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Full Length Research Paper

Characterization of Tanzanian elite sweet potato genotypes for sweet potato virus disease (SPVD) resistance and high dry matter content using simple sequence repeat (SSR) markers

Catherine Gwandu, Fred Tairo*, Emmarold Mneney and Alois Kullaya

Mikocheni Agricultural Research Institute (MARI), P.O Box 6226, Dar es Salaam, Tanzania.

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A total of 57 sweet potato genotypes with high dry matter content and resistant to sweet potato virus disease (SPVD) were characterized using four simple sequence repeat (SSR) markers. The germplasm included 20 genotypes identified as having high dry matter content and 25 accessions tolerant to SPVD in a study conducted in Tanzania in 2008. The total number of alleles within the 57 genotypes across 4 loci was 395, with an average of 4 alleles per locus. The unweighted pair group method with arithmetic mean (UPGMA) and analysis of molecular variance (AMOVA) using generated SSR data, grouped the 57 genotypes into two major clusters, with mean pair-wise genetic distance of 0.55. No specific grouping was observed in relation to SPVD resistance, dry matter content and geographic location. The four microsatellites markers distinguished the 57 Tanzanian sweet potato genotypes into two major clusters. The relatively high level of genetic diversity indicates broad genetic base for sweet potato breeding in Tanzania. The results obtained demonstrate the efficiency of SSR marker technique for the assessment of genetic relationships and distinguishing between Tanzanian sweet potato genotypes. The findings of this of this study, provide valuable information to breeders to facilitate cost effective germplasm conservation and development of improved sweet potato varieties resistant to SPVD and containing high dry matter.

Key words: Simple sequence repeat (SSR), *Ipomoea batatas*, cluster analysis, genetic diversity.

INTRODUCTION

Sweet potato virus disease (SPVD) is the most devastating virus–induced syndrome of sweet potato. It is a disease of economic importance because the affected plants produce non-usable root tubers (Gibson et al., 1998). The disease is caused by the synergistic interaction of aphid-transmitted Sweet potato feathery mottle virus (SPFMV: genus *Potyvirus*, family *Potyviridae*) and whitefly-transmitted Sweet potato chlorotic stunt virus (SPCSV: genus *Crinivirus*, family *Closteroviridae*) (Gibson et al., 1998). The consequences of SPVD are not only limited to reduction in root yield, but also undermine the ongoing efforts in genetic improvement for yield, quality and development of virus resistant cultivars. Currently, there is no part of the world that is growing cultivars immune to SPVD (Tairo et al., 2005). Since high levels of SPVD resistance are rare (Kreuze et al., 2009), selection and planting of tolerant and better performing genotypes commonly practiced by farmers in East

*Corresponding author. E-mail: fredtairo@yahoo.com. Tel: +255 22 2700554. Fax: +255 22 2775549 .

Abbreviations: SPVD, Sweet potato virus disease; **SSR,** simple sequence repeat; **UPGMA,** unweighted pair group method with arithmetic mean; **AMOVA,** analysis of molecular variance.

Africa has been a short-term measure to sustain production in the presence of SPVD, resulting in improved yield.

The development of host resistant to SPVD is a high priority for sweet potato breeding program in Tanzania. The most widely used approach to germplasm improvement in sweet potato is through mass selection (Jones, 1986). This fundamentally simple technique lends itself well to an allohexaploid crop that possesses few, if any, simply inherited traits (Buteler et al., 1999; Zhang et al., 2001). Since many of the agronomically important traits in sweet potatoes like root dry matter (DM) content are quantitative (Mcharo et al., 2001) several challenges complicate improvement of sweet potatoes with desirable traits such as disease resistance and/or high dry matter. For instance, self-incompatibility prevents introgression of desirable traits from genotypes or exotics into adapted material. In addition, the preponderance of quantitatively inherited traits in sweet potato (Jones, 1986), unstable trait expression due to genotypes × environment interaction and the encumbrance of two year breeding cycles undermine the breeding efforts. These factors underlie recent attempts to use trait-linked random amplified polymorphic DNA molecular markers in breeding programs.

