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Evaluation of sweetpotato accessions for end-user preferred traits improvement

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This study assessed the genetic diversity and differentiation in sweetpotato accessions in Ghana to guide selection for genetic improvement on beta-carotene, dry matter and sugar contents to promote increased utilization. One hundred and fifteen sweetpotato accessions from four different sources, which were the International Potato Centre (CIP) collection, local collection from farmers' field, local improved varieties, and local and exotic collections from the National Agricultural Research Programmes were studied using 40 agro-morphological and physico-chemical traits, and 25 SSR markers. Variability was obtained for 13 agro-morphological traits and all the physico-chemical traits. Significant genetic diversity indicates existence of a high degree of agro-morphological and physicochemical variation. Within Group variation (97%) accounted for most of the diversity indicating a broad genetic base. The divergence indicates that breeders can form different populations with significant levels of genetic variation to exploit heterosis and improvement of populations. A strong negative relationship was found for sugar content and dry matter content and indicates a possible development of non-sweet high dry matter sweetpotato varieties. However, developing non-sweet, high dry matter and high beta-carotene sweetpotato varieties could be challenging due to the strong negative association between dry matter content and beta-carotene content, and the positive association existing between beta-carotene and sugar content. This study has in addition confirmed the breeding potential of sweetpotato accessions in Ghana and the probability of providing useful genetic variation for the development of farmer preferred cultivars.

Key words: Analysis of Molecular Variance (AMOVA), diversity, end-user, simple sequence repeats (SSR) markers, Sweetpotato, traits.

INTRODUCTION

Sweetpotato is a major staple crop in developing countries all over the world because of its diverse uses. These include use in many food and industrial products such as starch, sweeteners, noodles, citric acid, soft

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drinks, desserts, flour, industrial alcohol, ethanol fuel and livestock feed. Despite its importance, the level of utilization in Ghana is very low and it is not well integrated into Ghanaian diets (Adu-Kwarteng et al., 2002). This is because consumers in Ghana prefer sweetpotato with dry mealy flesh, non-sweet, and high nutritive value (Sam and Dapaah, 2009; Baafi et al., 2015), but locally available varieties are sweet that limits consumption as a staple food (Missah and Kissiedu, 1994). In addition, the recently introduced orange-flesh genotypes, identified as a cheapER source of Vitamin A, are low in dry matter content. These factors have led to the low adoption of the 13 varieties released to date. There is, therefore, the need to incorporate non-sweetness, high dry matter, and/or high beta-carotene contents into the existing genetic background of high yielding and early maturing cultivars which are resistant to biotic and abiotic stresses.

A prerequisite for genetic improvement of sweetpotato is knowledge of the extent of genetic variation present in the germplasm. Information on genetic diversity guides selection of divergent parents to broaden genetic base of a breeding population and produce progenies with heterosis (Manosh et al., 2008). Identification of populations with high frequencies of favourable alleles for desirable traits is an important step in the development of improved varieties (Gasura et al., 2008). Understanding the genetic diversity is also critical to find new alleles for desirable traits (Warburton et al., 2002). Since the amount of genetic diversity within populations determines the extent of response in traditional breeding through selection, genetically diverse breeding populations are needed (Bos et al., 2000). Morphological characterization has been used extensively in diversity studies for various crop plants including sweetpotato (Bos et al., 2000; Kaplan, 2001; K'opondo, 2011). Agro-morphological and physicochemical traits are important diagnostic features for distinguishing among sweetpotato accessions. The use of these traits as genetic markers can speed up selection in sweetpotato improvement. SSR markers have been used to study genetic diversity in sweetpotato (Buteler et al., 1999; Diaz and Gruneberg, 2008; Tumwegamire et al., 2011; Somé et al., 2014). SSR markers are multi-allelic, highly polymorphic, highly reproducible, co-dominant and provide rich genetic information with good genome coverage (Kawuki et al., 2009; Sree et al., 2010). The SSR markers are affordable and amenable to most breeding procedures and applicable in public breeding programmes that may not be able to afford expensive diversity assessment techniques (Turyagyenda et al., 2012). Application of both phenotypic and genetic markers is important in obtaining full knowledge of genetic diversity in sweetpotato germplasm.

The objective of this work was to characterize sweetpotato germplasm in Ghana using phenotypic and SSR markers with focus on enhancing end-user characteristics of sweetpotato for increased utilization in Ghana.

MATERIALS AND METHODS

Agro-morphological and physico-chemical characterization

Germplasm collection and evaluation

Germplasm was collected from the major sweetpotato growing areas in Ghana in 2010. These were the Northern, Upper East, Upper West, Volta, Eastern, Central and the Brong Ahafo Regions. Collections from the CSIR-Crops Research Institute, Kumasi and the CSIR-Plant Genetic Resource Institute, Bunsu, were also included. In addition, accessions were collected from the Crop Science Department, University of Ghana and the International Potato Centre (CIP) gene bank in Accra and Kumasi. Thus, a total of 115 sweetpotato accessions (Table 1) were collected. These represent four groups, which were local accessions (32), local improved varieties (13), exotic and local accessions in National Agricultural Research Systems (NARS) or programmes (43), and exotic accessions from CIP, Kumasi germplasm (27). Evaluation of the sweetpotato germplasm was carried out under rain-fed conditions using Randomised Complete Block Design (RCBD) in three replications at CSIR-Crops Research Institute research fields at Fumesua (forest ecozone) in 2011, after carrying out planting material multiplication in 2010. Planting distance was 1 m between ridges and 0.3 m within row of ridge length 3.6 m.

Data collection

Data collection was done based on the sweetpotato descriptor for field phenotyping (CIP/AVRDC/IBPGR, 1991) as well as storage root quality traits as shown in Table 2. Harvesting was done at three and half months after planting. At harvest, data were taken on storage root yield and its components and a random sample of storage roots (one small, one medium and one large) were taken for physico-chemical analysis. Storage roots considered for the yield data were those over 0.3 m in diameter and without cracks, insect damage or rotten parts (Ekanayake et al., 1990). With the exception of the dry matter content, all the storage root quality traits were determined using the near-infrared reflectance spectroscopy (NIRS) which uses the work flow of the Quality and Nutrition Laboratory of CIP Lima, Peru. Fifty grams fresh sample was used. It was freeze-dried for 72 h using a freeze dryer. Dry matter content was determined after freeze drying as ratio of dry weight to fresh weight of sample expressed as a percentage. These were determined at CIP Laboratories in Kumasi, Ghana and Lima, Peru.

