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Evaluation of Selected Zambian Popular Sweet Potato Genotypes for Response to Sweet Potato Virus Disease

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Authors' contributions

This work was carried out in collaboration among all authors. Author JB undertook the research performed the statistical analysis, interpreted the data and writing of the manuscript. Authors PCC and LT helped in planning for execution of research, guided the analyses and interpretation process and proof read the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The objective of this study was to determine the effect of sweet potato virus disease (SPVD) on the beta carotene content, tuber weight and vine weight of selected popular sweet potato genotypes.
Study Design: The experiment was laid as a randomized complete block design (RCBD) with three replications.
Place and Duration of Study: The experiment was conducted for two cropping seasons (2015/16 and 2016/17) at the Zambia Agriculture Research Institute in Chilanga district of Zambia.
Methodology: The uninfected (control) genotypes of Kanga, Chiwoko and Chingovwa were evaluated alongside their SPVD infected genotypes. Genotypic infection was confirmed using molecular approaches, and data was collected at harvest on beta carotene content, tuber weight and vine weight.
Results: The results showed that SPVD affects the yield and beta carotene content of sweet

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potato. Significant differences (P< .001) for yield performance and beta carotene were observed. The yield reduction in percentage across seasons for all genotypes between the uninfected and infected genotypes ranged from 77% to 79% and 67% to 76% for tuber weight and vine weight respectively. Only Chiwoko exhibited higher levels of beta carotene among the genotypes. However, the SPVD infected Chiwoko genotype compared to the uninfected treatment produced mean beta carotene content of 39.1 µg/g and 91.5 µg/g respectively.

Conclusion: SPVD reduces the tuber weight, vine weight and beta carotene content in infected sweet potato genotypes.

Keywords: Ipomoea batatas; sweet potato feathery mottle virus; sweet potato chlorotic stunt virus; beta carotene; yield.

1. INTRODUCTION

Sweet potato (*Ipomoea batatas*) is the sixth most important food crop in the world after rice (*Oryza sativa* L.*),* wheat (*Triticum aestivum* L.*),* Irish potato (*Solanum tuberosum* L.), maize (*Zea mays* L.*)* and cassava (*Manihot esculenta* Crantz) [1]. The worldwide tuber sweet potato production is about 112,853,316 metric tonnes (FAO, 2017) [2]. It is an important subsistence food crop for human, and livestock consumption and it plays an essential role in farming and food systems in developing countries [3]. Storage roots of sweet potato contain 30% dry matter of which 70% is starch, 5% sugar, 5% protein and with vitamins A, C and B. Nutritionally, sweet potato is rich in dietary fibre (pectin, cellulose, hemicellulose and lignin), proteins, vitamins, energy, carbohydrates and Beta- carotene among others [4]. The leaves are consumed as a vegetable [5] and the root tubers as a source of starch [4]. In addition, the crop is used in many industrial processes to make a wide variety of products such as alcohol, liquor, noodles, candy, desserts, flour, and other starchy foods [3,6,7]. Orange fleshed sweet potato genotypes rich in beta carotene are also becoming increasingly popular [8,6].

In sub-Saharan Africa, sweet potato yields are low, averaging about 2 tonnes ha^{-1} due to biotic and abiotic stresses. The abiotic factors include soil type, nutrition, temperature, light intensity, and moisture [9]. On the other hand, the biotic factors include insect pests and diseases [10]. While single pathogen infection has been found to have an effect in crop plants and is well documented [11,12], the effect of co-infection for various pathogens is not yet fully exploited. Co-infection in crop plants with two or several different pathogens has been found to have a synergetic or antagonistic effect with the former causing severe disease than the sum effect of the infection [13-15]. With regards to the later, one pathogen suppresses the other or others.

Among the diseases, (caused by co-infection) sweet potato virus disease (SPVD) [resulting from the co-infection of sweet potato feathery mottle virus (SPFMV) and sweet potato chlorotic stunt virus(SPCSV)] [16] is an important disease that not only cause yield reductions of up to 90% but also affects the beta carotene content of sweet potato [10]. The effect of SPVD on sweet potato yield and beta carotene content has been found to vary depending on the infecting virus, virus strains, virus complexes, and sweet potato genotypes involved [17]. These effects may range from minimal to downright devastating.

