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Full Length Research Paper

Characterization of Kenyan sweet potato genotypes for SPVD resistance and high dry matter content using simple sequence repeat markers

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Simple sequence repeat (SSR) markers were used to characterize Kenyan sweet potato genotypes for resistance to the sweet potato virus disease (SPVD) and high dry matter content. Eighty nine (89) genotypes with a mean symptom severity score of between 1 and 1.5 were selected following graft inoculation with SPVD-infected scions and characterized using 6 SSR primers. The 6 SSR primer pairs had an average polymorphic information content (PIC) of 0.47. The average number of alleles within the 89 genotypes across the 6 loci was 13.52. Cluster analyses revealed a 50% variation among the 89 genotypes. The dendrogram did not reveal any unique clustering of the genotypes according to dry matter content and reaction to SPVD. The genetic differences among the SPVD resistant genotypes and those with high dry matter revealed by the distinct groups suggest a significant genetic variability and the presence of different sources of resistance to SPVD and high dry matter. This characterization will give valuable information for breeders and serve as a baseline for efficient development of new cultivars resistant to SPVD and containing high dry matter.

Key words: Polymorphic information content, SPVD, severity, cluster analysis.

INTRODUCTION

Sweet potato virus disease (SPVD), caused by the dual infection with sweet potato feathery mottle *potyvirus* (SPFMV) and sweet potato chlorotic stunt *crinivirus* (SPCSV) (Gibson et al., 1998), is the most harmful disease of sweet potato world-wide and especially in East Africa (Geddes, 1990; Karyeija et al., 2000; Njeru et al., 2004). The disease is characterized by chlorosis, small, deformed leaves, and severe stunting and can reduce yields of infected plants by over 90% (Gutierrez et al., 2003). Low production of sweet potato is also due to lack of high yielding varieties with consumer acceptable attributes, such as high dry matter. Consumers prefer sweet potato varieties with high dry matter as indicated in a survey of different regions in Kenya (Ndolo et al., 2006).

Molecular markers have shown important and critical applications in the assessment and conservation of genetic variation. Microsatellite or simple sequence repeat (SSR) markers exhibit high levels of polymorphism, and several such markers have been developed for sweet potato (Buteler et al., 1999; Hu et al., 2004) and used successfully for determining the genetic relationship between cultivars derived from hybrid or polycross breeding programs (Hwang et al., 2002) and for analyzing the genetic diversity of sweet potato landraces (Veasey et al., 2008). In sweet potato, DNA markers have been identified for economically important traits such as resistance to SPVD (Mwanga et al., 2002; Miano et al., 2008) and root knot nematodes (Mcharo et al., 2005b), dry-matter and starch content, yield and beta-carotene content (Cervantes-Flores, 2006).

Comparative studies in plants have shown that SSR markers, which are single locus markers with multiple alleles, are more variable than other markers and provide an effective means for discriminating between genotypes (Powell et al., 1996). To ensure that durable resistance to SPVD is maintained within the Kenyan sweet potato germplasm, there is a need to increase the levels of resis-

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tance within the genepool using additional sources of resistance with a wider genetic base. Several sweet potato cultivars are grown in Kenya (Gichuki et al., 2003), which have been shared by farmers through generations. These cultivars have large differences in susceptibility to SPVD, and good sources of resistance are present this germplasm (Aritua et al., 1998). This study therefore aimed at using SSR markers to identify Kenyan sweet potato germplasm resistant to SPVD and high dry matter content.

MATERIALS AND METHODS

SPVD resistance and dry matter content analysis

A total 314 sweet potato genotypes were collected from the main sweet potato growing regions of Kenya and propagated in an insect-proof screen-house at the Kenya Agricultural Research Institute's National Agricultural Research Laboratories (KARI-NARL). Once established, the apical portion of each plant was side grafted with scions from sweet potato plants pre-infected with SPVD. SPVD severity in each genotype was assessed weekly, for a period of eight weeks according to Njeru et al. (2004) using a subjective five-point severity rating scale of 1 to 5, where 1 = no visible symptoms and 5 = very severe symptoms of purpling / yellowing or mosaic on leaves, severe leaf distortion, reduced leaf size, severe stunting. A total of 89 sweet potato genotypes with SPVD severity ranging from 1 to 1.5 were selected and characterized using SSR markers. Out of the 89 genotypes, 20 that tested negative to SPCSV and SPFMV were re-inoculated with SPVD (20 plants per genotype). DM content was determined in freshly harvested roots of 314 sweet potato genotypes according to Kidmose et al. (2007).

