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Genetic relatedness based on morpho-agronomic traits and molecular marker (SSR) among seven Solanum (Eggplant) species and relatives

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ABSTRACT

Morpho-agronomic traits and microsatellite markers were used to survey genetic diversity among 43 accessions from seven *Solanum* species and two commercial tomato varieties (LBR 48 and Tanya). Fifty-six morpho-agronomic traits were studied. Field data of two consecutive years were subjected to principal component analysis. Dendrogram was constructed based on distance using the average linkage between group methods, with squared Euclidean phenotypic distance option Ward's as grouping criteria. Seventeen microsatellite markers (SSR) were also used to examine genetic diversity at molecular level that showed moderate to high polymorphism in the range of 0.38–0.87. Dendrogram based on squared Euclidean distances and UPGMA option ward analysis showed the species-specific clusters. There was genetic similarity among *S. aethiopicum* groups (Gilo and Shum), *S. integrifolium* (SOS-2) and *S. anguivi* (Fovembot). Genetic relationship between *S. aethiopicum* Aculeatum group (MM 457) and Gilo (Db_3) was reflective of the origin of Aculeatum. Overall, it was noted that accessions from the seven Solanum (eggplant) originating from different parts of the world did not form a distinct cluster associated with geographical origin, but were interspersed with each other. *Solanum aethiopicum* (S00916) was related to Tanya (*Solanum lycopersicum*), LBR 48 (*Solanum lycopersicum*) was similar to *S. melongena* (S0008 and S 00719). Arusha local related to MM 1616 (Shum) and S00017, while a landrace (Morogoro local) was similar to MM 196. The SSR markers returned significant variation in *S. macrocarpon*, conversely aggregation of *S. macrocarpon* was observed on the dendrogram based on morpho-agronomic traits. Both morpho-agronomic and microsatellites-SSR provided good insight of genetic diversity. Morpho-agronomic analyses exhibited similarities and plasticity among the entries.

Keywords: SSR markers, Genetic diversity, Polymorphism, Multivariate analysis, Solanum species.

INTRODUCTION

The *Solanaceae* is an important plant family that is distributed worldwide; it contains many domesticated species (tomatoes, pepper and potatoes) including the eggplant (*S. melongena*) and relatives (*S. aethiopicum, S. anguivi, S. macrocarpon, S. dasyphyllum, S. integrifolium* and *S. viarum*). *Solanum melongena* (brinjal eggplant), *S. aethiopicum* (scarlet eggplant), and *S. macrocarpon* (Gboma eggplant) are commonly referred to as eggplant (Mace *et al.,* 1999a). Other

Solanum species (*S. anguivi, S. dasyphyllum, S. integrifolium* and *S. viarum*) are least cultivated for consumption, in some cases they serve as medicinal purpose.

Genetic diversity is a prerequisite for improvement in *Solanum* (eggplant) species. This is essential for documentation of variability and identification of a section of the population that should be maintained to preserve maximum genetic diversity. Morphological (qualitative), agronomic (quantitative) traits and molecular markers are frequently used tools for estimation of diversity. The traditional method of morphological plants characterization is common, although it has some drawbacks, it is error-prone (Kumar *et al*., 2009). Collection and retention of germplasm based solely on morphology or agronomic traits can be misleading as both traits disparate accessions and may differ by one or two alleles. On the other hand consideration of only molecular genetic data is not a rationale for either germplasm management or breeding. A combination of these approaches could provide a guide to conservation and variety development in *Solanum* (eggplant) species. Simple sequence repeats (SSRs) have shown higher level of interspecies polymorphism in cultivated *S. melongena, S. turvum and S. dassyphylium* (Mace *et al.*, 1999a). SSR markers have proved to be useful for discrimination, assessment of genetic variation and protection of intellectual property (Plant Breeders' Rights). In sub Saharan Africa, morpho-agronomic and molecular analysis of diversity among *Solanum* species has not been utilized for purposes of breeding and crop improvement. There is a dearth of commercial and improved varieties compared to tomatoes (*Solanum lycopersicum*), onions (*A. sepa*) and peppers (*Capsicum annum*). The aim of this investigation was to investigate genetic diversity among accessions from seven *Solanum* (eggplants) species largely drawn from Africa based on morpho-agronomic traits and SSR markers, and to identify donor parents for intraspecies and inter species hybridization.

