African Crop Science Journal, Vol. 21, No. 1, pp. 37 - 49
ISSN 1021-9730/2013 $4.00

MORPHOLOGICAL DESCRIPTORS AND MICRO SATELLITE DIVERSITY AMONG SCARLET EGGPLANT GROUPS

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(Received 1 October, 2012; accepted 13 February, 2013)

ABSTRACT

Solanum aethiopicum L. groups is an important leaf and fruit vegetable, largely consumed in sub Saharan Africa. Genetic variation pattern among 35 accessions belonging to *S. aethiopicum* groups and sources of donor parents were investigated using morphological descriptors and SSR marker pairs. The Principal Component analysis showed great variations among accessions in terms of morphological traits. Leaf surface prickles, prickles underneath the leaves and fruit calyx pigmentation showed high discriminatory ability. Diversity analysis inferred distinctness, similarities and overlap among groups. The Kumba and Aculeatum groups are related for multi-locular characteristic of the fruits. The Nei's coefficients for morphological descriptors and SSR data indicated that *S. aethiopicum* groups showed a wide genetic base. MM1102 and MM457 showed high relatedness for pubescence and moderate prickles. These traits are useful during selection and intellectual right. The Kumba and Shum groups are morphologically and genotypically related. Inter-cluster hybridisation among clusters 2 and 3 may evolve new population for fruit colour. Based on SSR data, MM01160, MM1102, MM381, MM01108 are genetically related. The dispersion and grouping of accessions displayed aggregation of accessions from different geographical locations and group heterogeneity. Both morphological traits and SSR marker pairs sufficiently discriminated accessions belonging to *S. aethiopicum* groups and the outcome will be useful for conservation and genetic improvement in this specie.

Key Words: Fruit colour, genetic diversity, *Solanum aethiopicum*

RÉSUMÉ

Le groupe *Solanum aethiopicum* L. est une importante légume à feuille et fruit, largement consommés en Afrique sub Saharienne. La variation génétique parmi 35 accessions appartenant aux groupes *S. aethiopicum* et sources de parents donneurs étaient étudiés par l'utilisation des descripteurs morphologiques et des markeurs pairs de SSR. L'analyse de la principale composante a montré de grandes variations parmi les accessions en termes de traits morphologiques. Les épines à la surface foliaire, les épines en dessous des feuilles et la pigmentation de la calice du fruit ont montré une capacité discriminatoire élevée. L'analyse de la diversité a inferé des distinctions, similarités et coincidence parmi les groupes. Les groupes Kumba et Aculeatum sont associés aux caractéristiques multi-loculaires de fruits. Les coefficients de Nei's pour descripteurs morphologiques et des données de SSR ont indiqué que les groupes *S. aethiopicum* ont montré une large base génétique. MM1102 et MM457 ont montré une associativité élevée de la pubescence et des épines modérées. Ces traits sont utiles durant la sélection et le droit intellectuel. Les groupes Kumba et Shum sont morphologiquement et génotypiquement associés. L'hybridisation entre les groupes 2 et 3 pourrait évoluer en une nouvelle population de fruit en couleur. Basé sur les données de SSR, MM01160, MM1102, MM381, MM01108 sont génétiquement associés. La dispersion et le groupement des accessions ont montré l'aggrégation de différentes localisations géographiques et l'héterogéneité des groupes.

Les traits morphologiques et les markeurs des pairs SSR ont tous ensemble distingué les accessions appartenant aux groupes *S. aethiopicum* et le résultat sera utile pour la conservation et l'amélioration génétique dans ce milieu.

Mots Clés: Couleur du fruit, diversité génétique, *Solanum aethiopicum*

INTRODUCTION

Solanum aethiopicum L. (Scarlet eggplant) which belongs to the sub-family Solanoideae, family *Solanaceae*. *S. aethiopicum* L. (2n = 24), is classified in *Solanum* subgenus Leptostemonum, section Oliganthes, Dunal Bitter. The scarlet eggplant (*S. aethiopicum* L) comprises of four groups (Gilo, Shum, Aculeatum and Kumba). The Gilo is the most important and largely cultivated in sub-Sahara Africa (Shippers, 2002), and are predominantly self-pollinated. *Solanum aethiopicum* is important as a leaf and fruit vegetable largely consumed in Africa. The genus, *Solanum,* vary morphologically as they are diverse in number (Levin *et al*., 2005) and ecogeographical distribution (Wunderlin *et al*., 1993).

The collection and estimation of genetic dissimilarity among accessions can be useful in a breeding programme for directing crosses, evaluating available germplasm and for maintaining appropriate range of genetic diversity. Currently, there are various methods available for diversity studies; morphological traits (qualitative) is the oldest technique and has been used widely in determination of plant diversity. The traits are governed by oligogenic or monogenic action, stable, vary less within the supposed group and are not significantly modified by environment. On the other hand, agronomic traits are moderated by polygenic action, and are largely influenced by environmental variables, thus making predictability of performance for metric traits difficult.

Zewdie and Bosland (2003) reported additive and dominance gene action for seed colour in *Capsicum*, and single gene action for seed colour in water melon (*Citrullus lantus* Thunb) and *Brassica carinata* A. Braun. This emphasizes the need to understand genetic variation pattern for qualitative traits in *S. aethiopicum*; the outcome of this could complement selection activities.

Molecular markers have enormous potential to explore genetic diversity by detecting polymorphisms and are useful tools for breeding, genotype identification, determination of genome organisation and evolution in plants. Also, SSR markers are ideal for distinguishing accessions that are genetically very similar; their potential for automation and co-dominant manner are additional advantages when compared with other types of molecular markers. The identification of genetic variation within and among groups in *S. aethiopicum* through morphological and molecular analyses have not been sufficiently utilised for purposes of breeding and crop improvement; hence the dearth of market preferred commercial and improved varieties, and inadequate donor parents for single and multiple morphological traits for breeding, despite its widespread adaptation, cultivation, nutritional and economic importance in sub-Saharan Africa. The objective of this work was to estimate the genetic dissimilarity among *S. aethiopicum* groups through SSR marker pairs and morphological traits; and identify promising donor parents.

