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Heritability of reversion from *Sweet potato feathery mottle virus* infection in sweetpotato

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Sweet potato feathery mottle virus **(SPFMV) causes 50% yield losses in sweetpotato (*Ipomoea batatas*). However, some cultivars have been observed to become SPFMV free following initial infection, a phenomenon termed reversion. The heritability of reversion from virus infections in sweetpotato remains unclear. This study is aimed at determining heritability of reversion from SPFMV in progeny of crossed reverting and non-reverting sweetpotato cultivars 'New Kawogo' and 'Resisto,' respectively. Molecular diversity of the parents and progeny was also assessed using simple sequence repeats (markers) derived from coding regions of RNA dependent RNA polymerase 1 and dicer-like 1 gene that may be associated with reversion. Reversion potential varied among the progeny; 11 progeny reverted more effectively than 'New Kawogo' by 4 weeks after inoculation. Broad and narrow sense heritability was 76.42 and 42.4%, respectively. There was molecular variation among the 50 progeny, and markers ibDCL2b and ibDCL1a7F differentiated the progeny. These results indicate reversion was a heritable trait determined by multiple genes, and that genetic markers ibDCL2b and ibDCL1a7F may be used to differentiate the progeny. It is recommended that the progeny be tested in agro-ecological trials in virus-prone areas to identify those that deliver high yields and revert from SPFMV infection.**

Key words: *Sweet potato feathery mottle virus*, reversion, simple sequence repeats.

INTRODUCTION

Sweetpotato (*Ipomoea batatas*) is an important crop in sub-Saharan Africa, where it serves as a food security crop (Kapinga et al., 2007) and is a source of critical nutrients, including precursors of vitamin A (van-Jaarsveld et al., 2005). Despite its importance to human health, the crop is impacted by virus infections; one of the principal viruses is *Sweet potato feathery mottle virus* (SPFMV), which is prevalent in all regions where sweetpotato is cultivated (Parella et al., 2006). Single infections of SPFMV cause up to 50% yield losses; however, when it synergises with *Sweetpotato chlorotic stunt virus*, the resulting virus disease leads to yield losses of up to 98% (Gibson et al., 1998).

Recent studies have shown that some East African sweetpotato cultivars previously infected with SPFMV become virus-free (Gibson et al., 2014; Adikini et al., 2015) in a phenomenon termed 'reversion' that may represent a route to sustainable management of sweetpotato viruses. Reversion (a form of virus disease resistance) has been observed in a range of field-

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cultivated sweetpotato cultivars (Mukasa et al., 2003; Aritua et al., 2007; Adikini et al., 2016). However, occurrence of reversion in progeny derived from crosses of cultivars with high and low reversion potential, such as 'New Kawogo' and 'Resisto' (Gibson et al., 2014), respectively, and the wider heritability of reversion from SPFMV and molecular diversity of progeny remain unclear. The later can be evaluated using molecular markers of which simple sequence repeat (SSR) markers are key.

SSRs, in comparison to other markers, have many desirable genetic attributes such as linkage mapping (Akkaya et al., 1995), cultivar identification (Rongwen et al., 1995), marker-assisted selection (Fazio et al., 2003) and gene flow characterization (Aldrich and Hamrick, 1998; Chase et al., 1996) and have therefore been considered as marker of choice in plant research. SSRs are especially advantageous in genetic mapping research because of their simpler genotyping potential, co-dominance, reproducibility, mapping to similar locations in multiple populations (Cregan et al., 1999), and associating gene clusters with potential traits in plant genomes (Morgante et al., 2002). Simple sequence repeats have been identified as the most informative markers and can be derived from both coding and non-coding sequences of the genome (Parida et al., 2015).

Whereas SSR markers derived from coding sequences/regions have been reported to be less polymorphic than those from non-coding sequences, they have fundamental advantages: They are fast to obtain through *in silico* methods; repeat types are unbiased, occur in gene-rich regions of the genome and are abundant (Scott, 2001). Given that they signify the transcribed part of the genome, CDS-based SSR markers lead to the direct mapping of genes and have a high level of transferability among related species as they are located in more conserved regions of the genome (Varshney et al., 2002). Furthermore, SSRs are abundant in each organism, and it is becoming clear that such repeats are important in genomic organization and function and have been associated with disease resistance (Werner et al., 2003; Bhattarai et al., 2018; Collard and Mackill, 2008).

This paper reports on heritability of reversion from SPFMV in F_1 progeny derived from a cross between 'New Kawogo' and 'Resito' and quantification of molecular diversity of the progeny using SSR makers. This would improve understanding and potential for introgression of reversion in virus-susceptible cultivars in sweetpotato breeding programmes.

MATERIALS AND METHODS

Plant material and virus isolates

Plant materials from sweetpotato cultivars 'New Kawogo' and 'Resisto' were sourced from the virus-free sweetpotato collection at the Makerere University Agricultural Research Institute (MUARIK). 'New Kawogo' is a virus-resistant Ugandan cultivar (Gasura and Mukasa, 2010; Mwanga et al., 2016) with high reversion potential (Gibson et al., 2014), whereas 'Resisto' is a virus-susceptible American cultivar (Mwanga and Ssemakula, 2011) with low reversion potential (Gibson et al., 2014).