MATERIALS AND METHODS

Morpho-agronomic and molecular diversity was assessed in 43 accessions from seven *Solanum* species and two commercial tomato varieties (LBR 48 and Tanya (Table 1). Morpho-agronomic traits were monitored from vegetative through reproductive stages until harvest. Descriptors used conform to the guidelines of the Asian Vegetable Research and Development Center (http://www.avrdc.org). The experimental site is located at Latitude 4.8S Longitude 3°.7E; Altitude 1290 m (Asian Vegetable Research and Development Center, Arusha, Tanzania) with annual rainfall of 700 to 1000mm. Soil type was clay loam with a pH of between 6.0 and 6.5. Experimental plots were laid out in a randomized complete block design with three replications during 2008 through 2010. Each plot consisted of a double row of 7 meters and 0.75m between rows. Qualitative traits were measured on fifteen plants (five competitive plants per replicate). Vegetative traits were scored within fifteen days before flowering, during this time, maximum development of vegetative parts ought to have taken place. Traits related to colour were scored using the standard Royal Horticultural Society (1986) colour chart. Quantitative traits were consistently measured from fifteen plants. Plant height (m) was measured at 50% flowering and at maturity. By random sampling of 30 flowers per entry, petal length and width (cm), sepal length and width (cm), number of petals and sepals per flower (count) were estimated. Fruits per plant, fruits per infructescence and fruit infructescence per plant were counted at harvest. By random sampling of 22 fruits per replicate, fruit calyx length and width (cm), total acidity (pH) of the fruits at commercial ripeness and physiological maturity were

determined on a pH meter (meq citric acid/100 ml). Total Soluble Solids (TSS) was measured using hand held refractometer on ten randomly selected fruits per entry at commercial ripeness stage and at physiological maturity. Time to fruit browning (measured by visual estimation after 1min and 30 min) was determined on a stop watch. Harvested fruits from the net plot were weighed and processed for determination of fruit yield (t ha^{-1}) and seed yield (t/ha).

Plant material, DNA extraction and PCR reaction

Seeds of 43 accessions from seven *Solanum* species and two released tomato varieties (LBR 48 and Tanya) (Table 1) 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 disc samples were bulked in Eppendorf tubes and stored at a freezing temperature 24ºC for DNA extraction. Bulked leaf samples were supposed to combine equal amounts of DNA from each individual constituting the bulk.

For DNA extraction process, Wizard Genomic DNA Purification Kit, (Promega, Madison, WI, USA), was utilized. Sixteen SSR primer pairs were synthesized by Integrated DNA Technologies, Inc., 1A, USA and checked for amplification. The total genomic DNA was extracted from bulk 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 (Qiagen product) 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 include preliminary denaturation for 5 min. at 94ºC; 35 cycles at 94ºC for 30 s., 50ºC for 1 min., 72ºC for 1 min.; final extension for 5 min at 72ºC and held at 4ºC. For annealing temperature, estimation was done with ± 5ºC the melting temperature of the SSR primer pairs as indicated by Integrated DNA Technology, after series of troubleshooting, annealing temperature between 63ºC and 65ºC was applied. Amplification products were mixed with loading buffer containing bromophenol blue, separated electrophoretically on 2% agarose gel followed by ethidium bromide staining, and visualized on a UV Transilluminator. Each amplified fragment was visualized as distinct bands.

Morphological traits were coded, while agronomic traits were subjected to logarithm transformation before data analysis. Both were submitted to Principal Component Analysis using PROC PCR (SAS, 1998) and Cluster analysis using SPSS Version 16. Molecular base data generated by using 16 microsatellites primers on the accessions were scored in binary format, with the presence of a band scored as 1, and its absence as 0, thus generating a binary matrix. Total number of bands (TNB), number of polymorphic bands (NPB), number of monomorphic bands (NMB), and percent polymorphism (PP), (PP=NPBs/TNB) were recorded for each marker. Binary data were used to compute a pairwise similarity coefficient (Jaccard 1908). 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.

Table 1. List of 43 accessions from seven Solanum (Eggplant) species, and LBR 48 and Tanya (*Solanum* l*ycopersicum)* assayed with 16 SSR markers