MATERIALS AND METHODS

Plant material, DNA extraction and Microsatellite analysis. Field evaluation took place at the experimental site located at latitude 4.9°S, longitude 3.8°E; and altitude 1290 meters above sea level, at the Horticultural Training Research Institute, Arusha in Tanzania. The site has an annual rainfall of 700 to 1000 mm; and soil type of clay loam with a pH of between 6.0 and 6.5.

Experimental plots were laid out in a randomised complete block design, with three replications during 2009 through 2010. However, a single replication was used for data collection. Each plot consisted of a double row of 7 meters long and 0.75 m between rows. Qualitative traits were measured on fifteen plants (five competitive

plants per replicate). Morphological traits (Table 2) were scored within fifteen days before flowering; during which time, maximum development of vegetative parts ought to have taken place (Karamura and Karamura, 1995). Traits related to colour were scored using the standard Royal Horticultural Society (1986) colour chart.

Seed of 35 accessions of *S. aethiopicum* and LBR 48 (*Lycopersicon esculentum*) were assayed, seedlings were raised in trays; after five weeks leaf tissues were harvested from five plants by snapping the lid of the tube shut on a leaf. Leaf samples were bulked in eppendorf tubes and stored at a freezing temperature -24 °C for DNA extraction. Bulked leaf samples were supposed to combine equal amounts of DNA from each individual constituting the bulk.

For the DNA extraction process, the Wizard 2Genomic DNA Purification Kit, (Promega, Madison, WI, USA), was used. The protocol was applied with slight modifications . During grinding process, 600 µl Nuclei lysis solution was added to eppendorf tube (containing frozen leaf samples). Instead of directly using 600 µl Nuclei lysis solution to each tube, the amount was added in two steps. At first step, 250 µl of solution was used for grinding using plastic pestles. The remaining 350 µl was added, and was mixed several times for better homogeneity. Another modification was about centrifugation, instead of three minutes at 13.000 – 16.000 g, samples were spun at 10.000 g for five minutes and at 6th step in the protocol and 10.000 g for two minutes at 9th step. At the 10th step, ethanol washed samples were spun again at 10.000 g for two minutes. Sixteen SSR primers pairs (synthesized by Integrated DNA Technologies, Inc., 1A, USA) (Table 2) were used.

The total genomic DNA was extracted from a pool genomic DNA for each accession, PCR reactions were performed in a final volume of 20 µl per sample: 2.0 µl Go Taq flex buffer, 0.4 $MgCl_2$, DNA polymerase (Promega ®, Madison, W1, USA), 0.4 µl dNTP, 1.0 µl of Forward primer and Reverse primer; 0.15 µl Taq Polymerase; 11.15 µl PCR H_2O and 1.0 µl each genomic DNA. Amplifications were performed in a Perkin Elmer Thermocycler (Gene Amp 9600, Perkin Elmer Corporation, USA). PCR cycling conditions included preliminary denaturation for 5 min. at 94 °C; 35 cycles at 94 °C for 30 s., 50 °C for 1 min., 72 °C for 1 min.; final extension for 5 min at 72 °C and hold at 4 °C. Amplification products were mixed with loading buffer containing bromophenol blue, separated electrophoretically on 2% agarose gel followed by ethidium bromide staining, and visualised on a UV Transilluminator. Each amplified fragment was visualised as distinct bands.

Data analysis. Morphological traits were scored according to the World Vegetable Center (www.avrdc.org) and Bioversity (www.bioversity.org) descriptor for eggplants (*S. melongena*), with modifications (Table l). Characters that varied extremely on the same individual were eliminated; traits selected varied within species and exhibited high discriminatory ability. Traits were estimated from every part of the plant at vegetative, reproductive and maturity stages, presumably because different parts of the genome affect different suites of traits at different times.

In respect to coding, two-state qualitative traits, e.g. presence or absence of pubescence/ prickles, was coded as binary, e.g. by scoring one for presence and two for the absence of pubescence on plant parts. Ordered multistate qualitative traits were coded as series of discrete states. For example, if there were three flower colour (violet, red or cream), individuals were scored 1 = pink, 2 = red and 3 = cream. Non-ordered traits were coded as series of discrete states, because ratios do not give a true picture of their shapes. Traits related to colour (pigmentation gene) were scored using the standard Royal Horticultural Society (1986) colour chart. Qualitative traits (presence or absence of pubescence and prickles on plant parts) were measured on fifteen plants. In assessing similarities and differences among accessions, three steps were followed: (i) Selection of operational taxonomic unit (OTUs) and traits to be used in the study; (ii) Standardisation of traits; (iii) Similarities and dissimilarities between all parts of the OTUs to reveal any groupings present. To determine integrated diversity of multiple descriptors, traits (qualitative) with less variability for intra-specie and inter-specie diversity were removed.

