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GENETIC ANALYSIS OF YIELD AND FLESH COLOUR IN SWEETPOTATO

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ABSTRACT

Pre-breeding information on the inheritance mechanism of important sweetpotato (*Ipomoea batatas* L.) agronomic traits is still limited. This study aimed at assessing the inheritance of five sweetpotato agronomic traits, *viz.* marketable fresh root yield (MFRY) and number (MNR), total fresh root yield (TFRY) and number (TNR) and root β-carotene content (RBCC). A 5 x 5 full diallel was performed and F_1 progenies, evaluated in two environments alongside the parents. The data were subjected to ANOVA and DiallelSAS-05 Griffing's method 1. Simple Sequence Repeat (SSR) based genetic distance and cluster analysis were performed on the parental lines using Jaccard's coefficient and the unweighted pair group method with arithmetic averages (UPGMA). Significant differences ($P<0.01$) were detected among the genotypes for MFRY, MNR, TFRY, TNR and RBCC. Significant general and specific combining ability ($P<0.01$) effects were observed for all five traits. Additive gene action was predominantly involved in the inheritance of these traits. High broad sense heritability values were observed for the four yield parameters and for RBCC. The Jaccard's similarity coefficient indicated moderate to low genetic similarity distances among the parents, implying high diversity. The knowledge on the inheritance and diversity of the parental genotypes enables more effective choice of parents in breeding improved varieties.

Key Words: β-carotene, combining ability, heritability, root yield, genetic diversity

RÉSUMÉ

Les informations préliminaires sur le mode de transmission des caractères agronomiques importants chez la patate douce (*Ipomoea batatas* L.) sont encore très limitées. Cette étude vise à évaluer la transmission de cinq traits agronomiques chez la patate douce. *viz.* Le rendement de tubercules fraîches à valeur marchande (MFRY) et leur nombre (MNR), le rendement total de tubercules frais (TFRY) et leur nombre (TNR), de même que la teneur en β-carotene des tubercules (RBCC). Une série de croisement diallel 5 x 5 avec croisements réciproques a été réalisée et les descendants de génération F1 ont été évalués ensemble avec les parents dans deux environnements. Les données collectées ont été soumises à une analyse de variances et à une analyse DiallelSAS-05 méthode 1 de Griffing. Les distances génétiques basées sur des Répétitions de Séquences Simples (SSR) a été calculées et une classification numérique à été réalisée sur les lignées parentales. Le coefficient de Jaccard et la méthode des paires-groupes non pondérés avec la moyenne arithmétique (UPGMA) ont été utilisées à cet effet. Des différences significatives ($P<0.01$) ont été observées entre les génotypes pour MFRY, MNR, TFRY, TNR et RBCC. Des effets significatifs de l'habileté de combinaison générale ($P<0.01$) ont été observés sur tous les 5 traits. L'effet

additif des gènes était prédominant. Des valeurs d'héritabilité au sens large ont été observées pour quatre paramètres de rendements en tubercules et pour RBCC. Le coefficient de similarité de Jaccard a indiqué une des distances de similarité génétique faibles ou modérées entre les parents, ce qui suggère une grande diversité. Les connaissances sur le mode transmission et la diversité des lignées parentales permet des choix plus efficients des parents à utiliser dans un programme d'amélioration génétique.

Mots Clés: β-carotene, habileté de combinaison, héritabilité, rendement en tubercules, diversité génétique

INTRODUCTION

Sweetpotato (*Ipomoea batatas* L.) is the third most important root and tuber crop in the world, following potato (*Solanum tuberosum* L.) and cassava (*Manihot esculenta* Crantz). Over 95% of the world's sweetpotato is produced in developing countries, where it is considered to be a staple food crop (FAOSTAT, 2012). The root crop is very popular among poor farmers, and has the ability to grow in conditions with limited agricultural inputs and labour requirements (Lebot, 2009). Sweetpotato roots and leaves combine nutritional quality with high levels of edible energy, which is needed to combat food insecurity and malnutrition (Bovell-Benjamin, 2007). The incidence of vitamin A deficiency (VAD) in Africa and Asia is worrying. South Africa alone has reported that 43.6% of children between 1 and 5 years old (Shisana *et al.*, 2013) and 27% of women at the reproductive age (Labadarios *et al*., 2007) are vitamin A deficient.

