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Morphological and genetic characterization of sweetpotato F1 hybrids from *Sweet potato feathery mottle virus* infection-reverting and non-reverting genotypes in Uganda

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Abstract

Sweetpotato variably reverts from *Sweet potato feathery mottle virus* infections. In a particular study, crosses between sweetpotato genotypes with varying reversion potential yielded segregating F1 hybrids for the reversion trait. However, information on morphological and genetic diversity of these hybrids is lacking, yet this is important in breeding for reversion. Using standardized sweetpotato descriptors, a non-reverting parent (Resisto), reverting parents (New Kawogo, NASPOT 11 and NASPOT 1), hybrids from crosses (New Kawogo x Resisto, NASPOT 11 x Resisto and NASPOT 1 x Resisto), and random cultivars Ejumula and Tanzania were morphologically characterized. Subsequently, simple sequence repeats (SSRs) were used to genetically characterize parents, hybrids, cultivars Ejumula and Tanzania, and a wild highly virus-susceptible sweetpotato relative *Ipomoea setosa*. There was significant morphological diversity between hybrids, where analysis grouped the 121 hybrids into six clusters. The first five principal components contributed to 70.2% of total phenotypic variation.

Molecular variation was significant within and between parents, hybrids and cultivars Ejumula and Tanzania. Number of alleles for SSRs were 2 to 4, Shannon's information index was 0.325 to 1.061, diversity index was 0.180 to 0.642 and Nei's pairwise genetic distance was 0.2 to 0.8 indicating a high diversity between genotypes. Molecular analysis grouped hybrids into four clusters. The first four principal components contributed to 90.16% of total molecular variation. Number of leaf lobes, leaf lobe type, and primers IBS82, IBR12 and IBS166 were important in determining diversity within hybrids and these are recommended for identification of genotypes with reversion potential.

Key words: Diversity, reversion, SSR markers, sweetpotato descriptors

Introduction

Sweetpotato (*Ipomoea batatas* [L.] Lam) is an important food crop in much of the sub-Saharan region but its production is greatly constrained by virus infections, especially the crinivirus [*Sweet potato chlorotic stunt virus* (SPCSV)] and a potyvirus [*Sweet potato feathery mottle virus* (SPFMV)] (Ssamula *et al*., 2020). Over 95% storage root yield loss has been recorded in combined infections of SPCSV plus SPFMV. On the other hand, single infections can also lead to up to 50% storage root yield loss (Kiemo *et al.*, 2022).

Breeding for resistance is the best sweetpotato virus management option. Resistance to virus infections in crops is expressed as a reduction in symptom severity, recovery from symptoms, or 'dark-green-islands' – all marked with significant reduction in virus titer (Moore *et al*., 2001). Resistance can also be expressed as reversion, apparently the extreme natural phenomenon of recovery and 'dark-green-islands', in which plants completely heal from virus infections (Ssamula *et al*., 2020). Recovery, 'dark-green-islands' and reversion are as a result of gene silencing mechanism in the infected plant. During gene silencing, double stranded (ds) RNA of the replicating virus or from the secondary structure of the virus leads to the production of small interfering (si) RNAs through the action of RNAse III enzyme dicer to mediate the destruction of any mRNAs that are perceived aberrant by the plant (Moore *et al*., 2001).

The reversion phenomenon in sweetpotato is evidenced both indirectly and directly by previous researchers. For instance, despite the wide occurrence of sweetpotato viruses in East Africa, random surveys over the years have continued to show a big proportion of symptomless virus-free field plants (Njeru *et al*., 2008; Wasswa *et al*., 2011). Similarly, landraces such as Bitambi, Kyebandura and Kalebe of the 1960's in Uganda were still commonly grown by farmers throughout the 1990's due to their competitive yield (Bashaasha *et al*., 1995), which was probably because of reversion. Likewise, NASPOT 1 has maintained a high yield and has been maintained

by farmers since 1991 to date, and by 2003 it was the most popular variety in the Kagera region (Mwanga *et al*., 2003). Subsequently, experimental evidence showed reversion from potyviruses [SPFMV (Wasswa *et al*., 2011; Ssamula *et al*., 2020); *Sweet potato virus C* (Ssamula *et al*., 2020)]; and a begomovirus [*Sweet potato leaf curl virus* (Wasswa *et al*., 2011)] in cultivars such as New Kawogo, NASPOT 1, NASPOT 11, Tanzania and Dimbuka.

In the study done by Ssamula *et al*. (2019), crosses between the SPFMV-infection non-reverting and reverting cultivars resulted into first filial (F1) hybrids with varying potential to revert from SPFMV infections. Despite this effort, no work has been done on the morphological and molecular characteristics of such derived hybrids, yet this could help in the management of sweetpotato virus diseases through breeding for reverting sweetpotato genotypes. Leaf morphology is the first line of defense against viruses as it creates a physical and structural barrier against virus vectors. Key morphological barriers include waxy cuticle, epidermal thickness, trichomes, and stomatal size and density. Stomatal density can be determined by the leaf shape. Beyond the surface features, internal spatial organization such as vein density partly determined by the leaf shape, plays a crucial role in virus resistance. Dense venation provides an easier path for viruses for systemic spread (Palumbo *et al*., 2019).

Morphological attributes are crucial in differentiating sweetpotato varieties for example NASPOT 12 O and NASPOT 13 O with triangular and lanceolate shape of central lobe, respectively (Mwanga *et al*., 2016). Morphological diversity, however, measures the genotype by environment variation, and at times does not adequately reflect the segregation of F_1 hybrids. Despite this, a high morphological diversity among individuals can lead to a wide range of alleles increasing the potential expression of invisible traits such as reversion and other epistatic and pleiotropic interactions (Palumbo *et al*., 2019). Molecular diversity gives a more precise indication of genomic variation between sweetpotato genotypes, and this can provide information on the genomic regions (genes) that control various traits. Therefore, understanding diversity at molecular level for F_1 hybrids gives a more actual realization of variation that is heritable within a species population. Thus, combining phenotypic traits analysis with the genetic characterization in sweetpotato germplasm gives a better understanding of genetic diversity as influenced by the environment (Palumbo *et al*., 2019). This enables the selection of superior genotypes and optimization of breeding strategies, allowing breeding for the invisible traits such as reversion.