TABLE 1. *Solanum aethiopicum* accessions used in the study organised by group and country of collection

Acc Code	Sub-group	Country of collection
MM 01143	Gilo	Nigeria
MM 1186	Gillo	Unknown
MM 10213	Gilo	Unknown
MM 981	Gillo	Uganda
Db_3	Gillo	Tanzania
MM 148	Gillo	Ivory coast
MM 1381	Gillo	Brazil
MM 232	Gillo	Ivory coast
MM 1473	Gillo	Congo
MM 458	Gillo	Japan
MM 803	Gillo	Gabon
MM 368	Gillo	Chad
Acc 40	Gilo	Unknown
Acc 43	Gilo	Unknown
MM 1692	Kumba	Senegal
MM 10150	Kumba	Burkina Faso
MM 1107	Kumba	Burkina Faso
MM 267	Kumba	Mauritania
MM 674	Kumba	Senegal
MM 01108	Kumba	Burkina Faso
MM 10147	Kumba	Burkina Faso
MM 1207	Kumba	Senegal
MM 196	Kumba	Burkina Faso
MM 10078	Shum	Togo
MM 11008	Shum	Ivory coast
MM 1119	Shum	Togo
MM 1161	Shum	Benin
MM 01160	Shum	Benin
MM 1616	Shum	Unknown
S00817	Aculeatum	Yugoslavia
S00829	Aculeatum	Brazil
S00131	Aculeatum	Philippines
MM 1102	Aculeatum	Burkina Faso
MM 457	Aculeatum	Japan
MM 1158	Aculeatum	France

Morphological traits were submitted for two methods of multivariate analyses, i.e. Principal Component Analysis was done using PROC PCA procedure of (SAS, 1997) and Cluster analysis was performed using the unweighted pair group method of arithmetic averages (UPGMA) as described in Sneath and Sokal (1973), NTsys-PC software version 2.20 (Exeter Software, New York, USA). Molecular base data generated by using 16 microsatellites primers pairs on 35 accessions were scored in binary format, with the presence of a band scored as one, and its absence scored as 0, thus generating a binary matrix.

Binary data were used to compute pair wise similarity coefficient. The similarity coefficient matrix was subjected to cluster analysis by unweighted pair group method of arithmetic averages (UPGMA) as described in Sneath and Sokal (1973), NTsys-PC software version 2.20 (Exeter Software, New York, USA). Two way Mantel test (Mantel, 1967) was applied to test for association.

RESULTS

Morphological diversity. The PCA identified variables most responsible for the pattern of relationships observed among the accessions, based on morphological traits. The first three principal component (referred hereafter as PC) axes explained half (50%) of the total variation. The first PC accounted for 24% of the total variation, with high eigenvalue (7.62) (Table 4). Leaf surface prickles and prickles underneath the leaves contributed maximum (33%) variation, followed by fruit calyx pigmentation, which contributed 30% variation to genetic distance between accessions. However, leaf surface prickles and prickles underneath the leaves showed positive, high and low coefficients on PC 1 and 2. Equal weights were recorded for leaf vein prickles and sepal pigmentation on PC 1.

Leaf surface prickles, prickles underneath the leaves, fruit colour at commercial harvest, fruit colour at physiological harvest were inversely related on the first PC. The second PC accounted for 14% of variation unexplained in PC 1; altogether with the first PC, 40% of variation was explained. The second PC axis showed discriminatory power of pubescence on plant parts (stem pubescence, petiole pubescence, leaf pubescence and receptacle pubescence) compared with other traits. This axis demonstrated independent relationships among fruit length by breadth, fruit cross section, fruit apex shape and stem pigmentation. Traits of high discriminatory ability on the PC 2 recorded low positive and negative weights on PC 1 and 3. Fruit colour at commercial harvest and physiological maturity, and fruit colour distribution demonstrated high

TABLE 2. Summary of descriptor used for morphological diversity

Character	Character score	Number of variants
Stem pubescence	1= glabrous, 2 = sparse, 3 = medium,4 = dense	4
Leaf pubescence	1= glabrous, 2 = sparse, 3 = medium,4 = dense	4
Petiole pubescence	1= glabrous, 2=sparse, 3=medium,4 = dense	4
Stem prickles	1= glabrous, 2=1-2 spines (very few), 3= 3-5 spines (few), 5 = 6-10 (medium), 7 = 11-20 (many) 9= 20> (very many)	9
Leaf prickles	1= glabrous, 2=1-2 spines (very few), 3= 3-5 spines (few), 5= 6-10 (medium), 7 = 11-20 (many) 9= 20> (very many)	9
Petiole prickles	1= glabrous, 2=1-2 spines (very few), 3= 3-5 spines (few), 5= 6-10 (medium), 7 = 11-20 (many) 9= 20> (very many)	9
Leaf blade lobbing	1= very weak, 3= weak, 5=intermediate, 7=strong, 9= very strong.	9
Leaf blade tip angle	1=Very acute, 3= acute, 5= intermediate, 7= obtuse, 9=very obtuse	9
Leaf blade pigmentation	1= Green, 2= green+purple, 3= purple	3
Stem pigmentation	1= Green, 2= green+purple, 3= purple	3
Petiole pigmentation	1= Green, 2= green+purple, 3= purple	3
Leaf vein pigmentation	1= Green, 2= green+purple, 3= purple	3
Leaf mid rib pigmentation	1= Green, 2= green+purple, 3= purple	3
Fruit calyx pigmentation	1= Green, 2= green+purple, 3= purple	3
Calyx colour	1= Green, 2= green+purple, 3= purple	3
Fruit calyx prickles	1= glabrous, 2=1-2 spines (very few), 3= 3-5 spines (few), 5= 6-10 (medium), 7 = 11-20 (many) 9= 20> (very many)	9
Petal colour	1= greenish white, 3= white, 5= pale violet, 7=light violet, 9= bluish violet	9
Sepal colour	1= Green, 2= green+purple, 3= purple	3
Fruit peduncle pigmentation	1= Green, 2= green+purple, 3= purple	3
Flower size	1=small, 2= medium, 3= big	3
Fruit length/breadth	1= broader than long, 3= As long as broad, 5= slightly longer than broad, 7= Twice as long as broad, 8= Three times as long as broad, 9= Several times as long as broad	9
Fruit curvature	1= none, 3= slightly curved, 5=curved, 7= snake shape, 9= U shaped	9
Fruit apex shape	3= protruded, 5= Rounded, 7= Depressed	7
Fruit colour at commercial ripeness	1= Green, 2= Milk white, 3=Deep yellow, 4= Fired red, 5= scarlet red, 6=Lilac gray, 7= Purple, 8=Purple black, 9= black	9
Leaf blade length	Taken at flowering	7
Fruit colour distribution	1= Uniform, 3= mottled, 5= Netted, 7= Striped	9
Fruit colour at physiological maturity	1= Green, 2= Deep yellow, 3= Yellow orange, 4= Deep orange, 5= Fired red, 6= Poppy red, 7= Scarlet red, 8= Brown, 9= Black	9
Fruit cross section	1= Circular (no grooves), 3= Elliptic, no grooves, 5= Few grooves, 7=Many grooves, 9=Very irregular.	9
Seed colour	1= white, 2= light yellow, 3= grey yellow,4= brownish yellow, 5= brown, 6= brown black, 9= black	

