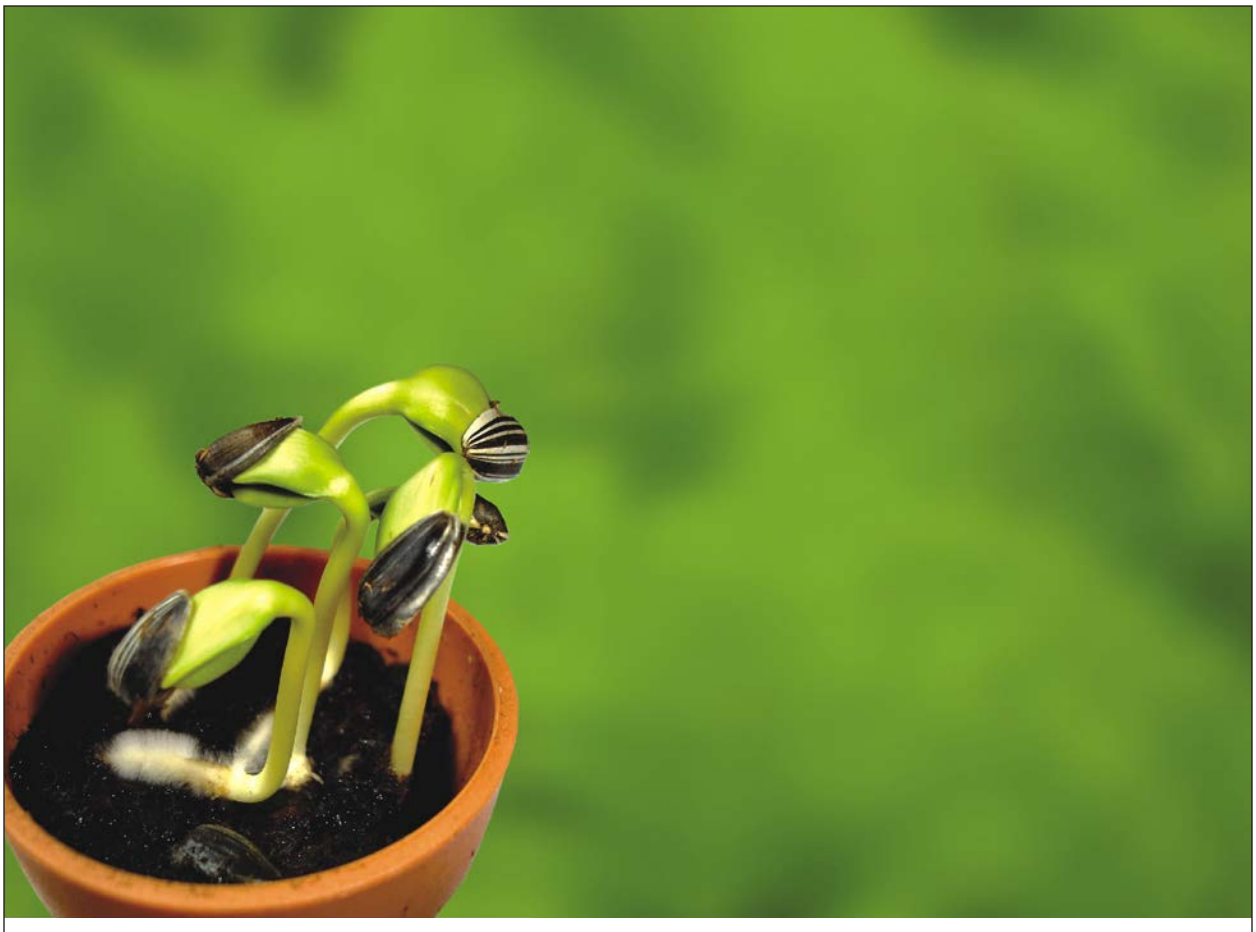

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Detection of Sweet Potato (*Ipomoea batatas* L.) Leaf Curl Virus (SPLCV) in Ghana Using Visual Symptomatology and PCR Technique

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ABSTRACT

Production of sweet potato (*Ipomoea batatas* L.), despite its high potential for food security, is constrained by viruses which reduce yield by up to 90%. It is therefore important to detect the type of viruses that affect the plant. In this study, Visual symptomatology and PCR-based techniques were used to identify *Sweet potato leaf curl viruses*. *Visual symptomatology revealed virus* associated symptoms including vein clearing, interveinal chlorosis, chlorotic spots, upward curling on leaf edges, leaf narrowing and distortion, purpling, blistering and general leaf yellowing in all 22 accessions grown on the field. Disease Incidence (DI) significantly ($p \leq 0.05$) varied between accessions with US003 having the lowest (20%) while ten accessions had the highest DI (90%) at the end of the study. Sweet potato viral disease symptom severity ranged from mild to moderate (1.70-2.19 mean severity score) in the accessions. However, the index of symptom severity of all plants (ISSap) ranged from 1.08±0.09 to 3.67±0.11 with VOTCR003 having the lowest, suggesting that it is a mildly susceptible accession while VOTCR002 had the highest thus suggesting that it is moderately susceptible to viral diseases. Contrarily, the index of symptom severity of diseased plants (ISSdp) ranged from 2.00±0.25 to 3.75±0.32. Visual symptomatology showed that VOTCR002 had the highest DI, ISSap and ISSdp, suggesting that it is highly susceptible to viral diseases. Ten severely infected accessions were tested for Sweet Potato Leaf Curl Virus (SPLCV) using PCR technique. PCR detected the virus in 30% of the accessions.