DNA extraction and amplification with SSR primers

Genomic DNA was extracted from fresh leaves of each genotype using a modified cetyl trimethyl ammonium bromide (CTAB) extraction method (CIP, 2000). Six labelled SSR primers were used for PCR amplification of the DNA samples (Table 1). Each 20 µl PCR reaction contained 10 pmol/µl of each primer, 2.5 mM $MgCl_2$, 10 mM dNTPs, 0.1 U Amplitaq Gold Polymerase (Applied Biosystems) and 5X PCR buffer (Applied Biosystems). Amplification was carried out using the Gene-Amp PCR system 9700 (Applied Biosystems) in following thermocycling conditions: 1 cycle of 94°C for 2 min, followed by 15 cycles of 94°C for 1 min, 60°C for 2 min, 72°C for 1.5 min, 94°C for 1 min, 50°C for 2 min and 72°C for 1.5 min and a final extension step of 10 min at 72°C to reduce the probability of false scoring of stutter bands as alleles. The PCR products of each sample were separated on a 2% agarose gel.

Capillary electrophoresis and fragment analysis

The amplified DNA fragments were screened by capillary electrophoresis on the ABI 3730 genetic analyzer (Applied Biosystems). The GeneMapper ver. 3.7 software (Applied Biosystems) was used to size peak patterns using the internal Genescan-500 LIZ size standard and Genotyper 3730 (Applied Biosystems) for allele calling.

Statistical analysis

The SSR data was analyzed using the SIMQUAL (Similarity for Qualitative Data) routine to generate jaccard similarity coefficients. These similarity coefficients were used to construct dendrograms using UPGMA and employing the SAHN (Sequential, Agglomerative, Hierarchical and Nested clustering) program from the Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc) version 2.11T (Rohlf, 2000).

RESULTS

SPVD resistance and dry matter content analysis

Analysis of variance showed highly significant ($p \leq 0.001$) differences in symptom severity among the 314 sweet potato genotypes. Average symptom severity of all the genotypes ranged from 1.0 to 4.1. Of the 314 genotypes evaluated for SPVD resistance, 89 had a mean SPVD severity score of between 1.0 and 1.5. Following re-inoculation of these 89 genotypes, 20 and 69 exhibited mean SPVD severity scores of between 1.0 and 1.5, and 1.6 and 3.0, respectively. Serological indexing by NCM-ELISA of the 89 genotypes showed that the 20 genotypes were free of SPFMV and SPCSV and were therefore regarded as resistant to SPVD (Table 2).

Analysis of variance showed significant ($p < 0.001$) differences in the DM content among the 314 sweet potato genotypes. The DM content ranged from 20.0 to 37.8%. The 89 genotypes selected for molecular analysis had a DM content ranging from 21.6 to 35.9%.

Polymorphism, allele variability, and observed heterozygosity in the 89 genotypes

The 6 SSR markers used to characterize the 89 sweet potato genotypes were polymorphic across all loci with an average polymorphic information content (PIC) average of 0.4. Marker IB-R19 revealed the highest PIC of 0.8 while marker IB-S07 had the lowest PIC of 0.3. A total of 23 alleles were detected with an average of 3.8 alleles per locus (Table 3). Observed heterozygosity ranged from 0.2 to 1.0 with a mean of 0.8 across the six SSR loci. The highest observed heterozygosity was in marker IB-R03 with a value of 1.0 while the lowest was 0.2 in marker IB-S07 (Table 3).

Genetic variability within the 89 sweet potato genotypes

The 89 genotypes used were classified into two groups namely resistant (20 genotypes) and susceptible (69 genotypes) based on their response to SPVD inoculation in the screen-house (data not shown). The number of alleles within the 89 genotypes across the 6 loci ranged from 10 to 17, with an average of 13.5. The highest number of alleles (17) was observed in genotypes SP33, SP66 and SP68, whereas the lowest was 10, which was observed in genotypes SP8, SP30, SP85, SP86 and

Table 1. Six sweet potato microsatellite primers used in the study.