Data analysis

Data were subjected to Principal Component Analysis (PCA) and Cluster Analysis using Genstat version 9.2.0.152 (Genstat, 2007). The PCA was done based on the correlation matrix. Data for beta-carotene, dry matter and total sugar contents were subjected to an Analysis of Variance (ANOVA) using Genstat version 9.2.0.152 (Genstat, 2007). Based on the mean performance of these traits, the top 10 and the bottom 10 accessions were selected to construct a dendrogram and a GGE Biplot using the most important traits for PC1 and PC2. The dendrogram was constructed based on the hierarchical, single link method using Euclidean test. The biplot was constructed to depict the phenotypic relationships among the accessions, their correlation with the traits significant for PC1 and PC2, as well as the association among the traits. The biplot was

Table 1. List of the 115 accessions collected and their source.

Local accessions	Local improved accessions	NARS accessions		CIP accessions
CRIWAC 01-10	SANTOMPONA*	TAG 03-019*	B-REGARD*	CIP 442903
CRIWAC 02-10	FARAA*	NS 001*	FIASO RED*	CIP 442291*
CRIWAC 03-10	TEKSANTOM	OK 03-015	TAG 03-030*	CIP 440069
CRIWAC 04-10	OGYEFO*	DOS 03-021	GWERI	CIP 440390*
CRIWAC 05-10*	OKUMKOM*	CARROT C	BD 96-029*	CIP 442462*
CRIWAC 06-10*	OTOO*	HUMBERCHERO*	FREMA*	CIP 442776
CRIWAC 07-10*	HISTARCH*	B/FASO 002*	DOS O3-006*	CIP 440062*
CRIWAC 08-10*	SAUTI*	FA 10-026*	NS 003	CIP 442589*
CRIWAC 09-10	APOMUDEN*	RESISTO*	AAT 03-004	CIP 442145
CRIWAC 10-10*	LIGRI*	NASPOT 1*	OK 03-021	CIP 442147*
CRIWAC 11-10*	BOHYE*	AAT 03-017	BOT 03-030*	CIP 440095*
CRIWAC 12-10*	PATRON*	OK 03-014	OK 03-017	CIP 441771
CRIWAC 13-10*	DADANUIE*	JONATHAN*	KAYIA WHITE	CIP 442901*
CRIWAC 14-10		H-ASIATOR*	UKEREWE*	CIP 443016*
CRIWAC 15-10*		TANZANIA	OK 03-018	CIP 440071*
CRIWAC 16-10		NINGSHU 1*		CIP 442896*
CRIWAC 17-10*		BOT 03-021		CIP 442162*
CRIWAC 18-10		KEMB 37		CIP 442775
CRIWAC 19-10*		BOT 03-028*		CIP 443027*
CRIWAC 20-10		BOT 03-020*		CIP 443129*
CRIWAC 21-10		J-ORANGE*		CIP 442264*
CRIWAC 22-10		BOT 03-027*		CIP 442654
CRIWAC 23-10*		ADA 001		CIP 443035*
CRIWAC 24-10*		DOS O3-017*		CIP 442913*
CRIWAC 25-10*		NAV 001		CIP 442237*
CRIWAC 26-10		AAT 03-025*		CIP 443019
CRIWAC 27-10*		B/FASO 001*		CIP 442850*
CRIWAC 28-10*		ZAMBEZI*		
CRIWAC 29-10*				
CRIWAC 30-10				
CRIWAC 31-10*				
CRIWAC 32-10*				

*List of the 76 sweetpotato accessions used for the molecular characterization.

constructed using GGE Biplot software (Yan and Kang, 2003).

Molecular characterization using SSR markers

Genetic material

A total of 76 sweetpotato accessions were used for the study (Table 1). These represent four groups, which were collections from International Potato Centre (CIP) gene bank in Ghana (19), local collection from farmers' field (19), local improved varieties (12), and local and exotic collections sourced from the National Agricultural Research Systems (NARS) or Programmes (26). These were planted at the CSIR-Crops Research Institute research field at Fumesua which is in the forest ecozone.

DNA extraction

This was done at the Molecular Laboratory of the CSIR-Crops Research Institute, Fumesua using the method of Egnin et al. (1998), in 2012. Two hundred milligram of young tender leaf tissue was weighed into 2 ml Eppendorf tube and was ground to powder after freeze drying with liquid nitrogen. Eight hundred microliter (800 µl) of buffer A [1M Tris HCl (pH 8) = 50 mM, 5 M NaCl = 300 mM, 0.5M EDTA (pH 8) = 20 mM, PVP = 20%, Sodium Metabisulphate = 1 g/100 ml, 20% Sercosine = 1.5] was added and incubated at 90°C for 10 min, and vortexed every 5 min. The suspension was cooled at room temperature for 2 min after which 400 µl of 5 M potassium acetate was added and then gently mixed by inversion 5 to 6 times. The suspension was then incubated on ice for 30 min with continuous shaking, followed by centrifuging at 13,000 rpm for 10 min. The upper phase was transferred to a new Eppendorf tube. One volume of cold isopropanol and $1/10^{th}$ of 3 M sodium acetate was added and mixed 10X by inverting the tube. This was followed by incubation at -20°C for 1 h, and centrifuging at 13,000 rpm for 10 min. The supernatant was poured off, the pellets were washed with 800 µl, 80% ethanol, and centrifuged at 14,000 rpm for 5 min. The alcohol was then discarded and the pellets were dried. Five

Table 2. List of agro-morphological descriptors and root quality traits.

Foliage descriptors	Agronomic descriptors and storage root morphology	Storage root quality descriptors
Vine inter-node length	Storage root shape (1 - 9)	Dry matter
Vine inter-node	Variability of storage root shape (3 - 7)	Fructose
Diameter	Storage root surface defects (0 - 8)	Glucose
	Storage root cortex thickness (1 - 9)	Sucrose
Vine colour (1 - 9)	Storage root skin colour (1- 9)	Maltose
Vine tip pubescence (0 - 7)	Storage root flesh colour (1 - 9)	Total sugars
Mature leaf size	Storage root formation (1 - 7)	Beta-carotene
Petiole length	Storage root stalk (0 - 9)	Starch
Petiole pigmentation (1 - 9)	Number of storage roots/plant	Protein
Vine weight	Number of storage root (marketable)	Calcium
General outline of leaf (1 - 7)	Number of storage root (unmarketable)	Magnesium
	Weight of storage root	Iron
	Weight of storage root (Marketable)	Zinc
	Weight of storage root (Unmarketable)	
	Variability of storage root size	
	Harvest index	
	Latex production in storage roots (3 - 7)	
	Oxidation in storage roots (3 - 7)	

Values in parenthesis indicate scale of measurement

hundred microliter (500 µl) of 1X TE buffer was used to dissolve the pellets, followed by the addition of 4 µl RNase A, and incubation at 37°C for 30 min. This was followed by addition of 250 µl of 7.5 M ammonium acetate. The suspension was incubated on ice for 3 min, and centrifuged at 13,000 rpm for five minutes, and then transferred into a new 1.5 ml tube. Seven hundred microliters (700 µl) of isopropanol was added, mixed by inversion (ice inversion), and centrifuged at 13,000 rpm for 15 min. The supernatant was discarded and the pellets were washed with 1 ml 80% ethanol by centrifuging at 14,000 rpm for five minutes. Again the supernatant was discarded, followed by drying of the pellets at room temperature. The DNA pellets were then dissolved in 200 µl 1X TE buffer, and its quality was checked on 0.8% agarose gel.