TABLE 3. SSR markers used in this investigation and marker characteristics

Primer name	Repeat motif (5' - 3')	Polymorphism (%)	Product size
EM 117	F-GAT CAT CAC TGG TTT GGG CTA CAAR- AGG GGA GAG GAA ACT TGA TTG GAC	54	120-220
EM 120a	F-GGA TCA ACT GAA GAG CTG GTG GTTR-CAG AGC TTC AAT GTT CCA TTT CAC A	89	100-218
EM120b	F- CAA AAG ATA AAA AGC TGC CGG ATGR-CAT GCG TGA GTT TTG GAG AGA GAG	76	80-240
EM 119	F-CCC CAC CCC ATT TGT GTT ATG TTR-ACC CGA GAG CTA TGG AGT GTT CTG	54	100-210
EM 141	F- TCT GCA TCG AAT GTC TAC ACC AAAR-AAA AGC GCT TGC ACT ACA CCT GAA T	41	100-260
EM 114	F-AGC CTA AAC TTG GTT GGT TTT TGCR-GAA GCT TTA AGA GCC TTC TAT GCA G	68	159-250
EM 127	F-CAG ACA CAA TGC TGA GCC AAA ATR-CGG TTT AAT CAT AGC GGT GAC CTT	62	150-230
EM 107	F-GGC CCT AGA CTG AGC CTG AAA TGT TR-TGC TAC AAC CAA CAC AAC CCT CAA	92	100-240
EM 116	F-TTA GAA ATT TCG GAA CAA AGA GAR-CCA CAT GAA ACT TGG ACC AAT GAG	32	150-230
EM 128	F- TAG CGG TGC TAG GTC CAT CAT CTC AR-TTC TCA AGA AGT TGC TCC AAA GGA	54	100-231
EM 133	F- GCG GAT CAC CTG CAG TTA CAT TACR- TCC TTT GAC CTA TAG TGG CAC GTA GT	52	120-220
EM 134	F-AGT AAG GGA AAG TGC TGA CGA AGGR-CAG AGT CAT CGT TAT GGG GAG GTT	62	120-300
EM 140	F-CCA AAA CAA TTT CCA GTG ACT GTG CR-GAC CAG AAT GCC CCT CAA ATT AAA	62	150-300
EM 145	F-TGA TTT GGC CCT TAA GCC TAA GTA TGR-GAC TCC TCA AGC CTT TAC CTC CAA	49	145-220
EM 146	F-GGA CCA AAG CGA AAT TTT CAC AACR-TTG CAC CAA TTG GGA AGT AAC ACA	78	120-350
EM 104a	F- TGG ATC TGC AAA GAA AAG GAG AAA GR- CGC AAA TCG GGT AGA CTT TCG AT	43	200-350
EM 139	F-TGC TAA GTC GTC ATC CAA CAA GAAR-GAT TTT GGC TCC TTG ACC ATT TTG	57	130-340

variation on PC 3 due to high and positive weights.

The projections of the accessions on the biplot of PC axes 1 by 2, which accounted for 40% of the total variation, showed the distribution of the accessions in four quadrants (Fig. 1). Three accessions of the Gilo group and two of the Aculeatum group were dispersed in the first quadrant. MM1158 and S00131 were widely separated from the others in this quadrant, they showed high and positive contribution to variability PC axes 1 and 2. The spread of accessions in this quadrant is attributed to discriminatory power of leaf prickles (on leaf surface and underneath) with positive loading on PCs 1 and 2. Accessions in the first quadrant showed positive coefficients on plant parts in PC 1 and 2. However, S00131 showed greater manifestation for pubescence and prickliness, followed by MM1158 (Table 5).

The second quadrant accommodated the Aculeatum (two accessions) and Kumba groups (four accessions), and showed discriminatory importance of pigmentation of leaf vein , petiole and receptacle. Two accessions (MM1102 and MM457) were moderately separated from the

TABLE 4. Eigen values and vectors for first three principal component axes estimated for 29 morphological traits among 35 accessions from four *Solanum aethiopicum* L groups

Traits	PC1	PC2	PC3
Stem pigmentation	-0.006	-0.21	0.11
Stem pubescence	0.02	0.38	0.04
Stem prickles	0.25	0.006	-0.02
Leaf pubescence	-0.03	0.34	0.02
Leaf surface prickles	0.33	0.03	-0.04
Leaf underneath prickles	0.33	0.04	-0.06
Leaf lobbing	-0.005	-0.02	-0.17
Leaf vein pigmentation	0.25	-0.15	-0.0003
Leaf mid rib pigmentation	0.29	0.10	-0.04
Leaf vein prickles	0.30	0.11	-0.07
Leaf tip angle	0.006	-0.11	0.06
Petiole pigmentation	0.16	0.26	0.14
Petiole pubescence	0.04	0.30	0.19
Petiole prickles	0.27	0.13	-0.12
Flower colour	0.14	-0.15	0.0006
Sepal pigmentation	0.30	0.03	-0.03
Receptacle pigmentation	0.26	-0.11	-0.06
Receptacle pubescence	0.11	0.33	0.04
Number of sepals	0.22	0.02	-0.09
Peduncle pigmentation	0.27	-0.12	0.08
Flower size	0.09	0.001	0.27
Fruit length by breadth	-0.009	0.12	0.03
Fruit apex shape	0.12	-0.22	0.20
Fruit colour at commercial ripeness	-0.04	0.13	0.32
Fruit colour at physiological maturity	-0.04	0.06	0.33
Fruit cross section	0.07	-0.26	0.11
Fruit colour distribution	-0.06	0.18	0.35
Seed colour	-0.02	0.03	0.008
Eigen value	7.62	5.07	3.55
Proportion	0.24	0.16	0.11
Cumulative (%)	24	40	51

