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Morphological markers cannot reliably identify and classify sweet potato genotypes based on resistance to sweet potato virus disease and dry matter content

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ABSTRACT
Objective: To characterize Kenyan sweet potato genotypes for resistance to sweet potato virus disease (SPVD) and dry matter content using morphological markers.
Methodology and results: Three hundred and fourteen genotypes were evaluated in the screenhouse for their reaction to sweet potato virus disease (SPVD) followed by serological analysis. Severity of SPVD was determined following graft-inoculation using a severity scale of 1- 5. Results showed that the genotypes responded significantly differently ($P<0.01$) to SPVD infection. Twenty genotypes were resistant to SPVD in the screenhouse. The 314 genotypes were planted in the field and characterized using 42 morphological characters. Tuber dry matter (DM) content was determined 5 months after planting in the field. The tuber DM content varied significantly ($P<0.01$) among the sweet potato genotypes. Phylogenetic analysis using morphological descriptors grouped the genotypes into two major clusters. None of the clusters clearly distinguished the 20 resistant genotypes from the 294 susceptible ones. Genotypes with highest and lowest tuber DM content were not distinguished from each other using the UPGMA phenogram generated.
Conclusions and application of findings: Our results indicate that morphological markers are not reliable in identifying and classifying sweet potato genotypes based on response to SPVD and dry matter content of the tubers. Morphological markers therefore need to be supplemented with molecular markers in identification of sweet potato germplasm with SPVD resistance and high dry matter content. This study has further shown that there is a significant amount of morphological variability among the SPVD resistant and high dry matter genotypes, which could be utilized in breeding to diversify resistance to the disease and generation of novel/new genotypes.

Key words: SPVD, severity, markers, phylogenetic analysis, serological analysis.

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INTRODUCTION

Sweet potato (*Ipomoea batatas*) plays an important role as a major component of diets and as a food security crop in many Kenyan households. It is also used as animal feed, with a value rated at 95-100% that of corn (Onwueme, 1978). Sweet potato yields in East Africa are about

one fifth of the potential yield (FAOSTAT, 2004). Constraints to sweet potato production include, pests, mainly sweet potato weevils (Carey *et al*., 1999) and viral diseases especially SPVD (Njeru *et al*., 2004). Other constraints include shortage of high quality planting materials, low yielding cultivars, short shelf life, limited processing outlets and marketing constraints (Mwanga, 2001). Sweet potato virus disease (SPVD) caused by the dual infection between sweet potato feathery mottle *potyvirus* (SPFMV) and sweet potato chlorotic stunt *crinivirus* (SPCSV) (Gibson *et al*., 1998) is a major constraint to production since it can reduce yields of infected plants by up to 98% (Gutierrez *et al*., 2003).

Kenyan sweet potato genotypes have shown marked differences in reaction to viral diseases with good sources of resistance being found (Miano *et al*., 2008). Cultivation of resistant cultivars is compatible with subsistence agriculture (Mwanga *et al*., 2001), and is the most effective means of reducing sweet potato losses due to SPVD. Apart from viruses, low production of sweet potato is also due to lack of consumer acceptable attributes such as taste and dry matter content. Taste acceptability of sweet potato is dependent on the dry matter content with high dry matter being preferred. Therefore, there is a need to identify sweet potato cultivars that are either SPVD resistant, or have high dry matter content or have both attributes combined. Sweet potato exhibits phenotypic diversity as reflected by the skin and flesh color of the tubers, the shape of roots, leaves and branches, the depth of rooting and maturity period, resistance to pests and diseases and dry matter content of the tubers (Austin & Huaman, 1996). Morphological characters have been used to identify the centre of origin and evolution of *Ipomoea batatas*, duplicates in sweet potato collections (Zhang *et al.,* 1996) and in establishment of core collections (Mok & Schmiendiche, 1999). Similarly, it is our opinion that morphological traits in sweet potato can be used to identify markers associated with resistance to SPVD. Although Kenyan sweet potato germplasm has superior characteristics such as resistance to diseases and high DM content, these superior genotypes have not been fully exploited in breeding programmes. Partly, this is due to the fact that Kenyan sweet potato germplasm has not been characterized to identify genotypes with SPVD resistance and/or high dry matter content. This study, therefore, aimed at using morphological markers to characterize Kenyan sweet potato genotypes for resistance to SPVD and high DM content.