Genotyping with simple sequence repeats (SSR) markers

The genotyping was carried out at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India in 2012. A 3 ng sample of total genomic DNA from each of the samples was used for the polymerase chain reactions (PCRs). Twenty-five pairs of SSR markers confirmed for sweetpotato DNA amplification (Buteler et al., 1999; Diaz and Gruneberg, 2008; Tumwegamire et al., 2011) were used (Table 3). A final volume of the reaction mixture of 10 µL, which contains 25 mM MgCl2, 10x buffer, 10 mM deoxyribonucleotide triphosphate (dNTPS), 1 µM M13 FORWARD 700/800, 1 µM forward primer, 1 µM reverse primer, 5 U µL–1 *Taq* polymerase, 3 ng µL–1 DNA, and a double distilled water were used for the PCR. The amplification conditions were set up at 94°C for four minutes and denaturation at 94°C for one minute; annealing at between 56.0 to 62.0°C (depending on the annealing temperature of the primer); and polymerization at 72°C for one minute. Step 2 annealing was 56.0 to 62.0°C (depending on the annealing temperature of the primer) and was repeated 30 times, and a final extension at 72°C for 7 min. Amplification products were analyzed and read on a computer automated Licor (4300) DNA Analyzer (Licor Biosciences, Lincoln, NE) for 25 pairs of SSR primers.

Simple sequence repeats data scoring and analysis

Accessions amplified were noted and used to estimate percent accessions amplified. The number of alleles for each marker was noted and recorded. Markers that showed variation in at least 25% of the accessions were noted and their alleles were recorded as unique alleles. Percent unique alleles were computed as the ratio of number of unique alleles to the total number of alleles. Genotypes were scored for the presence (1) or absence (0) of each fragment. NTSYSpc software version 2.1 (Rohlf, 1993, 2002) was used to run the binary data. Jacard's coefficients (Jaccard, 1908) were used to construct a similarity matrices from the binary data by using SIMQUAL algorithm. This was followed by construction of a dendrogram using the unweighted paired group method average (UPGMA) applying the SHUAN algorithm. Principal Coordinate Analysis (PCoA) was performed from Jacard's coefficients using Genstat (Genstat, 2007). The polymorphic information content (PIC) was determined based on the approach and method of Weir (1996) as presented below:

$$PIC = 1 - \Sigma P_i^2$$

Where, Pi is the frequency of the ith allele.

Analysis of Molecular Variance (AMOVA) was also performed using Arlequin 3.1 version computer software (Excoffier et al., 2005), to quantify the genetic variation and relationship existing between and among the sweetpotato and the four population groups studied.

Table 3. List and description of the 25 SSR markers used to characterize the sweetpotato accessions.

Marker	Repeat	Primer F	Primer R	Size	Temperature (°C)	Reference
Ib3/24	Not determined	TTTGGCATGGGCCTGTATT	GTTCTTCTGCACTGCCTGATTC	-	56	Tseng et al. (2002)
Ib-316	(CT)3C(CT)8	CAAACGCACAACGCTGTC	CGCGTCCCGCTTATTTAAC	150	58	Buteler et al. (1999)
Ib-242	(CT)3CA(CT)11	GCGGAACGGACGAGAAAA	ATGGCAGAGTGAAAATGGAACA	135	58	Buteler et al. (1999)
Ib-297	(CT)13	GCAATTTCACACACAAACACG	CCCTTCTTCCACCACTTTCA	134	58	Buteler et al. (1999)
IBCIP-1	(ACC)7	CCCACCCTTCATTCCATTACT	GAACAACAACAAAAGGTAGAGCAG	140-153	63	Yañez (2002)
IBCIP-2	(ACC)2+6	GTAACCTGTCAGCCATCTGT	CCTAGTGGGTATTTGCAGAG	268-290		Yañez (2002)
IbC12	(TTC)6	TCTGAGCTTCTCAAACATGAAA	TGAGAATTCCTGGCAACCAT	94-108	55	Solis et al. (2009)
IbS01	(AGA)10	TCCTCCACCAGCTCTGATTC	CCATTGCAGAGCCATACTTG	210-228	56	Benavides et al. (2005)
IbR03	(GCG)5	GTAGAGTTGAAGAGCGAGCA	CCATAGACCCATTGATGAAG	245-263	56	Benavides et al. (2005)
IbS07	(TGTC)7	GCTTGCTTGTGGTTCGAT	CAAGTGAAGTGATGGCGTTT	177-194	55	Benavides et al. (2005)
IbS10	(CT)12	CTACGATCTCTCGGTGACG	CAGCTTCTCCACTCCCTAC	253-298	60	Benavides et al. (2005)
IbS11	(TTC)10	CCCTGCGAAATCGAAATCT	GGACTTCCTCTGCCTTGTTG	217-242	60	Benavides et al. (2005)
IbS17	(GGA)4	CAGAAGAGTACGTTGCTCAG	GCACAGTTCTCCATCCTT	158-198	58	Benavides et al. (2005)
IbS18	(TAGC)4	CTGAACCCGACAGCACAAG	GGGAAGTGACCGGACAAGA	232-242	58	Benavides et al. (2005)
IbR12	(CAG)5A	GATCGAGGAGAAGCTCCACA	GCCGGCAAATTAAGTCCATC	331-393	60	Benavides et al. (2005)
IbR13	(TTC)6	GTACCGAGCCAGACAGGATG	CCTTTGGGATTGGAACACAC	205-258	60	Benavides et al. (2005)
IbR14	(CCT)6	CCTATGGCAATTCGGTCACT	GGAACATTGCCTACACTCTG	216 -222	58	Benavides et al. (2005)
IbR16	(GATA)4	GACTTCCTTGGTGTAGTTGC	AGGGTTAAGCGGGAGACT	196-215	60	Benavides et al. (2005)
IbR19	(CAG)5b	GGCTAGTGGAGAAGGTCAA	AGAAGTAGAACTCCGTCACC	192-213	60	Benavides et al. (2005)
IbR21	(GAC)5	GACAGTCTCCTTCTCCCATA	CTGAAGCTCGTCGTCAAC	169-186	58	Benavides et al. (2005)
IbR20	(GGC)5	CTTCACTCTGCTCGCCATTA	GTACTTGGACGGGAGGATGA	194-212	48	Benavides et al. (2005)
J175	(AATC)4	ATCTATGAAATCCATCACTCTCG	ACTCAATTGTAAGCCAACCCTC	-	58	Solis et al. (2009)
J10A	(AAG)6	TCAACCACTTTCATTCACTCC	GTAATTCCACCTTGCGAAGC	-	58	Solis et al. (2010)
J67	(GAA)5	CACCCATTTGATCATCTCAACC	GGCTCTGAGCTTCCATTGTTAG	-	58	Solis et al. (2011)
J116A	(CCT)6	TCTTTTGCATCAAAGAAATCCA	CCTCAGCTTCTGGGAAACAG	-	58	Solis et al. (2012)