Key words: DNA, *Ipomoea batatas*, PCR, symptomatology

INTRODUCTION

According to FAOSTATS Sweet potato (*Ipomoea batatas* L.) in 2010 plays a very vital role in food security in many countries worldwide providing food for over 350 million people in Africa. The importance of sweet potato as a food security crop will even be more significant in the wake of climate change and its associated drought and emergence of new pests and diseases since it is an early maturing crop and grows well on marginal soils. Despite the huge economic importance of sweet potato as domestic and export crop, its production is greatly constrained by viral diseases (Ndunguru *et al*., 2009) which is by-far a major concern to many farmers worldwide. According to Gibson *et al*. (2000), virus diseases rank second to weevil in causing yield reduction in sweet potato since these viral diseases spread simultaneously via traditional planting of vines. Virus diseases of sweet potato; therefore, cause significant yield loss up to 100% depending on the type(s) of infecting virus, plant cultivar, stage of infection and environmental conditions (Salazar and

Fuentes, 2001). The severity of virus symptoms varies with the number of different viruses infecting synergistically. Sweet potato virus disease (SPVD), a synergistic combination of *Sweet potato feathery mottle virus* (SPFMV) and *Sweet potato chlorotic stunt virus* (SPCSV), has been identified as the major cause of sweet potato yield loss in Africa (Ndunguru and Kapinga, 2007). With the exception of some strains of *Sweet potato leaf curl virus*, most sweet potato viruses express themselves visually (are symptomatic) as vein clearing, interveinal chlorosis, chlorotic mosaic, leaf curling, stunted growth, leaf narrowing and distortion (Gibson *et al*., 2000; Fuentes, 2010).

Although, the detection and identification of sweet potato viruses are a difficult procedure, complicated by frequent occurrence of mixed infections and synergistic complexes as in SPVD (Tairo *et al*., 2004), several virus detection methods including symptomatology, use of indicator plants, serology such as Enzyme-Linked Immunosorbent Assay (ELISA), DNA-based Polymerase Chain Reaction (PCR) and reverse-transcription polymerase chain reaction (RT-PCR) are used for virus indexing in sweet potato (Ghosh *et al*., 2008). According to Panta *et al*. (2007), PCR and RT-PCR techniques are the most reliable detection methods for viruses for which antibodies are not yet produced since sequencing conserved viral genome is much easier than producing the antisera used in ELISA detection. Therefore, the aim of this work was to employ Visual Symptomatology and Polymerase Chain Reaction (PCR) technique to detect *Sweet potato leaf curl virus* in Ghana.

MATERIALS AND METHODS

Twenty-two accessions of sweet potato, namely CRI027, SA-BNARI, UE007, Histarch, VOTCR004, UE017, BOT03028, UK-BNARI, CRI054, JON001, VOTCR003, US002, BOT0320, UE005, UE009, UE014, BOT03020, US001, US003, VOTVR009, VOTCR002 and BOT02020 were used in the present study at the Biotechnology and Nuclear Agriculture Research Institute (B.N.A.R.I.) of the Ghana Atomic Energy Commission (G.A.E.C.), Accra - Ghana. Fresh vine cuttings with five nodes per cutting were planted on ridges on the field with three replicates using a Randomized Complete Block Design (RCBD).

Virus assaying by visual observation: Eight weeks after planting, we indexed the plants for virus infection weekly for six weeks using visual observation of disease symptoms on young fresh leaves and scored on a six-point scale ranging from 1 to 6, as described by Fuentes (2010), with modifications:

- No visual symptom
- Presence of vein clearing or chlorotic spots
- Presence of chlorotic spots with mild chlorotic pattern over the entire leaf
- Presence of vein clearing, blistering, moderate mosaic pattern throughout the leaf, narrowing and distortion in the lower one-third of the leaflets
- Presence of vein necrosis, necrotic spots, mosaic distortion in two-third of the leaflets and general reduction in leaf size
- Presence of severe chlorosis, interveinal chlorosis, leaf curling, severe mosaic and distortion in the entire leaf

The symptom scores were used to estimate Disease Incidence (DI), index of symptom severity of all plants (ISSap) and index of symptom severity of diseased plants (ISSdp) using the method described by Njock and Ndip (2007) as:

$$\text{Index of symptom severity of all plants (ISSap)} = \frac{\sum_{s=1}^{3}(SX)}{\sum(X)}$$

$$\text{Index of symptom severity of diseased plants (ISSdp)} = \frac{\sum_{s=1}^{3}(SX)}{\sum(X)}$$

$$\text{Disease incidence (DI)} = \frac{Y}{X} \times 100\%$$

where, S is severity score; X is number of plants scored; Y is number of diseased plants and Z is total number of plants.