other entries in this quadrant. Accessions dispersed in this quadrant showed high relationships for leaf vein, receptacle and peduncle pigmentations, with positive and negative coefficients on PC 1 and 2. Members of the Shum and Gilo groups occupied the third quadrant, revealing high variation for fruit colour at physiological maturity, with negative relationships on PC1 and 2. Accessions of the Gilo group predominated the fourth quadrant; they showed high variation for stem pubescence and petiole pubescence. In general, the four quadrants displayed aggregation of accessions from different geographical locations and group heterogeneity.

The phenogram constructed among *S. aethiopicum* groups (Fig. 2) based on morphological data, showed that similarity coefficients ranged from 0.01 and 0.93; most of the accessions were grouped into three clusters at 45% similarity coefficient. Clusters 2 and 3 comprised of 16 accessions each; S00131 formed an outlier and was linked to clusters 2 and 3 at the lower end. The third cluster comprised of 16 accessions starting from accession 43 to MM196; members of the Kumba group were the most numerous (Kumba (8), followed by the Gilo (5) and Aculeatum (3) groups (Fig. 1). Members of this cluster showed resemblance for glabrous leaves and non-pigmented fruit sepals (Table 5).

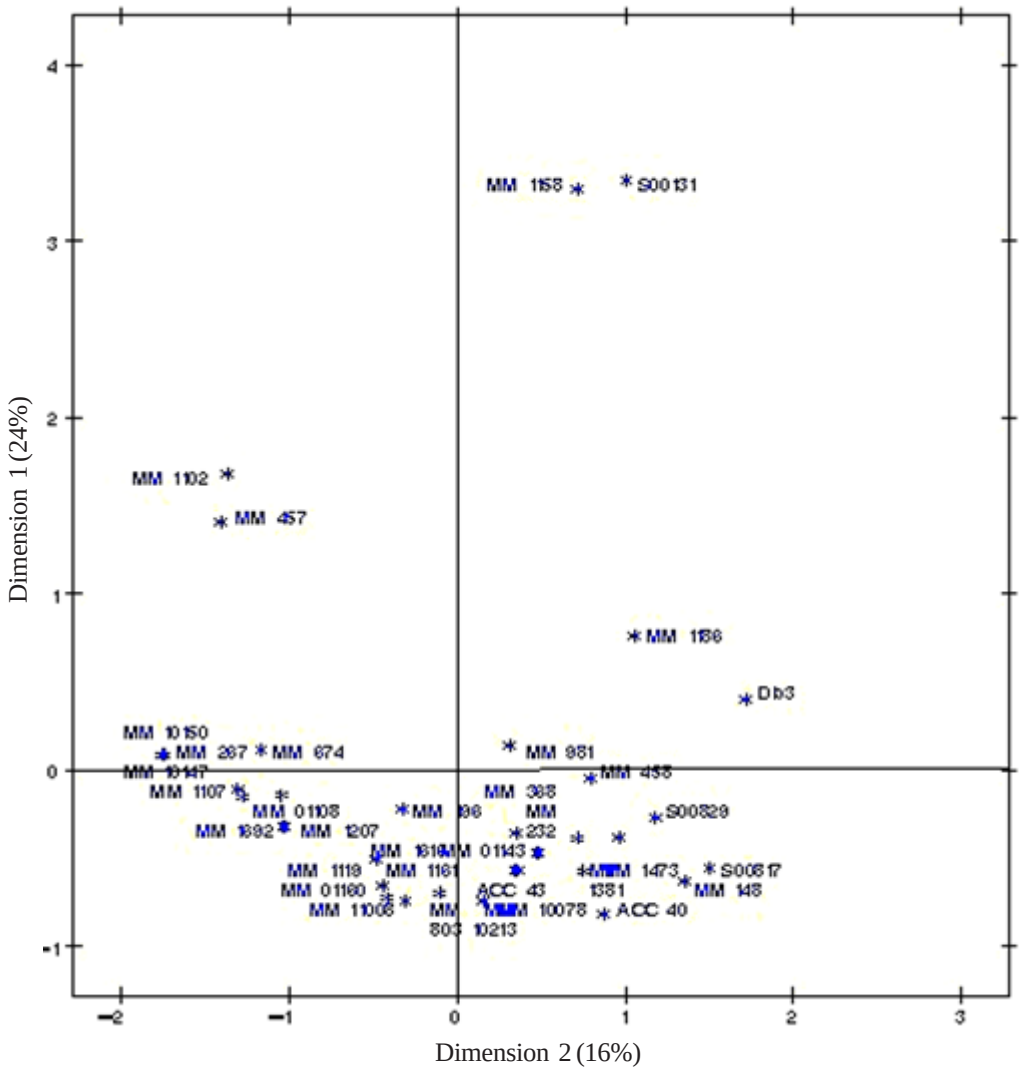

Figure 1. Plot of the first two principal components axes showing spatial distribution of 36 accessions belonging to *Solanum aethiopicum* based on morphological descriptors.