Mwanga et al. (2002a) estimated high broad-sense heritability for resistance to SPVD, suggesting that breeding for resistance is possible. Mwanga et al. (2002b) used linkage maps to associate DNA random amplified polymorphic DNA (RAPD) and amplification fragment of length polymorphism (AFLP) markers to SPFMV and SPCSV resistance, but attempts to identify markers associated with SPVD were unsuccessful because only few progenies in the defined population were resistant. The characterization of sweet potato germplasm in Tanzania has been done using several markers (Elameen et al., 2008; Tairo et al., 2008). For example, Elameen et al. *(*2008) used AFLP markers to estimate the genetic variations within and between sweet potatoes accessions maintained by the root and tuber research programme at the Kibaha Sugarcane Research Institute, Tanzania, while Tairo et al. (2008) characterized 280 sweet potato genotypes using morphological markers. Simple sequence repeat markers are particularly attractive to study because they are abundant in plants, have high level of polymorphism and are adaptable to automation (Donini and Stephenson, 1998). In sweet potato, efficiency of SSR markers has been demonstrated in identifying and characterizing the genetic diversity and relationships of sweet potato (Hu et al., 2003; Huang and Sun, 2002; Zhang et al., 2000; Yada et al., 2010). Recently, SSR markers have been used to characterize sweet potato genotypes for SPVD resistance and high dry matter content in the germplasm collection in Kenya (Karuri et al., 2009).

In the present study, a total of 18 SSR markers were used to characterize selected elite Tanzanian sweet potato genotypes. The objectives of the study were to: (1) determine the genetic diversity and relationship among selected genotypes, (2) assess specific grouping linked to SPVD resistance and/or dry matter content, and (3) explore the implications of SPVD resistance to sweet potato breeding work in Tanzania.

MATERIALS AND METHODS

Fifty-seven elite sweet potato genotypes used in this study were selected from a previous population of 280 sweet potato germplasm collected and maintained at Chambezi Field Station, Bagamoyo, Tanzania, as previously described by Tairo et al. (2008). These genotypes were selected based on their promising reactions to SPVD infection and high dry matter content (Table 1). The field studies were all based in tanzania while laboratory work SSR was done t the Bioscience for Eastern and Central Africa (BECA) in Nairobi, Kenya.

SPVD reaction and dry matter content

The experimental materials were screened in the field at Chambezi, a high diseased area for SPVD, for their reaction to SPVD following exposure to natural infection by vectors. A complete randomized block design (RCBD) with four replications was used. Each replication contained 57 accessions, grown onto four ridges of 7 m length per accession, with four plants per ridge at a spacing of 1 m × 0.3 m. SPVD severity in each genotype was assessed for the period of eight weeks repeatedly for two consecutive growing seasons in 2007/2008 using severity scale of 1 to 5, where 1 = no visible symptoms, and 5 is for very severe symptoms and stunting of the plant (Mukasa et al., 2005).

Of the 280 genotypes screened for SPVD reaction and high root dry matter content (Tairo et al., 2008) only 57 were selected as elite materials for subsequent use as parent materials for breeding purposes. The selected 57 genotypes were then verified for their virus status by nitro-cellulose membrane enzyme-linked immune-sorbent assay (NCM-ELISA) for SPFMV and SPCSV as per NCM-ELISA kit manufacturer instructions. Determination of root dry matter (DM) content was done according to Carey and Reynoso (1996) method on 400 g of root tuber flakes obtained from four undamaged tubers from four plants per accessions.

DNA extraction and polymerase chain reaction (PCR) analysis

Genomic DNA was extracted from 1 to 1.5 g of fresh leaves using the cetyltrimethylammonium bromide (CTAB) method described by Doyle and Doyle (1990). The amplification was performed in volume of 10 µL containing 25 ng/µL DNA template, 0.2 µM of each forward and reverse primer, 2.5 mM dNTPs, 25 mM $MgCl_2$, 10X PCR buffer, 0.5U Amplitaq Gold Polymerase (Applied Biosystems) and autoclaved sterile distilled water. Amplifications were carried out in a Gene-Amp PCR system 9700 thermocycler (Perkin Elmer, Wellesey, Mass, USA) using the following cycling conditions: initial denaturation for 15 min at 95°C, then 40 cycles for 1 min at 94°C for 30 s; 1 min at 58°C; 2 min at 72°C and final extension time of 20 min at 72°C. The amplification was checked on a 0.8% TBE (Tris-Borate-EDTA, pH 8.0) gel. Polymorphism was detected using automated capillary electrophoresis of fluorochrome-labeled PCR products. Each forward primer of the 18 polymorphic SSR primers was labeled with one of three fluorochrome moieties [FAM-6-carboxyfluorescein, HEX-hexachloro-6-carboxyfluorescein, NED-8'-

Table 1. List of 57 superior sweet potato genotypes selected for genotyping and their response to SPVD and root dry matter (DM) contents.