Virus detection by DNA-based PCR technique: Ten accessions which showed comparatively severe symptoms of virus infection on the field were screened for the presence of Sweet potato leaf curl virus using Polymerase Chain Reaction (PCR) technique. Genomic DNA was extracted using Sodium hydroxide (NaOH) extraction method as described by Wang *et al*. (1993). A hundred milligram of fresh young leaves was finely ground in 400 μL of 0.5 M NaOH using pestle and mortar. The solution was poured into a clean 2 mL eppendorf tube and centrifuged at 14000 rpm for 5 min. The supernatant was transferred to a new eppendorf tube and 5 μL of it was added to 495 μL of 100 mM Tris HCl (pH 8.0) to obtain the genomic DNA and amplified. The DNA amplification was performed in 20 μL reaction volumes using degenerate geminivirus primer pair SPG1/SPG2 and Sweet potato leaf curl virus strain-specific primer pair SPG3/SPG4. The reaction mixture for degenerate geminivirus detection contained 8.4 μL of PCR water, 4.0 μL of 5x *Taq* buffer, 2.8 μL of 25 mM $MgCl_2$, 0.5 μL of 10 mM dNTPs, 1.0 μL of 10 mM SPG1 (forward primer), 1.5 μL of 10 mM SPG2 (reverse primer) (Table 1), 0.3 μL of 0.09U *Taq* DNA polymerase and 1.5 μL of 11.06 μg mL^{-1} genomic DNA. For detection of Sweet potato leaf curl virus, the reaction mixture contained 8.8 μL of PCR water, 4.0 μL of 5x *Taq* buffer, 2.8 μL of 25 mM $MgCl_2$, 0.5 μL of 10 mM dNTPs, 1.0 μL each of 10 mM SPG3 (forward) and 10 mM SPG4 (reverse) (Table 1) primers, 0.3 μL of 0.09U *Taq* DNA polymerase and 1.6 μL of 11.06 μg mL^{-1} genomic DNA. The touchdown PCR amplification conditions used involved 11 cycles of initial denaturation of 94°C for 40 sec, 61°C for 30 sec and 72°C for 90 sec, 24 cycles of denaturation at 94°C for 40 sec, primer annealing at 60°C for 40 sec and elongation at 72°C for 90 sec. The final elongation was done at 72°C for 10 min. The PCR products were electrophoresed on 1% agarose solution (w/v) using a protocol described by Sambrook *et al*. (1989). Eight microliter aliquot of each PCR product was mixed with 2 μL of bromophenol blue loading dye (Sigma, USA) and run on the gel at a constant voltage of 90 V for 45 min and autoradiographed with ultraviolet transilluminator (UVP, Cambridge, UK).

Table 1: Primer sequence used for PCR amplification of genomic DNA

Target virus	Forward and Reverse primers	Primer sequence (5'→ 3')	Expected fragment length	Reference
Geminivirus degenerate	SPG1	CCCCKGTGCGWRAATCCAT	912 bp	Lotrakul *et al*. (1998)
	SPG2	ATCCVAAYWTYCAGGGAGCTAA		
SPLCV	SPG3	ACTTCGAGACAGCTATCGTGCC	1148 bp	Lotrakul *et al*. (1998)
	SPG4	AGCATGGATTCACGCACAGG		

RESULTS AND DISCUSSION

Viral disease symptoms observed on field grown sweet potato: The sweet potato cuttings sprouted eight days after planting and by the eighth week, all the accessions showed varied symptoms of virus disease infection. These symptoms which were observed on young as well as mature leaves were upward curling on leaf edges (Fig. 1a), blistering on entire leaf surface (Fig. 1b), vein clearing, interveinal chlorosis and chlorotic spots on the entire leaf (Fig. 1c), purpling on entire leaf surface (Fig. 1d), leaf narrowing and distortion (Fig. 1e) and general yellowing of the entire

Fig. 1(a-f): Sweet potato accessions showing (a) Severe upward leaf curling along the edges of leaves of UE007, (b) Severe blistering on leaf surface and stunted growth on OK03018, (c) Interveinal chlorosis and mild chlorosis on entire leaves of UE014, (d) Purpling on entire leaf surface of US002, (e) Purpling and leaf narrowing on VOTCR006 and (f) Healthy leaves of UK-BNARI

plant. Vein clearing, interveinal chlorosis and chlorotic spots on entire leaf were the most common observed symptoms in all the accessions. However, leaf curling was localized only in accessions UK-BNARI and UE007 in replicate one. Fuentes (2010) observed similar symptoms in sweet potato accessions grown in Ethiopia and this relates favorably to the observations made in this research.