Sn	Accession	Species	Origin	Sn	Accession	Species	Origin
1	MM 1161	*S. aethiopicum* Shum	Benin	24	S 02865	*S. viarum*	Lao Peoples Republic
2	MM 10213	*S. aethiopicum* Gilo	Ghana	25	MM 457	*S. aethiopicum* Aculeatum	Japan
3	MM 1164	*S. dasyphylium*	Togo	26	MM 01160	*S. aethiopicum* Shum	Benin
4	MM 1616	*S. aethiopicum* Shum	Unknown	27	MM 01108	*S. aethiopicum* Kumba	Burkina Faso
5	MM 1144	*S. macrocarpon*	Nigeria	28	S 0718	*S. melongena*	Indonesia
6	MM 10147	*S. aethiopicum* Kumba	Burkina Faso	29	S02223	*S. viarum*	India
7	MM 714	*S. macrocarpon*	Zimbabwe	30	MM 1158	*S. aethiopicum* Aculeatum	France
8.	S0014474A	*S. viarum*	Thailand	31	MM 1102	*S. aethiopicum* Aculeatum	Burkina Faso
9	S0017	*S. melongena*	Malaysia	32	SOS 2	*S. Integrifolium*	Nigeria
10	S00023	*S. melongena*	India	33	MM 10256	*S. macrocarpon*	Ghana
11	MM 1186	*S. aethiopicum* Gilo	Unknown	34	MM 12126	*S. dasyphylium*	Uganda
12	MM 1207	*S. aethiopicum* Kumba	Mali	35	S 0023	*S. melongena*	India
13	MM 981	*S. aethiopicum* Gilo	Uganda	36	MM 1619	*S. aethiopicum* Gilo	Ivory Coast
14	MM 1127	*S. macrocarpon*	Benin	**37**	MM 196	*S. aethiopicum* Gilo	Ivory Coast
15	Taumbot 3	*S. anguivi*	Cameroon	38	MM 905	*S. macrocarpon*	
16	Taumbot	*S. anguivi*	Cameroun	39	MM 148	*S .aethiopicum* Aculeatum	Unknown
17	S00017	*S. melongena*	Malaysia	40	Morogoro	Landrace	Tanzania
18	S00022	*S. melongena*	India	41	Tanya	*S. lycopersicum*	Tanzania
19	MM 12209	*S. macrocarpon*	Zaire	42	Arusha local	Landrace	Tanzania
20	Acc 43	*S. aethiopicum* Gilo	Unknown	43	S00916	*S. aethiopicum*	Indonesia
21	MM 01143	*S. aethiopicum*	Nigeria	44	S0008	*S. melongena*	Indonesia
22	LBR 48	*S. lycopersicum*	Tanzania	45	S 0017	*S. melongena*	Malaysia
23	DB3	*S. aethiopicum* Gilo	Tanzania				

RESULTS

Morpho-agronomic diversity

There were significant mean squares for all traits, the mean squares for year (Y) and genotype x year interaction (GYI) were significant or insignificant depending on the trait under consideration. The principal component analysis returned 1st to 9th principal axes to have eigenvalues greater than 2.0, altogether accounted for 76% of total variation (Table 3). The first to six principal components had eigenvalues greater than 3.00; they summarized 64% of the total variance. Eigen value was highest (9.96) in the first PC; it accounted for 18% of the total variance, and demonstrated high variation for leaf, petiole and receptacle pubescence. Both number of petals and sepals marked equal and positive weights on PC 1, though negative on PC 2. The second PC axis recorded eigenvalue of 8.60 and summarized 15% of the total variation (Table 3); it depicted discriminatory power of prickle spines under the leaves, midrib and petiole and leaf vein.