SN	Sample number	Cultivars name	*Agro-ecological zone	DM (%)	**SPVD severity	SN	Sample number	Cultivars name	Agro-ecological zone	DM (%)	**SPVD severity
1	1	Carroti	EZ	40.54	1	38	22	Gairo	EZ	-	4
2	2	Mwanahanga	EZ	42.26	1	39	23	Chanika-Orange	EZ	36.26	4
3	3	Moshi	LZ	36.52	1	40	24	Pananzala	EZ	38.25	4
4	4	Misalaba	LZ	37.20	1	41	25	Kanshabari	EZ	33.61	5
5	5	Fraisca	LZ	37.28	1	42	26	Kibisi-3	EZ	-	5
6	6	Kibakuli 2	EZ	39.00	1	43	27	Lubisi	SZ	39.25	5
7	7	Kamusoma	LZ	35.25	1	44	28	Za Wasukuma	SZ	38.27	5
8	8	Canada-C	EZ	34.50	1	45	29	Vumilia	EZ	40.50	5
9	9	Jitihada	LZ	34.89	1	46	30	Kabuganda	LZ	38.37	5
10	10	Mwanatatata	LZ	41.00	1	47	31	Mkono wa Nyerere	LZ	37.50	5
11	11	Butundwe	EZ	34.00	1	48	32	Aveline	EZ	-	5
12	12	Unknown-Katulika	LZ	35.00	1	49	33	Uwanja wa Ndege-1	SZ	36.50	5
13	13	Butundwe	LZ	32.94	1	50	34	Kenya	SZ	39.50	5
14	42	Mbeya-2	SZ	37.35	1	51	35	Rehema-2	EZ	39.21	5
15	47	Ex-Ipungu-1	SZ	38.00	1	52	36	440144	EZ	35.00	5
16	48	Ex-Lipumba-2	SZ	40.12	1	53	37	Bongoman	EZ	40.00	5
17	50	Ikumbi-1	SZ	36.50	1	54	38	Shinamugi	EZ	45.25	5
18	51	Viazi Mayai	SZ	36.48	1	55	39	Berena	EZ	31.45	5
19	52	Ikumbi-3	SZ	-	1	56	40	Simama	LZ	36.48	5
20	53	Mbeya	SZ	40.00	1	57	45	Mkombozi	SZ	37.75	5
21	55	Furahisha	EZ	-	1						
22	57	Simama-1	EZ	36.48	1						
23	43	Roiyailoya	LZ	31.00	1						
24	46	SP/93/13	EZ	38.50	1						
25	54	Kisangani	SZ	33.50	1						
26	49	Canada-M	EZ	34.50	2						
27	41	Matako Mapana	EZ	41.90	2						
28	56	Kibaha	EZ	44.18	3						
29	14	Mtoto wa shule	LZ	31.75	3						
30	15	NASPOT-1	LZ	38.00	3						
31	16	Mwaniweyegeke	LZ	34.00	3						
32	17	Hali Mtumwa Mayai	EZ	40.66	3						
33	18	Ukerewe	LZ	-	3						
34	19	Mwaniweyegeke	LZ	34.00	3						
35	44	Unknown Ex-Pangani	EZ	33.00	3						
36	20	Kupiga Wasami	EZ	39.60	4						
37	21	Polista	LZ	37.50	4						

*EZ, Eastern zone; LZ, lake zone; SZ, southern zone; **SPVD severity scores is the mean score of four plant assessed per accession.

Table 2. Primer sequences, size range, alleles per locus and quality index of the four primer pairs used for analysis.

Marker	Dye	Primer 5'-3'	Primer reverse 5'-3'	Quality index	Allele/ locus	Allele size
IB-R16	VIC	GACTTCCTTGGTGTAGTTGC	AGGGTTAAGCGGGAGACT	0.5314	11	161 - 237
SSR 07	PET	TTTTCAACGACAAGCCTCTTGC	TCAAAGGTCCGCATGGAAATC	0.5201	19	160 - 200
SSR 09		AAGTTAATCTAAGGTGGCGGGG	CGTCGATTCCAGTCTAATCCAATCC	0.5201	22	57 - 201
690524	VIC	AAGGAAGGGCTAGTGGAGAAGGTC	CAAGGCAACAAATACACACACACG	0.5372	14	240 - 315

benzo-5-fluoro-2 and 4,7-trichloro-5-carboxyfluorescein (Applied Biosystems) (Table 2). Triplex PCR products were separated with an ABI 3730 96-channel DNA sequencer (Applied Biosystems) and fragments sized by GeneMapper ver 3.7 software (Applied Biosystems). Peak detection and size matching was achieved by internal Genescan-500 LIZ size standard and Genotyper 3730 (Applied Biosystems) for allele calling.