Marker name	Dye	Primer forward (5'-3')	Primer reverse (5'-3')	Repeat motif	Tm (°C)	Expected product size
IB-R03	PET	GTAGAGTTGAAGAGCGAGCA	CCATAGACCCATTGATGAAG	(GCG)5	73	243-258
1B-S07	FAM	GCTTGCTTGTGGTTCGAT	CAAGTGAAGTGATGGCGTTT	(TGTC)7	69	162-178
IB-R12	NED	GATCGAGGAGAAGCTCCACA	GCCGGCAAATTAAGTCCATC	(CAG)5A	71	303-342
IB-R16	VIC	GACTTCCTTGGTGTAGTTGC	AGGGTTAAGCGGGAGACT	(GATA)4	76	201-213
1B-R19	PET	GGCTAGTGGAGAAGGTCAA	AGAAGTAGAACTCCGTCACC	(CAG)5b	76	190-208
IB-CIP13	NED	CGTGCTTGAGGTCTGAGTAGAA	TTCCCTAGAAGCTGCGTGAT	ACC)3+(CCG)2+(TGC)3+(GTC)2	68	196-373

SP87 all of which appeared resistant to SPVD except SP30. All the 89 genotypes (both resistant and susceptible groups) had the same alleles of 206, 374 and 175 at loci IB-R16, IBCIP-13 and IB-S07, respectively. At locus IB-R19, the most abundant alleles were 193 and 208 bp for the resistant and susceptible genotypes, respectively. At locus IB-R12, the resistant and susceptible genotypes had 339 and 327 bp, respectively, as the most common allele. Similarly, at IB-R03, the most common/abundant alleles were 252 and 258 bp for the resistant and susceptible genotypes, respectively. The rare allele of 191 bp at locus IB-S07 was observed in 6 genotypes namely SP79 (resistant), SP72 (resistant), SP78 (resistant), SP24 (susceptible), SP35 (susceptible) and SP40 (susceptible), whereas the rare allele of 206 bp at locus IBCIP-13 was observed in 5 genotypes namely SP54, SP42, SP55, SP53 and SP56, all of which were susceptible.

Genetic relationships among the 89 sweet potato genotypes

The genetic similarity among the 89 genotypes ranged from 0.3 to 1.0 with an average of 0.6. The genetic similarity matrix showed that the most closely related genotypes were SP8, SP85, SP86 and SP87; SP48, and SP49; SP5, SP23, SP20 and SP81; SP1, SP25, SP31, and SP73; and SP33, SP36, SP70 and SP74; SP9 and SP49; and between SP7 and SP77. The most distant relationship was observed between the genotypes SP5 and SP27, SP23 and SP28, SP5 and SP28, SP28 and SP81 and SP28 and SP20 with minimum (low) genetic similarity matrix of 0.3 (data not shown). The similarity matrices showed that the 20 genotypes that appeared resistant to SPVD were not genetically similar. Similarly, the genotypes with very high dry matter were not genetically similar.

Phylogenetic analysis

The UPGMA analysis clustered 89 genotypes into 2 main clusters namely A and B and 5 sub-clusters (Figure 1). The Jaccard's coefficient ranged from 0.5 to 1, accounting for 50% variation among the 89 genotypes. Cluster A contained 72 genotypes of which 6 had appeared resistant to SPVD, whereas Cluster B contained 17 genotypes of which 14 had appeared resistant to SPVD. Cluster A, sub-cluster I contained 41 genotypes of which SP38, SP44, SP67, SP72, SP79 and SP75 were resistant to SPVD. This sub-cluster contained 3 (SP21, SP24 and SP33) and 12 genotypes with very high and high dry matter, respectively. Cluster A, sub-cluster II and III contained 7 and 24 genotypes, respectively, all of which were susceptible to SPVD. Cluster A sub-cluster III had 11 genotypes with high dry matter content. Cluster B, sub-cluster I had 9 genotypes of which 3 namely SP48, SP59 and SP71 were susceptible to SPVD. Cluster B, sub-cluster II contained 8 genotypes which had appeared resistant to SPVD with 4 genotypes namely SP19, SP39, SP65 and SP87 containing high dry matter. Generally, genotypes with high dry matter content were distributed across all the sub-clusters in the UPGMA dendrogram generated. Of the 20 SPVD-resistant genotypes, 10 had high dry matter content and were not clustered together in the dendrogram.

Principal component analysis (PCA) based on genetic distances of the 89 genotypes

Although no distinguishable pattern of clustering of genotypes on the basis of resistance to SPVD, most of the genotypes that appeared resistant to SPVD grouped in the same area of the plot. The first and second principal components accounted for 24.7 and 13.8% of the total variation, respectively. The PCA scatter plot revealed two major cluster groups (Figure 2). Generally the

Table 2. Reaction of sweet potato genotypes to infection with SPCSV and SPFMV.