Molecular (genetic) markers have shown important and critical application in the assessment and conservation of genetic variation, as well as the assessment of genetic diversity among germplasm. Simple sequence repeats (SSRs) in particular have been widely used for studying genetic diversity in sweetpotato populations (Yada *et al.,*

2015). In this study, we provide a morphological and molecular diversity report on sweetpotato F1 hybrids with varying SPFMV infection-reversion potentials. This would provide an understanding of those characters that would be important in breeding for reversion trait for the management of sweetpotato virus diseases.

Materials and methods

Plant materials

In 2019, Ssamula *et al*. (2019) performed a full diallel cross for parental accessions Resisto, New Kawogo, NASPOT 11 and NASPOT 1. These cultivars are reported to variably revert from SPFMV infections; NASPOT 11 is highly reverting, NASPOT 1 and New Kawogo are moderately reverting while Resisto does not revert. The diallel cross of the parents generated 121 F1 hybrids. All the parents, F1 hybrids, other two random sweetpotato cultivars Tanzania (tolerant to SPFMV infections) and Ejumula (susceptible to SPFMV infections), and a sweetpotato wild relative *Ipomoea setosa* (highly susceptible to SPFMV infections) were used in this study for morphological and molecular evaluations. Five vines were obtained from each cultivar/hybrid and individually planted in 1-litre capacity buckets. For *I. setosa*, five pre-germinated seeds were planted in 1-litre capacity buckets, each bucket carrying a seed. Plants were grown in a screen house for 8 weeks in sterilized potting mixture containing loam soil, lake sand and cow manure in a 3:1:1 ratio, and watered daily. The insecticide Imidacloprid, was applied weekly at a rate of 30ml/20L of water to control any aphid and whitefly vectors.

Morphological diversity evaluation

Morphological diversity evaluation was done on the parental accessions Resisto, New Kawogo, NASPOT 11 and NASPOT 1, the 121 F1 hybrids consisting of 50 hybrids from New Kawogo x Resisto (NKxRe) cross; 53 from NASPOT 1 x Resisto (N1xRe) cross and 18 from NASPOT 11 x Resisto (N11xRe) cross, and two random cultivars Tanzania and Ejumula. Morphological evaluation was done using the 16 descriptors on a scale of 0-9 developed by the International Potato Center (CIP) (CIP *et al*., 1991). The 16 parameters included shape of central leaf lobe, number of leaf lobes, size of mature leaves, general outline of the leaf, pigmentation of abaxial veins, twining ability, colour of immature leaves, colour of mature leaves, diameter of vines, length of vine internode, vine tip hairiness, predominant colour of vine, type of leaf lobe, length of petiole, pigmentation of petiole, and secondary colour of vine.

Molecular diversity evaluation

The parents (Resisto, NASPOT 11, New Kawogo and NASPOT 1), hybrids of crosses NKxRe, N1xRe and N11xRe, two random cultivars Tanzania and Ejumula, and *I. setosa* were evaluated for molecular diversity. For the hybrids, a total of 30

F1 hybrids (10 randomly selected hybrids from each of the 50 NKxRe hybrids, 53 N1xRe hybrids and 18 N11xRe hybrids) were used for molecular diversity evaluation. This subset (30 F1s) was used because genotyping all 121 hybrids was not feasible with available resources (limited consumables and PCR reagents), and hybrids were therefore sampled as 10 plants per cross (stratified random selection) to ensure balanced representation of each F1 population of the three hybrid groups. Within each cross, hybrids were assigned unique identification numbers (IDs) and 10 individuals were selected using a random-number method without replacement.

DNA extraction

A leaf sample was collected from the five plants/vines of each of the 4 parents, 30 hybrids, the two sweetpotato cultivars Tanzania and Ejumula, and *I. setosa* grown in the screen house. Five leaves from the five vines of each plant made a composite sample. Genomic DNA from the leaf samples was extracted using acetyl trimethyl ammonium bromide method (Maruthi *et al*., 2002). A NanoDrop-ND-1000 spectrophotometer (Thermo Scientific, Image Care - Kampala, Uganda) was used to determine the quantity of DNA. All samples were then diluted, using high-purity laboratory grade water, to a concentration of 10 ng/µl before downstream DNA amplification.

DNA amplification

The total PCR reaction mix of 10µl consisted of 5 µl of PCR mix (HyLabs Ready Mix (×2), Image Care - Kampala, Uganda), 3 µl of high-purity grade water, 0.5 µl of each primer (10 pmol) and 1 µl DNA (10ng/µl). The six primer sets used in this study for PCR amplification of the DNA template were adopted from studies by Karuri *et al.* (2010); Koussao *et al.* (2014) and Yada *et al.* (2015), where they were used during the diversity assessment of sweetpotato genotypes. These markers were selected due to their high polymorphism and co-dominance showed in these reference studies. The six SSR primers included IBU5F (5'-TTT CAA GGA TGT GGC TAA TG-3') and IBU5R (5'-CCT GCT AAT ATA ACC CAA CCT C-5'); IBY53F (5'-CCA CGA TCT CGG AAA CCG CCA T-3') and IBY53R (5'-GGG GCA AAA GGT CTT ATT CAT AT-3'); IBS82F (5'- GAC ATA ATT TGT GGG TTT AGG G-3') and IBS82R (5'- GAA ATG GCA GAA TGA GTA AGG G-3'); IBU4F (5'-GGC TGG ATT CTT CAT ATT TAG C-3') and IBU4R (5'-GCT TAA TGG ATC AGT AAC ACG A-3'); IBS166F (5'-TCC GTC TTT CTT CTT CTT CTT C-3') and IBS166R (5'-ATA CAC TAA CTG CAT CCA AAC G-3'); and IBR12F (5'-GAT CGA GGA GAA GCT CCA CA-3') and IBR12R (5'-GCC GGC AAA TTA AGT CCA TC-3'). The DNA was denatured at 94°C for 4 minutes; followed by 30 cycles of 94 °C (denaturation) for 30 seconds, 50 °C (annealing) for 45 seconds and 72 °C (extension) for 30 seconds. The reaction was ended with a

final extension step at 72°C for 10 minutes. The reaction was then held at 4°C for another 10 minutes.

Gel electrophoresis

Amplicons were separated on a 1.5% agarose gel in 0.5% Tris ethylene diamine tetra acetic acid buffer, stained with ethidium bromide. The gel was run at voltage of 90 for 30 minutes and products viewed under ultraviolet light in an OmniDoc gel documentation system (Clever Scientific, Image Care - Uganda).