Statistical data analysis

AlleloBin software (Idury and Cardon,1997) was used to adjust allele sizes for scoring inconsistencies. The output file was then used by ALS Binary to convert the allele sizes from the bulks into binary format (1,0). Analysis of genetic relationship among individual was achieved by pair-wise comparison among all 57 genotypes using Jaccard's similarity index in the SIMQUAL program of NTSYS-pc 2.1 (Rolfs, 1993). Principal component analysis (PCA) was used to analyze the genetic relationship among individual genotypes. The generated similarity index matrix was then used to cluster groups of genotypes using Nei and Li's similarity coefficient (Nei and Li, 1979), with options of unweighted pair group method using arithmetic averages (UPGMA) algorithm of NTSYS pc software version 2.2 (Rohlf, 1993) to generate a dendrogram. Quantification of the diversity level and the genetic relationship among the 57 genotypes was achieved by analysis of molecular variance (AMOVA) using Gen AIEX 6.4 software.

RESULTS AND DISCUSSION

Reaction of genotypes to SPVD and dry matter content

With respect to reaction of the 57 genotypes to SPVD infection, √25 √(43.8%) genotypes had low level of susceptibility to SPVD with infection scores of 1, 10 (17.5%) genotypes showed mild infection with scores of 2 and 3, while 22 (38.7%) genotypes had high level of susceptibility with infection scores of 4 and 5 (Table 1). The dry matter content of the 57 genotypes ranged from 31 to 44%, with mean dry matter content of 37.3%. The DM content of the genotypes showing the lowest SPVD infection scores ranged from 31 to 42%, with a mean of 36.9%, while the DM content of those showing mild infection ranged from 31 to 44% (mean 36.9%). The most susceptible genotypes had DM content of 31 to 40%, with a mean of 37.9%. The relatively high dry matter contents shown by this study to some genotypes were due to the state of tubers during harvesting and processing for DM determination. Some of the tubers were shriveled and reduced in size following a prolonged period of drought prior to harvesting. Thus, during oven drying of samples, dry weight became too small over the fresh weight, thus resulting in an elevated DM for some genotypes which are known to have low DM such as carrot, NASPOT 1 and Mayai.

East African sweet potato genotypes have several unique important characteristics like high dry matter content, high resistance to virus diseases and vigorous foliage cover, although they have low root beta-carotene content (Gichuki et al., 2003). In this study, the absence of SPFMV and SPCSV were confirmed in the resistant genotypes by repeated grafting on universal indicator plant for sweet potato viruses *Ipomoea setosa.* The presence of viruses was rechecked with NCM-ELISA and reverse transcriptase (RT)-PCR using virus specific primers for the respective viruses as described by Tairo et al. (2005). However, due to the absence of real time PCR machine, the levels of virus titers in the resistant materials could not be checked. The large number of genotypes (43.8%) showing low level of SPVD infection suggests that there is a source of resistance to SPVD within Tanzania gene pool, which can be explored for breeding for SPVD resistance. Most of the sweet potatoes grown in Africa are local genotypes derived from chance seedling. Farmers often grow several genotypes in the same season, allowing direct competition and comparison to select and maintain vegetatively asymptomatic genotypes (Karyeija et al., 1997). By so doing they maintain SPVD resistant genotypes.

Allelic diversity

Although 18 SSR primers were screened in this study, the majority (14) had low quality index. Only four SSR primers (Table 2) with quality index of 0.5 were used for the analysis. These were polymorphic with more than three bands across the loci of the few genotypes used for

screening. The number of alleles per locus ranged between 11 and 22 for the four SSR markers. This is in agreement with the study by Karuri et al. (2010), which amplified 10 to 17 per primer (slightly less than this study) using SSR markers. In this study, the primer SSR-09 had the maximum number of allele (22) (Table 2). The results showed a high level of polymorphism with all four microsatellite loci analyzed, thus suggesting wide genetic diversity. The high degree of variation can be attributed to the polyploidy and outcrossing nature of sweet potato. In total, the four SSR loci detected 66 SSR variants with an allele size ranging from 161 to 315 bp. The four highly polymorphic markers adopted for analysis were powerful enough to distinguish between 161 geno-types used in this study. Our results are in agreement with previous results (Zhang et al., 2000; Gichuru et al., 2006) in which only six and four primers successfully discriminated 119 Latin American and 57 sweet potato cultivars in East Africa, respectively.