RESULTS

Phenotypic variation

The first six Principal Components (PCs) with Eigen values greater than 1.0 jointly explained 54.86% of the total variation in the accessions based on the 40 agro-morphological and physicochemical traits studied (Table 4). The traits of importance for the first component involved root traits of commercial interest. Beta-carotene, dry matter and total sugar contents were of importance for PC2.

The mean performance of the top 10 and the bottom 10 selected accessions for beta-carotene, dry matter and sugar contents are presented in Table 5. Significant differences were observed between the accessions for the traits. The range of values obtained for beta-carotene content was 6.83 - 33.67 (mg/100 g) DW. For dry matter content the range was 27 - 50%, and for sugar content the range was 9.83 - 30.34%. Ogyefo and Apomuden had the lowest and highest values for beta-carotene content. Apomuden had the lowest dry matter content whilest FA-10-026 had the highest dry matter content. CRIWAC 19-10 and CIP 442850 gave the lowest and highest sugar

Table 4. Principal component analysis of the agro-morphological and physico-chemical traits.

Trait	PC1	PC2	PC3	PC4	PC5	PC6
Root weight	-0.371	-0.091	-0.133	0.005	-0.028	-0.033
Marketable root wgt.	-0.362	-0.082	-0.139	0.021	-0.003	-0.008
Unmarketable yield.	-0.370	-0.094	-0.128	-0.002	-0.038	-0.043
β-carotene	0.168	-0.310	-0.128	-0.050	-0.030	0.024
Calcium	0.035	-0.300	0.205	0.155	-0.143	-0.063
Dry matter	0.168	-0.310	-0.128	-0.050	-0.030	0.024
Iron	0.172	-0.035	-0.416	0.063	-0.126	-0.099
Fructose	0.041	-0.259	-0.056	-0.257	0.343	0.173
Glucose	0.023	-0.316	0.020	-0.212	0.307	0.143
Maltose	-0.046	-0.284	0.310	0.119	-0.197	0.009
Magnesium	0.086	-0.308	-0.140	0.043	-0.049	-0.086
Rt. Oxidation	-0.064	0.020	-0.111	0.069	-0.032	0.414
Protein	0.114	0.071	-0.401	0.107	-0.178	-0.125
Starch	-0.141	0.144	0.361	0.201	-0.133	-0.018
Sucrose	0.018	-0.235	0.145	0.191	-0.336	-0.143
Total sugar	0.029	-0.404	0.133	0.030	-0.012	-0.025
Zinc	0.157	-0.021	-0.364	0.147	-0.249	-0.140
Outline of leaf	0.087	0.017	0.091	0.106	-0.052	0.090
Harvest index	-0.245	0.016	-0.012	-0.323	-0.165	-0.165
Latex in roots	-0.001	0.037	-0.015	-0.005	-0.153	0.128
Mature leaf size	-0.165	-0.031	-0.010	0.301	0.125	0.037
Storage root no.	-0.371	-0.091	-0.133	0.005	-0.028	-0.033
Marketable roots no.	-0.325	-0.043	-0.097	-0.087	-0.089	-0.26
Unmarketable rt. no.	-0.144	-0.101	-0.087	0.164	0.102	0.094
Petiole length	-0.119	0.079	-0.085	0.184	0.226	-0.156
Petiole pigmentation	-0.021	0.115	-0.001	-0.185	-0.153	0.260
Cortex thickness	-0.007	0.122	-0.051	0.104	-0.006	0.014
Flesh colour	0.118	0.124	0.061	0.023	0.040	-0.335
Root formation	0.070	0.014	-0.022	-0.025	-0.150	0.039
Root shape	-0.038	0.034	0.062	0.045	0.056	-0.310
Root skin colour	-0.011	-0.013	-0.114	0.008	-0.239	0.346
Root stalk	0.113	0.075	-0.029	0.129	0.146	0.144
Root surface defects	-0.056	-0.066	-0.131	-0.020	0.113	-0.083
Root shape	0.057	0.070	0.011	0.038	0.047	-0.148
Root size variability	-0.079	0.038	0.053	0.116	-0.201	0.168
Vine colour	-0.032	0.164	-0.009	-0.116	-0.261	0.254
Inter-node diameter	-0.105	-0.012	-0.051	-0.116	0.194	0.015
Inter-node length	-0.037	-0.067	-0.075	0.200	0.011	0.185
Vine tip pubescence	0.013	-0.017	-0.026	0.300	0.129	0.096
Vine weight	0.023	0.001	-0.039	0.441	0.156	0.137
Latent roots (Eigen vectors)	6.304	4.501	3.688	2.817	2.419	2.215
Variability (%)	15.76	11.25	9.22	7.04	6.05	5.54
Cumulative (%)	15.76	27.01	36.23	43.27	49.32	54.86

*Values in bold indicate the most relevant characters (>0.3) that contributed most to the variation of the particular component.

contents, respectively.

The dendrogram separated the selected accessions with a Euclidean similarity distance ranging from 1.00 to 0.93 (Figure 1). At 1.00 level of similarity, all the accessions were distinct from each other except BOT 03-030 and CIP 442896. Conversely, at about 0.93 levels of significance, two clusters were identified with all the accessions being similar except for CRIWAC 12-10. Five

Table 5. Performance of the top 10 and bottom 10 accessions selected based on beta-carotene, dry matter and sugar contents for construction of dendrogram and GGE biplot.