The cultivars (New Kawogo' and 'Resisto') were used as parents in a full diallel cross with reciprocals considered (Griffing, 1956), and resulting seeds were harvested and planted in pots containing a sterile potting mix (3:1:1 ratio of black soil:manure:sand) in an insect-proof screenhouse at MUARIK; imidacloprid and cypermethrin were applied weekly as spray, to control whiteflies and aphids. Each germinated seed was considered a progeny (50 progeny were obtained) and was subsequently grown for 2 months before it was multiplied using vine cuttings.

The East African strain of SPFMV (GenBank accession no. FJ795762) (Tugume et al., 2010) was used. The SPFMV isolate was sourced from the reserve virus collections at MUARIK that was partially sequenced using primers (Table 1) to confirm identity. The SPFMV isolate was stored in 'Beauregard', which is an American cultivar susceptible to SPFMV, until required for infection of study plants, when a leaf of the host plant was side-grafted to a 1-week old *Ipomoea setosa* plant. *Ipomoea setosa* is used as a universal indicator for sweetpotato virus infections (Moyer et al., 1989; Fuentes, 2010), and it was used in this study to multiply and increase virus titre/concentration to ensure inoculation of experimental material.

Evaluation of reversion inheritance

Fifteen vines cuttings, each of approximately 25-cm-long, of the two parents, 50 progeny and 3 outliers (*I. setosa* and sweetpotato cultivars 'Tanzania' and 'Ejumula') were established in pots containing sterile soil, as described previously, in an insect-proof screenhouse. The pots were laid in a completely randomized design. After two weeks, the plants were side-graft inoculated using a leaf of SPFMV-infected *I. setosa*. Once the graft was established, a section of the original plant growing above it was excised, planted in a new pot of the soil mix and allowed to re-establish for one week. Infection status of the cuttings was measured, using quantitative reverse-transcription PCR (RT-qPCR), from a composite sample of leaf discs taken from the basal, middle and top positions of the plant.

Ten plants (each of 2 parents, 50 progeny and 3 Outliers) that had tested positive for SPFMV were selected to monitor reversion at 2, 4 and 6 weeks post-virus inoculation using a composite sample of leaf discs taken from three fully expanded leaves below the apical leaf. Plants that tested negative for presence of the virus were further tested at weeks 8 and 10 to confirm full reversion (virus negative). One plant of each parent, progeny and outlier was graft inoculated with virus-free *I. setosa*, using the procedure described above, as a control.

RNA extracted from the leaf discs using the cetyl trimethyl ammonium bromide (CTAB) method, modified by Maruthi et al. (2002) was used in detection of SPFMV. RNA was quantified using a NanoDrop-ND-1000 spectrophotometer (Thermo Scientific; Bargal Analytical Instruments, Airport City, Israel) and evaluated on a 1.5% agarose gel.

Complementary DNA (cDNA) was synthesised from 100 ng to 1 µg of RNA, using a Verso cDNA synthesis kit (Thermo Scientific, Tamar, Israel) following the manufacturer's instructions, and the synthesised cDNA was used as template in a RT-qPCR reaction to confirm SPFMV infection. The RT-qPCR reaction comprised a 20 µL volume of PCR master mix that contained 0.8 µL each of forward and reverse SPFMV primers (10 µM) (Table 1), 10 µL of 2× PCBIO SYBR mix (Eisenberg Brothers Ltd, Airport City, Israel), 6.4 µL of nuclease free water and 2 µLof cDNA (diluted in a 1:4 ratio of

Table 1. Primers used to detect and confirm SPFMV.

Purpose	Primer	Sequence (5'-3')	Annealing temperature (°C)	Fragment size (bp)	Reference
Sequence confirmation	SPFMV-ILF	CTCCACCACCCACAATAACTG	60	800	This study
	SPFMV-ILR	CAGTTGTCGTGTGCCTCTCCG			
Virus detection	qSPFMV-Uni-818F	CGCATAATCGGTTGTTTGGTTT	60	107	Wasswa (2012)
	qSPFMV-Uni-925R	GGTTATGTATATTTCTAGTAACATCAG			
	qSPCOX_Fwd	ACTGGAACAGCCAGAGGAGA	60	159	Park et al. (2012)
	qSPCOX_Rev	ATGCAATCTTCCATGGGTTC			

cDNA:water).ART-qPCR master mix that contained primer COX (Table 1) was prepared and used as a control. The RT-qPCR was performed using duplicated samples with a negative control (molecular grade water), positive control and housekeeping gene (*cytochrome oxidase*) (Park et al., 2012) on each plate on a PE 7900 Sequence Detection System (ABI PRISM, Applied Biosystems, Warrington, UK). A 96-well skirted PCR microplates (twin.tec, Thermo Scientific, Dubai UAE) sealed with optical adhesive covers (Applied Biosystems) was used. The RT-qPCR thermal cycler conditions were 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 15 s and annealing/ extension at 60°C for 60 s. Reversion among the progeny was tested using repeated measures analysis of variance in XLSTAT (Addinsoft, 2017).