MATERIALS AND METHODS

Three hundred and thirty sweet potato genotypes were collected in 2007 as vine cuttings from Kakamega, Vihiga, Bungoma and Busia districts in Western province; Homabay, Migori, Kisii and Rachuonyo districts in Nyanza province; Thika and Kirinyaga districts in Central province; Embu, Makueni and Machakos districts in Eastern province and Kwale, Malindi and Kilifi districts in Coast province. The plants were propagated in an insect-proof screenhouse at the Kenya Agricultural Research Institute's National Agricultural Research Laboratories (KARI-NARL). Morphological identification of duplicates was done in the green house 3 months after planting according to Huaman (1992). Three hundred and fourteen genotypes were identified as unique and established in the screenhouse, the apical portion of each plant was side grafted with scions (Beetham & Mason, 1992) from sweet potato plants pre-infected with SPVD. Five plants per variety were graft-inoculated, with one extra plant grafted with a healthy scion to act as a control. SPVD severity was assessed weekly for a period of eight weeks 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 and severe stunting (Njeru *et al*., 2004). The SPVD severity data was subjected to analysis of variance (ANOVA).

Eighty nine (89) sweet potato genotypes with a mean SPVD severity score of between 1 and 1.5 were selected and re-inoculated with scions pre-infected with SPVD followed by serological analysis eight weeks after inoculation by nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA). Polyclonal antibodies specific to SPFMV

and SPCSV as well as NCM strips pre-spotted with sap from virus-infected and non-infected control plants obtained from the International Potato Center (CIP, Lima, Peru) were used. Twenty genotypes that tested negative to SPCSV and SPFMV were re-inoculated again with SPVD (20 plants per genotype).

The 314 sweet potato genotypes were planted in a field at KARI-NARL and allowed to grow for five months. Morphological characterization was done using CIP, AVRDC, IBPGR, (1991), 5 months after planting. A total of 42 characters/descriptors were used in the evaluation of each genotype (Table 1). The phenotypic data was converted into a binary data matrix and cluster analysis was done using the Nei and Li coefficients and the UPGMA algorithm with Treecon version 1.3b (Van de Peer & De Wachter, 1994). Principal component analysis was done using XLSTAT 2008 (Agresti, 1990, New York).

Table 1: Descriptors used to assess the vegetative, floral and storage root traits of sweet potato genotypes.

Plant part	Observed trait
Vine	Twining, plant type, ground cover, vine internode length and diameter, vine pigmentation (predominant and secondary vine color), vine tip pubescence
Leaf	General outline of the leaf, leaf lobe type, leaf lobe number, shape of central leaf lobe, mature leaf size, abaxial leaf vein pigmentation, foliage color (mature and immature leaf color), petiole pigmentation, petiole length
Storage root	Root shape, root surface defects, root skin color (predominant and secondary skin color, intensity of predominant skin color), root flesh color (predominant and secondary flesh color, distribution of secondary flesh color), root formation, root cracking, latex production and oxidation in roots, quality characteristics of boiled storage root (consistency, undesirable color, texture and sweetness of boiled storage root)
Flower	Flower color, shape of limb, equality of sepal length, sepal pubescence, sepal color, color of stigma and style, stigma exertion

Source: CIP, AVRDC, IBPGR, (1991).

Dry matter content was determined in freshly harvested roots of the 314 sweet potato genotypes. Roots were selected randomly, washed, dried and then peeled. The middle sections of the roots were sliced and 25g (fresh weight) was obtained in three replicates. The weighed slices were dried at 80ºC for 20 h in a heating cabinet. After drying the samples were weighed immediately (final dry weight). Dry matter content (% DM) was calculated as: Percentage DM = [Final dry weight (g)/ Initial fresh weight (g)] X 100.