Fruits were characterised by uniform fruit colour distribution at commercial ripeness, fired red fruit colour at maturity and seeds were cream in colour. Also, cluster members displayed variation for fruit cross section (elliptic, circular, few grooves), fruit colour at commercial ripeness (cream or green) and fruit length by breadth. Within cluster 3, accessions 43, MM457 and MM1102 were related for non-pigmented stem (green), and green fruits at commercial ripeness, which turned scarlet red at physiological maturity. Also, fruits had uniform colour distribution at commercial harvest and white seed colour. However, MM1692 and MM1473 were similar for glabrous leaves and absence of prickles and non-pigmented leaf vein (green). Accession 40 and MM674 were similar for non-pigmented petiole (green) and sepals (green), and scarlet red fruit colour at physiological maturity. MM267, MM237, MM458, MM101047 and MM10150 were related for glabrous leaves and absence of prickles on plant parts and pigmented petiole (violet). In addition, S00131 (Aculeatum group) was characterised by intermediate pubescence and prickles on the stem, leaves and receptacle, and uniform fruit colour distribution, and fruits turned fired red

TABLE 5. Average inter-cluster genetic diversity for morphological traits among accessions of *Solanum aethiopicum* groups

Cluster	Cluster members	Morphological traits
1	S00829, MM129	Stem pubescence (2), stem prickles (1), petiole pigmentation (3), flower colour (3), flower size (2), fruit length and breadth (1), fruit curvature (1), fruit colour at commercial harvest (1), fruit colour at physiological maturity (5), seed colour (1)
Cluster 2	MM10213, MM01143, MM1381, MM368, MM805, MM1161, MM1616, MM10078, MM01160, MM 11008, MM01108, MM 1186, MM 981, DB3, S00817, MM 148	Fruit curvature (1), seed colour (1), fruit cross section (1, 3, 5), stem pigmentation (1,3), stem prickles (1), leaf prickles (1), sepal colour (1,3), receptacle prickles (1,3), flower size (1,2), fruit colour distribution (1,3,7), fruit colour at maturity (3,4,5)
Cluster 3	Acc 43, MM 457, MM 1102, MM1692, MM1473, Acc 40, MM 674, MM267, MM237, MM458, MM10147, MM 10150	Stem prickles (1,3),leaf vein pigmentation (1,3), leaf surface prickles (1), leaf under leaf (1), petiole pigmentation (1,3), leaf pubescence (1,2,3), sepal pigmentation (1), receptacle pig mentation (1,3), fruit colour at commercial ripeness (1,2), fruit colour at maturity (5), fruit curvature (1), fruit cross section (1, 5, 7,9), fruit colour distribution (1), seed colour (1)
Outlier	S00131	Stem pubescence (2), stem prickles (7), leaf prickles (7), leaf vein pigmentation (3), leaf surface spines (5), petiole spines (3), fruit apex (7), fruit colour at commercial ripeness (1), fruit colour at physiological ripeness (5)

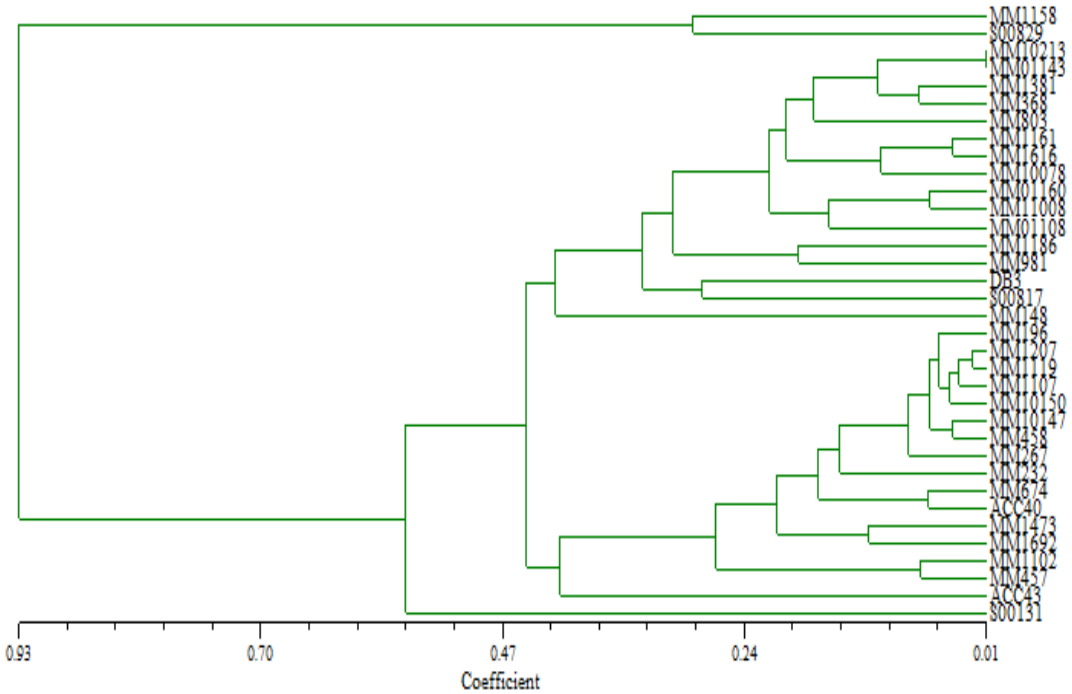

Figure 2. Dendogram constructed from morphological data using jaccard's coefficient of similarity and UPGMA clustering.

colour at physiological maturity with grey yellow seed colour (Table 5).

Accessions grouped in cluster 3 spread over quadrants 3 and 4 on the PC biplot (Fig. 1) indicating negative contribution to variability on PC 1 and 2, positive and negative contribution to variability on PC 1 and 2. The second cluster accommodated 16 accessions flanked on both sides by accessions of the Gilo group, six accessions of the Shum group displayed intermediate phenotype, and were intermingle with members of the Gilo group. Cluster members were related for seed colour (white), absence of fruit curvature, glabrous leaves, non-prickled stem and moderate leaf lobbing. MM10213 and MM01143 were phenotypically related; MM1381, MM368, MM805 showed intermediate stem pubescence, non-prickled leaves and stems, white seed colour, non-pigmented receptacle and sepals (green).