Genetic diversity of progenies

Simple sequence repeat (SSR) markers designed according to Ssamula et al. (2019) were used to assess genetic diversity. Primers were screened to determine and differentiate polymorphic from monomorphic primers using DNA extracted from parental genotypes; polymorphic primers were subsequently used to evaluate the genetic diversity of F_1 progeny.

Genomic DNA was isolated from parental and progeny genotypes and outliers (*I.setosa* and sweetpotato cultivars Tanzania and Ejumula). This was done using a modified CTAB method (Maruthi et. al., 2002). DNA quality was analysed using a NanoDrop-ND-1000 spectrophotometer (Thermo Scientific) at wavelength of 260/280 and 260/230 nm ratio of between 1.8 and 2.0, and visualised on 1% agarose gel; then the DNA was diluted/standardised to 50 ng for downstream analysis.

Annealing for amplification of polymorphic bands of derived primers was optimised along a gradient of eight temperatures using a PCR machine (Clever Scientific, Image Care, Kampala, Uganda) with a 10-µL PCR master mix containing 3 µL of water, 5 µL of PCR mix (HyLabs Ready Mix (×2), HyLabs, Rehovot, Israel), 0.5 µL each of forward and reverse primer (10 pmol) (Table 2) and 1 µL of DNA (50 ng). The PCR conditions for SSR amplification comprised an initial denaturation at 94°C for 4 min, 35 cycles of denaturation (94°C) for 30 s, annealing at 50°C for 45 s, extension at 72°C for 30 s, followed by a final extension at 72°C for 10 min. The SSR bands were visualised on a 3% agarose gel, prepared using 1× TBE buffer and stained with ethidium bromide. The gel was run at 50 Vfor 60 min and bands visualised on a gel documentation system (Clever Scientific). Band scoring was based on Zeinalzadeh-Tabrizi et al. (2018) and Guo et al. (2015) using binary counts of presence/absence (1/0). Data were used to evaluate distance matrices, as described by Nei and Li (1979), in XLSTAT, and a dendrogram produced based on Euclidian distances in a dissimilarity index using UPGMA (Addinsoft, 2017). Polymorphic information content (PIC), principal component analysis (PCA) and genetic diversity parameters for the SSRs in the progeny were evaluated using GenAlex software (Peakall and Smouse, 2012).

Estimation of heritability

Data obtained from reversion of parents, progeny and outliers was considered as phenotypic variability and polymorphic markers were considered as genotypic variability, from which broad (H^2) and narrow (h^2) sense heritabilities were estimated. The H^2 for polymorphic markers was calculated as the proportion (percent) of genotypic to phenotypic variability, according to Andrade et al. (2015). The h^2 was calculated using the restricted maximum likelihood method in R (R Core Team, 2003) and took into consideration both phenotypic (reversion observations/counts) and genotypic (SSR marker data) variation for each progeny. This used the mixed model y = Xb + Zu + e, where y is observed reversion from SPFMV, X is the incidence matrix for fixed effects (grand mean, µ; week, environment) and Z is the incidence matrix for random effects (progeny genotype and SSR marker). The vectors of best linear unbiased estimates (b, u and e) were the fixed effects and vector for best linear unbiased predictions of the random effects and random error, respectively.

RESULTS

Reversion from SPFMV

Reversion differed (P<0.001) among cultivars and

Table 2. Reversion from *Sweet potato feathery mottle virus* among parents, progeny and outliers at 2, 4 and 6 weeks after inoculation.

Variable	df	F	P
Week 2	54	9.09	<0.001
Week 4	54	13.59	<0.001
Week 6	54	17.49	<0.001