Genetic variability

Among the 57 genotypes studied, the similarity index ranged from 0.00 to 0.98, with an average of 0.55. Similarity values obtained for each pair-wise comparison of SSR allele among the 57 sweet potato accessions were used to construct a dendrogram based on hierarchical clustering and the results are presented in (Figure 1). Most genetic distance (GD) coefficients ranged from 0.21 to 0.25 (Figure 1) and account for more than 60% of the pair-wise distance coefficients. The highest mean pairwise genetic distance was 0.55, and was between genotypes NASPOT-1 and Kanshabari, Ukerewe and Kanshabari. The most closely related genotypes were Kibaha and Canada-M and Mtoto wa shule and Katulika, with matching GD coefficient of 0.22 (Figure 1).

The highest genetic diversity (0.55) revealed in this study was higher than in the previous work by Tairo et al. (2008), but slightly less than 0.57 reported by Yada et al. (2010) and 0.60 by Karuri et al. (2009). The small number of SSR markers and the genotypes used in this study may have caused these differences. In contrast to previous authors, the majority of genotypes in this study (60%) showed relatively low similarity coefficient of 0.22 compared to Ugandan genotypes (Yada et al., 2010). However, our results correspond to the findings of Elameen et al. (2008) who analyzed 69 Tanzania sweet potato germplasm using 6 AFLP markers. The high genetic diversity of sweet potato is attributed to self-incompatibility and cross-pollination that result into different individual progenies. The mean genetic distance of 0.55 obtained in this study is almost similar to the values of 0.60, 0.57 found among sweet potato germplasm of Kenya and Uganda, respectively (Karuri et al., 2009, Yada et al., 2010). Our results are also in agreement with results of Fajardo et al. (2002) who found that the genotypes collected in a given region often displayed molecular variability similar to that observed over the entire sampled area. This is true here, since all three countries in East Africa belong to the same gene pool.

Similarly, Zhang et al. (2000) found high diversity of 0.58 among accessions from South America, which is similar to those of East Africa. This is anticipated since genetic diversity is expected to be higher in the center of the diversity (South America) (Elameen et al., 2008). East Africa can also be considered as a potential area for search for genetic diversity for sweet potato since after its introduction in the 15th century, it is possible that new cultivars have evolved post introduction. However, in this study, although similar diversity to that of Zhang et al. (2000) was obtained on 57 accessions, this diversity (0.55) is far smaller compared to the diversity that exists in for example Peru alone with 2,476 landraces (Loebenstein and Thottappilly, 2009).

Cluster analysis

The UPGMA analysis clustered 57 genotypes in two major clusters (Figure 2). Cluster A contains 17 genotypes of which six are highly susceptible to SPVD with severity score of 4 to 5, while six showed severity score of 1 and five genotypes severity score of 3. Cluster B comprises 31 genotypes of which 18 are resistant to SPVD with severity score of 1, while 13 genotypes showed moderate to high susceptibility. The nine remaining genotypes did not group in any cluster (Figure 2). Principle component analysis was also done to analyze the genetic relationships among the individual accessions (Figure 3). The results were similar to cluster analysis.

The clustering of the sweet potato genotypes into two major groups, as also reported by Elameen et al. (2008), may possibly be explained by the findings of Kapinga et al. (1995) that two separate gene-pools were introduced into East Africa, one early by Portuguese and the other one later by the British. The cluster analysis did not reveal specific grouping of the 57 genotypes according to their geographic location or their reaction to SPVD and DM content (Figure 2). However, eleven (64.7%) of the genotypes in cluster A showed low to medium SPVD infection. Surprisingly though, all the nine genotypes that were not grouped into any of the two clusters were all highly susceptible to SPVD with severity scores of 4 and 5 (Table 1). Since tagging SPVD resistance and dry matter is still problematic, efforts are under way to develop robust molecular markers (Kreuze et al., 2009).