Three accessions (MM1161, MM1616 and MM10078) were grouped in cluster 2; and were related for greenish-white petals. Two accessions (MM01180 and MM11008) of the Shum group and one accession (MM01108) of the Kumba formed a group in cluster 2. They were related for non-pigmented petiole, they showed variation for fruit length by breadth varied and fruit shape. Two accessions (MM1158 and S00829) from France and Brazil formed cluster 1; they belonged to Aculeatum group. Cluster members had pigmented petiole (greenish-violet), with varying intensity of pubescence and are non-prickled (Table 5). Fruits had cream colour at commercial harvest and scarlet red colour at physiological maturity.

The 35 accessions of *S. aethiopicum* were fingerprinted through a total of 16 microsatellite primer sets. The number of bands detected through ranged from 3 to 23 (123 bp to 240 bp). The proportion of polymorphism ranged from 0.20 (EM 128) and 0.73 (EM 141) (Table 3). The dendogram scale produced by Jaccard's coefficient and UPGMA clustering method varied from 0.68 and 1.00, with mean similarity of 0.84 (Fig. 2). The 35 accessions were ordered into 6 clusters at 70% similarity coefficient. Cluster 1 comprised of 16 accessions, starting from MM1161 to MM1692; cluster members showed geographic and group heterogeneity. MM1161, MM1692 MM10213 and MM232 were most related at 100% similarity coefficient. MM1161, MM11008, MM1119, MM10213, MM232 and MM805 (members of Shum and GIlo) formed a group in this cluster. Also, accession 43 (Gilo) and MM1692 (Kumba) constituted another group within cluster 1. MM1616 (Shum), MM10147 (Kumba), MM1186 (Gilo) and MM01143 (Gilo) were ordered into a separate group.

The second cluster comprised of five accessions, separated into two groups; members of the Gilo group predominates this cluster. The third cluster accommodated two accessions each for Aculeatum, Shum, and Gilo groups, while LBR 48 grouped with S00817 in cluster 4. Three and two accessions of Kumba and Gilo groups respectively were grouped in 5th cluster; while one accession each of Aculeatum (MM 1158) and Kumba (MM 1107) were grouped in the 6th cluster.

The goodness-of-fit of the UPGMA dendrogram generated for SSR data tested by 2-way Mantel test (Mantel, 1967) showed a moderate support for clustering patterns for SSR data (r=0.70). Similarly, the goodness-of-fit of the UPGMA dendrogram generated with morphological and SSR data tested by 2-way Mantel test (Mantel, 1967) showed a moderate support for clustering patterns (r = 0.77).

The phenogram constructed for based on morphological and molecular data were consistent in showing morphological overlaps and genome sharing among the groups in *S. aethiopicum*. The grouping pattern observed for members of the Shum group in cluster 2 (Fig. 2) showed resemblance with cluster 1 (Fig. 3). In addition, the molecular based dendogram showed aggregation of accessions of the Kumba group from different geographical locations into cluster 5, with relatedness to the Gilo group (Fig. 3). While morphological data revealed that accessions of the Kumba group were interspersed among the Gilo and Shum groups (Fig. 2). Two accessions (MM10213 and MM232) are related at the molecular level (Fig. 2), but were distinct and separated into different clusters based on morphological data. MM1161 and MM11008 are related based on SSR data (Fig. 3), although both accessions were moderately separated in cluster 2 (Fig. 2). Two entries (MM232 and MM10213) were morphologically identical, but were

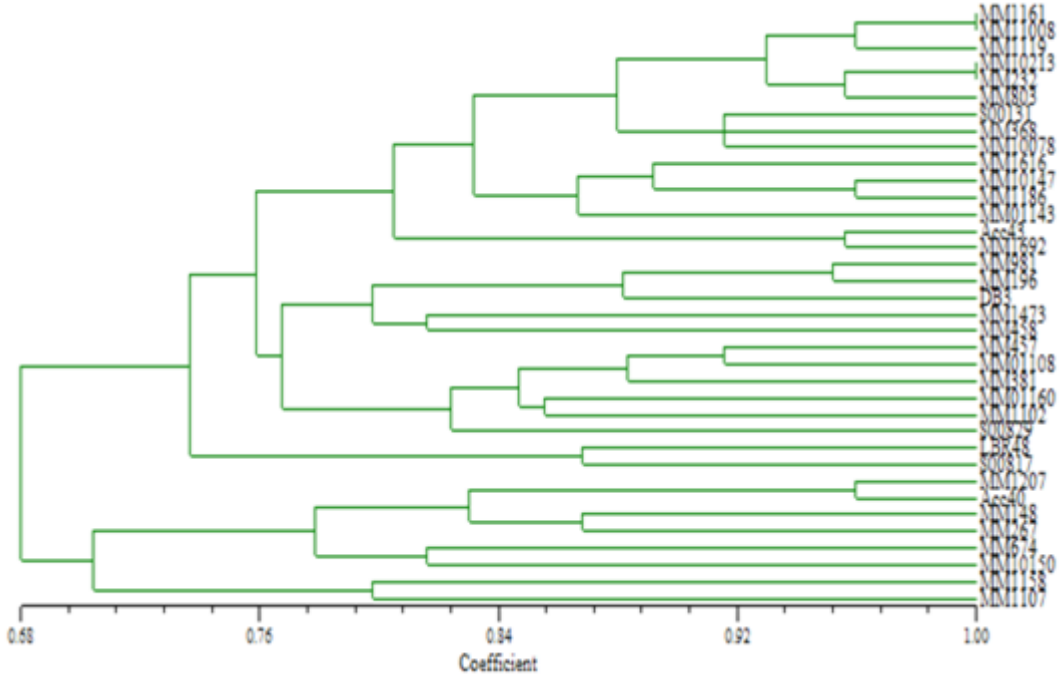

Figure 3. Dendogram constructed from SSR data using jaccard's coefficient of similarity and UPGMA clustering.

genetically unrelated considering SSR data. Accessions grouped in cluster 3 (Fig. 2) in dendogram based on morphological data, were ordered into cluster 1 in the dendogram (Fig. 3) constructed based on SSR data, except for MM1119, MM232, MM 1616 and MM10147.