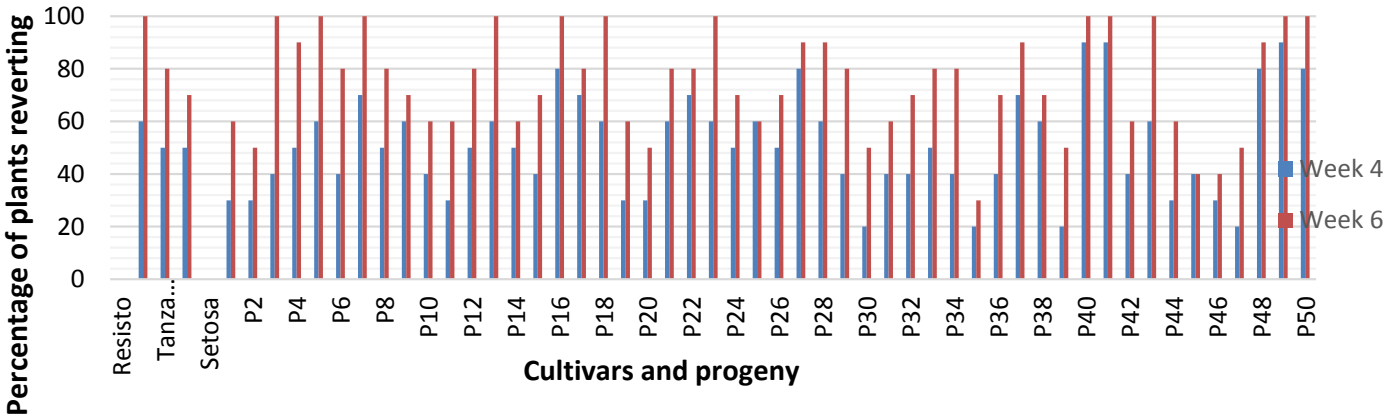

Figure 1. Proportion (%) of sweetpotato cultivars and progeny reverted from *Sweet potato feathery mottle virus* (SPFMV) at 4 and 6 weeks post-virus inoculation.

tended to increase with time (P<0.001). Reversion in progeny further varied within cultivars for weeks 4 (P<0.001) and 6 (P<0.001) (Table 2), where it was 60 and 100% in 'New Kawogo', 50 and 80% in 'Tanzania' and 50 and 70% in 'Ejumula', respectively. Reversion was not observed in 'Resisto' or *I. setosa* (Figure 1). All plants that tested negative by week 6 maintained their healthy status at weeks 8 and 10 (data not shown).

Simple sequence repeats

Two hundred and twenty-two SSR-based primer sets were designed (supplementary information containing SSRs is attached as an excel sheet), and 63 primers, which covered all chromosomes that contained defence genes and their variants, were selected based on the number and length of repeat motifs. The genes comprised *I. batatas* RNA dependent RNA polymerase 1 (*IbRDR1*), *I. batatas* dicer-like 1 (*IbDCL1*) and *I. batatas* dicer-like 2 (*IbDCL2*). Nine of the 63 SSR primers were polymorphic and were assigned as identifier markers (Table 3) for the evaluation of molecular diversity.

Genetic diversity

Analysis of SSRs identified 719 alleles among the progeny, ranging from 1.33 to 2.67 alleles per marker (mean = 2). Mean PIC was 0.682 (Table 4), with the greatest PIC for marker A (0.931) and lowest (0.511) for marker B. Mean number of effective alleles per marker was 1.526, and was greatest for marker I (2.015) and lowest for marker A (1.028) (Table 4).

Genetic diversity parameters associated with the SSR primers varied with loci (Table 4). The number of alleles per locus (Na) ranged from 2 to 4 and the number of effective alleles (Ne) ranged from 1.028 (marker A) to 2.015 (marker I) (mean: 1.526 ±0.099). Shannon's information index (I) ranged from 0.056 to 0.777 (mean: 0.424 ±0.073), the diversity index (h) ranged from 0.026 to 0.500 (mean: 0.269 ±0.046), and the unbiased diversity index ranged from 0.026 to 0.744 (mean: 0.389 ±0.075. The genetic diversity varied within the population. Markers B, D, E, F, H and I contributed significantly (P≤0.05) to the variability in genetic diversity among the populations (Table S1).

Relatedness of SSR markers

Cluster analysis based on Euclidian distance for UPGMA revealed five clusters, where four clusters comprised sweetpotato cultivars (including two outliers) and the fifth comprised *I. setosa* (Figure 2). Cluster 1 contained the largest number of genotypes of 'New Kawogo' with 38

Table 3. Polymorphic SSRs and respective markers derived from *RDR1* and *DCL* defence genes associated with RNA silencing.

Gene	Variant and chromosome (Chr)	Primer name	Repeat	Primer sequence (5′- 3′) forward and reverse	Identifier
ibRDR1	*IbRDR1a1* (Chr 8)	*IbRDR1a1_3*	(TTTATT)2	GGCCACATGGTAAATGAAGTAT GTGTTTTGAGGGCTGTTAATGT	Marker A
	IbRDR1a2 (Chr 8)	*IbRDR1a2_17*	(TA)11	AAGCTGTAAGCACGGAGTAAAA AGAAGAAGAAGAAGAAGGAGGG	Marker B
	IbRDR1a2 (Chr 8)	*IbRDR1a2_74*	(A)13	GCATTAGCGCATTACTGGTT AACACGATAAAGAAGATGAGGC	Marker C
IbDCL1	*IbDCL1a* (Chr 1)	*IbDCL1a_7*	(TTCAA)2	GGGTTGAAACACCTAGTAATGC AGCTGTGTGGAGGGTTAGTTTA	Marker D
	IbDCL1a (Chr 1)	*IbDCL1a_15*	(TA)10	GGGGTCATTTCTGTATGTGATT GTCCCTGCTTCAAAGGTAAGAT	Marker E
	IbDCL1b (Chr 9)	*IbDCL1b_23*	(AGTAGC)2	TTAACTGAAACCCTAGCCTCAC GCATCAAGTCAACTCAACTCAA	Marker F
	IbDCL1b (Chr 9)	*IbDCL1b_24*	(ATA)6	TTAACTGAAACCCTAGCCTCAC GCATCAAGTCAACTCAACTCAA	Marker G
IbDCL2	*IbDCL2b* (Chr 13)	*IbDCL2b_3*	(AGTAAA)2	GCAAGAATCGAATTTAGTGCTC TTCCCGAAATGTCTACTGCTAT	Marker H
	IbDCL2c(Chr 6)	*IbDCL2c_2*	(AGTAAA)2	GCAAGAATCGAATTTAGTGCTC TTCCCGAAATGTCTACTGCTAT	Marker I

Table 4. Genetic diversity parameters across the nine different SSR loci showing allele number, number of effective alleles, balanced and unbalanced diversity, and polymorphic information content (PIC).