Furthermore, the lack of geographical association among Tanzanian genotypes corresponds to the findings of Yada et al. (2010) on Ugandan sweet potato geno-

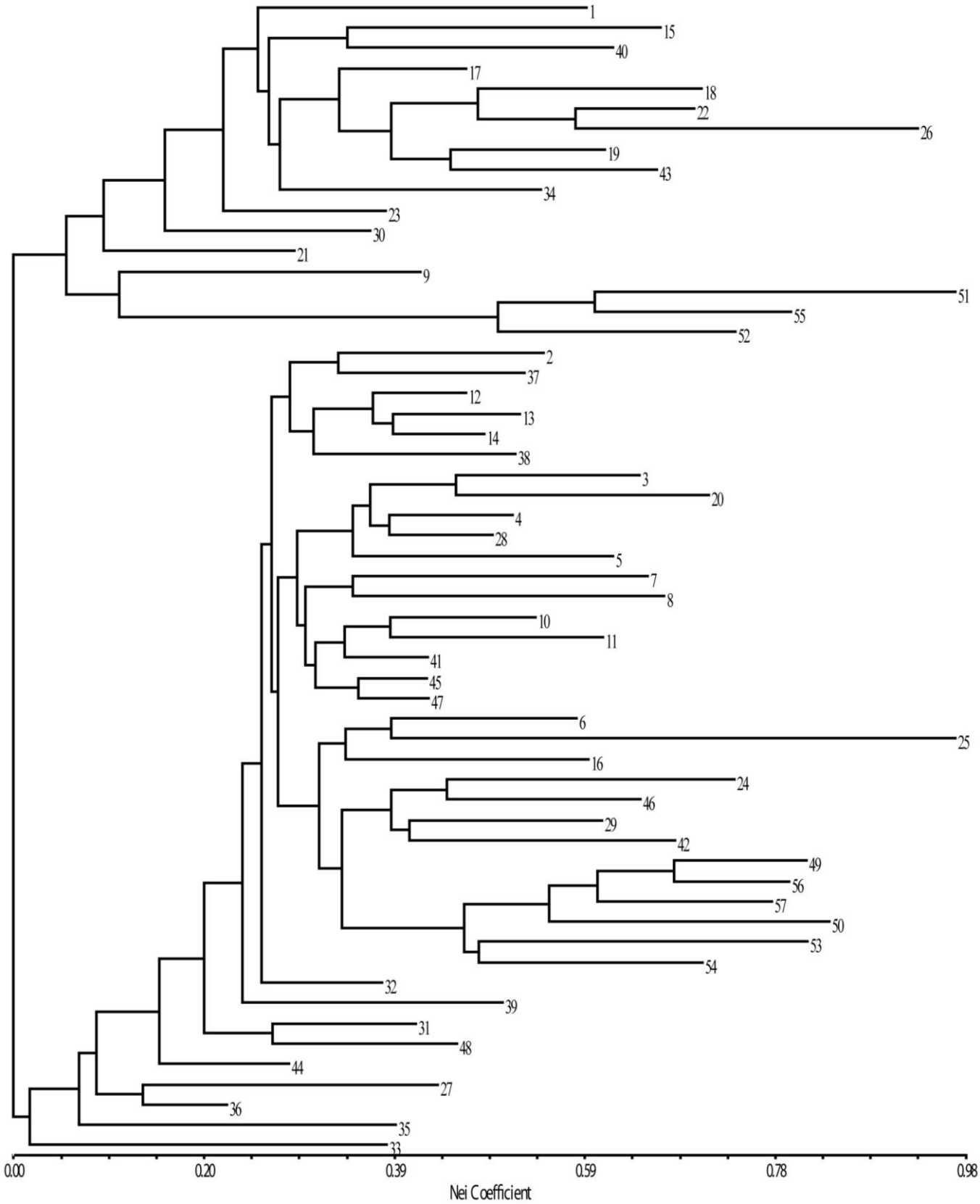

Figure 1. Dendrogram showing genetic relationships among 57 sweet potato genotyeps.