Disease incidence and symptom severity on the field: All the accessions showed various degrees of viral disease incidence (DI) ranging from mild to severe (Fig. 2a and b) depending on the accession and age of the plants. At eight weeks after planting, three accessions namely BOT03028, VOTCR003 and US003 showed mild DI of 15, 10 and 15% respectively while seven accessions: BOT0320, UE009, US002, HISTARCH, VOTCR004, VOTVR009 and US001 showed severe DI of 80, 75, 90, 80, 75, 75 and 70%, respectively (Fig. 2). The remaining accessions showed moderate (mean of 49.17%) disease incidence. In spite of this varied observations, there was no significant difference ($p \geq 0.05$) in DI between all accessions at this stage. The variation in DI from 10-90% could be due to several factors such as virus concentration in the vine cuttings used as planting materials and the genetic constitution of the individual accessions. Ndunguru and Kapinga (2007) have made similar observation in sweet potato accessions planted in Southern Tanzania. They observed a disease incidence of 3-100% which compares favorably with the results obtained in this study.

The disease incidence increased steadily from the eighth week to the thirteenth week. By the thirteenth week, all the accessions showed symptoms of virus infection (Fig. 2b) suggesting that

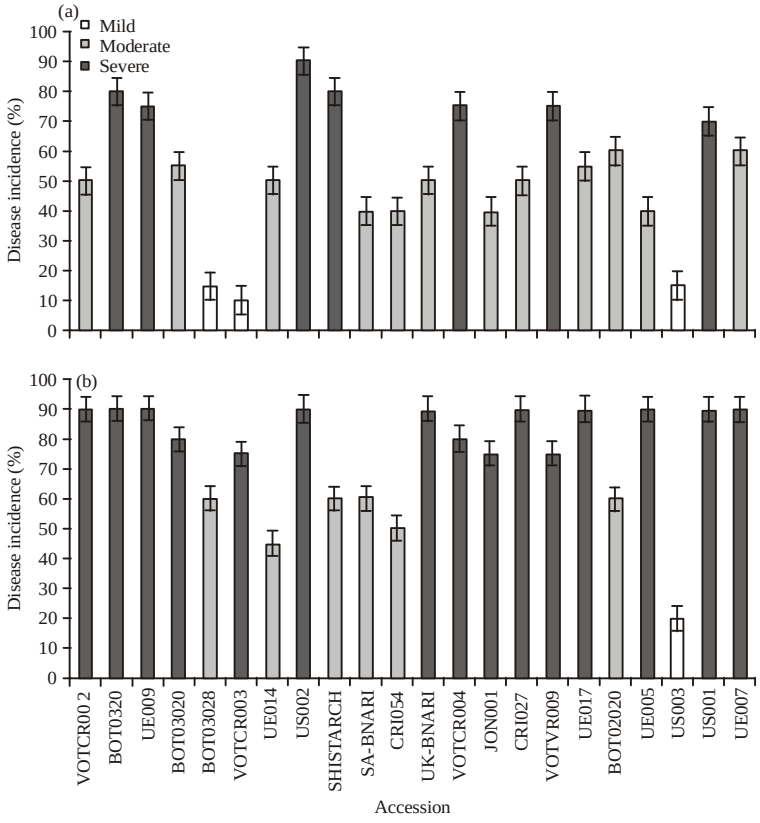

Fig. 2(a-b): Viral disease incidence (DI) of 22 accessions of sweet potato (*Ipomoea batatas* L.) at (a) Eight weeks and (b) Thirteen after planting

they were susceptible to the virus but at different degrees. Accession US003 showed mild symptom with DI of 20% while BOT03028, UE014, Histarch, SA-BNARI, CRI054 and BOT02020 showed moderate symptoms with DI of 60, 45, 60, 60, 50 and 60%, respectively. The remaining fifteen accessions showed severe (mean of 85.67%) disease incidence (Fig. 2b) indicated by stunted growth, severe leaf distortion and leaf necrosis. At this stage, most accessions which showed mild and moderate DI at eight weeks after planting increased to severe DI indicating a possible corresponding increase in virus concentration. Accessions JON001, UE005, BOT03020, VOTCR002, UK-BNARI, CRI027, UE017 and UE007 which showed moderate DI of 40, 40, 50, 50, 50, 55, 55 and 60% at eight weeks after planting increased to severe DI of 75, 90, 90, 90, 90, 80, 90 and 90%, respectively, by the end of the thirteenth week. Similarly, VOTCR003 which showed DI of 10% at eight weeks after planting increased to 75% by 13 weeks after planting (Fig. 2a, b). However, US003, showed the least DI of 15 and 20% in weeks 8 and 13, respectively (Fig. 2a, b) indicating that it is mildly susceptible to the viral infection and, therefore, could be planted by farmers depending on tuber size and yield. Thus, the accessions used for the study could be classified as mildly, moderately or highly susceptible cultivars.