SSR locus	Identifier	Repeat	Genetic diversity parameter					Alleles per locus	PIC
			Na	Ne	I	h	uh		
IbRDR1a1_3	A	(TTTATT)2	1.333	1.028	0.056	0.026	0.026	2	0.931
IbRDR1a2_17	B	(TA)11	2.333	1.863	0.599	0.371	0.542	4	0.511
IbRDR1a2_74	C	(A)13	2.000	1.615	0.480	0.319	0.489	3	0.680
IbDCL1a_7	D	(TTCAA)2	2.000	1.586	0.459	0.310	0.480	3	0.667
IbDCL1a_15	E	(TA)10	1.333	1.106	0.135	0.080	0.082	2	0.780
IbDCL1b_23	F	(AGTAGC)2	1.667	1.144	0.178	0.101	0.103	3	0.732
IbDCL1b_24	G	(ATA)6	2.333	1.702	0.556	0.337	0.415	4	0.535
IbDCL2b_3	H	(AGTAAA)2	2.333	1.678	0.572	0.378	0.620	3	0.763
IbDCL2c_2	I	(AGTAAA)2	2.667	2.015	0.777	0.500	0.744	4	0.534
Mean			2.000	1.526	0.424	0.269	0.389	3	0.682
SE			0.192	0.099	0.073	0.046	0.075		

PIC: Polymorphic information content; Na: number of different alleles; Ne: number of effective alleles; I: Shannon's information index; h: genetic diversity index; and uh: unbiased diversity.

progeny, cluster 2 contained nine progeny, cluster 3 contained 'Resisto' with three progeny, and cluster 4 contained 'Ejumula' and 'Tanzania.' *Ipomoea setosa*was in a cluster of its own (cluster 5). The shortest distance was between clusters 1 and 2 (2.093), and the greatest distance was between clusters 3 and 4 (6.042).