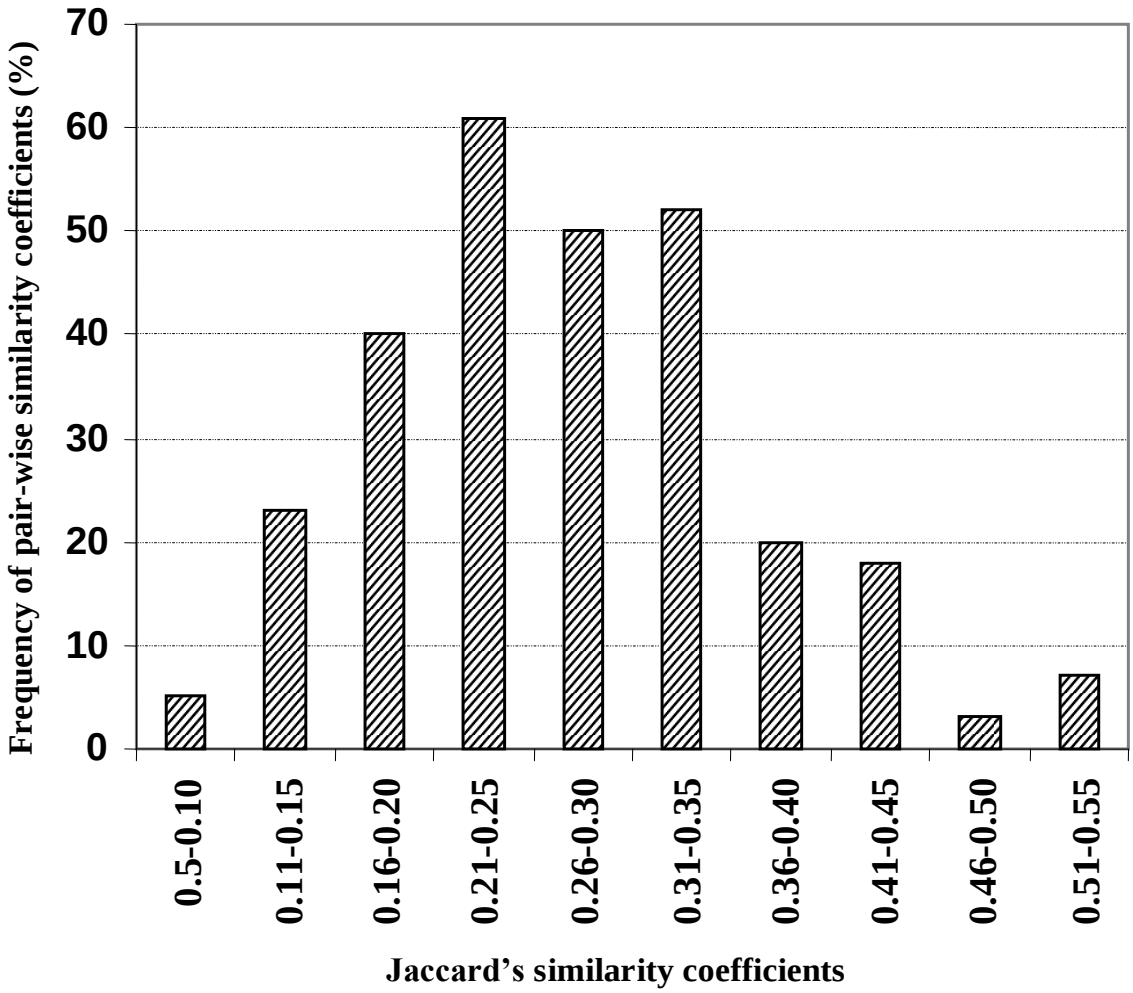

Figure 2. Frequency distribution of pair-wise genetic similarity coefficinet estimated among 57 sweet potato genotypes from Tanzania.

types using SSR markers. The lack of specific geographic grouping may be explained by short distances between agroecological zones in Tanzania. Moreover, farmers have routinely shared planting materials thus promoting occurrence of gene flow across agroecological zones over the years of sweetpotato cultivation.

Analysis of molecular variance

To quantify the diversity level and the genetic relationship among the 57 genotypes, analysis of molecular variance (AMOVA) was done. The genotypes were classified based on their reaction to SPVD. Among the groups, variation accounts for only 3% of the total molecular variance and was highly significant. This explains why clustering of the genotypes according to SPVD was not evident. The within group variation accounts for 97% of the total molecular variance and was also statistically significant.