The mean DI value (53.41% at the eighth week) indicating the presence of the virus as early as eight weeks after planting could be due to the presence of the viral particles in the planting materials. Similarly, a mean disease incidence of 64.55% has been reported by Ndunguru *et al.* (2009) in sweet potato grown in Central Uganda. This observation can be attributed to the fact that in most vegetatively propagated crops including sweet potato, virus transmission is mostly through cuttings. The increased mean DI (74.55%) at the end of the thirteenth week could be as a result of increased virus titre due to viral transmission from vectors.

The mean indexes of symptom severity for all plants (ISSap) and symptom severity for diseased plants (ISSdp) increased throughout the study period (Table 2 and 3). Accession VOTCR003 had the lowest ISSap of 1.08 while VOTCR002 had the highest value of 3.67 (Table 2). At week eight,

Table 2: Index of symptom severity of all plants (ISSap)

	Weeks					
Accessions	8	9	10	11	12	13
VOTER	2.00±0.09^{cdefg}	2.17±0.09^{def}	2.50±0.09^{fgh}	3.25±0.10^{h}	3.42±0.11^{e}	3.67±0.11^{e}
BOT0320	2.08±0.09^{efg}	2.75±0.09^{f}	2.83±0.09^{h}	2.92±0.10^{gh}	3.00±0.11^{de}	3.17±0.11^{de}
UE009	1.92±0.09^{cdefg}	2.08±0.09^{def}	2.58±0.09^{gh}	2.58±0.10^{fgh}	2.42±0.11^{bcde}	2.42±0.11^{bcd}
BOT03020	2.42±0.09^{g}	2.42±0.09^{ef}	2.17±0.09^{defgh}	2.17±0.10^{cdefg}	2.42±0.11^{bcde}	2.50±0.11^{bcd}
BOT03028	1.33±0.09^{abc}	1.33±0.09^{abc}	1.33±0.09^{abc}	1.33±0.10^{ab}	1.67±0.11^{ab}	1.67±0.11^{ab}
VOTCR003	1.08±0.09^{a}	1.08±0.09^{a}	1.08±0.09^{a}	1.25±0.10^{a}	2.92±0.11^{cde}	2.92±0.11^{cde}
UE014	1.50±0.09^{abcdef}	1.50±0.09^{abcd}	1.50±0.09^{abcde}	1.67±0.10^{abcde}	1.67±0.11^{ab}	1.67±0.11^{ab}
US002	2.00±0.09^{defg}	2.00±0.09^{def}	2.00±0.09^{cdefgh}	2.00±0.10^{bcdef}	2.00±0.11^{abcd}	2.00±0.11^{abc}
HISTARCH	2.17±0.09^{fg}	1.75±0.09^{bcde}	1.92±0.09^{bcdefg}	2.08±0.10^{cdefg}	1.75±0.11^{ab}	1.75±0.11^{ab}
SA-BNARI	1.50±0.09^{abcde}	1.58±0.09^{abcd}	1.58±0.09^{abcde}	1.42±0.10^{abcd}	2.00±0.11^{abc}	2.00±0.11^{abc}
CRI054	1.50±0.09^{abcdef}	1.50±0.09^{abcd}	1.83±0.09^{abcdefg}	1.92±0.10^{abcdef}	1.75±0.11^{ab}	1.92±0.11^{ab}
UK-BNARI	1.50±0.09^{abcdef}	1.75±0.09^{bcde}	1.75±0.09^{abcdef}	1.75±0.10^{abcdef}	2.17±0.11^{bcd}	2.17±0.11^{bcd}
VOTCR004	1.83±0.09^{cdefg}	1.83±0.09^{cde}	1.83±0.09^{bcdefg}	1.83±0.10^{abcdef}	2.17±0.11^{bcd}	2.17±0.11^{bcd}
JON001	1.42±0.09^{abcd}	1.17±0.09^{ab}	1.25±0.09^{ab}	1.42±0.10^{abc}	2.00±0.11^{abcd}	2.00±0.11^{abc}
CRI027	1.67±0.09^{abcdef}	1.83±0.09^{cde}	1.75±0.09^{abcdef}	2.17±0.10^{defg}	2.17±0.11^{bcd}	2.17±0.11^{bcd}
VOTVR009	1.92±0.09^{cdefg}	2.00±0.09^{def}	2.08±0.09^{defgh}	2.08±0.10^{cdefg}	2.08±0.11^{abcd}	2.08±0.11^{abc}
UE017	1.67±0.09^{abcdef}	2.00±0.09^{def}	2.17±0.09^{efgh}	2.25±0.10^{efg}	2.42±0.11^{bcde}	2.42±0.11^{bcd}
BOT02020	1.67±0.09^{abcdef}	1.67±0.09^{abcd}	2.00±0.09^{bcdefg}	2.00±0.10^{abcdef}	2.00±0.11^{abc}	2.00±0.11^{abc}
UE005	1.75±0.09^{bcdefg}	2.00±0.09^{def}	2.00±0.09^{cdefgh}	2.00±0.10^{bcdef}	2.00±0.11^{abcd}	2.08±0.11^{abc}
US003	1.17±0.09^{ab}	1.25±0.09^{abc}	1.42±0.09^{abcd}	1.50±0.10^{abcde}	1.33±0.11^{a}	1.33±0.11^{a}
US001	1.83±0.09^{cdefg}	2.08±0.09^{def}	2.08±0.09^{defgh}	2.08±0.10^{cdefg}	2.33±0.11^{bcd}	2.33±0.11^{bcd}
UE007	1.67±0.09^{abcedf}	1.83±0.09^{cde}	1.92±0.09^{bcdefg}	2.00±0.10^{bcdef}	2.00±0.11^{abcd}	2.00±0.11^{abc}