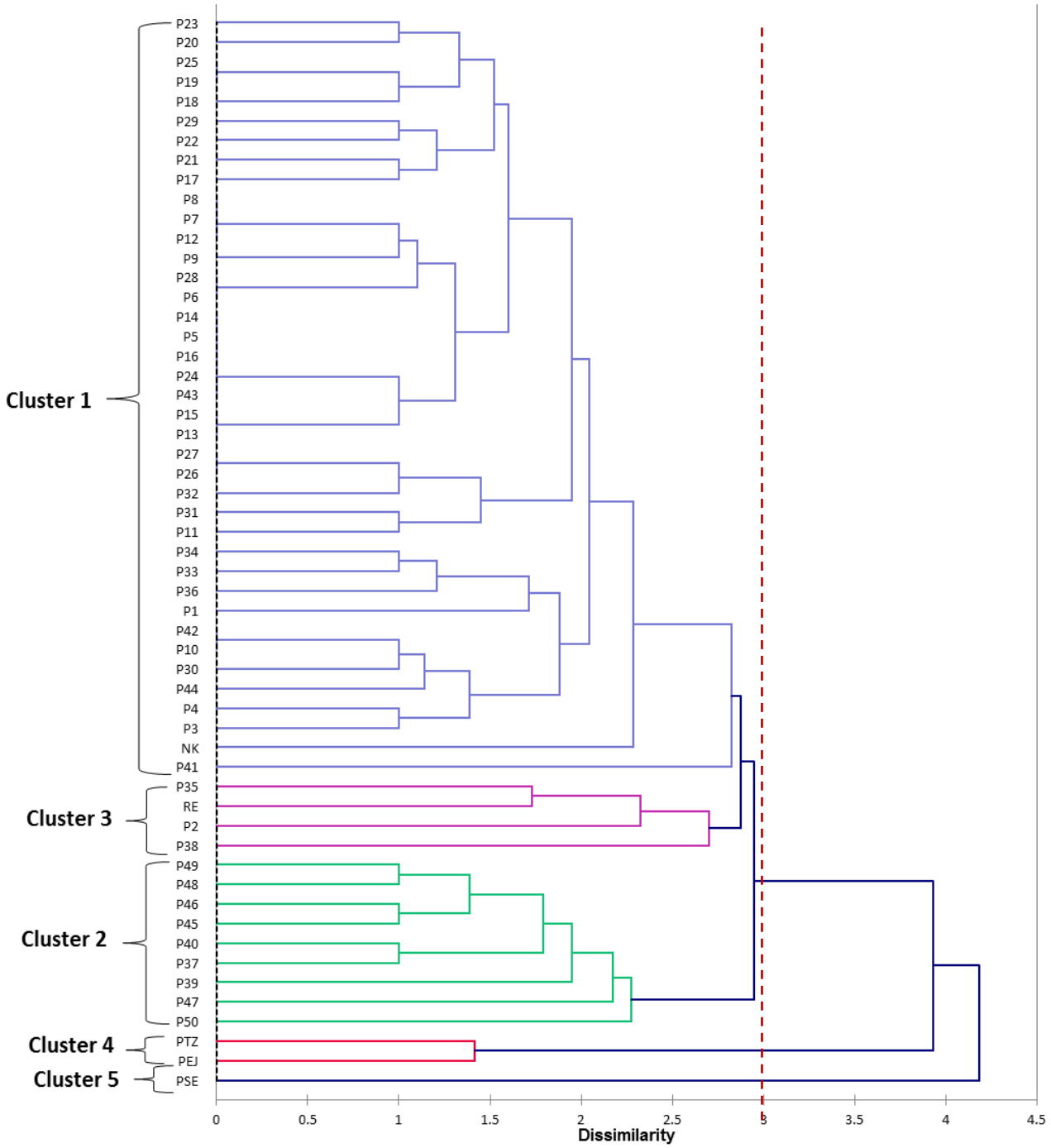

Figure 2. Dendrogram showing multivariate clustering due to SSR markers of sweetpotato defence genes from parents ('New Kawogo' - NK and 'Resisto' - Re) progeny (P1 - P50) and outliers ('Tanzania' - PTZ, 'Ejumula' - PEJ and '*I. setosa*'- PSE).

The PCA was used to test the relative importance of the SSR markers in genetic variability of the progeny. The first two principal components (PCs) accounted for 53.59% of the total variability (Table 5 and Figure 3). Variability in PC1 was largely accounted for by markers E (0.699) and F (0.623), and markers C (0.682) and A (0.339) accounted for much of the variability in PC2 (Table 5).

It was further observed that the genetic markers led to clustering of parents, progeny and outliers along PCs 1 and 2. In this regard, five clusters were derived (Figure 3). This thus complements observations from evaluations

Table 5. Contribution of the nine SSR markers in the first three principal components (PCs) to the variation in genetic diversity of sweetpotato parents, progeny and outliers.

Marker	Principal components			
	Identifier	PC1	PC2	PC3
IbRDR1a1_3	A	0.138	**0.339**	0.189
IbRDR1a2_17	B	0.261	0.006	**0.499**
IbRDR1a2_74	C	0.027	**0.682**	0.036
IbDCL1a_7	D	**0.338**	0.300	0.130
IbDCL1a_15	E	**0.699**	0.001	0.078
IbDCL1b_23	F	**0.623**	0.007	0.015
IbDCL1b_24	G	0.253	0.102	0.018
IbDCL2b_3	H	**0.385**	0.192	0.086
IbDCL2c_2	I	**0.384**	0.087	0.231
Eigen value		3.107	1.716	1.284
Variability (%)		34.525	19.064	14.262
Cumulative (%)		34.525	53.589	67.851

Values in bold indicate the PC factor for which the squared cosine value was the greatest.