Conclusion

The four microsatellites markers used were able to distinguish the 57 Tanzania sweet potato genotypes. The relatively high level of genetic diversity is an indication of the broad genetic base for sweet potato breeding in Tanzania. The results demonstrate the efficiency of SSR marker technique for assessment of genetic relationships among Tanzanian sweet potato genotypes. Analysis of the 57 genotypes against SPVD infection revealed that a high proportion (43.8%) of genotypes has promising resistance against SPVD. This study has therefore produced useful information with respect to root DM content and reaction to SPVD that can facilitate cost-

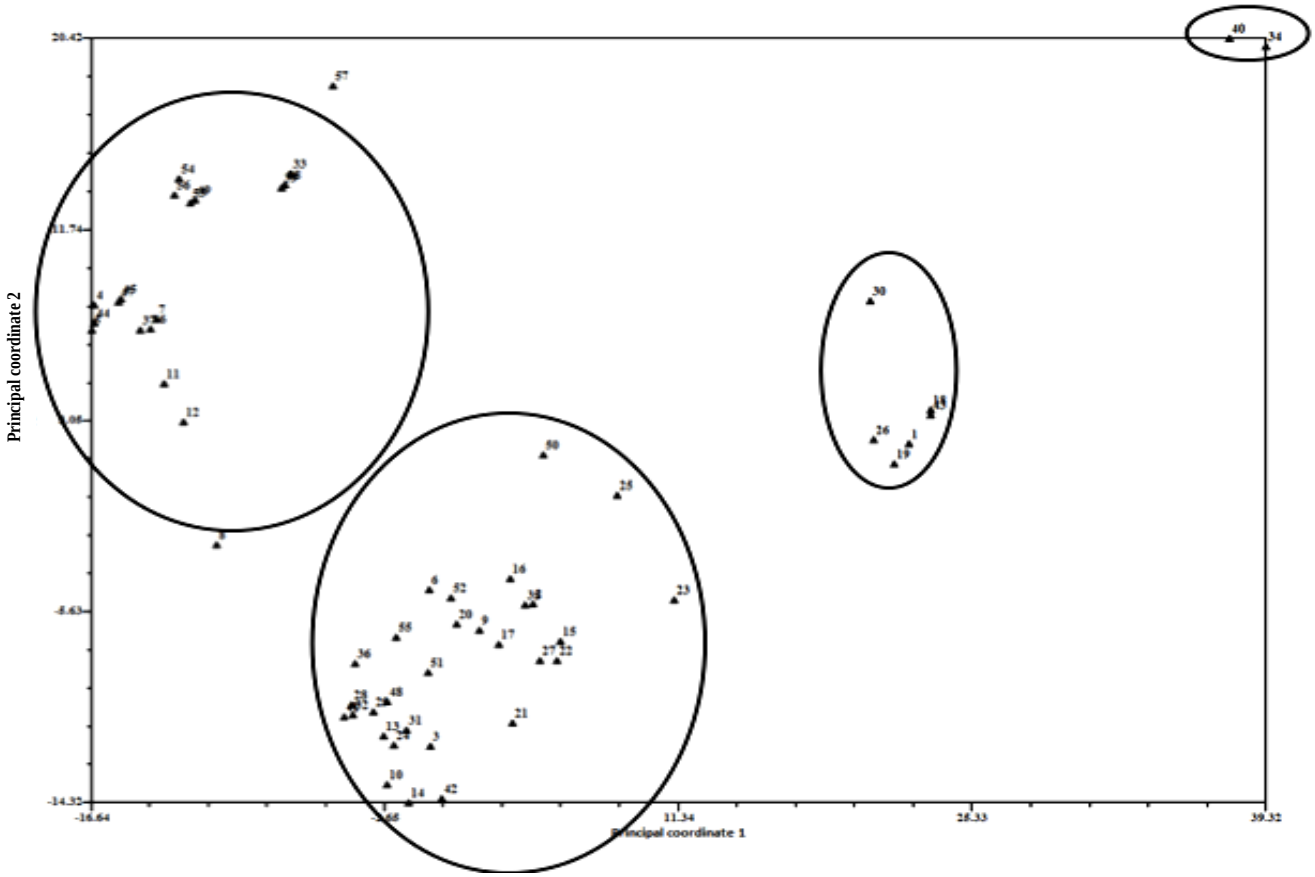

Figure 3. Principal coordinate analysis of 57 sweet potato landraces with varying level of SPVD resistance and dry matter content included in this analysis based on SSR markers. Grouping corresponds to Figure 2 cluster analysis.

effective germplasm conservation and development of improved sweet potato varieties by breeders.

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