As a cheaper and sustainable measure to combat and prevent VAD, orange-fleshed sweetpotato (OFSP) cultivars rich in β-carotene (precursor of pro-vitamin A) are being used to mitigate micronutrient deficiency in Africa (Nagujja and Yanggen, 2005; Van Jaarsveld *et al.*, 2005; Laurie and Faber, 2008) and West Asia (Mukherjee and Ilangantileke, 2001). Although bio-fortification of sweetpotato in South Africa through conventional breeding has resulted in the release of superior cultivars, the pre-breeding information on the inheritance mechanism of the essential traits is still limited.

Due to self- and cross-incompatibility in sweetpotato, caused by incompatibility alleles (Shiotani and Kawase, 1987), polycross and diallel crossing are the mating designs commonly used in the breeding (Hwang *et al.*, 2002; Laurie *et al.*, 2009) combined with genetic analysis (Mwanga *et al.*, 2002; Chiona, 2010; Shumbusha *et al.*, 2014; Musembi *et al.*, 2015). Diallel analysis is a breeding tool that makes use of direct crosses between selected genotypes, in order to obtain information on the combining abilities, which are then used to predict the performance of the progenies (Buteler *et al.*, 2002; Bertan *et al.*, 2007). Diallel analysis has been used in sweetpotato to determine the general combining ability (GCA) and specific combining ability (SCA) of yield, flesh colour, dry matter content and harvest index in the root (Chiona, 2010; Tumwegamire *et al.*, 2011; Shumbusha *et al.*, 2014).

The understanding of the genetic relationship between parental lines is particularly important in the selection of lines capable of combining and creating superior progeny in a hybrid combination. To maximise successful improvement and genetic gains in the breeding programmes, genetic variation is necessary for effective selection of parental genotypes to be included in the breeding programs (Tumwegamire *et al.*, 2011).

Both morphological and molecular analyses have been used in the past, to select parental lines that would maximise the desired traits in the resulting progenies. However, molecular markers remain the most reliable method, as these are not affected by the environment. Simple Sequence Repeat (SSR) markers have become tools of choice for many crops, due to their analytical resolution (Edward and McCouch, 2007), easy scoring and interpretation of results, especially in crops with high ploidy levels such as sweetpotato (hexaploid, 2n=6x=90; Jones, 1965).

Although over 1600 EST-based SSR markers have been identified for sweetpotato (Schafleitner *et al.*, 2010), only a limited number of SSR markers have been tested and used in diversity analysis of sweetpotato by various authors (Zhang *et al.*, 1999; Hwang *et al.*, 2002; Gichuru *et al.*, 2005;

Elameen *et al.*, 2008; Veasey *et al.*, 2008; Karuri *et al.*, 2010; Tumwegamire *et al.*, 2011; Gwandu *et al.*, 2012).

In this study SSR markers were used to establish genetic relationships among selected sweetpotato parental lines that were subsequently used in a 5 x 5 diallel cross. Furthermore, the combining abilities of the parents and heritability of yield and flesh colour in sweetpotato root were determined across two environments.

MATERIALS AND METHODS

Parental lines and crossing. Eight sweetpotato breeding lines, commonly used by the Agricultural Research Council-Vegetable and Ornamental Plants (ARC-VOP), Roodeplaat Experimental Station (25.604 p S 28.345 p E). sweetpotato breeding programme were selected according to yield and flesh colour attributes (Table 1). The parental lines were hand-crossed in all possible ways. Due to cross incompatibility between some genotypes, a full diallel was obtained from five parental genotypes (Monate, Ndou, Khano, Resisto and W-119). These generated 20 first generation families (F_1). The F_1 seeds were scarified by immersion in 98% sulphuric acid for 20 to 25 minutes, in order to remove the hard seed coating to enhance germination (Montelaro and Miller, 1951). The seeds were thoroughly rinsed with running tap water and then placed on wet filter paper, in petri-dishes kept in the dark for 48 hours to sprout. These were then planted in trays filled with 50:50 mixture of Hygromix growing medium (Hygrotech, South Africa) and vermiculite. At three to four nodes stage, the seedlings were transplanted to the field for bulking of vine material.

Field evaluation. Five individuals from each cross combination were selected from the bulking plots, based on the plant vigour and best chance of survival. Field trials were established at two diverse agro-ecologies *viz*. Roodeplaat (North of Pretoria, Gauteng; 25.604 p S 28.345 p E) and Owen Sithole College of Agriculture (Empangeni, Kwa-Zulu Natal; 28. 725 p S 31.898 p E) in South Africa in 2013 (Table 2). The experiments were conducted in a randomised complete block design (RCBD), with three replications. Standard agronomic practices for sweetpotato production were followed (Van den Berg and Laurie, 2004). NPK (1:0:1) and 12% Calsiphos (Petrow Agri, South Africa) were broadcast in the field at a rate of 500 kg ha^{-1}, potassium chloride at 200 kg ha^{-1} and 28% limestone ammonium nitrate (LAN, 28) (Petrow Agri, South Africa) at a rate of 120 kg ha^{-1}.