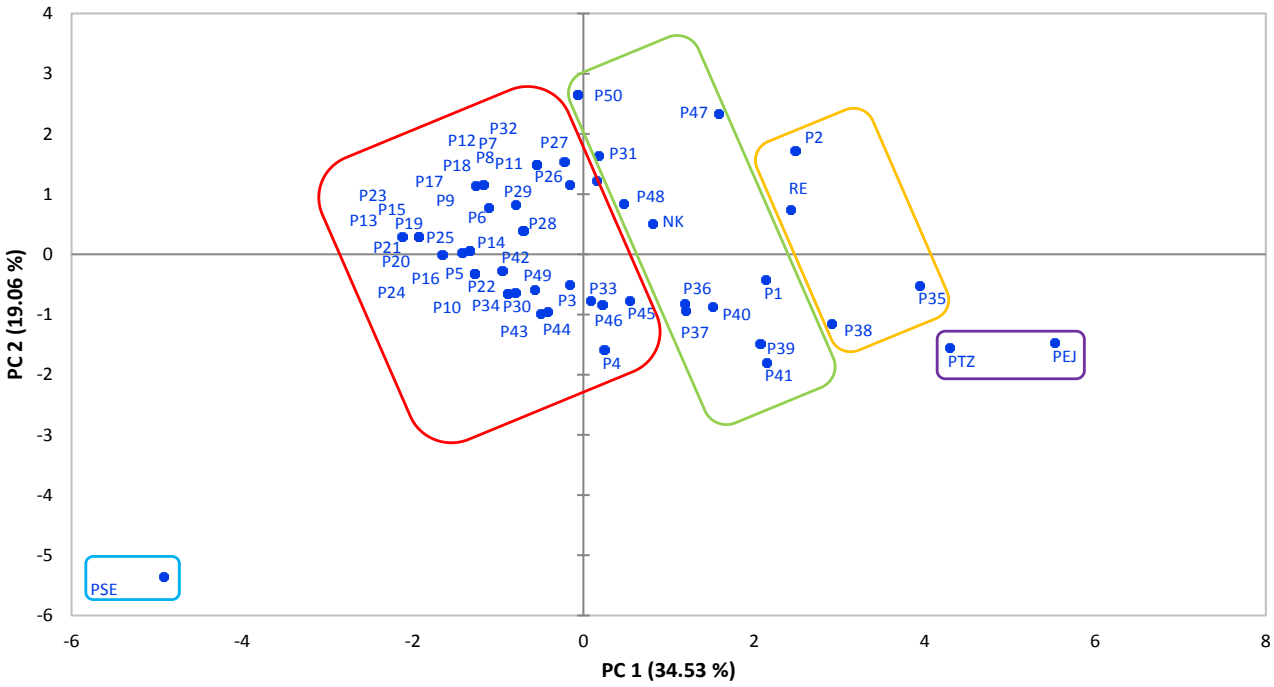

Figure 3. Ordination of genetic diversity of parents, progeny and outliers along principal components (PCs) 1 and 2.

of Euclidian distance in Figure 2.

Estimated heritability

The SSR markers contributed to a H^2 of 76.42%; the greatest H^2 (20.76%) was for marker B, and was lowest (1.04%) for marker A. The H^2 for markers C, D, E, F, G, H and I was 8.37, 6.94, 3.24, 5.39, 12.94, 5.22 and 12.52%, respectively. The overall $h^2$was estimated to be 42.4%.

DISCUSSION

This study sought to determine reversion from SPFMV in 50 progeny derived from a cross of 'New Kawogo' and

'Resisto' and test whether it was heritable, and to quantify the molecular diversity of the derived progeny.

It was found that varying levels of reversion from SPFMV among the progeny (Figure 1). Reversion is considered a form of resistance against virus infections in sweetpotato (Gibson and Krueze, 2014), and similar trends of resistance have been observed in 190 accessions of bottlegourd (*Lagenaria siceraria*) germplasm against *Zucchini yellow mosaic virus* (Ling and Levi, 2007) and in cassava half-sib progenies against *Cassava mosaic virus* (Musungayi et al., 2018). This variation in reversion (or resistance) to SPFMV may be attributed to genetic differences caused by introgression/crossing events of virus-targeting genes. Gallois et al. (2018) noted that introgression of genes from virus-resistant genotypes to susceptible genotypes – such as 'New Kawogo' and 'Resisto', respectively – led to greater genetic diversity, as observed in the present study for some progeny. As a result, resistance to SPFMV and potential introduction of defence genes varied among the progeny. There may have been an associated loss in susceptibility to viral infection (Gallois et al., 2018), particularly for progeny in which reversion was lower at week 4 than week 6. Variation in reversion may also be attributed to incomplete penetrance of resistance genes from 'New Kawogo'. Similar observations were made for resistance genes in studies of *Capsicum chinense* against *Tomato spotted wilt tospovirus* (Boiteux, 1995; Moury et al., 1997), the *RYMV3* gene in *Oryza glaberrima* against *Rice yellow mottle virus* (Pidon et al., 2017) and the *RPV1* gene in *Arabidopsis thaliana* accessions against *Plum pox virus* infection (Poque et al., 2015).

SSR markers derived from coding regions of virus defence genes were analysed, and it was found that nine of the 63 markers were polymorphic; there were 719 alleles with a mean of two per marker. These observations were similar to those reported by Chipungu et al. (2017) for Malawian sweetpotato, but contrasted to those reported by Gichuru et al. (2006), who found 2 to 5 alleles in 57 sweetpotato landraces from Kenya, Uganda and Tanzania, and by Kiarie et al. (2016), who found 18 alleles, with an average of three per marker. The low mean number of alleles per marker in this study may be attributed to genetic predisposition or the derivation of SSRs from different genomic regions in the various studies. Further, Chipungu et al. (2017) reported that genetic diversity is the most important limiting factor of average number of alleles per SSR marker during screening, and Legesse et al. (2007) suggested that factors, such as number of SSR marker and repeat types and methodologies used to detect polymorphic markers, influence allelic differences, as was observed in this study.

In the present study, the mean PIC was found to be 0.682, indicating that the SSRs effectively discriminated among the progeny. According to Botstein et al. (1980), PIC >0.5 indicates high diversity for the marker/SSR in use, and the mean PIC in this study was higher than values reported for sorghum (Geleta et al., 2006), cucumber (Danin-Poleg et al., 2001), potato (Ashkenazi et al., 2001), Kenyan sweetpotato (Karuri et al., 2010), East African sweetpotato (Ochieng et al., 2015), sweetpotato (Naidoo et al., 2016) and orange fleshed sweetpotato (Kiarie et al., 2016). Shannon's information index and Nei genetic distances indicated moderate genetic diversity among the progenies, and Chipungu et al. (2017) reported similar values in Malawian sweetpotato genotypes. Although moderate to low gene diversity values have been found in crops, such as mulberry (h = 0.20) (Zhao et al., 2006) and *Medicago citrina* (h = 0.15) (Juan et al., 2004) populations, very high h values have been observed in sweetpotato from different countries, including in Mesoamerica (h = 0.71), Venezuela–Colombia (h = 0.70) and Peru–Ecuador (h = 0.52). These studies utilised a wider range of genotypes (parents), hence the high levels of diversity (Zhang et al., 2000); however, contrary to the present study which used only two parents ('New Kawogo' and 'Resisto').