In Zambia, the SPFMV and SPCSV virus strains exist. However, their effect on Zambian popular sweet potato genotypes when co-infected is yet to be understood. The objective of this study was therefore to determine the effect of sweet potato virus disease (SPVD) on the beta carotene content, tuber weight and vine weight of selected popular sweet potato genotypes.

2. MATERIALS AND METHODS

2.1 Phenotypic Evaluation

2.1.1 Experimental site and germplasm used

This study was conducted at the Zambia Agriculture Research Institute (ZARI) Mount Makulu Research Station in Chilanga (15^{o} 33' S, 28^{o} 11' E) for two cropping seasons; 2015/16 and 2016/17 cropping seasons. The soil type was sandy clay loam. Three sweet potato genotypes sourced from Mansa Agriculture Research Institute – Root and Tuber Improvement Programme were used in this study. These were; Chingovwa, Kanga and Chiwoko. The characteristics of these genotypes are as shown

in Table 1. Genotypic selection was based on popularity and preferred taste. Forty (40) cuttings of each genotype which were virus free indexed following the molecular approach methods in section 2.2 were selected for the experimental study.

2.1.2 Inoculation of germplasm and conduct of the experiment

Inoculation of the three sweet potato genotypes (Table 1) with SPVD was done in the screen house by grafting 20 cuttings of each genotype with spreader cuttings infected with SPVD, resulting from the co-infection of SPFMV and SPCSV. After grafting, the plants were left in the screen house for two months for vine multiplication, before transferring to the open field for evaluation. The SPVD infected plants were proof-checked by the molecular approach in section 2.2 for dual presence of SPFMV and SPCSV.

The experiment was laid as a randomized complete block design (RCBD) with three infected genotypic treatments and their controls each replicated three times. Each treatment and control was assigned a 5 row plot of 5 meters long. The soil was treated with mancozeb, dazomet and carbofuran before planting to prevent soil-borne diseases. A weekly spraying programme using emamectin benzoate and acetamiprid insecticides was followed to avoid whiteflies and aphids from transmitting viruses among plants. The vines were planted on top of the ridges in a straight line. During planting, three vine nodes were buried into the soil, leaving two nodes outside to establish as root and shoot systems respectively. The planting was done at a depth of 15 cm with a spacing of 25 cm between vines, and each row had 20 plants giving 100 plants per plot.

2.2 Molecular Marker Analysis

Artificially Infected SPVD genotypes of Kanga, Chiwoko and Chingovwa with diseased spreader plants were confirmed positive to SPVD in the laboratory at Mount makulu research institute in Chilanga. Samples of the artificially inoculated plants were taken to the molecular lab to confirm dual infection of SPCSV and SPFMV. RNA was extracted using the method by Lodhi et al. [18] and cDNA was synthesized using reverse transcription as described in section 2.2.1.

2.2.1 Reverse transcription-polymerase chain reaction

The RNA from the infected sweet potato genotypes was diluted to 10 ng/µl for cDNA synthesis. Reverse transcription of the RNA extract was performed at 37°C for 45 minutes, according to Fenby et al. [19] using Omni script Reverse Transcription Kit (Qiagen, USA). Primers used are as presented in Table 2. Polymerase chain reaction (PCR) was performed using a thermorcycler (Techen 500) and the gel was visualized using the gel documentation system (Gel Doc XR, Bio-rad, UK)".

2.3 Data Collection

The crop was harvested 20 weeks after planting. Data on vine weight, tuber weight and the beta carotene content were then collected on all treatments. The vine and tuber weights were measured using an electronic digital scale while the beta carotene content was measured using the high-pressure liquid chromatography (HPLC) machine as described by Rodriguez-Amaya and Kimura [22].