Data analysis for morphological diversity evaluation

The average value of a given descriptor from the five vines was computed and used for further analysis. The morphological variation indices were analyzed using XLSTAT software version 18.0 (Addinsoft, 2018) and Genetic Analysis in Excel (GenAlEx) software (Peakall and Smouse, 2006). The percentage distribution of hybrids belonging to specific descriptors were established. Pearson's correlation and algometric hierarchical cluster analysis were done. Cluster analysis involved grouping study populations using a dendrogram based on Euclidean dissimilarity (Euclidean distance) computed from the 16 CIP morphological descriptor scores (0-9) and the unweighted pair group method with arithmetic mean (UPGMA) to visualize patterns of phenotypic variation among accessions. Euclidean genetic distances method was used for its ability to measure the shortest distance between points, and this is important in morphometric analysis and for understanding the spatial arrangement and configuration of biological specimens. Euclidean distance provides an intuitive measure of overall multivariate phenotypic dissimilarity, and it is the default and widely accepted metric for agglomerative hierarchical clustering of quantitative/ordinal score matrices in XLSTAT using UPGMA. Therefore, clusters were defined using XLSTAT's optimal classification from the UPGMA dendrogram, supported by the variance decomposition output (within-class *versus* between-class variation). Principal component analysis (PCA) was done to determine the most important variability factors. PCA components were retained for interpretation based on XLSTAT outputs using variance explained and variable representation quality i.e., squared cosines (cos^2). Components where multiple variables had high and maximal cos^2 (typically ≥ 0.25) were emphasized. No interpretation was done for components with consistently low cos^2.

Data analysis for molecular diversity evaluation

SSR profiles were scored from agarose gels based on band presence at distinct positions (fragment-size classes). Each distinct band position was treated as an allele and recorded as 1 (present) or 0 (absent) for each genotype. Because allele calling was gel-based (no precise base-pair sizing), the total number of bands per well were additionally counted to estimate the number of alleles per locus used in downstream diversity analyses. Only clearly resolved bands were scored; faint, smeared, or

ambiguous bands were excluded (treated as missing) to minimize scoring error. Molecular diversity analyses were performed using XLSTAT and GenAlEx statistical software. Analysis involved analysis of molecular variance (AMOVA) to quantify molecular variation. Shannon's information index (I), diversity index (H) (Shannon, 1948)], Nei's genetic identity or distance (Nei, 1978), agglomerative hierarchical clustering (AHC) and PCA were performed to analyze genetic diversity and the most variability factors. The relative importance of the SSR markers in distinguishing the genotypes was determined from Nei's diversity index, $H = 1 - \Sigma Pi^2$, where Pi is the frequency of the i^{th} allele at a given locus.

Results

Morphological diversity

Character distribution among the F1 hybrids

Highly significant ($P<0.001$) morphological variation in general leaf outline, leaf lobe number, central lobe shape, abaxial leaf vein pigmentation, petiole color, and vine predominant colour was observed among the F1 hybrids. Significant ($P<0.05$) variation in leaf lobe type was also observed between the hybrids. Considering F1 hybrids of the N1xRe cross and looking at the highest and lowest percentage values, the leaf outline varied among hybrids (Fig. 1a, b and c), with 50.9% being triangular and 7.5% divided (Fig. 1, more information in supplementary files). Close to half (49.1%) of the hybrids had a single toothed central lobe while none had an elliptic central lobe. N1xRe hybrids also exhibited different leaf lobe types where 49.1% had very slight leaf lobes while 5.7% had deeply lobed leaves. 92.5% of the hybrids had green abaxial veins while less than 2% had purple abaxial veins. With petiole colour, 86.8% were green. Majority of the hybrids (94.3%) had a green predominant vine colour with no secondary colour.

Considering NKxRe cross and still looking at the highest and lowest percentage values, 52% of the hybrids had a triangular leaf outline, whereas only 2% were hastate (trilobular and spear-shaped) (Fig. 1, more information in supplementary files). These hybrids mostly had very slight leaf lobe type (Fig. 1d and e), where 48% of the hybrids had very slight teeth and with only 2% of these being deeply lobbed. The abaxial veins were partially purple (32%), whereas 2% of the leaves had their main rib mostly or totally purple. With petiole colour, 38% were green with purple near leaf. Many of the hybrids (46.0%) had a green predominant vine colour with purple nodes.

For the N11xRe hybrids, 94.4% had triangular leaves (Fig. 1f; more information in supplementary files), whereas only 5.6% had hastate leaf outline. 88.9% of the hybrids

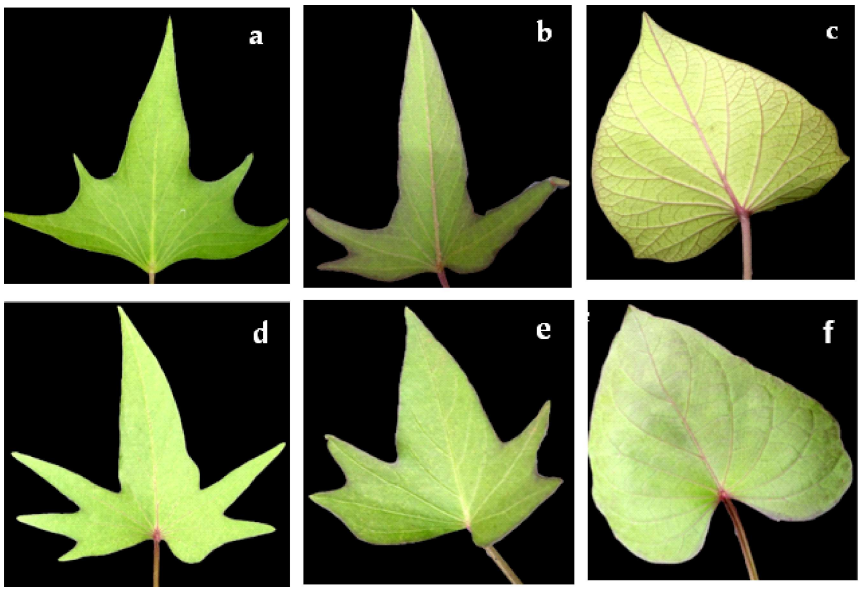

Figure 1. Sample sweetpotato leaves showing varying leaf outlines and venation colouration of the different sweetpotato hybrids: (a-c) represent NASPOT 1 × Resisto hybrids where, (a) has slightly lobbed and green leaves, (b) has slightly lobbed and purple leaves and (c) has triangular and purple leaves; (d and e) represent New Kawogo × Resisto hybrids where, (d) has a green leaf margin and (e) has a purple leaf margin; (f) represents NASPOT 11 × Resisto hybrids with triangular general leaf outline and purple leaf margin.

had single very slight leaf lobes while 11.1% had averagely three slight leaf lobes. 94.4% of the hybrids had a toothed central lobe while only 5.6% were triangular. The abaxial veins were either green (33.3%) or mostly purple (33.3%). With petiole colour, 33.3% were green. Many of the hybrids (72.2%) had a green predominant vine colour.