RESULTS

The 314 sweet potato genotypes exhibited varying reactions when challenged with SPVD. Analysis of variance showed highly significant ($P \leq 0.001$) differences in SPVD severity among the genotypes. The mean disease severity ranged from 1.0 to 4.0 with most (>71%) of the genotypes being highly susceptible to SPVD. Following re-inoculation of the 89 genotypes which had a severity score of between 1.0-1.5, 20 and 69 had mean SPVD severity scores of between 1 - 1.5, and 1.6 - 3.0, respectively. Serological tests of the 89 genotypes showed that 49 were infected by both SPFMV and SPCSV, 62 tested positive for SPFMV and 55 tested positive for SPCSV. In sum, twenty (20) genotypes tested negative for both viruses by NCM-ELISA (Table 2). The twenty genotypes exhibiting an SPVD severity rating of between 1.0 and 1.5 were selected as resistant to SPVD.

Table 2: Reaction of sweet potato genotypes to infection with sweet potato chlorotic stunt virus (SPCSV) and sweet potato feathery mottle virus (SPFMV).

No.	Genotype	SPVD severity	Serological test*		Dry Matter 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

*Test was done by Nitrocellulose Membrane Enzyme Linked Immunosorbent Assay (NCM-ELISA). Sweet potato virus disease (SPVD) severity score was determined following a 1 - 5 scale where; 1 = no visible symptoms, 5 = very severe symptoms (Njeru *et al*., 2004).

The 314 sweet potato genotypes that were characterized showed significant variation in vine, leaf, root and floral characters. Principal component analysis (PCA) revealed 13 principal components which had eigen values greater than 1 and accounted for 68.7% of the total variation (Table 3). The first 3 principal components accounted for 28.1% of the variation. The first, second and third principal components, respectively, accounted for 12.7, 8.6 and 6.9% of the variation.

Large variation was observed in the dry matter content as well as the predominant storage root flesh color of the 314 genotypes. The dry matter content ranged from 20 to 37.8%. The white/cream, yellow and orange root flesh colored genotypes had a dry matter content ranging from 20 to 37.8%, 23.1-35.6% and 22.5-32.9%, respectively. Most of the white/cream (70.3%) and yellow fleshed varieties (61.7%) had dry matter content greater than 35% (Fig. 1).

Phylogenetic analysis of the sweet potato genotypes resulted in two major clusters A and B (Fig. 2). Cluster A was further sub-divided into 7 sub-clusters whereas cluster B was sub-divided into two sub-clusters (Fig 2; Table 4). The phylogenetic analysis did not reveal any unique cluster(s) of the sweet potato genotypes on the basis of dry matter content, since genotypes with high DM content were grouped in the same sub-clusters with those having low DM.

Table 3: Eigen values, total variation and cumulative variation of the 13 principal components for 314 sweet potato genotypes.

Principal component	Eigen value	Variation (%)	Cumulative variation (%)
1	4.3	12.7	12.7
2	2.9	8.6	21.2
3	2.3	6.9	28.1
4	2.2	6.4	34.5
5	1.9	5.6	40.1
6	1.5	4.3	44.4
7	1.4	4.0	48.4
8	1.3	3.8	52.3
9	1.2	3.6	55.9
10	1.2	3.5	59.4
11	1.1	3.3	62.7
12	1.1	3.1	65.8
13	1.0	3.0	68.7