Irrigation during the growing season was performed once to twice a week, depending on the rainfall. Each plot contained five cuttings, with a spacing of 30 cm x 1 m. Border plants were added at each end of the plot.

At maturity (approximately 165 days after transplanting), data were collected on total fresh root mass and number (TFRY and TNR), and marketable fresh root mass and number (MFRY and MNR). Marketable roots were considered as roots between 100 and 1200 g and with a regular shape. The root β-carotene content (RBCC) was estimated by cutting the fresh root longitudinally, comparing the flesh colour to the colour chart used by Burgos *et al.* (2009), and recording the corresponding β-carotene value indicated by the colour chart.

Statistical analysis. The data were subjected to analysis of variance (ANOVA) using GenStat® 16th Edition (VSN International Ltd., Hempstead). General combining ability (GCA) and specific combining ability (SCA) were estimated following Model 1 of Griffing (1956), including the parents, F_1 progenies and reciprocal effects, in DiallelSAS-05 (Statistical Analysis Software Institute; Zhang *et al*., 2004). DiallelSAS-05 uses a linear model for estimating combining ability, which apportions the total genotypic variance into variance components (Zhang *et al.*, 2004), including GCA, SCA and reciprocal effects. The model is represented by Equation 1:

$$Y_{ijkml} = \mu + S_i + B_{j(i)} + GCA_K + GCA_l + SCA_{kl} + S * GCA_{ik} + S * GCA_{il} + S * GCA_{ikl} + R_{ijklm} \quad \text{..... Equation 1}$$

Where:

Y_{ijklm} = the *m*th observation of the *j*th block for *kl*th cross in the *i*th site; μ is the overall mean; *Si* = the th fixed site (environment) effect *i* = *l* to *t*; $B_{j(i)}$ = the fixed effect of the *j*th block within the *i*th site, *j* = *l* to *b*; GCA_k, GCA_l = the random general combining ability (GCA) effect of the *k*th female or *l*th more ~ Normally, Independently Distributed (NID) (0, σ^2_G), *k, l* = 1 to *p* and *k*<*l*; SCA_{kl} = the random specific combining ability (SCA) effect of the *k*th and *l*th parents (*k*=*l*) NID (0, σ^2_S); $S*GCA_{ik}$, $S*GCA_{il}$, = the random GCA by site interaction ~ NID (0, σ^2_{TG}); $S*GCA_{jkl}$ = the random SCA by site interaction effect ~ NID (0, σ^2_{TS}) and R_{ijklm} = the random error term ~ NID (0, σ^2_{TS}).

The predominant gene effect in the inheritance of a trait was determined according to the function proposed by Baker (1978):

$$2\sigma^2_{gca}/(2\sigma^2_{gca} + \sigma^2_{sca} \quad \text{..... Equation 2}$$

Where σ^2_{gca} is the genetic variance component for general combining ability, and σ^2_{sca} is the genetic variance component for specific combining ability.

The narrow sense heritability (h^2) for MFRY, MNR, TFRY, TNR and RBCC was estimated using Equation 3 (Dudley and Moll, 1969):

$$h^2 = \frac{Cov\,(G,\ P)}{\sigma^2_P} = \frac{\sigma^2_G}{\sigma^2_G + \frac{\sigma^2_e}{rl} + \frac{\sigma^2_{GL}}{l}} \quad \text{..Equation 3}$$

The genotypic variance (σ^2_G) was computed as:

$$\sigma^2_G = \sigma^2_F = \frac{MS_F - MS_{FL}}{rl}; \quad \text{…..… Equation 4}$$

Where:

σ^2_F represents the family variance; MS_F and MS_{FL} = the mean squares for family and family by location, σ^2_e = the error variance; σ^2_{GL} = the genotypic by site (environment) variance interaction, represents the number of replicates (blocks) and the number of sites (environments).