Algometric hierarchical cluster analysis

The Euclidean dissimilarity coefficient based on the UPGMA method grouped the parents, F1 hybrids and cultivars Tanzania and Ejumula into six clusters (Fig. 2). The distance between class centroids ranged from 6.901 to 14.819. Variation within classes was 53.36% and between classes was 46.64%. Cluster II was the largest with 63 plants, and consisted the non-reverting parent Resisto, 28 N1xRe hybrids, 25 NKxRe hybrids, and nine N11xRe hybrids. Cluster I was the second largest with 39 plants, and constituted cultivars Ejumula and Tanzania, reverting parents NASPOT 1 and New Kawogo, 19 NKxRe hybrids, 15 N1xRe hybrids, and only one N11xRe hybrid. Cluster III was the third largest with 14 plants, and these consisted of highly reverting parent NASPOT 11, eight N11xRe hybrids, three N1xRe hybrids, and two NKxRe hybrids. Cluster IV constituted only hybrids (9 plants) including six

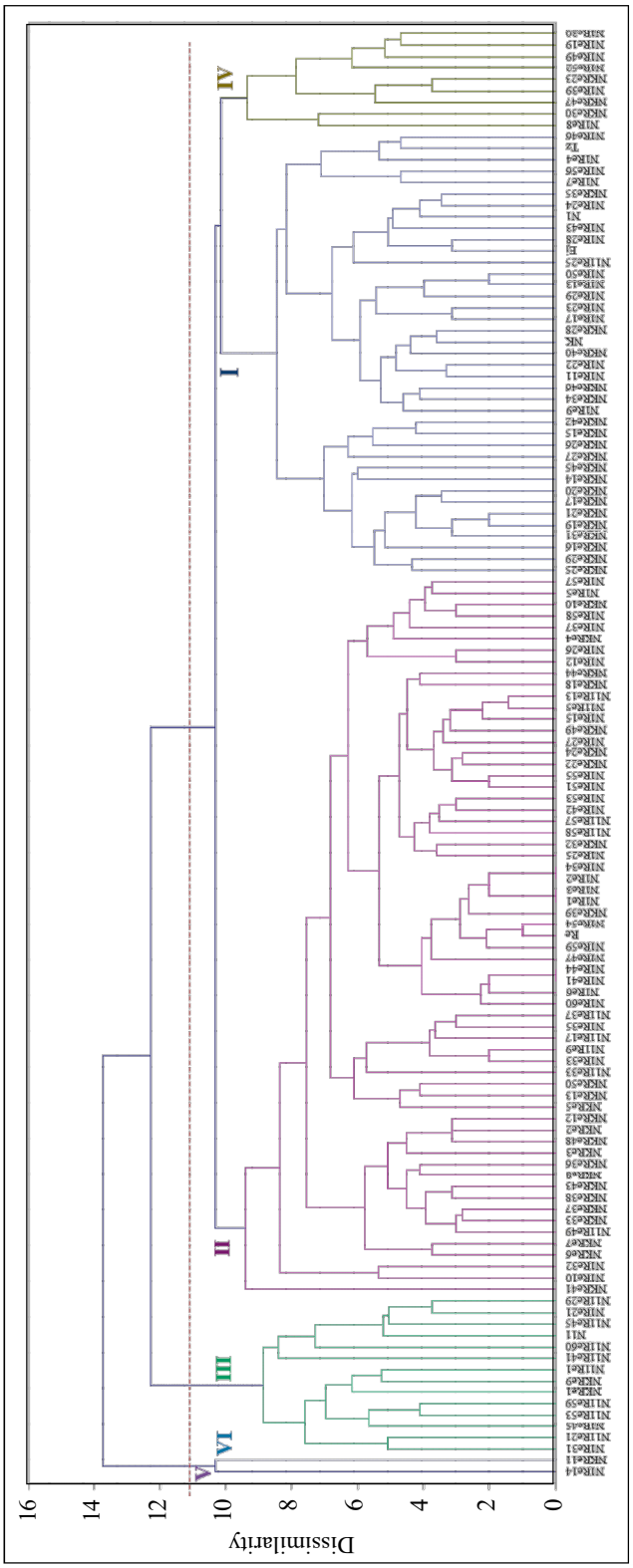

Figure 2. Multivariate clustering of the sweetpotato parents, hybrids and the random sweetpotato cultivars Tanzania and Ejumula using the 16 phenotypic traits based on Euclidean distance by UPGMA method. The method grouped the parents, hybrids and cultivars Tanzania and Ejumula into six clusters; I, II, III, IV, V and VI. N1Re represents hybrids from NASPOT 1 x Resisto, NKRe represents hybrids from New Kawogo x Resisto, and N11Re represents hybrids from NASPOT 11 x Resisto.

N1xRe hybrids and three NKxRe hybrids. The 14 hybrids of N1xRe cross and 11 hybrids of NKxRe cross constituted their own clusters V and VI, respectively (Fig. 2).

Principal component analysis for phenotypic traits

The first five principal components (PC) contributed to 70.2% of the cumulative variability, with Eigen value of 1.19, indicating the lowest relative discriminating power. On the other side, PC1 contributed to 25.32% of the cumulative variability, and had Eigen value of 4.051, thus showing the highest relative discriminating power. Principal component 2 had the second highest relative discriminating power (2.845) at variability of 17.78% (Table 1). The factor loading scores show that PC1 had the highest importance for distinction since it was differentiated by highest number of variables including type of leaf lobe and number of leaf lobes. These traits therefore had the highest discriminating power.