The analysis of SSRs formed five groups, with *I. setosa* forming a unique group (cluster 5) and four clusters contained sweetpotato progeny, outliers ('Tanzania' and 'Ejumula') and parents. More progeny (38) clustered with 'New Kawogo' (cluster 1) than with 'Resisto' (cluster 3), possibly as a result of gene flow or crossing, where 'New Kawogo' contributed more virus defence genes than 'Resisto', as evidenced by all progeny showing better reversion from SPFMV than 'Resisto'. Gene flow leading to resistance to weevils was observed by Rukarwa et al. (2013), and varying levels of gene flow led to development of resistant progeny in beans to common bacterial blight (Urrea et al., 1999) and in inheritance of downy mildew resistance in table grapes (Maurus et al., 1999). It was found that PCs 1 and 2 contributed 34.53 and 19.06% to the total variability within the progenies, respectively, where markers E and F were the main contributors. According to Yada et al. (2010) and Rodriguez-Bonilla et al. (2014), such markers are the most useful in detecting molecular variation within accessions.

The broad sense heritability (H^2) was 76.42% (with contribution of different genes markers ranging from 1.04 to 20.76%). These heritability observations were within the range of some AFLP markers observed by Mwanga et al. (2002) in a cross of 'Tanzania' and 'Wagabolige', where $H^2$of SPFMV resistance markers e36m59.a and e32m36.f was 9.21 and 8.90%, respectively, but was lower than the SPFMV AFLP marker e39m32.f and SPFMV RAPD marker S12.1130 that had H^2 of 41.02 and 205.83%, respectively. The $H^2$of the different markers in this study is low (Robinson et al., 1949), possibly due to the small contribution of many genes to a character/trait (Hill, 2010), such as reversion. For example, in maize, H^2<0.3% was observed for 50 markers associated with oil

content (Laurie et al., 2004). The low H^2 observed in this study supports the supposition that reversion is due to various genes that each contributes a specific action directed towards virus infection during RNA silencing (Gibson and Krueze, 2014). The h^2 of 44.84% recorded in this study is within the range of observations (31 to 41%) for Sweet Potato Virus Disease in sweetpotato (Mwanga et al., 2002). Thus, the observations indicate reversion is a heritable trait that is likely associated with multiple genes.

Conclusion

Reversion varied among the parents, progeny and outliers. There was also genetic variation among the studied material, indicating the SSR primers used may be used during breeding programmes aimed at developing or screening virus-resistant genotypes. The broad sense heritability was high with contributions of each gene being low, highlighting the importance of genes in reversion. Also the narrow sense heritability estimation indicated that reversion was a heritable character. Our findings may be used in agro-ecological trials assessing sweetpotato root quality in virus-prone areas to enable appropriate recommendations of progenies for use by sweetpotato farmers.

CONFLICT OF INTERESTS

The authors have no conflict of interests to declare.

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Table S1. Analysis of among, within and total population genetic variation in sweetpotato parents, progeny and outliers at nine loci.

Locus	Source of variation	df	SS	MS	Est. Var.	Variation (%)	F	P
	Among	2	0.007	0.004	0.000	0	0.240	0.934
Locus A	Within	52	1.920	0.037	0.037	100		
	Total	54	1.927		0.037	100		
	Among	2	4.927	2.464	0.368	33	0.329	0.035
Locus B	Within	52	39.000	0.750	0.750	67		
	Total	54	43.927		1.118	100		
	Among	2	0.416	0.208	0.000	0	0.075	0.561
Locus C	Within	52	16.020	0.308	0.308	100		
	Total	54	16.436		0.308	100		
	Among	2	4.429	2.215	0.418	61	0.611	0.002
Locus D	Within	52	13.880	0.267	0.267	39		
	Total	54	18.309		0.685	100		
	Among	2	3.362	1.681	0.336	74	0.744	0.001
Locus E	Within	52	6.020	0.116	0.116	26		
	Total	54	9.382		0.452	100		
	Among	2	2.909	1.455	0.271	59	0.585	0.017
Locus F	Within	52	10.000	0.192	0.192	41		
	Total	54	12.909		0.463	100		
	Among	2	3.770	1.885	0.295	37	0.365	0.052
Locus G	Within	52	26.667	0.513	0.513	63		
	Total	54	30.436		0.808	100		
	Among	2	4.313	2.156	0.427	72	0.718	0.003
Locus H	Within	52	8.725	0.168	0.168	28		
	Total	54	13.038		0.595	100		
	Among	2	4.341	2.170	0.357	41	0.413	0.020
Locus I	Within	52	26.387	0.507	0.507	59		
	Total	54	30.727		0.865	100		