2.4 Data Analysis

The genotypic responses for vine weight, tuber weight and the beta carotene were evaluated using analysis of variance (ANOVA) assuming a mixed model with genotype fixed and season random. The means for the genotypic main effects were separated using Fisher protected least significant difference (LSD), at a significance level of $\alpha = .05$. The yield reduction in percentage was computed as follows; $P = [(X_i - X_f)/ X_i] * 100$. Where P is the yield reduction percentage; X_i is the genotypic parameter measurement from the genotypic control treatment; X_f is the genotypic parameter measurement from the infected genotypic measurement.

Table 1. Characteristics of sweet potato genotypes used in the study

Genotype	Leaf shape	SC	BCC (µg/g)	FC
Kanga	Broad and large	Beige	< 1	White
Chiwoko	Palm lobbed	Beige	> 50	Orange
Chingovwa	Palm lobbed	Beige	< 1	Yellow

SC – Skin colour, BCC – Beta carotene content, FC – Flesh colour, µg/g – microgram per gram

Table 2. Primers used in reverse transcription – polymerase chain reaction for the detection of sweet potato viruses at Mt Makulu Agriculture Research Institute

TV	PN	Sequences 5' - 3'	AS (kb)	References
SPCSV	F: CP1	5'CTGCTAGATTAGAAA3'	1.2	Alicai et al., [20]
	R: CP2	5'TATATGAATATAGTTC3'		Alicai et al., [20]
SPFMV	F: FM1360	5'GACCAAGCCCCATACAATGA3'	1.3	Tairo et al., [16]
	R:10820R	5'GGCTCGATCACGAACCAAA3'		Tairo et al., [21]

TV- Target Virus. SPFMV-sweet potato feathery mottle virus. SPCSV-sweet potato chlorotic stunt virus. F- Forward. R- Reverse. AS- Amplicon size. VN – virus name. PN – primer name

3. RESULTS

3.1 Molecular Detection of Sweet Potato Genotypes

SPFMV and SPCSV infected plants were detected and visualized as DNA band sizes of 1.3 kb and 1.2 kb respectively. Fig. 1 displays a gel electrophoresis image showing band from infected SPCSV source plant. Infected SPVD genotypes were confirmed at a molecular level and bands associated with both SPFMV and SPCSV. Uninfected genotypes didn't show any band.

3.2 Phenotypic Analysis

The SPVD symptoms started appearing on infected genotypes from week 4 (four) up to the time the crop was harvested in week 20 (Twenty). These included distortion in leaf shape, size and stunted plant growth and sometimes complete death of the infected plant. The infected plants also exhibited a prominent appearance of feathery, purple patterns on the leaves (Plate 1).

Significant differences (P<.001) were obtained among the Genotypic main effects across seasons for beta carotene, root yield and vine weight main effect. No significant differences were obtained between seasonal main effects across genotypes. Equally Genotype x Season interaction effect was not significant (p>0.05) (Table 3). Further analysis showed that there was a significant yield reduction between the infected genotypes when compared to their control (uninfected) genotypes (Table 4).

Furthermore, only Chiwoko had a significant amount of beta carotene of 91.5 µg/g and 39.1 µg/g for uninfected and infected plants, respectively (Table 5), thus representing a reduction percentage of 57%.

The yield reduction percentages for both tuber weight and vine weight ranged from 77% to 79% and 67% to 76% respectively, for all genotypes employed (Table 5).

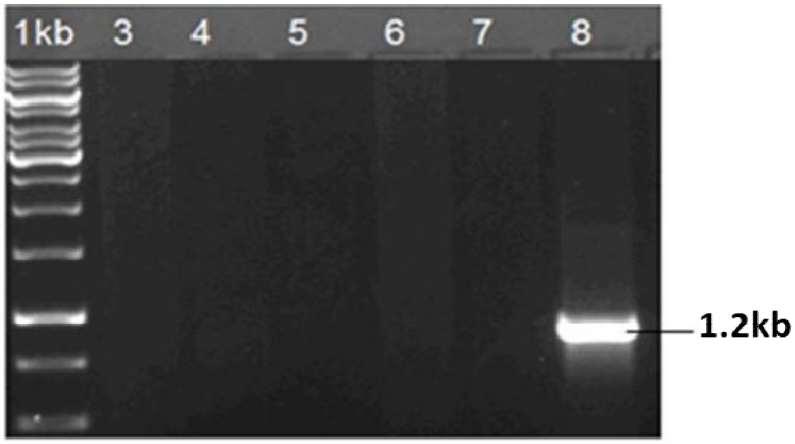

Fig. 1. A sample obtained image result, depicting identified SPCSV infected genotype. cDNA obtained from reverse transcription. PCR using primers-CP1/CP2, Identity plant number 8 infected by SPCSV. First lane, ladder- 1 kb. Band size 1.2 kb