Table 1. Eigen values of the first seven principal components showing the relative importance of the 16 morphological markers in determining variability within the sweetpotato parents, F1 hybrids and random sweetpotato cultivars (Tanzania and Ejumula)

Variable	Principal components						
	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Twining ability	0.054	0.007	0.118	**0.366**	0.041	0.003	0.211
General leaf outline	**0.778**	0.092	0.003	0.005	0.020	0.001	0.005
Leaf lobe type	**0.825**	0.099	0.004	0.005	0.003	0.002	0.001
Number of leaf lobes	**0.833**	0.089	0.001	0.015	0.008	0.000	0.000
Central lobe shape	**0.768**	0.073	0.020	0.006	0.012	0.003	0.000
Mature leaf size	0.026	0.088	0.113	0.010	**0.326**	0.066	0.121
Abaxial leaf vein pigmentation	0.053	**0.573**	0.083	0.051	0.039	0.030	0.001
Mature leaf colour	0.030	0.073	0.017	**0.314**	0.205	0.038	0.069
Immature leaf colour	0.023	0.174	0.129	**0.259**	0.065	0.097	0.008
Petiole pigmentation	0.086	**0.653**	0.125	0.008	0.001	0.001	0.002
Length of petiole	0.117	0.049	**0.292**	0.080	0.099	0.012	0.062
Diameter of vine	0.001	0.073	**0.321**	0.121	0.123	0.001	0.001
Length of vine internode	0.133	0.000	**0.193**	0.178	0.181	0.002	0.008
Vine predominant colour	0.148	**0.417**	0.167	0.002	0.002	0.037	0.003
Vine secondary colour	0.069	0.295	0.000	0.014	0.000	**0.456**	0.006
Hairiness	0.105	0.087	0.123	0.000	0.070	0.163	**0.315**
Eigen value	4.051	2.845	1.712	1.433	1.194	0.909	0.813
Variability (%)	25.317	17.781	10.698	8.953	7.463	5.681	5.081
Cumulative %	25.317	43.099	53.796	62.750	70.213	75.894	80.975

Values in bold indicate variables with largest squared cosine for a given principal component

Molecular characterization

AMOVA to quantify genetic variation

SSR markers IBS82 (P = 0.013), IBR12 (P = 0.007) and IBS166 (P = 0.003) revealed significant variation between and within populations of the parents, F1 hybrids, random cultivars (Tanzania and Ejumula) and *I. setosa*. In particular, the following percentage variations were observed; 32% (IBR12), 28% (IBS82) and 34% (IBS166) between populations and 68% (IBR12), 72% (IBS82) and 66% (IBS166) variation within populations (Table 2). The overall variation within populations (76%) was higher than the variation between populations (24%).

Genetic diversity analysis and genetic distance

The genetic diversity associated with F1 hybrids varied across the different SSR markers (Table 3). Overall, a total of 43 polymorphic bands were generated from 121 F1 hybrid population. Four polymorphic bands from the F1 hybrids of NASPOT 11 x Resisto cross were produced using the IBS82 marker locus, showing the highest polymorphism. This same marker locus produced low polymorphism for the other F1 hybrids from crosses New Kawogo x Resisto and NASPOT 1 x Resisto. The lowest polymorphism (only 2 polymorphic bands) was generated for all SSR markers for some crosses except marker loci IBS166 which produced three polymorphic bands for all the crosses (New Kawogo x Resisto, NASPOT 1 x Resisto and NASPOT 11 x Resisto). On average, each marker locus generated 2.39 alleles. The lowest number of effective alleles was 1.220 for marker loci IBU5 for New Kawogo x Resisto hybrids and IBY53 for New Kawogo x Resisto hybrids while the highest was 2.793 for IBS166 SSR marker for NASPOT 11 x Resisto hybrids, giving an average of 1.738 ± 0.109. The lowest Shannon's information index was 0.325 for SSR markers IBU5 for New Kawogo x Resisto hybrids and IBY53 for New Kawogo x Resisto hybrids while the highest was 1.061 for marker locus IBS166 for NASPOT 11 x Resisto hybrids, with an average of 0.634. The lowest diversity index was 0.180 for SSR markers IBU5 for New Kawogo x Resisto hybrids and IBY53 for New Kawogo x Resisto hybrids while the highest was 0.642 for SSR marker IBS166 for NASPOT 11 x Resisto hybrids, with an average of 0.380. The lowest unbiased diversity index was 0.200 for SSR markers IBU5 for New Kawogo x Resisto hybrids and IBY53 for New Kawogo x Resisto hybrids while the highest was 0.722 for SSR marker IBS166 for NASPOT 11 x Resisto hybrids, giving an average of 0.435.

Principal component analysis for molecular markers

The scree plot showed that PC5 and PC6 had no significant contribution in explaining variability from any SSR marker, and these components had Eigen values of less than 0.5. Only the first four principal components played a significant role in explaining variability (Fig. 3a) The first four components contributed to 90.16% of the total

Table 2. AMOVA between and within populations of reverting and non-reverting sweetpotato parents, F1 hybrids, random sweetpotato cultivars and *I. setosa* evaluated using SSR markers

Marker	Source of variation	Df	SS	MS	Est. Var.	% Var	F-Stat	F-Prob
IBU4	Between populations	4	1.677	0.419	0.008	2	0.022	ns
	Within populations	32	11.567	0.361	0.361	98		
	Total	36	13.243		0.370	100		
IBU5	Between populations	4	0.744	0.186	0.000	0	-0.061	ns
	Within populations	32	10.067	0.315	0.315	100		
	Total	36	10.811		0.315	100		
IBS82	Between populations	4	7.154	1.788	0.187	28	0.283	0.013**
	Within populations	32	15.117	0.472	0.472	72		
	Total	36	22.270		0.659	100		
IBR12	Between populations	4	7.908	1.977	0.216	32	0.324	0.007***
	Within populations	32	14.417	0.451	0.451	68		
	Total	36	22.324		0.667	100		
IBY53	Between populations	4	2.160	0.540	0.037	12	0.119	0.174^{ns}
	Within populations	32	8.867	0.277	0.277	88		
	Total	36	11.027		0.314	100		
IBS166	Between populations	4	15.873	3.968	0.442	34	0.341	0.003***
	Within populations	32	27.317	0.854	0.854	66		
	Total	36	43.189		1.295	100		

Df = degrees of freedom, SS = sum of squares, MS = mean squares, Est. Var. = estimated variance, % Var = percentage variance, F-Stat = F statistical value, F-Prob = F probability value, *** = significance at P<0.001, ** = significance at P<0.01 and ns = non-significant

variability, with Eigen values greater than 0.6. The relative discriminating power was highest for PC1 (2.983) at 49.72% followed by PC2 (1.043) at 17.38%; and these accounted for 67.10% of the total variability (Table 4 and Fig. 3b). The factor loading scores also showed that PC1 had the highest importance for distinction since it could be differentiated by four markers; IBR12 (0.636), IBU5 (0.620), IBS166 (0.513), and IBY53 (0.488). Marker IBU4 (0.539) and IBS82 (0.442) largely contributed to the second and fourth components, respectively. The third, fifth and sixth principal components were not important for distinction since they could not be differentiated by all the markers (Table 4).