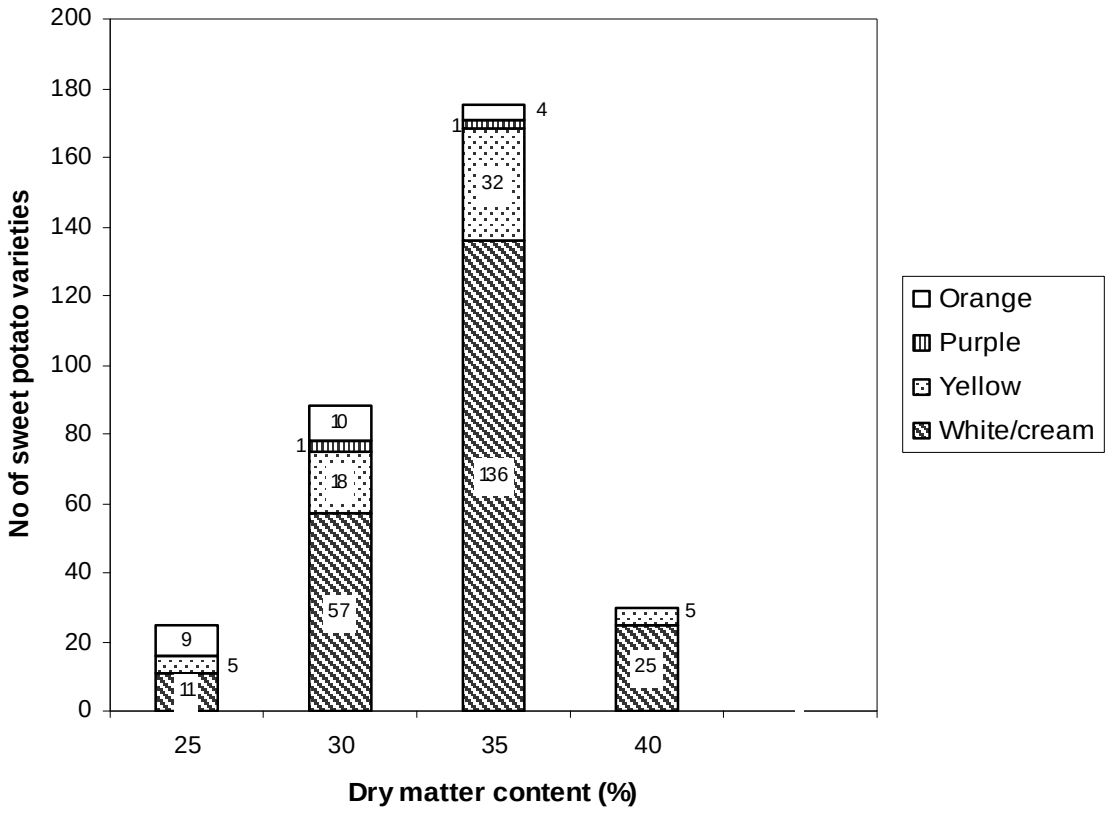

Figure 1: Frequency distribution of dry matter content and flesh colour of 314 sweet potato genotypes.

Table 4: Phenotypic characters used to separate sweet potato genotypes.

Cluster	Sub-cluster	No. of genotypes	Genotypes resistant to SPVD in cluster	Phenotypic characters
A	I	11	None	Green mature leaves with five lobes and semi-elliptic central leaf lobe, an erect plant type with thin vines and purple nodes as the secondary vine colour and green petioles with purple at both ends.
	II	9	TVT/02/2007, MKN/04/2007	Green mature leaves moderately lobed with five leaf lobes and semi-elliptic central lobe and storage roots that were slightly sweet when cooked.
	III	11	KKFS 56682/03 (1), Naspot , Kikanda (1), Kikuyu (3)	Green mature leaves, moderately lobed with a semi-elliptic central lobe. Absence of secondary vine colour and storage roots were soft when cooked.
	IV	7	WFTC/03/2007, SPK 004 (Katumani)	Green mature leaves, moderately lobed with green abaxial veins and short green petioles. Non-twining green vines with very short vine internode length.
	V	5	MCK/21/2007, Kikamba (2).	Green mature leaves with five lobes, semi-elliptic central lobe, petioles were green with purple near the leaf and abaxial veins with a purple spot at the base of the main rib. Very thin green vines with few purple spots and no secondary colour. White fleshed storage roots formed in a dispersed manner and soft and sweet when boiled.
	VI	11	YS Kemb 10	Green mature leaves, moderately lobed with five leaf lobes. Storage roots were soft with no undesirable colour when boiled.
	VII	13	KAK/04/2007, YS Sopalla, Nyanguyegwo	Green mature leaves that were almost divided with five leaf lobes and an elliptic central leaf lobe. Absence of secondary vine colour.
B	I	8	OP-LNA-006-08, KKFS Mwavuli	Green mature leaves with a single leaf lobe, triangular outline, toothed central leaf lobe and no lateral leaf lobes. Short petioles with purple colour at both ends, thin vine internode diameter, purple nodes as the secondary vine colour.
	II	14	Marooko (1), Marooko (3), Katumani (2) and Katumani (7)	Green mature leaves with a single leaf lobe, triangular leaf outline, toothed central lobe and green petioles. Non-twining vines with no secondary colour.