SSR-based genetic relationships. For DNA extraction, leaf samples were obtained from the eight parental lines (Monate, Resisto, W-119, Ndou and Khano, Purple_Sunset, Mvuvhelo, 1999_5_1), collected from the glasshouse of ARC-VOPI. Two to three leaf discs from the younger leaves were harvested in 2 ml Eppendorf tubes (Sigma-Aldrich, Missouri, USA), and kept in ice for DNA extraction. DNA was isolated using the modified CTAB method, described by Edwards *et al.* (1991). The DNA concentration for each sample was determined using the nanodrop 1000 ND reader (Thermo Scientific, Wilmington, USA), and compared to three dilutions of λ standard DNA (150 ng, 75 ng and 37.5 ng); and visualised using electrophoresis on 1% agarose gel containing ethidium bromide (Sigma-Aldrich, Missouri, USA).

The DNA samples were subjected to genotyping, using 20 published SSR markers (Table 2). A 25 µl Polymerase Chain Reaction (PCR) containing 0.2 units of MyTaq polymerase (Biogenotype, Massachussetts, USA), 1 x MyTaq reaction buffer (5 mM dNTPs and 15 mM $MgCl_2$), 0.2 µM of forward and reverse primer and 10 ng of DNA was performed for every primer pair. Individual reactions were amplified in the Gene Amp® PCR system 2700 machine (Applied Biosystems, California, USA), with the following conditions: an initial denaturation at 95 °C for 3 minutes, followed by 35 cycles of denaturation 95 °C for 15 seconds, primer annealing at optimal temperature for 15 seconds and primer extension at 72 °C for 20 seconds.

A final extension at 72 °C for 5 minutes was included. Thereafter, 3 µl of 3 x STR loading solution (10 mM NaOH, 95% formamide, 0.05 % bromophenol blue, 0.05% xylene cyanol) was added to each PCR product before electrophoresis through a 3 % (w/v) agarose gel at 90 Volts for 30 minutes to confirm amplification of each primer alongside a 50 bp and 100bp ladder [Thermo Scientific, Lithuania (EU)]. Five micro-litres of each successful amplicon was further separated on 6% non-denaturing polyacrylamide gels, for 3 hours at 600 volts and visualised by silver staining. All SSR markers (Table 3) were screened across all genotypes listed in Table 1.

Amplification bands were numbered according to their migration within the gel. It was assumed that bands of the same migration distance in different genotypes, were identical.

TABLE 1. Origin, yield classification and flesh colour of parental genotypes selected for the study

Entry	Line	Flesh colour	Yield	Source
1.	Monate	Cream (C)	High	ARC
2.	Ndou	Cream	High	ARC
3.	Khano	Orange (O)	High	ARC
4.	Resisto	Orange	Low	USA – CIP collection
5.	W-119	Orange	Low	USA – CIP collection
6.	Mvuvhelo	Cream	High	ARC
7.	Purple sunset	Orange	Medium	ARC
8.	1999_5_1	Orange	Medium	ARC

ARC = Agricultural Research Council of South Africa; CIP = Centro Internacional de la Papa (International Potato Center); USA = United States of America

TABLE 2. Location and environmental conditions of the two experimental sites (Pretoria, Roodeplaat and KwaZulu-Natal, Empangeni) in South Africa during the growing period

Sites	Roodeplaat	Empangeni
Location	25.604 p S 28.345 p E	28. 725 p S 31.898 p E
Planting date	21 November 2012	18 January 2013
Harvesting date	24 June 2013	2 September 2013
Climatic area	Warm temperate	Humid subtropical
Altitude (m)	1168m	105m
Average temperature (R"C)	18.3	20.3
Total rainfall (mm)	299.95	350.2
Humidity (%)	51.7	71.1
Soil type	Clay loam	Sandy loam

Source: Climatic database ARC-Institute for Climate and Water, South Africa in 2014

For each genotype, the presence of each band was determined and designated "1", and "0" when absent. Markers that produced the expected size (100–500 bp) of amplification product were recorded and the polymorphism information content (PIC) calculated using the formula proposed by Roldàn-Ruiz *et al.* (2000):

$PIC = 2fi(1 - fi)$ …..…… Equation 5

Where:

i = the marker in study, fi is the frequency of the allele (band) present and $(1\text{-}fi)$ = frequency of the null allele. *PIC* values vary from zero (0) to 0.5, with zero being the minimum and 0.5 the maximum value.