Algometric hierarchical cluster analysis

The Euclidean dissimilarity coefficient based on UPGMA method grouped the sweetpotato parents, F1hybrids, random sweetpotato cultivars Tanzania and Ejumula, and *I. setosa* into four clusters (Fig. 4). Variation within the classes was 51.13% and

Table 3. Genetic diversity across the different SSR marker loci within the sweetpotato F1 hybrids from crosses of sweetpotato genotypes with varying SPFM-infection reversion potential

SSR locus	F1 hybrid	Genetic diversity parameters				
		No. of alleles	No. of effective alleles	Shannon's information index	Diversity index	Unbiased diversity index
IBR12	NK × Re	3	2.632	1.030	0.620	0.689
	N1 × Re	2	2.000	0.693	0.500	0.556
	N11 × Re	2	1.528	0.530	0.346	0.389
IBS166	NK × Re	3	2.174	0.898	0.540	0.600
	N1 × Re	3	1.588	0.684	0.370	0.417
	N11 × Re	3	2.793	1.061	0.642	0.722
IBS82	NK × Re	2	1.724	0.611	0.420	0.467
	N1 × Re	2	1.471	0.500	0.320	0.356
	N11 × Re	4	2.077	1.003	0.519	0.583
IBU4	NK × Re	2	1.923	0.673	0.480	0.533
	N1 × Re	2	1.528	0.530	0.346	0.389
	N11 × Re	2	1.246	0.349	0.198	0.222
IBU5	NK × Re	2	1.220	0.325	0.180	0.200
	N1 × Re	2	1.528	0.530	0.346	0.389
	N11 × Re	2	1.246	0.349	0.198	0.222
IBY53	NK × Re	2	1.220	0.325	0.180	0.200
	N1 × Re	3	1.852	0.802	0.460	0.511
	N11 × Re	2	1.528	0.530	0.346	0.389
Mean		2.389	1.738	0.634	0.389	0.435
Standard Error		0.143	0.109	0.057	0.034	0.038

NK x Re = New Kawogo x Resisto cross, N1 x Re = NASPOT 1 x Resisto cross, N11 x Re = NASPOT 11 x Resisto cross

between classes was 48.87%. The distance between class centroids ranged from 1.852 to 5.648. Cluster II was the largest with 20 plants, and consisted of the sweetpotato cultivars Ejumula and Tanzania, *I. setosa*, three NKxRe F1 hybrids, six N11xRe F1 hybrids, and seven N1xRe F1 hybrids. Cluster I was the second largest with 15 plants and constituted all the four parents, seven NKxRe F1 hybrids, three N11xRe F1 hybrids, and only one N1xRe F1 hybrid. Cluster III and Cluster IV constituted only one F1 hybrid each from N1xRe cross and N11xRe cross, respectively (Fig. 4).

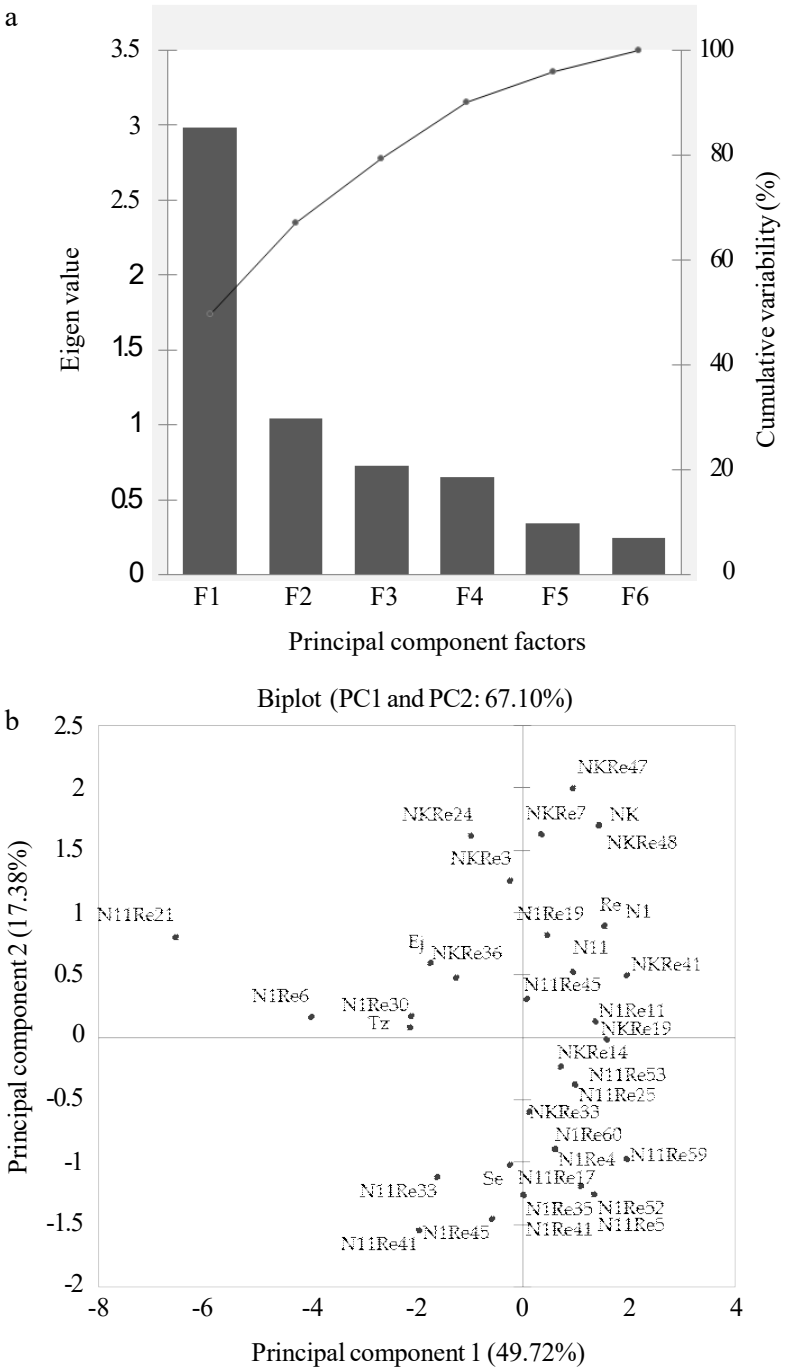

Figure 3. (a) Scree plot indicating relative contribution of each principal component in explaining variability. Principal components one and two showed the highest contribution to variability (b) Biplot of the first two principal component scores showing the 37 plants consisting of sweetpotato parents, F1 hybrids, random sweetpotato cultivars Tanzania and Ejumula and *I. setosa*.