S/N	Genotype	SPVD severity	Serological test*		DM content (%)
			SPFMV	SPCSV	
1	OP-LNA-006-08	1.4	-	-	29
2	TVT/02/2007	1.1	-	-	28.2
3	WFTC/03/2007	1.3	-	-	34.7
4	YS sopalla	1.4	-	-	27.8
5	Marooko (1)	1.4	-	-	33.1
6	KKFS Mwavuli	1.2	-	-	31.6
7	YS Kemb 10	1.2	-	-	29.9
8	YS Nyanguyegwo	1.1	-	-	34.3
9	Marooko (3)	1.4	-	-	33.8
10	KAK/04/2007	1	-	-	26.3
11	KKFS 56682/03 (1)	1.1	-	-	34.1
12	Kamau (1)	1.4	-	-	33.1
13	Naspot	1.4	-	-	27.4
14	MKN/04/2007	1.5	-	-	34.8
15	Katumani (2)	1.5	-	-	31.7
16	Kikuyu (3)	1.4	-	-	32.8
17	Katumani (7)	1.5	-	-	25.4
18	Kikanda (1)	1	-	-	27.9
19	Kikamba (2)	1	-	-	28
20	SPK 004 (Katumani)	1.2	-	-	32.4
21	Mugande	5	+	+	37.8
22	Wamuciri	4.7	+	+	36.1
23	KKFS NK-L-22	4.4	+	+	35.9

*NCM-ELISA. SPVD severity score determined following the scale of 1-5 where; 1 = no visible symptoms, 5 = very severe symptoms (Njeru et al., 2004).

Table 3. Quality indices and polymorphism detected by 6 SSR markers in 89 sweet potato genotypes.

Marker name	Quality Index	Total No. of alleles	Allele size range	Abundant Allele (%)	Rare Allele(s) (≤ 5%)	[a]PIC values	[b]Observed heterozygosity
IB-R16	0.0005	4	202-214	41.55	None	0.69	0.99
IB-R19	0.0005	6	190-208	26.62	None	0.81	0.87
IBCIP-13	0.0005	3	206-374	56.08	206	0.53	0.87
IB-R12	0.001	3	318-339	41.18	None	0.65	0.56
IB-SO7	0.0005	3	175-191	87.91	191	0.33	0.21
IB-R03	0.0005	4	243-258	29.08	None	0.74	1.00
Mean	0.00058	3.83		47.07		0.47	0.75

[a]PIC = 1 - $\Sigma(pi^2)$ where Pi is the frequency of the i^{th} allele detected.
[b]Frequency at which heterozygous individuals occur in a population at a given locus.

PCA scatter plot, detected trends similar to the clustering illustrated in the dendrogram.

DISCUSSION

The present study is the first genetic characterization of Kenyan sweet potato genotypes for resistance to SPVD and high dry matter content using microsatellite markers. Results showed that microsatellite markers were highly polymorphic in sweet potato similar to many important crop plants. The high level of polymorphism associated with SSR markers may be a function of unique replication slippage mechanism responsible for generating SSR allelic diversity (Pejic et al., 1998). Five out of the 6 SSR markers revealed a high discriminatory power (PIC of

