in vitro* planting material over the Farmer material. Yield advantage of 30% - 50% [35] and even as high as 118% [36] in using healthy planting material have been reported.

The results on the varieties also showed that when viral load was higher, as in

the case of Farmer material, Bohye variety was the best among the four varieties though difference was not significant. However, in the healthier planting material as observed under *in vitro*, Bohye variety was not the top variety, Apomuden and Dadanyuie recorded higher root yield though the difference was not significant. It could be seen from the result that root yield of the varieties increased with increasing health (virus-wise) of the planting material. In sweetpotato study by [31], they reported lowest root yield in Dadanyuie, about 7.6 ton/ha but in our study 20 - 23 ton/ha was recorded for this variety under Field and *in vitro* planting materials respectively. The difference in yield could be attributed to provision of irrigation for the crops in our study.

9. Conclusion

The study revealed that Field planting material that has been rogued of infected material is better than farmer material that is kept for many years without cleaning. The best planting material is the one that has been virus indexed to give specific virus free planting material, in our case *in vitro* planting material. Sweetpotato chlorotic fleck virus (SPCFV) was the most prevalent across the four varieties and should be indexed before commercial use of any planting material. Healthier planting material gave higher vine and root yield. In general, the *in vitro* was better than Field which also outperformed Farmer planting material in vine and root yield. Apomuden and Dadanyuie are recommended when using Field and *in vitro* planting material while in using Farmer planting material Bohye is recommended. Field symptoms showed that there were virus in the planting material and this was confirmed by serology test.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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