Plate 1. Symptoms of sweet potato virus disease. X- exhibiting small, narrow and severly stunted leaves with abnornal pigmentation (purple in colour). The leaves are also crickled and distorted in shape

Table 3. Genotypic mean squares for analysis of variance for measured parameters on sweet potato (*Ipomoea batatas*) across seasons

Source of Variation	df	BCC	R Y	V W
Reps	2	804.80	74849	70919
Genotypes	5	8312.30***	1336521***	1226266***
Seasons	1	2.20^{ns}	401^{ns}	215^{ns}
Season x Treatment	5	41.40^{ns}	17896^{ns}	15273^{ns}
Error	22	195.90	53433	43056

****- significant (p<.001). df-degrees of freedom. BCC-beta carotene content. R Y-root yield. V W- vine weight*

Table 4. Genotypic means of beta carotene content, vine weight and tuber weight across seasons

Genotype	Parameters		
	VW(g/ plot)	TW(g/ plot)	BCC(µg/g)
$Chiwoko^{I}$	248	229	39.10
$Kanga^{i}$	275	263	0.30
$Chingovwa^{i}$	376	225	0.50
$Chiwoko^{u}$	1043	1001	91.50
$Chingovwa^{u}$	1138	1071	1.60
$Kanga^{u}$	1172	1205	0.70
$LSD_{(\alpha=0.05)}$	**248.4**	**276.8**	**16.8**

I - infected. U - Uninfected. VW – Vine weight. TW – Tuber weight. g – Grams.µg/g – microgram per gram. LSD-Fisher protected least significant difference at a significance level of α = .05

Table 5. Reduction percentages for the measured parameters

Parameter	Genotype	YRP
Tuber weight	Chiwoko	77%
	Chingovwa	79%
	Kanga	78%
Vine weight	Chiwoko	76%
	Chingovwa	67%
	Kanga	76%
Beta carotene	Chiwoko	57%
	Chingovwa	N
	Kanga	N

N – Negligible, YRP - Yield reduction percentage

4. DISCUSSION

4.1 Effect of SPVD on Sweet Potato Yield (Tuber Weight and Vine Weight)

The productivity of sweet potato is hampered by the effect of SPVD among other production constraints. In this study, reduced sweet potato yield (tuber weigh and vine weight) (Table 4) in infected genotypes could be attributed to a decrease in size of photosynthetic organs resulting from the severe effect of SPVD due to the synergistic interaction. In synergistic interactions of SPFMV and SPCSV, SPFMV is enhanced by SPCSV virus [15]. This may trigger a devastating tissue invasion which a single virus cannot archive on its own. This may result in the increased replication and accumulation in the phloem and xylem that may affect the movement of water and food hence resulting in reduced photosynthesis and ultimately plant growth [23].

Though previous studies agree that SPVD causes a remarkable reduction in yield (30 to 69%) [24,25,26], the yield reduction percentage in this study was much higher (over 76%). The differences obtained could be due to differences in genetic make-up and the environment under-study. It could be possible that the utilized genotypes are relatively more susceptible to SPVD, but this can only be confirmed if a trial including other reported genotypes is undertaken alongside the genotypes used in this study. For now, this is an area for further research. The fact that there were no significant differences with regards to yield among tested infected genotypes (Table 4), implies that the level of resistance of the tested genotypes to SPVD is probably similar. Hence we can only hypothesize that there is a likelihood of sharing a common ancestry among these genotypes.