Values in same column followed by same superscript(s) are not significantly different at ($p \leq 0.05$) according to Tukey's pair wise comparison

Table 3: Index of symptom severity of diseased plants (ISSdp)

	Weeks					
Accessions	8	9	10	11	12	13
VOTCR002	3.75±0.32^{ab}	2.75±0.27^{abc}	2.50±0.27^{bcd}	3.25±0.26^{de}	3.42±0.25^{d}	3.67±0.25^{cd}
BOT0320	2.18±0.32^{b}	2.75±0.27^{bc}	2.83±0.27^{bcd}	2.92±0.26^{cde}	3.00±0.25^{cd}	3.17±0.25^{cd}
UE009	2.83±0.32^{b}	2.83±0.27^{bc}	3.50±0.27^{d}	3.50±0.26^{e}	3.17±0.25^{cd}	3.17±0.25^{cd}
BOT03020	2.58±0.32^{b}	3.50±0.27^{c}	3.08±0.27^{cd}	3.08±0.26^{bcde}	2.67±0.25^{bcd}	2.61±0.25^{bcd}
BOT03028	2.00±0.32^{a}	2.00±0.27^{a}	2.00±0.27^{a}	2.00±0.26^{a}	2.00±0.25^{ab}	2.00±0.25^{ab}
VOTCR003	2.00±0.32^{a}	2.00±0.27^{a}	2.00±0.27^{a}	2.00±0.26^{ab}	3.08±0.25^{cd}	3.17±0.25^{cd}
UE014	2.00±0.32^{b}	2.00±0.27^{bc}	2.00±0.27^{ab}	2.00±0.26^{ab}	2.00±0.25^{ab}	2.00±0.25^{ab}
US002	2.00±0.32^{b}	2.00±0.27^{bc}	2.00±0.27^{bcd}	2.00±0.26^{bcde}	2.00±0.25^{bcd}	2.00±0.25^{bcd}
HISTARCH	2.25±0.32^{b}	2.00±0.27^{bc}	2.00±0.27^{bcd}	2.17±0.26^{bcde}	2.08±0.25^{bcd}	2.08±0.25^{bcd}
SA-BNARI	2.12±0.32^{ab}	2.00±0.27^{bc}	2.11±0.27^{bcd}	1.92±0.26^{bcde}	2.33±0.25^{bcd}	2.33±0.25^{bcd}
CRI054	2.17±0.32^{ab}	2.17±0.27^{ab}	2.50±0.27^{abc}	2.33±0.26^{bcde}	2.50±0.25^{abc}	2.50±0.25^{abc}
UK-BNARI	2.00±0.32^{b}	2.00±0.27^{bc}	2.00±0.27^{bcd}	2.00±0.26^{bcde}	2.33±0.25^{bcd}	2.33±0.25^{bcd}
VOTCR004	2.00±0.32^{b}	2.00±0.27^{bc}	2.00±0.27^{bcd}	2.00±0.26^{bcde}	2.33±0.25^{bcd}	2.33±0.25^{bcd}
JON001	2.0±0.32^{ab}	2.00±0.27^{ab}	2.00±0.27^{ab}	2.50±0.26^{abcd}	2.33±0.25^{bcd}	2.33±0.25^{bcd}
CRI027	2.17±0.32^{b}	2.08±0.27^{bc}	2.00±0.27^{bcd}	2.17±0.26^{bcd}	2.17±0.25^{bcd}	2.17±0.25^{bcd}
VOTVR009	2.08±0.32^{b}	2.17±0.27^{bc}	2.25±0.27^{bcd}	2.25±0.26^{bcde}	2.25±0.25^{bcd}	2.25±0.25^{bcd}
UE017	2.00±0.32^{b}	2.00±0.27^{bc}	2.08±0.27^{bcd}	2.25±0.26^{bcde}	2.42±0.25^{bcd}	2.42±0.25^{bcd}
BOT02020	2.00±0.32^{ab}	2.00±0.27^{ab}	2.50±0.27^{abc}	2.50±0.26^{abcd}	2.50±0.25^{abc}	2.50±0.25^{abc}
UE005	2.00±0.32^{ab}	2.00±0.27^{bc}	2.00±0.27^{bcd}	2.00±0.26^{bcde}	2.00±0.25^{bcd}	2.08±0.25^{bcd}
US003	2.00±0.32^{a}	2.00±0.27^{ab}	2.00±0.27^{bcd}	2.17±0.26^{abc}	2.33±0.25^{a}	2.33±0.25^{a}
US001	2.08±0.32^{b}	2.08±0.27^{bc}	2.08±0.27^{bcd}	2.08±0.26^{bcde}	2.33±0.25^{bcd}	2.33±0.25^{bcd}
UE007	2.00±0.32^{ab}	2.00±0.27^{bc}	2.00±0.27^{b}	2.00±0.26^{bcde}	2.00±0.25^{bcd}	2.00±0.25^{bcd}