DISCUSSION

The accessions of *S. aethiopicum* (Gilo, Kumba, Shum and Aculeatum) groups trailed in this investigated displayed large amount of variation for most morphological traits taking into consideration the multivariate techniques applied (Tables 4 and 5; Fig. 1). Both the PCA and dendrogram analyses applied to this species showed distinctness, similarities and overlap morphological traits within and among groups, and genotypic relatedness based on SSR data. Three morphological traits (leaf surface prickles, prickles underneath the leaves and fruit calyx pigmentation) demonstrated high discriminatory ability among accessions of *S. aethiopicum* groups. These traits are essential for documentation, conservation and crop improvement in this species. In general, members of the *S. aethiopicum* groups showed discrete variation for pubescence and prickliness on the plant parts. Both prickles and pubescence on plant parts are essentially used in breeding program to reduce incidence of insect pests and associated diseases. In Nagpur (Central India), the presence of prickles on the calyxes is linked with good fruit organoleptic quality (Levin *et al*., 2005). In contrast, prickles are aggressive towards the workers hands during handling (Aubert *et al*., 1989a, 2002). In addition, pubescence on plant parts caused a strong sensation on the skin and adversely affects the respiratory system of people who handle the plants. Specifically, one accession (S000131) recorded many prickles and medium pubescence on plant parts, and could be selected as donor parent in breeding programme targeting these traits.

The discriminatory ability associated with fruit calyx pigmentation (pigmented and non pigmented fruit calyxes) lends weight to previous report of Collonnier *et al.* (2001) among *Solanum* species. Variability observed for fruit colour at commercial ripeness and physiological maturity implied the possibility of identifying donor parent for breeding work. Fruit pigmentation is a

complex trait; it combines two pigmentation and distribution pattern genes (Daunay *et al.*, 2001). Fruit colour differences at commercial ripeness among the groups in *S. aethiopicum* are basically due to two colour pigments' their effects on appearance are controlled by more than one gene (Frary *et al.*, 2007).

The spread of accessions on the PC biplot (Fig. 1) and grouping (Fig. 2) on the dendrogram considering morphological traits implied that accessions belonging to the Gilo, Aculeatum and Kumba groups displayed higher genetic variability within each group than among groups. The morphological biplot (Fig. 1) and dendrogram (Fig. 2) consistently revealed overlap morphological traits and geographical heterogeneity among the groups. Morphological proximity and similarities observed among accessions of the Kumba and Aculeatum groups was reflective of relatedness associated with multi locular characteristic of the fruits, this is associated with the number of filaments observed for each group (Schippers, 2000). This trait is predominant in the Kumba group compared to the Aculeatum group. Further, within the Aculeatum group, fruits are largely green compared to cream or white at commercial ripeness, while fruits are either cream or green in the Kumba group. Based on morphological data, the proximity between accessions of the Gilo and Shum groups was reflective of relatedness for seed colour (white), absence of fruit curvature, glabrous leaves, non-prickled stem and moderate leaf lobbing.

Molecular profiling with SSR markers pairs reinforced a wide genetic background among *S. aethiopicum* groups. The phylogenetic tree (Fig. 3) resulting from the analysis of the matrix of dissimilarities sufficiently discriminated accessions belonging to the four groups; this was evident by the degree of chaining on the phylogenetic tree. The tree constructed using SSR data set revealed that accessions from the *S. aethiopicum* groups had moderate to high genetic variation. The grouping pattern observed for MM1102 and MM457 (Aculeatum group) showed consistence for morphological descriptors (pubescence, prickles and fruit colour) and at genotypic relatedness (based on SSR data). Additionally, genome sharing between these accessions was possible, though these entries were received from Burkina Faso and Japan respectively. Both accessions could be selected as donor parent (pubescence and prickles).

Findings based on morphological and SSR data implied that the accessions of the Kumba and Shum groups are morphologically and genetically related. Morphological traits associated with this grouping are; absence of pigmentation on vegetative parts and glabrous leaves. Also, considerable segregating population might be developed for fruit colour (cream) and other associated traits, if inter cluster hybridisation was undertaken among members of clusters 2 and 3 (Fig. 2). Based on SSR data, three accessions (S00131, MM10078, and MM368) showed high genetic relatedness, and possible morphological trait are pubescence on plant parts; and to a lesser extent is prickles on plant parts. These accessions belong to the Aculeatum, Shum and Gilo groups respectively. In contrast, both MM 10078 and MM 368 were grouped in cluster 2 (Fig. 2), while S00131 was an outlier considering morphological traits. MM1102 and MM457 were morphologically related, though showed wide variation at the genotypic level.

The application of morphological and microsatellite marker pairs showed that accessions from groups in this species are intermingled and group specific clusters were absent. This support phenotypic and genotypic overlaps among the groups in *S. aethiopicum*. The magnitude of morphological variation pattern reported in this study implied that these accessions are important and should be conserved for future genetic studies. Also the application of morphological traits and molecular markers used in this investigation provided a reliable evaluation and robust characterisation of diversity among *S. aethiopicum* groups. The high morphological variation in the materials studied is matched by high levels of molecular diversity. The lack of correlation between morphological and molecular data may be caused by the fact that both types of markers follow different evolutionary paths. Overall, it was noted that accessions from *S. aethiopicum* groups originating from different parts of the world did

not form a distinct cluster associated with geographical origin, but were interspersed with each other indicating no association between morphological traits or SSR marker pattern and geographical origin of the accessions. Therefore, the present study suggested that morphological traits and molecular markers could be able to achieve a reliable evaluation and robust characterisation of diversity among *S. aethiopicum* groups.

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