Table 4. Squared cosines and Eigen values of the six principal components showing the relative importance of the six SSR markers in determining the total variability within the sweetpotato parents, F1 hybrids, random sweetpotato cultivars (Tanzania and Ejumula) and *I. setosa*

Marker	Principal components					
	PC1	PC2	PC3	PC4	PC5	PC6
IBU4	0.294	**0.539**	0.080	0.027	0.010	0.050
IBU5	**0.620**	0.005	0.274	0.000	0.000	0.100
IBR12	**0.636**	0.085	0.066	0.000	0.212	0.001
IBY53	**0.488**	0.040	0.227	0.178	0.049	0.018
IBS82	0.432	0.055	0.053	**0.442**	0.018	0.000
IBS166	**0.513**	0.319	0.030	0.007	0.057	0.075
Eigen value	2.983	1.043	0.731	0.653	0.346	0.244
Variability (%)	49.717	17.382	12.175	10.890	5.772	4.063
Cumulative %	49.717	67.099	79.274	90.164	95.937	100.000

The bolded values are the marker's principal component factor for which the squared cosine is the largest

Discussion

This study, for the very first time, characterized the morphological and molecular diversity of F1 hybrids derived from crossing of sweetpotato genotypes with varying reversion potential. Sweetpotato is a hexaploidy crop with a complex genome and this poses challenges on diversity interpretation (Gao *et al*., 2020). SSR are highly polymorphic and codominant with a high discriminatory power between genotypes. Besides, phenotypic analysis scoring models greatly enhance SSR marker analysis and provides a robust approach to diversity evaluations, thus overcoming the challenges of heterogeneous hexaploidy sweetpotato (Gao *et al*., 2020). According to Yada *et al*. (2010), analysis of morphological and molecular diversity is key to plant breeders since it reveals important traits for crop improvement. In the current study, sweetpotato phenotypic characters showed high variability within F1 hybrids, where PC1 and PC2 cumulatively accounted for 43.10% of the total variability (Table 1). This observation is similar to the one recorded in a study by Koussao *et al*. (2014) with 41.16%. This is however lower than the percentage value of 60% observed in a study by Yada *et al*. (2010), but higher than 28.37% observed in South African sweetpotato by Laurie *et al*. (2013). This variability in observations confirms that sweetpotato is highly segregating (Xiao *et al*., 2023) and values obtained with one cross vary widely from values obtained with another cross. Indeed, the first

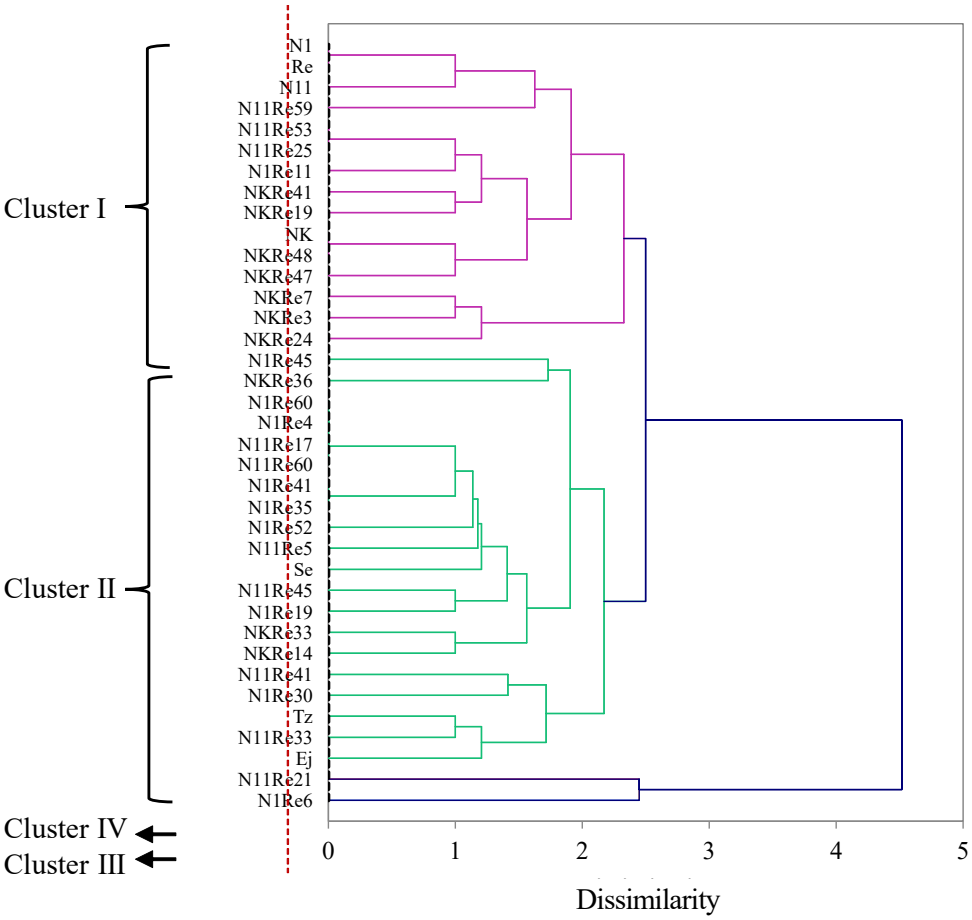

Figure 4. Multivariate clustering of sweetpotato parents, F1 hybrids, random sweetpotato cultivars Tanzania and Ejumula, and *I. setosa* using SSR markers.

five principal components accounted for up to 70.2% of total morphological variability. This is an indicator for high variability, thus high diversity among F1 hybrids.