Values in same column followed by same superscript(s) are not significantly different at ($p \leq 0.05$) according to Tukey's pair wise comparison

the mean ISSap for all accessions was 1.70 and increased to 2.20 by the end of the thirteenth week indicating mild viral disease infection of all the accessions at their developmental stages (Table 2). The ISSap for VOTCR002 and BOT0320 increased beyond 2.00 at week 13 suggesting that they are moderately susceptible to viral diseases while US002 remained constant throughout the study period. However, the non-significant difference ($p>0.05$) in ISSap in all accessions at the end of the thirteenth week suggests that all the accessions are susceptible to the virus infection.

Among all the accessions, VOTCR002 showed the highest ISSdp (3.75) throughout the study while fifteen accessions showed the lowest ISSdp of 2.0 (Table 3). The mean ISSdp, eight weeks after planting, was 2.19 and increased to 2.30 by the end of the thirteenth week. However, the ISSdp value for US002, BOT03028, UE014, US002 and UE007 remained the same at 13 weeks after planting. In the present study, sweet potato viral disease symptom severity ranged from mild to moderate (1.70 -2.19 mean severity score) in the accessions. According to Gutierrez *et al*. (2003), SPFM and SPCSV do interact in co-infected plants synergistically causing the severe Sweet potato virus disease that is more damaging to the crop than would be expected if an individual virus was present. Thus, mild to moderate symptoms observed in this study could be due to lack of synergism. However, not all moderate to severe symptoms on sweet potato are due to lack of synergistic effect of mixed infections as was observed in Barbados by Salazar and Fuentes (2001). Whiteflies differ considerably in abundance on sweet potato crops and these differences may be important in determining the severity of sweet potato viral diseases.

Disease symptoms were sometimes absent on young leaves of accessions which resulted in the sudden fluctuation in DI, ISSap and ISSdp in UE014, UE009, BOT03020, Histarch, SA-BNARI, CRI054, JON001, CRI027, US003 and VOTCR002 (Table 2 and 3). These fluctuations suggested that these accessions recovered from the virus infection at some periods in their developmental stages. This could be due to plant cells use defense mechanisms against diseases to prevent the spread of infection by microbial pathogens following an earlier localized exposure to a pathogen.

These mechanisms are known as Hypersensitive Resistance (HR) (Iakimova *et al*., 2005) and/or Systemic Acquired Resistance (SAR) (Song *et al*., 1995). These mechanisms also account for the constant ISSap for US002 and ISSdp values for US002, BOT03028, UE014, US002 and UE007 throughout the study period. The recovery from the virus, however, complicated the assessment of sweet potato viruses as plants that had recovered were excluded from virus symptomatology diagnosis. Ndunguru and Kapinga (2007) have made similar observations in sweet potato accessions planted in Southern Tanzania. They observed that most young leaves which emerged after the death of an old leaf did not show any symptom of virus infection; thus, were excluded in virus assessment.

Detection of SPLCV using PCR amplification: Although viral symptomatology is an easy and low cost technique for viral detection, it has serious limitations as it is influenced by subjectivity as well as environmental factors such as nutrient deficiency. This shortfall of symptomatology can be overcome by the use of polymerase chain reaction diagnostics technique due to its high sensitivity and specificity. Thus, PCR diagnostic technique with virus primer pairs SPG1/SPG2 and SPG3/SPG4 was used to identify Sweet potato leaf curl virus in 10 sweet potato accessions which showed moderate symptoms of virus infection and high disease incidence on the field. The DNA extraction method using sodium hydroxide (NaOH) allowed for fast processing of samples and produced quality DNA for PCR amplification in the ten accessions.

Both primer pairs SPG1/SPG2 and SPG3/SPG4 amplified their corresponding fragments sizes at ~912 and ~1148 bp, respectively (Fig. 3 and 4) in the accessions UK-BNARI, CRI054 and UE007 indicating the presence of the virus. The amplification of the genomic DNA by strain-specific primer pair SPG3/SPG4 suggested that the virus was a *Sweet potato leaf curl virus* (SPLCV). The positive control was also amplified at corresponding fragment sizes for both SPG1/SPG2 and SPG3/SPG4, respectively confirming the presence of the virus.