Accession	Total sugars (%)	Accession	Beta-Carotene (mg/100 g) DW	Dry matter (%)
Top 10 accessions			**Top 10 accessions**	**Bottom 10 accessions**
CIP 442850	30.34	APOMUDEN	33.67	27
APOMUDEN	28.97	RESISTO	27.53	38
B/FASO 002	24.04	B-REGARD	24.31	32
CIP 440062	23.30	CRIWAC 03-10	23.32	32
B-REGARD	22.90	CIP 442850	20.21	27
CRIWAC 12-10	22.84	CIP 443035	19.75	36
B/FASO 001	22.69	CRIWAC 05-10	19.00	39
TAG 03-030	21.92	BOT 03-028	17.83	38
CIP 440071	21.84	ZAMBEZI	17.58	40
UKEREWE	21.10	BOT 03-020	17.35	39
Bottom 10 accessions			**Bottom 10 accessions**	**Top 10 accessions**
CRIWAC 25-10	12.54	FA 10-026	16.75	50
CRIWAC 30-10	12.45	HISTARCH	9.85	45
DOS O3-006	12.35	CIP 442264	7.74	45
AAT 03-025	12.26	ABAIDOO 01	7.00	44
CRIWAC 11-10	12.26	BD 96-029	12.97	43
CIP 440095	12.06	OGYEFO	6.83	42
OGYEFO	11.67	FARAA	12.27	42
CIP 442264	11.06	CRIWAC 31-10	9.74	41
HISTARCH	10.43	CIP 442896	11.27	40
CRIWAC 19-10	9.83	BOT 03-030	17.35	39
SED (P<0.05)	2.62	SED (P<0.05)	1.52	3.00

main clusters A, B, C, D, and E at 94.5% (0.945) level of significance were identified. The first four clusters contained 1 to 5 accessions per cluster while the fifth cluster (E) had 26 accessions.

The distribution of PC1 and PC2 among the correlated traits, the selected accessions as well as between the selected accessions and the correlated traits are shown in Figure 2. Three groups were observed for the correlated traits. Beta-carotene, fructose, total sugars, calcium (Ca), and magnesium (Mg) were grouped together in Quadrant 1. Storage root yield traits were grouped in Quadrant 2, while only dry matter was found in Quadrant 3. Four groups were detected for the accessions. Beauregard and Apomuden were the most distantly related accessions in Quadrant 1, whilest CIP 440032 and CIP 442264 were the most distantly related accessions in Quadrant 2. The most distantly related accessions in the third and fourth quadrants were Histarch and Ogyefo, and CIP 442850 and TAG 03-030, respectively.

Genotypic variation

Out of the 25 SSR markers used to assess the genetic diversity of the sweetpotato accessions, only 20 produced amplifications. The five markers that did not produce amplification were IbS01, IbS07, IbS10, IbCIP2 and IbR20. A total of 87 polymorphic alleles were observed across the accessions and loci. These ranged from two to six with mean of 4.25. Markers IbS18 and IbR21 recorded the lowest number of alleles while Ib3/24, Ib316, Ib-297, IbC12, IbS11, J10A and J116A recorded the highest number of alleles (Table 6). Out of the 87 alleles revealed by the 20 SSR markers across accessions and loci, 40 (45.98%) were unique alleles and the average number of unique alleles was two. IBCIP-1, IbC12 and J67 produced no unique alleles while Ib3/24 recorded the highest number (5) of unique alleles followed by Ib-297 and J10A with 4 unique alleles. However, Ib3/24 obtained the highest percent polymorphism (83.33%), followed by IbR14 (75.00%). The range and the average percent polymorphism were 0 to 83.33 and 45.50%, respectively. The PIC values were high and ranged between 0.62 for J67 and 0.96 for IbR16 and IbR19, with a mean of 0.84. The highest amplification was recorded by IbR14 (90.91%) followed by IbR316 and J67 with value of 77.92%. IbR16 recorded the lowest amplification. Base range for the markers was highest and lowest for IbR03 (262-277) and J175 (133-147).

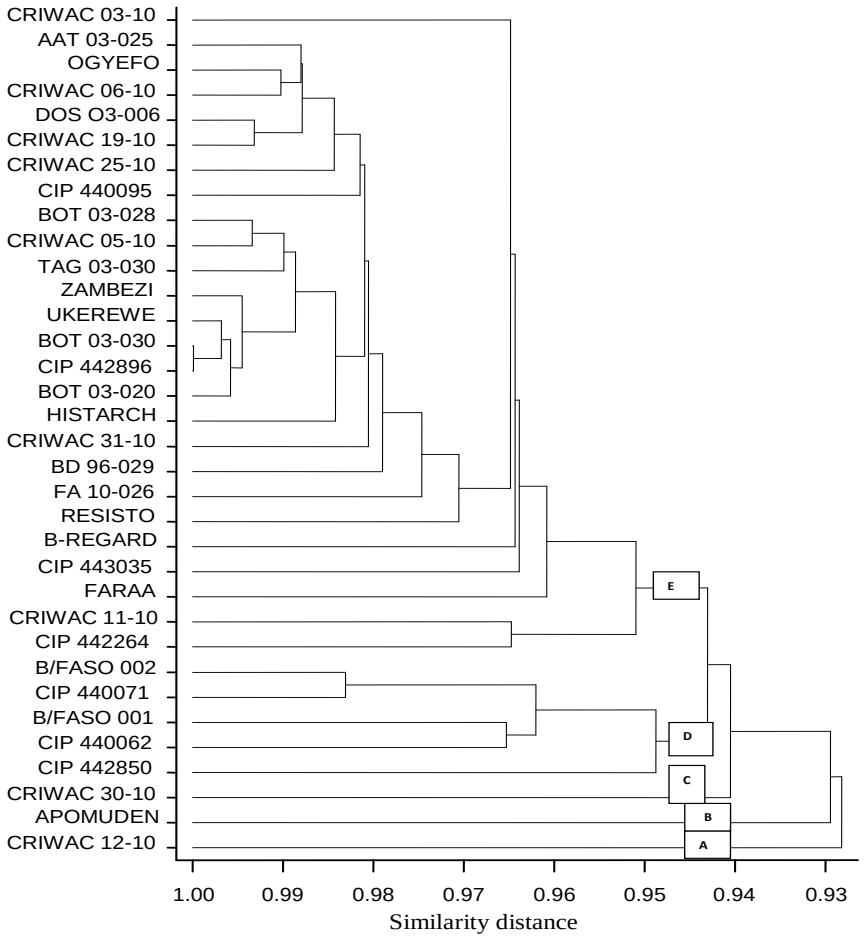

Figure 1. Dendrogram constructed based on the selected accessions and traits important for PC1 and PC2.