The extent of morphological variability within species is important in achieving breeding objectives through selection (Mwanga *et al*., 2016; Palumbo *et al*., 2019). PCA results consistently indicated that leaf-shape descriptors had the highest discriminating power among the evaluated genotypes. In particular, type of leaf lobe and number of leaf lobes were the most important traits for differentiating F1 hybrids, with additional separation supported by general leaf outline and central lobe shape. These phenotypic traits with high discriminating power reflect the high diversity between the tested reverting and non-reverting sweetpotato genotypes (and their hybrids), and thus show indirect implications for the potential inheritance of the invisible traits such as reversion (Palumbo *et al*., 2019). These morphological descriptors therefore provide practical

phenotypic markers for rapid differentiation of sweetpotato genotypes with reversion potential. Leaf shape can influence virus resistance indirectly in a number of ways. For instance, different leaf shapes can variably affect the shading intensity and moisture retention by leaves thereby influencing the microenvironment and thus, virus vector activities. Leaf morphology can also influence the venation density thereby affecting virus local movement within the leaf and subsequent systemic spread through vascular tissues (Palumbo *et al*., 2019).

The F1 hybrids were grouped into six clusters (Fig. 2). The lowest distance for cluster centroids was 6.901 and the highest was 14.819, giving a high range between cluster centroids, which further implies high variability. Also, the variation between clusters was 46.64% and within the clusters was 53.36%, further indicating a high level of diversity among hybrids within each cluster (Xiao *et al*., 2023), thus suggesting that the reversion trait is highly heritable. The high morphological variation may be attributed to the fact that these traits in sweetpotato are quantitative and controlled by multiple genes, which are significantly influenced by environmental effects (Xiao *et al*., 2023). As such, results from the screen house may not fully reflect trait expression under diverse field conditions. Therefore, field validation of the key discriminating traits across multiple seasons and locations is recommended to confirm the stability of these traits and their usefulness for reversion trait selection in sweetpotato hybrids in Uganda.

Overall, 43 marker alleles - ranging from 2 to 4 alleles for each marker – were detected in six SSR loci analyzed throughout the 121 F1 hybrid population. On average, each pair of primers generated 2.389 polymorphic alleles per locus (Table 3). This observation is different from the observation by Yada *et al*. (2015) where, the lowest number of alleles per locus was 4 and the highest was 6. In the current study, markers IBR12 (for NKxRe hybrids), IBS166 (for NKxRe, N1xRe, and N11xRe hybrids), IBY53 (for N1xRe hybrids) and IBS82 (for N11xRe hybrids) had 3 or 4 alleles per locus; this was lower than the 5 and 7 alleles per locus observed in studies by Yada *et al*. (2010) and Koussao *et al*. (2014). However, similar to our findings, Karuri *et al*. (2010) while working on Kenya sweetpotato germplasm observed 3 alleles per locus for IBR12 marker. The low average allele number of 2.389 observed in the current study is similar to the observations by Ssamula *et al*. (2019), and this consistence in allele number could be attributed to narrow geographic zone of collection of genotypes in these studies. This trend was further observed from averages for effective alleles (1.738), Shannon's information index (0.634) and diversity index (0.389) (Table 3), which were all lower than those obtained from Tanzanian sweetpotato genotypes that were collected over a large geographical zone (Ngailo *et al*., 2016).

There was generally a high molecular variation within the populations of parents, hybrids and sweetpotato cvs Tanzania and Ejumula for IBS82, IBR12 and IBS166 SSR markers, with marker IBS82 contributing to the highest significant variation (72%) (Table 3). Therefore, these markers are suitable for detecting molecular diversity within sweetpotato germplasm (Yada *et al*., 2010). Understanding that these sweetpotato genotypes are diverse as revealed by morphological and molecular analysis is vital for breeding sweetpotato that can withstand SPFMV infections. For these significant markers, AMOVA results indicated higher variation (mean square values) among than within sweetpotato populations of parents, hybrids and the random sweetpotato cultivars. This is similar to the findings obtained by Koussao *et al*. (2014). Also, consistent with another study by Ngailo *et al*. (2016) in Tanzanian, sweetpotato variation among individuals (87%) was greater than that within individuals (9%). On the contrary, findings obtained by Xiao *et al*., 2023 in China showed higher sweetpotato variation within populations (99%) than between populations (1%). Also, our findings are different from David *et al*. (2018) observations in East African sweetpotato cultivars, where variations of 85% and 14% within and between pools, respectively, were observed. As observed for morphological variation, this variability in observations confirm that sweetpotato is highly heterogeneous and segregating (Xiao *et al*., 2023). As a hexaploid and highly heterogeneous crop, sweetpotato gives F1 hybrids with diverse variation types (Xiao *et al*., 2023).

Nei's pairwise genetic distance of 0.2 to 0.8 observed in this study is similar and largely consistent with that of 0.2 to 0.9 obtained among New Kawogo × Beauregard hybrids in a study by Yada *et al*. (2015). The genetic distance observed in the current study, however, was lower than that of 0.1 to 1.0 observed by Yada *et al*. (2010) while working with the Ugandan sweetpotato germplasm. The genetic distance observed in the current study suggests the uniqueness of the hybrids used.

The first principal component and PC2, with Eigen values greater than one, accounted for 67.10% of the total molecular variability, mainly attributed by SSR markers IBR12, IBU5, IBS166, and IBY53 (Table 4) - which is an indicator for high variability within the hybrids. This variability is higher than that observed by Yada *et al*. (2010) and Yada *et al*. (2015), with 40.7% and 53.99% respectively. Results from cluster analysis of the SSR markers indicated four clusters of the hybrids (Fig. 4), which also indicates a high genetic diversity. In addition, the distance between class centroids ranged from 1.852 to 5.648; with variation within the classes of 51.13% and between classes of 48.87%. This distance and variation further suggest genetic diversity. Both cluster and principal component analysis results using the SSR markers in the current study indicated that some hybrids were genetically different from the parents. This could be due to random re-assortment of alleles in the diverse bi-parental crosses

(Yada *et al*., 2015). Overall, observations made from the morphological and molecular studies reveal diversity within the F1 hybrids.

Conclusion and recommendations

Significant morphological and molecular variation was observed in sweetpotato population of parents, their hybrids and the random sweetpotato cultivars Tanzania and Ejumula. Seven phenotypic traits were sufficient in differentiating F1 hybrids of which, type of leaf lobe and number of leaf lobes were the most important. Overall, SSR markers IBS82, IBR12 and IBS166 significantly showed the highest level of genetic diversity within the hybrids. The high level of genetic diversity implies a broad genetic base for the reversion trait within hybrids. Therefore, type of leaf lobe and number of leaf lobe and SSR molecular markers (IBS82, IBR12 and IBS166) are suitable for future identification and diversity analyses for the reversion trait in sweetpotato germplasm.

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