The strain specific primer pair SPG3/SPG4 confirmed the presence of virus in all the infected accessions, as already detected by the geminivirus degenerate primer pair SPG1/SPG2 (Fig. 3 and 4), indicating that both primers are equally sensitive to the degenerate geminivirus in the PCR assay. Li *et al*. (2004) used the same primers to detect geminivirus in sweet potato accessions collected from different parts of the world and grown in the U.S.A and this compares favorably with the result in this study. The primer pair SPG1/SPG2 had been used to identify

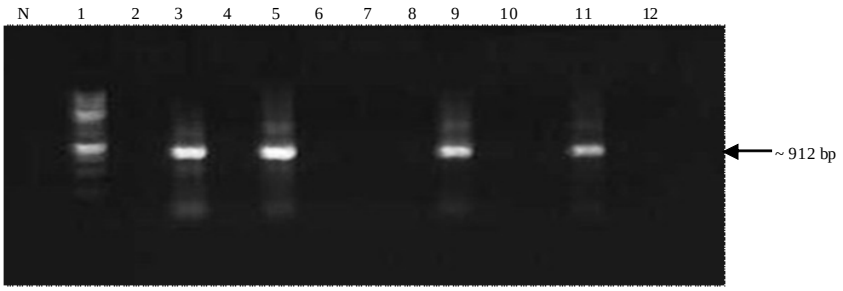

Fig. 3: PCR amplification products from genomic DNA of ten accessions of sweet potato using geminivirus degenerate primer pair SPG1/SPG2. Lane N: 1kb DNA ladder, 1: UE017, 2: UK-BNARI, 3: BOT02020, 4: CRI054, 5: SA-BNARI, 6: US001, 7: CRI027, 8: UE007, 9: VOTCR002, 10: BOT03028, 11: Infected sweet potato (positive control), 12: Healthy sweet potato (negative control)

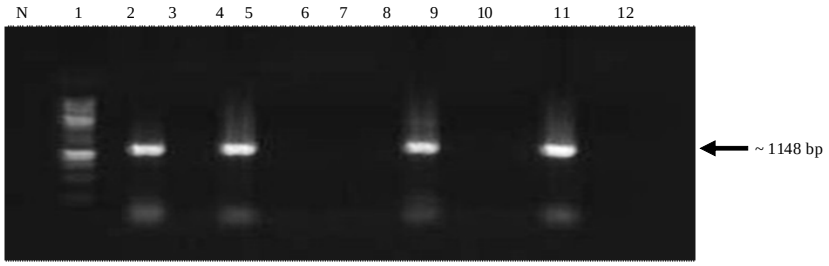

Fig. 4: PCR amplification products from genomic DNA of ten accessions of sweet potato using SPLCV specific primer pair SPG3/SPG4. Lane N: 1kb DNA ladder, 1: UE017, 2: UK-BNARI, 3: BOT02020, 4: CRI054, 5: SA-BNARI, 6: US001, 7: CRI027, 8: UE007, 9: VOTCR002, 10: BOT03028, 11: Infected sweet potato (positive control), 12: Healthy sweet potato (negative control)

SPLCV due to its high sensitivity (Lotrakul *et al*., 1998). Its sensitivity may be attributed to its annealing regions of Open Reading Frames (ORFs) AC2 and AC1 which are highly conserved in geminiviruses infecting sweet potato while SPG3/SPG4 binds the coat protein gene V1 and C3 of regions of ORF. However, the strain-specific primer pair SPG3/SPG4 may not be able to amplify every isolate of SPLCV because coat protein gene C3 is the least conserved ORF in the genome of the geminivirus (Lotrakul *et al*., 1998).

While it was not possible to discriminate, with accuracy, symptoms in plants tested positive for SPLCV from non-infected plants at early stages of growth on the field, the PCR technique was able to detect virus in symptomless accession. In this study, CRI054 did not show any symptom of leaf curling on the field, yet, it tested positive using the PCR technique. However, the remaining accessions, namely UE017, BOT02020, SA-BNARI, US001, CRI027, VOTCR002, BOT03028 and the negative controls were not amplified suggesting the absence or probably low titre of the virus in the genomic DNA (Fig. 3 and 4).

CONCLUSION

In this present study, all the sweet potato accessions, independent of their origin, expressed symptoms of virus infection as vein clearing, interveinal chlorosis and chlorotic spots on entire leaf, upward leaf curling, leaf narrowing and distortion, purpling, leaf blistering, reduction of leaf blades and general yellowing of entire plant on the field. However, VOTCR002 was most susceptible while US003 was less susceptible to virus infection. The PCR technique with primer pairs SPG1/SPG2 and SPG3/SPG4 revealed the presence of Sweet potato leaf curl virus in some of the severely diseased accessions. The technique was, therefore, a more effective and reliable tool for virus detection as it did not only confirm the presence of the virus as detected by visual observation but also, revealed the presence of Sweet potato leaf curl virus in accession CRI054 which did not show symptom of curling on the field.

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