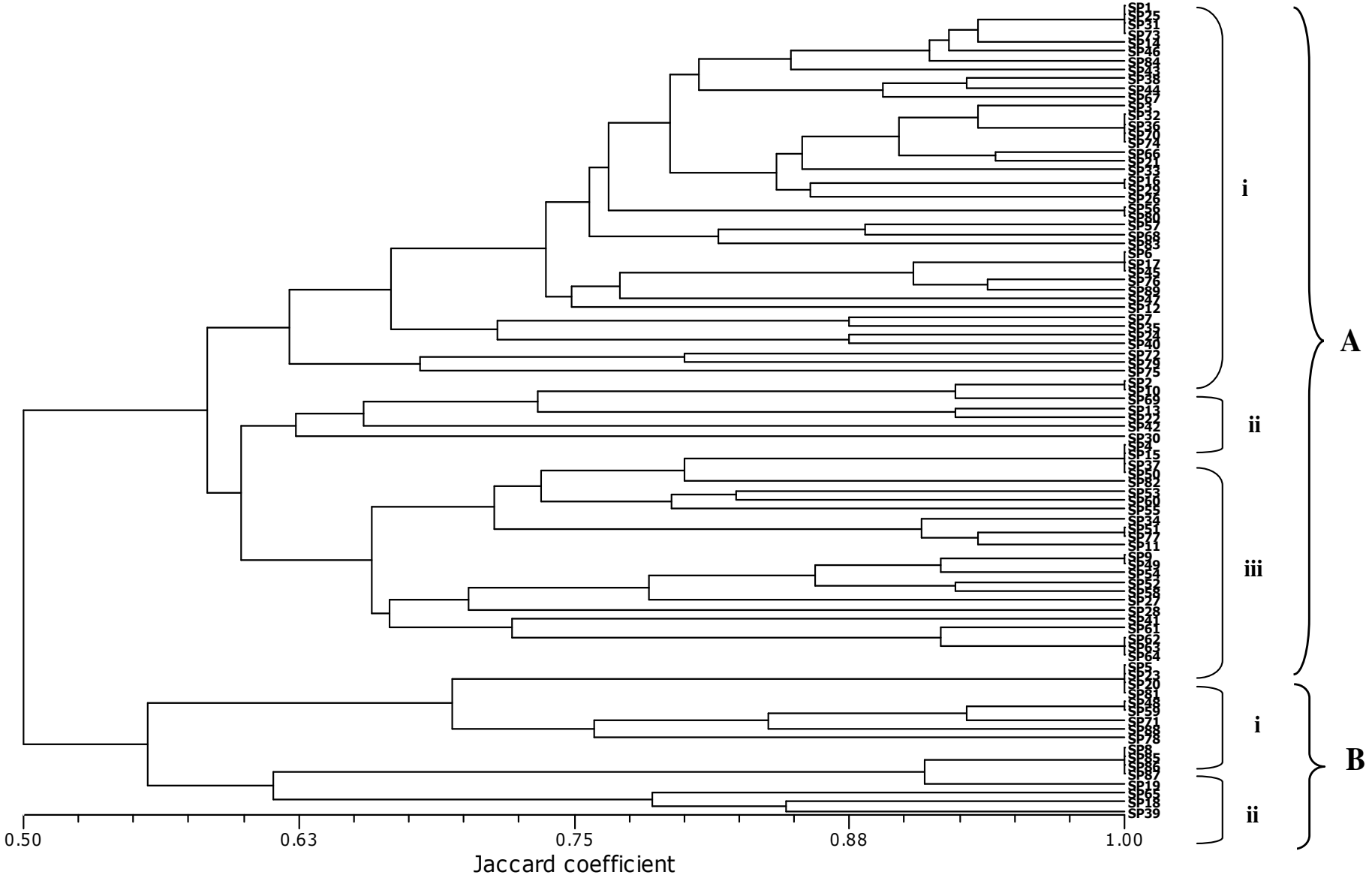

Figure 1. UPGMA dendrogram of 89 sweet potato genotypes based on genetic similarity matrix calculated from SSR markers. Cluster A contained 72 genotypes of which 6 had appeared resistant to SPVD, whereas Cluster B contained 17 genotypes of which 14 had appeared resistant to SPVD genotypes of which 14 had appeared resistant to SPVD.