4.2 Effect of SPVD on the Beta Carotene Content of Sweet Potato

This study showed that only Chiwoko's (the orange-fleshed genotype) treatment exhibited meaningful beta carotene content of 91.5µg/g and 39.1µg/g, for the control and infected genotypes, respectively. The SPVD had a significant effect on the beta carotene content of the infected Chiwoko genotype (Table 4) exhibiting a carotene reduction percentage of 57% across seasons (Table 5). Similar results have been previously obtained. A study done by Kapinga et al. [17] on a high beta carotene content genotype showed a reduction percentage of 37%. The reduction in the beta carotene content for the studied genotype could be as a result of a disturbance in the plastids were the synthesis of carotenes takes place via the 1-deoxy-D-xylulose-5-phosphate pathway [27]. This disturbance in the plastids could occur in the following ways; an overall decrease of chloroplast numbers, chloroplast clustering, distorted, loosened, or dilated thylakoid and disorganized grana scattering into the cytoplasm [27]. However, differences in the carotene content reduction in experimental trials of 57% (obtained in this trial) and 37% (previously obtained from another study) may imply that genetic differences could occur among germplasm with regards to coding for beta carotene content. In addition, virulence levels of SPVD may differ depending on the environments and the strains for SPFMV and SPCSV involved. Future research may need to be undertaken to look into this aspect. The fact that Chiwoko was the only genotype evaluated with a reasonable content of beta carotene may imply that beta carotene content is associated with specific genotypes. Other genotypes such as Olympia, Kokota and Zambezi have been found to contain high beta carotene [28]. It remains to be established how the beta carotene characteristic

trait is inherited for easier exploration in sweet potato improvement.

5. CONCLUSION

SPVD reduces the tuber weight and vine weight of the infected sweet potato genotypes. In addition, this study showed that the level of genotypic resistance for Kanga, Chiwoko and Chingovwa to SPVD was probably similar. Furthermore, it was noted that in comparison, the beta carotene content of the uninfected Chiwoko genotype was reduced by 57% in the infected genotype due to SPVD, implying that SPVD is a devastating disease which requires attention.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. International potato centre (C.I.P); 2010. Available:http://www.cipotato.org/sweetpotato [Accessed 20 November 2016].
2. FAO. FAOSTAT. Food and agriculture organization of the United Nations, Rome, Italy; 2017.
3. Antiabong E, Bassey E. Characterization and evaluation of six sweet potato varieties for quantitative and qualitative characters and tolerance to *Cylas puncticollis,* both in high and low humid environments of southeastern Nigeria. Journal of Agriculture Research. 2009;4: 17-21.
4. Luban TF, Peace K, Robert M, Maggiore K, Hellen M, Muhumuza J. Participatory agronomic performance and sensory evaluation of selected range-fleshed sweet potato varieties in southwestern Uganda. Global Journal of Science Frontier Research. 2015;15: 25-30.
5. Johnson M, Pace RD. Sweet potato leaves: properties and synergistic interactions that promote health and prevent disease. Nutrition Reviews. 2012; 68:604-615.
6. Hazra P, Chattopadhyay A, Karmakar K. Dutta S. Sweet potato. In: Modern technology in vegetable production. New Delhi*:* New India Publishing Agency. 2011; 358-370.
7. Lebot V. Root and Tuber Crops - Sweet Potato. Handbook of plant breeding. New York: Springer Science Business Media. 2010;97-126.
8. Mekonnen B, Tulu S, Nego J. Orange fleshed sweet potato (Ipomoea batatas L.) varieties evaluated with respect to growth parameters at jimma in Southwestern Ethiopia. Journal of Agronomy. 2015;14: 164-169.
9. Mark GL, Dean AK. Air temperature affects biomass and carotenoid pigment accumulation in Kale and Spinach grown in controlled environment. Plant sciences department, The University of Tennessee, Knoxville. Horticulture Science. 2005; 40(7):2026-2029.
10. Mukasa SB, Rubaihayo PR, Valkonen JPT. Incidence of viruses and virus-like diseases of sweet potato in Uganda. Plant disease 87,329–35. Journal of Plant Diseases. 2003;87:329-335.
11. Parthasarathy S, Thiribhuvanamala G, Prabakar K. Diseases of field crops and their management. London: CRC Press; 2020.
12. Tembo L, Asea G, Gibson PT, Okori P. Assessment of Uganda's farmers' perception and knowledge on maize cob rots towards breeding for resistance. Journal of Agriculture and Crops. 2016; 2:1-8.
13. Chilipa LNK, Lungu DM, Tembo L. Multiple race inoculation as an option in breeding for resistance to *C. lindemuthianum* in common beans. Journal of Agriculture and Crops. 2016;2:45-50.
14. Tembo L, Asea G, Gibson PT, Okori P. Resistance breeding strategy for *Stenocarpella maydis* and *Fusarium graminearum* cob rots in tropical maize. Journal of Plant Breeding. 2013;132:83-89.
15. Mukasa SB, Rubaihayo PR, Valkonen JPT. Interactions between a crinivirus, an ipomovirus and a potyvirus in co-infected sweet potato plants. Plant pathology. 2006;55:458-467.
16. Tairo F, Jones RAC, Valkonen JPT. Potyvirus complexes in sweetpotato: Occurrence in Australia, serological and molecular resolution, and analysis of the sweet potato virus 2 (SPV2) component. Journal of Plant Diseases. 2006;90:1120–1128.