Binary data were used to generate Nei similarity matrix through the similarity qualitative data (SimQual) function (Sneath and Sokal, 1973) using the Jaccard's similarity coefficient between parental pairs (Mohammadi and Prasana, 2003):

$$GS = \frac{2N_{ij}}{N_i + N_j} \quad \text{…….....…… Equation 6}$$

Where:

N_{ij} = the number of bands common to parent i and j and N_i and N_j the number of bands specific to parent i and j, respectively. SSR based genetic diversity *(GD)* is given as: $GD = 1 - GS$. Cluster analyses were computed using the unweighted

TABLE 3. SSR primers pairs used to amplify SSR regions in the DNA of sweetpotato genotypes

Primer name	Primer sequence	Microsatellite repeat	Ta[1] (°C)	Expected size	Reference
IBSSR18	F: GATCTTGAATTAGCCCAC R: AGATGGATGACCGTATGC	$(GA)_7(AG)_5(GA)_4$	58	90-110	Hu *et al*. (2004)
IBSSR19	F: GCGAATCAAGTCTTTTGTCCAC R: GGGACTGTCCTTTGGGTATG	$(CA)_{25}$	76	171-195	Hu *et al*. (2004)
IBSSR21	F: AAACAACCAACGGGTCTTTGC R:CTCTAGGGTCGCCATAAAAATCAC	$(CA)_{14}$	64	215-240	Hu *et al*. (2004)
IBSSR24	F: CCATGCCCTCTGATTGAACG R: GAACCCAATGAACTTCGCCAC	$(GA)_{10}N(GA)_5$	66	180-195	Hu *et al*. (2004)
IBSSR25	F: TCTTGCTTCCTAGTCGGCTG R:CAGGTGAACCAAGTGACCT	$(GT)_{15}T2(TG)_9$	60	110-140	Hu *et al*. (2004)
IBSSR27	F: GTGTTTATCACATCGTTTTCTG R: GGCTCGTACAATTTTCAAAG	$(TA)_6(CA)_{16}(TA)_3$	60	120-154	Hu *et al*. (2004)
IB-242	F: GCGGAACGGACGAGAAAA R: ATGGCAGAGTGAAAATGGACCA	$(CT)_3CA(CT)_{11}$	58	95-135	Buteler *et al*. (1999); Tumwegamire et al. (2011)
IB-248	F: GAGAGGCCATTGAAGAGGAA R: AAGGACCACCGTAAATCCAA	$(CT)_9(CT)_8$	62	164 -177	Buteler *et al*. (1999); Veasey *et al*. (2008)
IB-255	F: CGTCCATGCTAAAGGTGTCAA R: ATAGGGGATTGTGCGTAATTTG	$(CT)_{14}$	53	210-255	Buteler *et al*. (1999); Gwandu *et al*. (2012)
IB-286	F: AGCCACTCCAACAGCACATA R: GGTTTCCCAATCAGCAATTC	$(CT)_{12}$	50	90 – 122	Buteler *et al.* (1999)
IB-297	F: GCAATTTCACACACAAACACG R: CCCTTCTTCCACCACTTTCA	$(CT)_{13}$	58	130 - 200	Buteler *et al.* (1999)
IB-318	F: AGAACGCATGGGCATTGA R: CCCACCGTGTAAGGAAATCA	$(CT)_{9C}(CT)_5$	54	120- 135	Buteler *et al*. (1999); Veasey *et al*. (2008)
IBR-16	F: GACTTCCTTGGTGTAGTTGC R: AGGGTTAAGCGGGAGACT	$(GATA)_4$	58	131 - 237	Karuri *et al.* (2010); Gwandu *et al.* (2012)
IBR-19	F: GGCTAGTGGAGAAGGTCAA R: AGAAGTAGAACTCCGTCACC	$(AG)_{5b}$	60	190 - 208	Karuri *et al.* (2010)
690524	F: AGGAAGGGCTAGTGGAGAAGGTC R: AAGGCAACAAATACACACACACG		57	240 - 315	Gwandu *et al*. (2012)

F – Forward primer sequence (5' – 3'), R – Reverse primer sequence (5' – 3'), Ta[1] - optimal annealing temperatures

pair group method with arithmetic averages (UPGMA) through Numerical Taxonomy Multivariate System (NTSYS-pc) Version 2.21c (Rohlf, 2009).

RESULTS

Combining ability for root β-carotene and yield. The ANOVA of the combined data across the two locations (Table 4), showed highly significant differences (P<0.0001) among the genotypes for all five traits, (RBCC, MFRY, MNR, TFRY and TNR). Highly significant (P<0.0001) interactions between genotypes and the environment were observed for RBCC and MFRY (P<0.05). Both GCA and SCA were highly significant (P<0.0001) for almost all the traits, except the SCA effect for TNR (P<0.05). The reciprocal and maternal effects were significant (P<0.0001) for RBCC (Table 4). The interactions between the GCA, SCA and reciprocal effects with the environment were found in some traits. High narrow sense heritability was observed for all the traits.