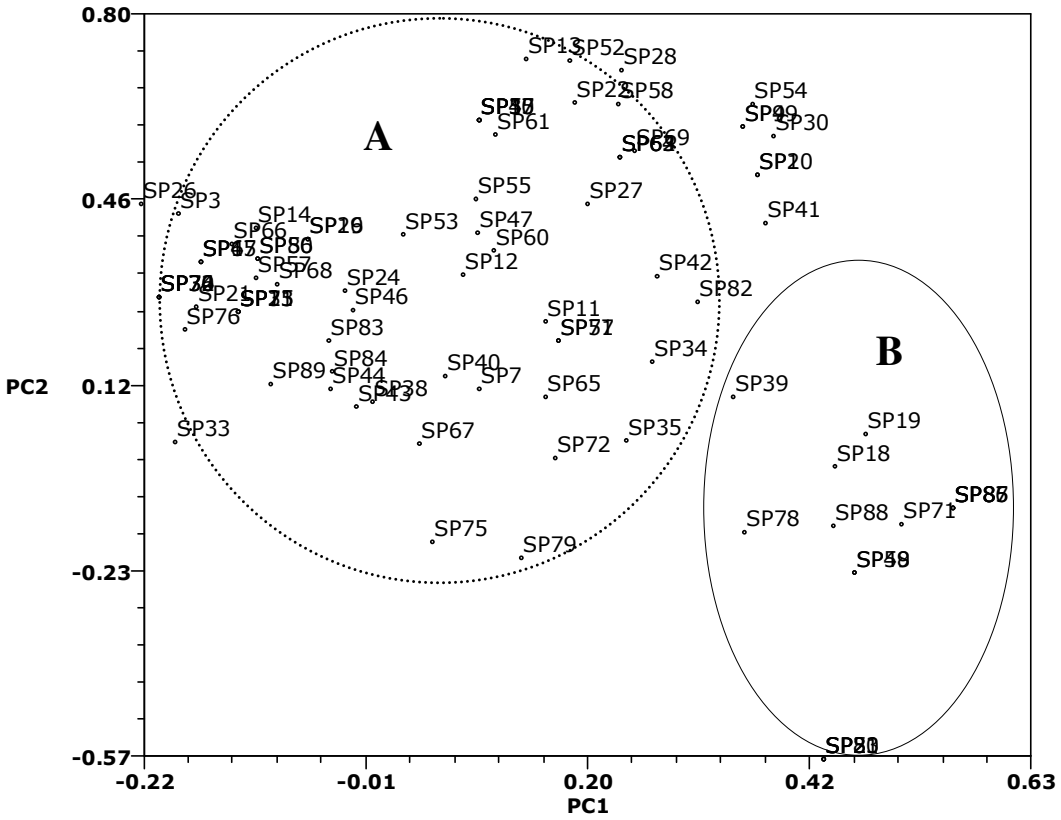

Figure 2. Principal component analysis of 89 sweet potato genotypes using 6 SSR markers. PC1 and PC2 represent 24.72% and 13.82% of the total variation, respectively.

greater than 0.5) and hence were highly informative. The low PIC value of 0.33 for IB-SO7 was due its highly monomorphic nature.

The number of alleles amplified per primer pair from the 89 sweet potato genotypes ranged from 10 to 17 showing a relatively high level of microsatellite polymorphism in sweet potato. One possible reason for the high degree of variation among the genotypes may be related to the mating system of sweet potato; a cross pollinating and hexaploid species (Ozias-Akins and Jarret, 1994). Rare alleles were observed in several genotypes probably due to the high rate of mutation in SSR loci (Henderson and Petes, 1992).

High levels of heterozygosity were observed in the genotypes, and varied greatly across the six loci, ranging from 0.21 (IB-S07) to 1.00 (IB-R03), with a mean of 0.75. This could be attributed to the out breeding nature of sweet potato, where the proportion of heterozygous loci is likely to be high. It has also been reported that self-incompatibility in the flowers results in allogamy, increasing genetic heterozygosity (Thompson et al., 1997).

The mean genetic similarity of 0.62 obtained in this study is lower than values of 0.69 and 0.71 found among sweet potato cultivars in Taiwan and Tanzania, respectively, (Tseng et al., 2002; Elameen et al., 2008), and higher than that found among accessions from South America (0.58) (Zhang et al., 2000). This is not surprising since the genetic diversity is expected to be higher in the center of the diversity (South America), and the introductions both to Africa and Asia probably have involved just a few genotypes. The genetic differences among the SPVD resistant genotypes revealed by their clustering into distinct groups suggest the presence of different sources of resistance to SPVD. Hierarchical UPGMA analysis and PCA, revealed three groups of SPVD resistant genotypes. Generally, the relationship between genotypes in the cluster groups could not be attributed to their resistance to SPVD or high dry matter. The PCA analysis further provides information about associations between genotypes, which are useful to formulate better strategies for breeding. The absence of strong associations among the genotypes in the groups implies significant diversity within each cluster group, and the dominant independent role in the cluster groups along each separate principal component implies significant diversity between the groups. It is therefore envisaged that, combining genotypes from the different groups as parents in breeding would result in diversifying SPVD resistance genes in the breeding population.

Incorporating SPVD-resistant genotypes which have other desirable agronomic and consumer quality traits such as high dry matter content, from the different cluster

groups into the breeding programmes as parents, would ensure the diversification of resistance to the disease while creating new genotypes. In addition, by combining different genes that relate to different sources of resistance, epistatic interaction may be identified such that higher levels of resistance can be developed to protect the crop. This study has shown that despite the damaging effects of the disease on the crop, there is a significant amount of genetic variability among the SPVD resistant and high dry matter genotypes, which could be utilized in breeding to diversify resistance to the disease. This molecular characterization of sweet potato will give valuable information for breeders and lead to a more efficient development of new cultivars resistant to SPVD and high dry matter. Since the SSR markers used in this study were developed to determine genetic diversity of sweet potato germplasm, there is need to use markers linked to the genes responsible for resistance to SPVD and high dry matter in order to facilitate the effective identification of quantitative trait loci linked to these two traits.

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