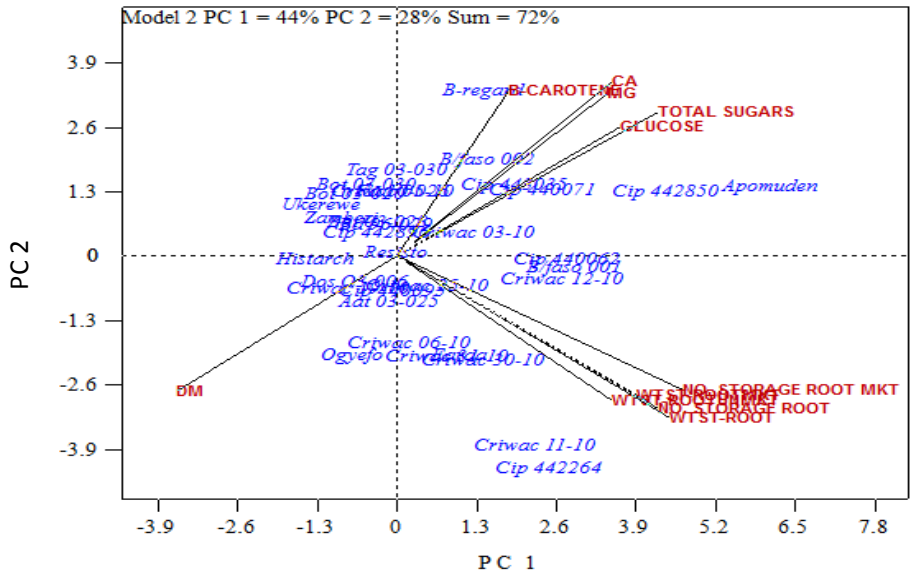

Figure 2. Biplot showing relationship between correlated traits and selected accessions.

Table 6. Polymorphism and base range of the 20 SSR markers.

Marker	Accessions Amplified	Accessions Amplified (%)	No. of Alleles	Loci across Accessions	No. of unique Alleles	Percent Polymorphism	PIC	Base Range
Ib3/24	47	61.04	6	1 - 2	5	83.33	0.87	136 - 150
Ib-316	60	77.92	6	1 - 4	2	33.33	0.66	152 - 168
Ib-242	39	50.65	4	1 - 4	2	50.00	0.90	135 - 155
Ib-297	40	51.95	6	1 - 4	4	66.67	0.86	151 - 183
IBCIP-1	38	49.35	4	1 - 4	0	0.00	0.89	154 - 166
IbC12	54	70.13	6	2 - 6	0	0.00	0.72	108 - 123
IbR03	43	55.84	4	1 - 4	1	25.00	0.86	262 - 277
IbS11	47	61.04	6	1 - 6	2	33.33	0.88	241 - 256
IbS17	51	66.23	5	1 - 3	3	60.00	0.84	181 - 202
IbS18	40	51.95	2	1 - 2	1	50.00	0.87	249 - 253
IbR12	57	74.03	4	1 - 3	2	50.00	0.73	336 - 357
IbR13	32	41.56	4	1 - 4	2	50.00	0.91	222 - 231
IbR14	70	90.91	4	1 - 2	3	75.00	0.75	179 - 188
IbR16	30	38.96	3	1 - 3	2	66.67	0.96	220 - 230
IbR19	31	40.26	3	1 - 3	1	33.33	0.96	212 - 223
IbR21	42	54.55	2	1 - 2	1	50.00	0.84	182 - 203
J175	46	59.74	3	1 - 3	2	66.67	0.93	133 - 147
J10A	38	49.35	6	1 - 4	4	66.67	0.91	192 - 220
J67	60	77.92	3	1 - 3	0	0.00	0.62	191 - 212
J116A	50	64.94	6	1 - 5	3	50.00	0.84	206 - 229
Mean	45.75	59.42	4.35	1.1 - 3.4	2	45.50	0.84	192.1 - 208.7

IbS11 recorded the highest number of loci (1- 6) across accessions followed by IbC12 (2 - 6). The lowest number of loci (1-2) across accessions was produced by Ib3-24, IbS18, IbR14 and IBR21.

Principal coordinate analysis (PCoA), which was determined from the similarity coefficients is graphically presented in Figure 3 (showing diversity in sweetpotato accessions), and Figure 4 (showing diversity in the group structure of the sweetpotato accessions). The two axes explained 45.21% of the total similarity (54.79% of total variation) with the first axis (PCoA1) accounting for 28.08% and the second (PCoA2) accounting for 17.13%. The 76 sweetpotato accessions investigated by PCoA did not form clear groups according to the group structure both within and between.

The dendrogram constructed separated the 76 sweetpotato accessions into major clusters at different similarity levels ranging from 0.00 to 1.00 (Figure 5). At slightly greater than 0.00 similarity level, two major clusters were observed. CIP 6 (CIP 442462) constitutes the first cluster while the second cluster consisted of the other 75 accessions. At 0.25 similarity level, seven major clusters were observed while 17 were found at 0.50 similarity level. The markers fully discriminated the 76 sweetpotato accessions by the 1.00 level of similarity except for two improved cultivars LOCIMP2 (Santompona) and LOCIMP10 (Otoo). The primers, however, did not fully discriminate the accessions into the different group structures.

Significant differences were observed between the sweetpotato accession within the groups ($P<0.01$) as well as between the groups ($P<0.05$)