GCA effect of the parental lines. GCA effects were highly significant (P<0.0001) for RBCC, for all parents; and positive GCA effects were observed for orange fleshed parents (Khano, Resisto, W-119) (Table 5). Parents, Monate and Ndou, were good general combiners with positive and significant (P<0.01) contribution to GCA effect for yield related traits (MFRY, MNR and TFRY). Khano, Resisto and W-119, orange-fleshed breeding lines were not good general combiners, for the yield related traits. Parent W-119 had a significantly negative contribution to the GCA effect for MFRY, MNR, TFRY and TNR. Parental lines, Khano and Resisto had positively significant GCA effect for TNR meaning that these lines contributed to the high number of total roots (Table 5).

SCA effect of the crosses. Majority of the crosses showed more SCA effect for RBCC than any other trait (Table 6). Significant SCA effects were particularly found in crosses involving orange- and cream-fleshed parents. The highest and positive significant SCA effect was found in the combination of W-119 x Monate with 1.69 mg 100 g^{-1} fw. The parents with highest GCA, Khano and Resisto (Table 5), did not necessarily result in the highest SCA when crossed. The cross

TABLE 4. Analysis of variance (mean square values) for combining ability of root β-carotene content (RBCC), marketable fresh root mass and number (MFRY, MNR) and total fresh root mass and number (TFRY, TNR) across two locations

Source of variation	RBCC (mg 100 g^{-1}fw)	MFRY (kg $plant^{-1}$)	MNR (roots $plant^{-1}$)	TFRY (kg $plant^{-1}$)	TNR (roots $plant^{-1}$)
Genotypes (G)	39.20***	0.11***	3.03***	0.23***	7.84***
Environments (E)	6.98**	2.47***	16.68***	5.10***	0.05
G x E	3.27***	0.05*	0.87	0.06	2.45
GCA	176.66***	0.21***	6.99***	5.89***	23.14***
SCA	12.20***	0.13***	3.12***	3.55***	5.33*
Reciprocal	11.20***	0.04	1.36	0.85	4.22
Maternal	9.79***	0.01	0.54	0.22	1.72
GCA x E	2.66	0.02	0.19	0.84	8.39**
SCA x E	2.10	0.06**	0.72	1.51**	1.41
Reciprocal x E	4.69**	0.03	1.28	0.83	1.10
Error	1.22	0.02	0.77	0.05	2.19
GCA:SCA	0.97	0.76	0.82	0.77	0.89
H	0.96	0.75	0.94	0.77	0.99
CV (%)	45.78	37.72	33.22	23.17	28.88

*, **, *** = Significant at the 0.05, 0.01 and 0.001 probability levels, respectively. GCA = General combining ability; SCA = Specific combining ability; H = Broad sense heritability; CV = Coefficient of variation; fw = fresh weight

combination of Monate x W-119 was the highest as well as the only one with significant positive SCA, namely for MFRY. No positive and significant SCA effect occured for MNR among the cross combinations. The lowest and significant SCA for MNR and MFRY was observed in the cross Ndou x Monate, despite both being high yielding parents. The lowest significant SCA for MNR and MFRY observed in the cross Ndou x Monate contributed negatively to the yield. The SCA effect in the cross W-119 x Resisto and the reciprocal cross Ndou x Khano were negative and significant for TNR, which had a contribution in reducing the number of small roots produced by their progenies (Table 6).

TABLE 5. General combining ability effect for root β-carotene and yield related traits

Parental line	RBCC (mg 100 g^{-1}fw)	MFRY (kg $plant^{-1}$)	MNR (roots $plant^{-1}$)	TFRY (kg $plant^{-1}$)	TNR (roots $plant^{-1}$)
Monate	-2.24***	0.061**	2.7**	0.30**	0.1
Ndou	-1.42***	0.068**	2.6**	0.36***	-0.2
Khano	1.32***	-0.047**	-0.9	-0.26**	0.6**
Resisto	1.46***	-0.026	0.9	-0.11	0.4*
W-119	0.89***	-0.056**	-5.3***	-0.28**	-0.9***

*, **, *** = Significant at the 0.05, 0.01 and 0.001 probability levels, respectively; fw = fresh weight; RBCC = root β-carotene content; MFRY = marketable fresh root yield; MNR = marketable number of roots; TFRY = total fresh root yield; TNR = total number of roots

TABLE 6. Specific combining ability effect for root β-carotene and yield related traits