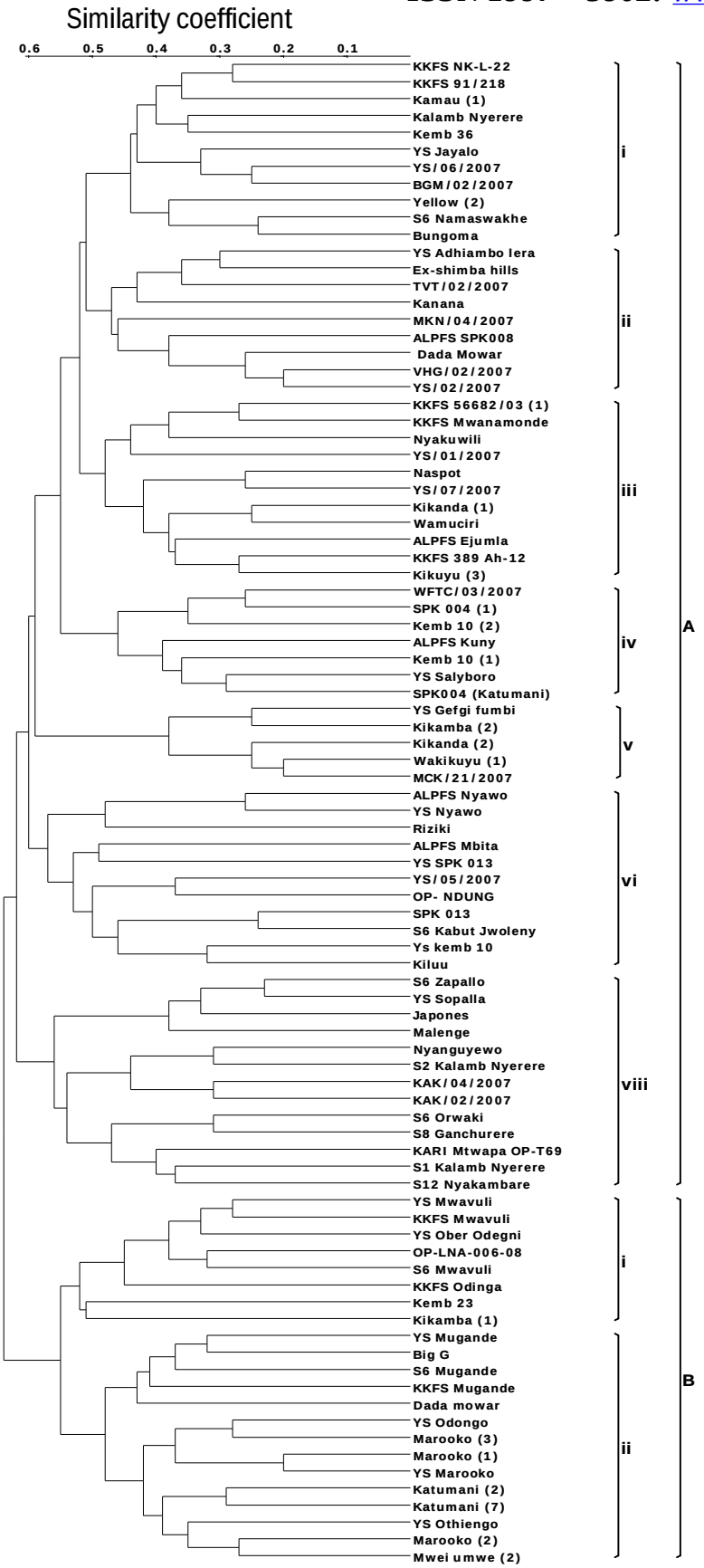

Figure 2: Cluster analysis of sweet potato genotypes using morphological traits.