17. Kapinga RS, Tumwengamire J, Ndunguru JS. Catalogue of orange-fleshed sweet potato varieties for sub-Saharan Africa. Lima, Peru: International Potato Centre; 2009.
18. Lodhi MA, Guang-Ning Y, Weeden N, Fand Reisch BI. A simple and efficient method for DNA extraction from grapevine cultivars and vitis species. Plant Molecular Biology Reporter. 1994;12:6-13.
19. Fenby NS, Seal SE, Gibson RW, Foster GD. RNA extraction from virus diseased sweet potato for RT-PCR analysis. Journal of Molecular Biotechnology. 1998;10:187–90.
20. Alicai T, Fenby NS, Gibson RW, Adipala E, Vetten, HJ, Foster GD, Seal SE. Occurrence of two serotypes of sweet potato chlorotic stunt virus in East Africa and their associated differences in coat protein and HSP70 homologue gene sequence. Plant pathology. 1999;48:718-726.
21. Tairo F, Mukasa SB, Jones RAC, Kullaya A, Rubaihayo PR, Valkonen JPT. Unraveling the genetic diversity of the three main viruses involved in Sweet Potato Virus Disease (SPVD) and its implications. Mol. Plant Pathol. 2005; 6:199-211.
22. Radriguez – Amaya DB, Kimura M. Harvest Plus handbook for carotenoid analysis. Washington, DC and Cali Columbia: Harvestplus Technical Monograph; 2004.
23. Karyeija RF, Gibson RW, Valkonen JPT. Comparisons of coat protein gene sequences show that East African isolates of sweet potato feathery mottle virus form a genetically distinct group. Archives of Virology. 2000;145:567-74.
24. Adiniki S, Mukasa SB, Mwanga ROM, Gibson RW. Effects of sweet potato feathery mottle virus and sweet potato chlorotic stunt virus on the yield of sweet potato in Uganda. Journal of Phytopathology. 2015;164:242–254.
25. Ling LDE, Goszczynski JR, McFerson, Gonsalves D. Nucleotide sequence and genome organization of grapevine leafroll associated virus-2 are similar to beet yellows virus, the closterovirus type member. Journal of General Virology. 1998;82:1-7.
26. Carroll HW, Villordon AQ, Clark CA, La Bonte DR Hoy MW. Studies on beauregard sweet potato clones naturally infected with viruses. International Journal of Pest Management. 2003;50: 101–106.
27. Zhao J, Zhang X, Hong Y, Liu Y. Chloroplast in plant-virus Interaction. Front. Journal of Microbiology. 2016;7:15-65.
28. Chiona M, Shanahan P, Mwala M. Experience with hand pollination of sweet potato in Zambia. Biotechnology, Breeding and Seed Systems for African Crops; 2007.

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