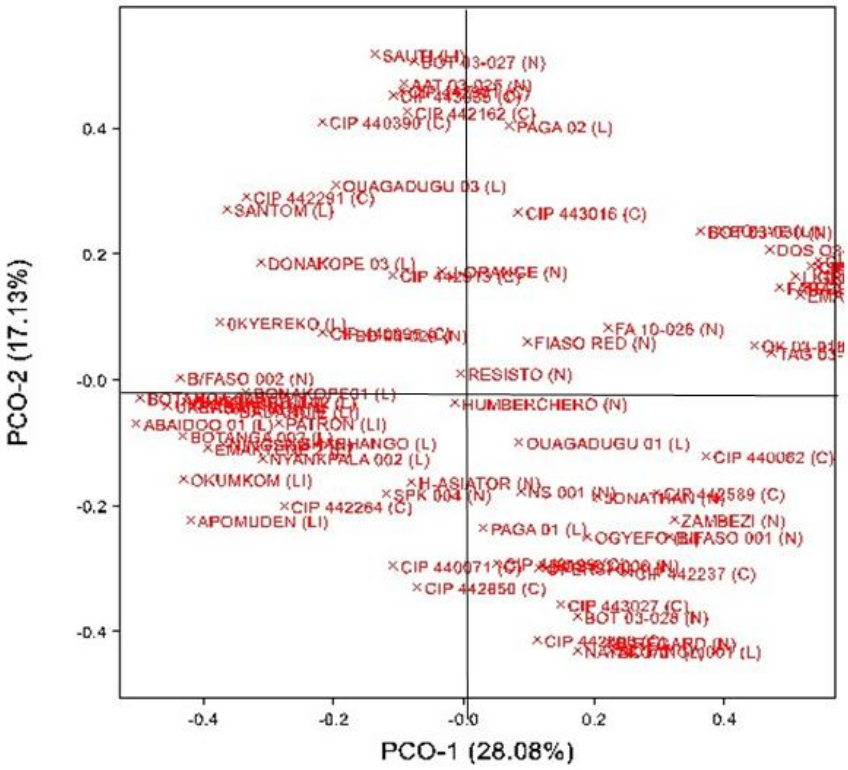

Figure 3. Principal coordinates analysis from similarity coefficients showing diversity in the 76 sweetpotato accessions.

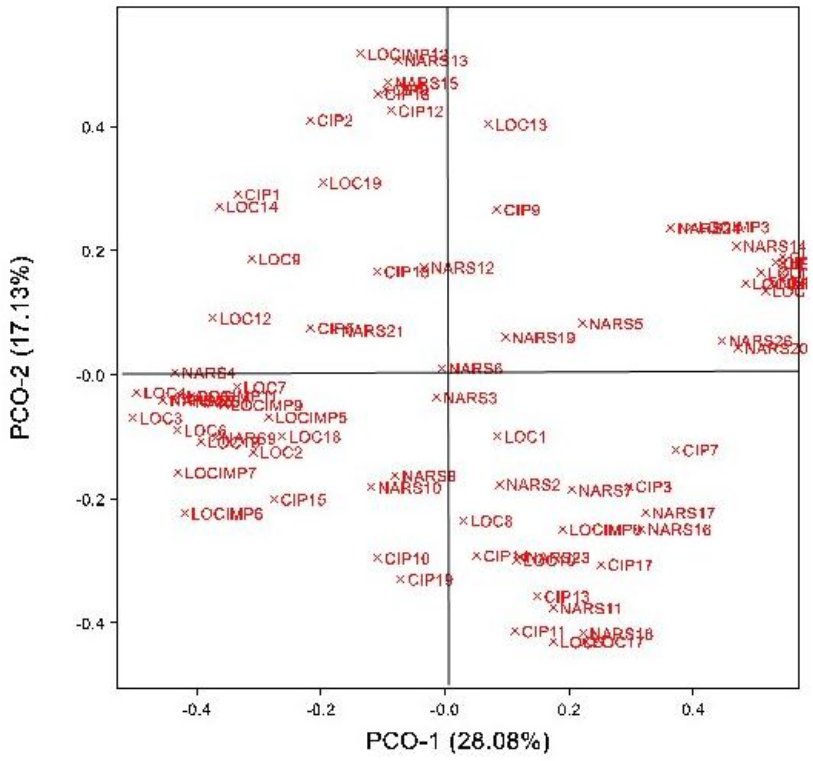

Figure 4. Principal coordinates analysis from similarity coefficients of 76 sweetpotato accessions showing the diversity in the group structure.