DISCUSSION

Several sweet potato genotypes commonly grown by farmers in Kenya were collected and evaluated for reaction to SPVD. Following graft-inoculation with SPVD, the genotypes were observed to greatly differ in the severity of symptoms in the screenhouse. Variations in severity of SPVD symptom expression could be associated with differences in the rate of virus replication among the genotypes (Kuhn *et al*., 1981). Only 20 (6%) of the 314 genotypes had mean SPVD severity scores of between 1.0 and 1.5 and tested negative for both SPFMV and SPCSV, indicating their resistance to SPVD and their ability to suppress virus multiplication.

DM content is an important quality attribute in sweet potato as it is directly linked to consumers' preference for a particular genotype. Farmers grow a wide range of sweet potato cultivars depending on the needs of a particular market segment. There was a significant variation in DM content among the 314 genotypes, ranging from 13.6 to 48.6%. DM content greater than 35% was observed in the white/cream and yellow fleshed genotypes whereas orange fleshed genotypes which consisted of exotic genotypes had DM less than 30%. These data confirmed earlier reports by Brabet *et al*. (1998) that orange fleshed sweet potato genotypes have lower DM content than the white/cream and yellow fleshed genotypes.

Of the 20 sweet potato genotypes that were apparently resistant to SPVD, 55% had DM content less than 30%. The genotypes that had high DM content were severely affected by SPVD, for instance cv. Mugande had the highest DM content but was highly susceptible with a mean score of 5. Since DM content is an important quantitative trait of direct interest to the consumer, there is need to breed for high DM content and SPVD resistance.

Following principal component analysis (PCA), vegetative descriptors that contributed to the diversity of sweet potato included predominant vine colour, leaf lobe type, shape of central leaf lobe, abaxial leaf vein pigmentation, and immature leaf colour and petiole pigmentation. This confirms earlier reports that variation in Kenyan (Njuguna, 2005) and Tanzanian (Tairo *et al*., 2008) sweet potato germplasm is expressed based on the shape of the central leaf lobe. Two storage root descriptors namely the predominant root skin and flesh colour are other expressions of the crop's diversity. The predominant flesh color was also observed as an expression of genetic diversity in sweet potato genotypes from Brazil (Oliviera *et al.,* 2000).

The phylogeny of the sweet potato genotypes using 42 traits was mainly influenced by the general outline of the leaf. Using the general leaf outline, Gichuru *et al*. (2004) separated cultivars from Kenya, Uganda and Tanzania into two clusters using UPGMA. In this study, cluster analysis showed no formation of defined groups based on resistance to SPVD or high DM content. The hypothesis that the genotypes with SPVD resistance should be classified in a common cluster or sub-cluster was not observed as resistant genotypes were distributed into 8 of the 9 sub-clusters formed. Similarly, genotypes KKFS NK-L-22, Kemb 36, S6 Namaswakhe, YS/01/2007, ALPFS Nyawo, S2 Kalamb Nyerere and S6 Mugande that had high DM (>35%) were grouped in different sub-clusters together with genotypes YS/02/2007, KKFS Mwanamonde, Riziki, YS Sopalla, Malenge, Big G and Marooko (2), that had with low DM (<30%).

In this study, no correlations were observed between the reaction of genotypes to SPVD and DM content and morphological markers in the 314 genotypes. The results confirm earlier reports by Ivancic and Lebot (2000) that agronomically desirable traits are not always expressed as morphological characters or linked to them. Consequently morphological markers may not be relied on while identifying and classifying sweet potato genotypes as resistant or susceptible to SPVD or on the basis of DM content. Use of molecular markers could be a more reliable way to identify genotypes that are resistant to SPVD and with high DM.

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