Crosses Female x Male	RBCC (mg 100 g^{-1} fw)	MFRY (kg $plant^{-1}$)	MNR (roots $plant^{-1}$)	TFRY (kg $plant^{-1}$)	TNR (roots $plant^{-1}$)
Ndou x Monate	0.18	-0.14**	-0.51*	-0.70**	0.04
Khano x Monate	-1.38***	-0.05	-0.47*	-0.31	-0.68
Resisto x Monate	-1.33***	0.03	0.30	0.18	0.45
W-119 x Monate	1.69***	-0.003	-0.11	-0.05	0.43
Khano x Ndou	0.96**	-0.03	0.30	-0.17	0.14
Resisto x Ndou	0.22	-0.06	-0.11	-0.34	0.18
Resisto x Khano	0.19	0.04	-0.04	0.24	-0.15
W-119 x Ndou	-0.65*	-0.06	-0.32	-0.36*	-0.54
W-119 x Khano	0.29	0.03	0.03	0.18	-0.31
W-119 x Resisto	0.13	-0.06*	-0.44*	-0.33	-1.09**
Reciprocal					
Monate x Ndou	0.10	0.03	0.05	0.08	-0.61
Monate x Khano	1.05**	-0.03	-0.10	-0.16	0.19
Monate x Resisto	0.21	-0.04	-0.39	-0.18	-0.69
Monate x W-119	-1.11**	0.12**	0.59	0.60**	0.02^{n}
Ndou x Khano	1.59***	0.003	-0.05*	0.02	-1.19**
Ndou x Resisto	-1.34***	0.03	0.15	0.10	0.68
Ndou x W-119	-0.73*	-0.09*	-0.55*	-0.42	-0.68
Khano x Resisto	0.45	-0.03*	-0.49	-0.15	-0.27
Khano x W-119	-0.03	-0.05	-0.13	-0.22	-0.42
Resisto x W-119	-1.36***	-0.03	-0.12	-0.12	0.003

*, ** = Significant at the 0.05 and 0.01 probability levels, respectively; fw = fresh weight; RBCC = root β–carotene content; MFRY = marketable fresh root yield; MNR = marketable number of roots; TFRY = total fresh root yield; TNR = total number of roots

TABLE 7. Simple Sequence Repeat (SSR) amplification and PIC of 12 SSR markers amplified across eight sweetpotato genotypes

Primers	Total number of alleles	Polymorphic alleles	% polymorphism	PIC
IBSSR 01	5	5	100	0.49
IBSSR04	7	7	100	0.46
IBSSR07	5	4	80	0.39
IBSSR10	7	7	100	0.39
IBSSR17	6	6	100	0.50
IB-242	4	3	75	0.32
IB-248	6	4	66.7	0.46
IB-255	3	2	66.7	0.10
IB-286	6	5	83.3	0.48
IB-297	9	8	100	0.49
IB-318	6	5	83.3	0.49
IB-R19	5	5	100	0.49
Total	69	61	-	-
Average	5.75	5.08	87.92	0.42

Genetic relationships among parental lines. Out of the 20 SSR markers tested, 12 markers showed positive amplification and scorable alleles (Table 7). A total of 60 alleles (bands) was scored across eight sweetpotato genotypes, with an average of 5.75 alleles per SSR locus. The number of allele varied from three to nine per SSR locus. The Polymorphism Information Content (PIC) (Roldàn-Ruiz *et al.*, 2000) varied from 0.1 to 0.5. For most of the SSR markers, PIC values were above 0.4.

The UPGMA dendrogram revealed sufficient genetic diversity between the eight genotypes, with a maximum of 60% similarity observed between two orange-fleshed genotypes, Purple Sunset and Resisto (Fig. 1). The cream-fleshed genotypes (Monate and Mvuvhelo) had the second highest genetic similarity (58%), further revealing that there was more diversity across genotypes with contrasting morphological features, especially with regards to the â-carotene content (flesh colour).

Genetic similarity and combining ability. Although successful crosses were not obtained between the two most distantly related parents, 1999_5_1 and Monate, the highest and significant MFRY and TFRY was observed among hybrids from the cross between Monate and W-119, also distantly related parents, when Monate was used as the female parent (Table 6). A similar pattern was observed in the SCA effect for RBCC for the cross between Monate and W-119, when W-119 was used as the female. A negative correlation was observed between SCA effect for RBCC and the yield related traits.

DISCUSSION

Combining ability for root β-carotene content and yield. The presence of genetic variation among the parental genotypes and progenies across the two locations (Table 4), confirmed the usefulness of diallel crosses in creating genetic variation in the breeding populations of sweet potato. Previous studies in sweetpotato reported high genetic variability resulting from diallel crosses (Kanju, 2000; Chiona, 2010; Zulu *et al.,* 2012), indicating an extensive genetic pool for selection purposes in breeding of a crop (Pierce, 2012).