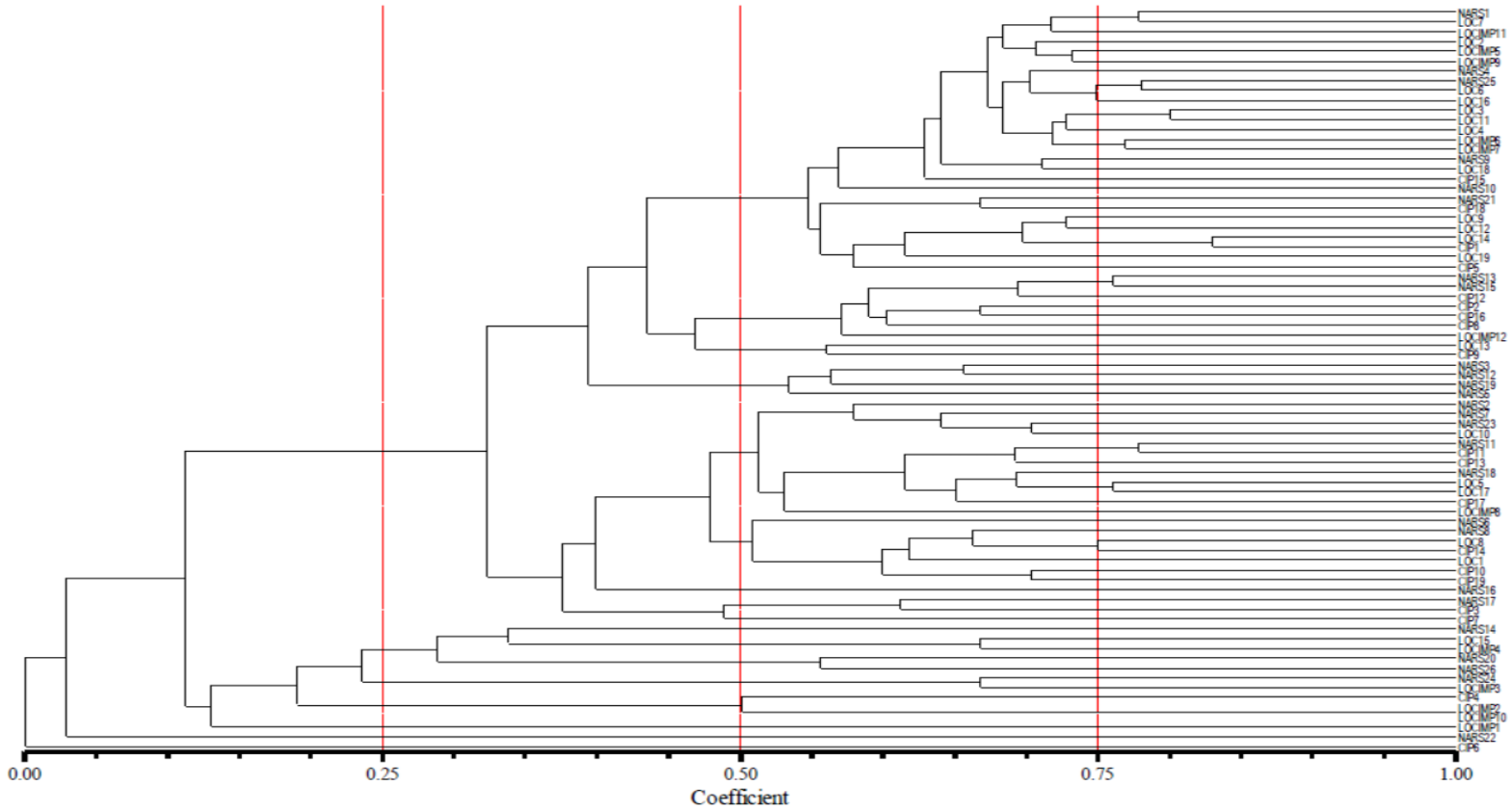

Figure 5. Dendrogram showing genetic relationships among 76 sweetpotato accessions.

as shown in Table 7. The differences observed within the groups however accounted for a greater percentage (97.12%) of variation observed than that found between the groups (2.88%).

DISCUSSION

Variability was observed in all the physico-chemical traits and 20 out of the 27 agro-morphological traits. This indicates a high degree of agro-morphological and physicochemical polymorphism among the accessions. Diversity in flesh colour (beta-carotene content) of sweetpotato cultivars has been reported (Warammboi et al., 2011). Sugar content in sweetpotato is also reported to be cultivar-dependent (Ravindran et al., 1995; Aina et al., 2009), and showed high levels of polymorphism with SSR markers. This confirms the discriminatory capacity of the SSR markers on sweetpotato (Gichuru et al., 2006; Tumwegamire et al., 2011). High level of polymorphism was observed in this study with an allele range of two to six alleles per SSR marker and this is in agreement with Yada et al. (2010). Buteler et al. (1999) obtained high polymorphism with an allele range of 3 to 10. Somé et al. (2014), also reported 1 to 8 alleles. A range of 2 to 11 alleles was reported by Tumwegamire et al. (2011). A lower level of polymorphism, ranging between one and

Table 7. Analysis of molecular variance (AMOVA) for the 76 sweetpotato accession.

Source of variation	Df	Sum of squares	Variance components	Percentage of Variation
Among groups	3	55.894	0.35619*	2.88
Within groups	72	865.198	12.01664**	97.12
Total	75	921.092	12.37284	-

*Significant at 0.05 **Significant at 0.01.

four alleles per SSR locus has also been reported (Hwang et al., 2002). Differences observed may be attributed to the use of different SSR primers, sweetpotato genotypes and annealing temperatures. Varying number of SSR primers used in diversity studies may also account for the differences in observations.

Hwang et al. (2002) attributed high level of polymorphism to large genome size and heterozygosity of sweetpotato. It should also be noted that genetic diversity due to heterozygosity in sweetpotato is driven by both the mating system (outcrossing in combination with self-incompatibility) and the high ploidy level of the crop (autohexaploid) (Tumwegamire et al., 2011). The AMOVA and ANOVA results also indicated significant differences within and between the different sweetpotato groups studied. These results demonstrate significant genetic diversity and indicates that meaningful selection and improvement of these traits is possible (Mohammed et al., 2012; Nwangburuka and Denton, 2012). Furthermore, these demonstrate the existence of diversity at the individual genotype level that can be exploited to obtain trait combinations in specific varieties. In addition, the divergences indicate that it is possible to select contrasting parents from these accessions for improvement of beta-carotene, sugar and dry matter contents in sweetpotato. These results agree with results of other researchers (Zhang et al., 2000; 2001; Gichuki et al., 2003; Gichuru et al., 2006; Abdelhameed et al., 2007; Grüneberg et al., 2009; Tumwegamire et al., 2011).

PIC is a measure of the discriminatory capacity of a marker (Jia et al., 2009). According to Heng-Sheng et al. (2012), a PIC value greater than 0.5 is high, and any marker with such value may be effective in genetic diversity study. In this study, the PIC value for all the markers that showed amplification were greater than 0.5. This implies that the values which ranged from 0.62 to 0.96 with mean of 0.84 were very high indicating a high discriminating power of the SSR markers used. These values are greater than range and mean of 0 to 0.88, and 0.72 reported by Somé et al. (2014). Based on the number of unique alleles and the PIC values, all the SSR markers that showed amplification were very effective in discriminating among the sweetpotato accessions. In spite of this, the markers did not discriminate between cultivars LOCIMP2 (Santompona) and LOCIMP10 (Otoo) at 1.00 level of similarity even though these cultivars are agro-morphologically distinct. It is probable that no were repeats found that could differentiate the two cultivars and therefore, more SSR markers need to be used in the future to have a full diversity study.

Genetic relationships between traits may result from pleiotropic gene effects, linkage of two genes, linkage disequilibrium and epistatic effects of different genes or environmental influences (Falconer and Mackay, 1996). The strong negative relationship found for sugar content and dry matter content as depicted in the GGE biplot indicates that it is possible to develop non-sweet high dry matter sweetpotato varieties. A similar observation was made by Gruneberg et al. (2009), who also reported that development of non-sweet sweetpotato varieties should not be too difficult. However, developing non-sweet, high dry matter and high beta-carotene sweetpotato varieties could be challenging due to the strong negative association between dry matter content and beta-carotene content, and the positive association existing between beta-carotene and the sugar content. Breeding for such cultivars may require many cycles of selection and hybridization to break genetic linkages associated with the traits. However, beta-carotene seems to be controlled by a limited number of genes and should be easy to manipulate.

Conclusion

This study provides estimate on the level of genetic variation among sweetpotato accessions in Ghana. Significant genetic diversity was found between the accessions for dry matter, beta-carotene and sugar content. This information can be used in sweetpotato germplasm management and improvement in Ghana. The study also affirmed the discriminatory capacity of the SSR markers, and the agro-morphological and physico-chemical markers for sweetpotato characterization especially for breeding programmes with limited resources. Sufficient useful genetic variation is present in the accessions studied which may be exploited to provide for substantial amount of improvement through selection of superior genotypes. The strong negative association between dry matter and sugar content indicates that it is feasible to develop non-sweet high dry matter sweet potato cultivars which are the preferred sweetpotato varieties in Ghana. However, developing non-sweet, high dry matter and high beta-carotene sweetpotato varieties

may require many cycles of selection due to the strong negative association between dry matter content and beta-carotene content.

Conflict of Interests

The authors declare they have no conflict of interests.

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