GCA and SCA effects. The magnitudes of GCA and SCA variances (Table 4) indicated that additive and non-additive gene action were important in determining the inheritance of MFRY, MNR, TFRY, TNR and RBCC. The high GCA:SCA

ratio observed in the β-carotene and yield related traits (Table 4), suggests that the additive gene action was predominant over non-additive gene action (Baker, 1978). This confirmed the results reported by Kanju (2000) and Chiona (2009) where additive gene action were predominant in the inheritance of root yield and β-carotene content in sweetpotato. Although in a different root crop, Da Silva (2008) and Njenga *et al.* (2014) also reported predominance of additive gene action in the inheritance of cassava. The significant differences observed in the performance of reciprocal crosses for RBCC implied that the maternal effect was involved in determining the magnitude of the trait. The presence of maternal effect is an indication of the cytoplasmic effect (Pierce, 2012), which also confirms the findings by Chiona (2010) on the occurrence of maternal effects in sweetpotato parental lines.

The high broad sense heritability (Table 4) found for all the traits (RBCC, MFRY, MNR, TFRY and TNR) showed that there was a high degree of resemblance between parents and off-springs (Pierce, 2012). Studies by Chiona (2010) and Tumwegamire *et al.* (2011) also obtained high values for broad sense heritability for fresh root β-carotene and root fresh yield in sweetpotato. Chiona (2009) found that the broad sense heritability of sweetpotato for β-carotene was 0.99 and for yield was 0.97. Tumwegamire *et al.* (2011) obtained 0.96 for β-carotene, which conforms with the present findings on the occurrence of high broad sense heritability estimates for β-carotene content in the root of sweetpotato. Moderate narrow sense heritability for yield related traits was previously reported (Jones, 1986; Kanju, 2000; Martin, 1988), confirming the present findings of low to moderate heritability estimates for yield. In contrast, Ernest *et al.* (1994) reported high narrow sense heritability for yield, and moderate narrow sense heritability for the number of roots. Thus, high estimates of narrow sense heritability indicate that high response to selection and genetic gains is more likely to be achieved through combining two parents with high GCA effects for RBCC (Pierce, 2012).

Progenies from the crosses with the highest SCA effects are useful in producing superior hybrids (Griffing, 1956). As sweetpotato is a vegetatively propagated crop, progenies displaying genetic gains for a specific trait, can be maintained through stem propagation to retain the genotype superiority (Da Silva, 2008). Selection of the best performing progenies, displaying high SCA effects could potentially result in the identification of a hybrid with superior traits for commercial release at the ARC-VOPI. Selection of parents should be based not only on the highest GCA effects, since not all the best general combiners result in highest SCA effects in the progenies. Selection of parents to be included in future breeding programmes should also consider its positive contribution to the SCA effects in the progenies. Therefore, parents should not be discarded solely based on their negative GCA effect, instead, performance of the parent within a cross combination should also be considered during the selection process. Careful consideration oughts to be placed on the role of parents when planning future crosses, as cytoplasmic maternal effects are involved in the inheritance of certain traits such as colour in sweetpotato. Parents displaying the best possible quality of the trait of interest that is influenced by the cytoplasmic maternal effect should then be used as the mother parent.

Genetic relationship among parental lines. The SSR markers used in our study successfully fingerprinted the eight sweetpotato parental lines. Similarly, the study by Gichuru *et al.* (2005) and Karuri *et al.* (2010) reported the usefulness and robustness of SSR markers in fingerprinting this polyploid. High genetic variability was found among the lines, which implies the existence of diversity among the parental lines. High diversity has also been found in the sweetpotato collections of East Africa (Gichuru *et al.*, 2005; Tumwegamire *et al.*, 2011; Yada *et al.*, 2010). The high diversity found in sweetpotato is attributed to its high ploidy levels (hexaploid) (Ozias-Akins and Jarret, 1994) and its high outcrossing nature (Shiotani *et al.*, 1990).

CONCLUSION

High genetic diversity exists among the sweetpotato genotypes in the local sweet potato programme. Genetic analysis is crucial in the selection of genotypes to be used as parental

lines for improvement of important traits. Hybrids from the crosses between distantly related parental genotypes Monate and W-119 were display high performance in terms of MFRY, TNR and RBCC were selected and included for further field evaluation on new varietal development.

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