Table 2. List of SSR markers used in this investigation

Primer name	Repeat motif (5' – 3')	Primer sequence 5'-3'	Motif length	Annealing temperature	product size (reference)	Expected product size	% GC	Repeat types
EM 117	F-GAT CAT CAC TGG TTT GGG CTA CAA	$(AC)_{19}$ $(AT)_{11}$	24	65	160	120-220	45.8	Imperfect
	R- AGG GGA GAG GAA ACT TGA TTG GAC		24				50	
EM 120a	F-GGA TCA ACT GAA GAG CTG GTG GTT	$(AC)_{16}$	24	65	160	100-218	50	Perfect
	R-CAG AGC TTC AAT GTT CCA TTT CAC A		25				40	
EM120b	F- CAA AAG ATA AAA AGC TGC CGG ATG	$AC)_{16}$	24	65	248	80-240	41.6	Perfect
	R-CAT GCG TGA GTT TTG GAG AGA GAG		24				50	
EM 131	F- TCT GGG ACA CCA AGT GAA AAA TCA	(AT)5(AC)3A(AC)14(AT)7GTA(TG)5(TA)3	24	65	213	120-220	41.6	Compound
	R- TGC GTT TTT GGC TCC TCT ATG AAT		24				41.6	
EM 119	F-CCC CAC CCC ATT TGT GTT ATG TT	$(GGAGG)_5$ $(AT)_8$	23	65	210	100-210	47.8	Imperfect
	R-ACC CGA GAG CTA TGG AGT GTT CTG		24				54.1	
EM 141	F- TCT GCA TCG AAT GTC TAC ACC AAA	$(AT)_{16}$ $(GT)_{19}$	24	65	228	100-260	41.6	Imperfect
	R-AAA AGC GCT TGC ACT ACA CCT GAA T		25				44	
EM 114	F-AGC CTA AAC TTG GTT GGT TTT TGC	$(AC)_{13}$	24	65	221	159-250	41.6	Perfect
	R-GAA GCT TTA AGA GCC TTC TAT GCA G		25				44	
EM 127	F-CAG ACA CAA TGC TGA GCC AAA AT	$(AC)_{13}(AT)_{13}$	23	65	200	150-230	43.4	Imperfect
	R-CGG TTT AAT CAT AGC GGT GAC CTT		24				45.8	
EM 107	F-GGC CCT AGA CTG AGC CTG AAA TGT T	$(AC)_{13}(AT)_7$	25	65	214	100-240	52	Imperfect
	R-TGC TAC AAC CAA CAC AAC CCT CAA		24				45.8	
EM 116	F-TTA GAA ATT TCG GAA CAA AGA GA	$(AC)_{12}(AT)_8$	23	62	246	150-230	30.4	Imperfect
	R-CCA CAT GAA ACT TGG ACC AAT GAG		24				45.8	
EM 128	F- TAG CGG TGC TAG GTC CAT CAT CTC A	(CA)26(TA)19	25	60	295	100-231	52	Imperfect
	R-TTC TCA AGA AGT TGC TCC AAA GGA		24				41.6	
EM 133	F- GCG GAT CAC CTG CAG TTA CAT TAC	(AC)13(AT)4	24	65	177	120-220	50	Imperfect
	R- TCC TTT GAC CTA TAG TGG CAC GTA GT		26				46.1	
EM 134	F-AGT AAG GGA AAG TGC TGA CGA AGG	$(GT)_2GC(GT)_6$	24	65	168	120-300	50	Imperfect
	R-CAG AGT CAT CGT TAT GGG GAG GTT		24				58.3	
EM 140	F-CCA AAA CAA TTT CCA GTG ACT GTG C	$(AC)_4GC(AC)_5T(AC)_3ATGC(AC)_4$	25	65	268	150-300	44	Compound
	R-GAC CAG AAT GCC CCT CAA ATT AAA	$AT(AC)_6(AT)_5G(TA)_{13}$	24				41.6	
EM 145	F-TGA TTT GGC CCT TAA GCC TAA GTA TG	$(TG)_3TA(TG)_8$ $(TA)_6$	26	65	165	145-220	42.3	Compound
	R-GAC TCC TCA AGC CTT TAC CTC CAA		24				50	
EM 146	F-GGA CCA AAG CGA AAT TTT CAC AAC	$(AC)_{19}(AT)_{11}AC(AT)_2$	24	63	288	120-350	41.6	Compound
	R-TTG CAC CAA TTG GGA AGT AAC ACA		24				41.6	
EM 104a	F- TGG ATC TGC AAA GAA AAG GAG AAA G	(TC)9(AC)38(AT)19	25	60	246	200-350	40	Compound
	R- CGC AAA TCG GGT AGA CTT TCG AT		23				47.8	
EM 139	F-TGC TAA GTC GTC ATC CAA CAA GAA	(AC)6AT(AC)11(AT)10	24	65	258	130-340	45.8	Compound
	R-GAT TTT GGC TCC TTG ACC ATT TTG		24				41.6	

Table 3. Eigen values, proportion of variance, cumulative variance and vectors for nine principal component axes estimated for 53 morphological and agronomic descriptors among seven Solanum (eggplant) species

Principal Component Analysis	Eigen value	Proportion of variation accounted for (%)	Percentage cumulative	Eigen vectors				
1	9.96	18	18	leaf pubescence (0.23)	Petiole pubescence (0.24)	Receptacle pubescence (0.24)	Number of sepals (0.21)	Number of petals (0.21)
2	8.60	15	33	Spines under the leaf (0.27)	Mid rib spines (0.27)	Spines on leaf vein (0. 25)	Petiole spines (0.26)	Petal width (-0.20)
3	5.89	11	44	Flower size (0.30)	Receptacle pigmentation (0.28)	fruit cross section (0.27)	Sepal colour (0.26)	Seed colour (-0.25)
4	4.69	8	52	Plant height at flowering (0.28)	Calyx width (-0.28)	Petal length (-0.27)	Petal width (-0.25)	Fruit colour at commercial ripeness (0.24)
5	3.52	6	58	Stem prickles (-0.39)	Fruit colour at commercial ripeness (0.29)	Seed yield (-0.24)	Total soluble solid(-0.23)	Seed yield (0.23)
6	3.31	6	64	Fruit per cluster (0.41)	Calyx length (0.27)	Plant height at flowering (0.27)	Fruit yield per hectare (0.24)	Fruit colour at commercial ripeness (-0.22)
7	2.52	5	69	Leaf tip angle (0.31)	Day to 50% flowering (-0.31)	Leaf lobbing (-0.27)	Petiole colour (-0.25)	Leaf width (0.24)
8	2.18	4	73	P^H at commercial ripeness (0.37)	Taste (0.32)	Number of filament (0.31)	Seed yield per hectare (0.29)	Fruit apex shape (-0.27)
9	2.02	4	77	Fruit yield (0.30)	Seed yield per plant (0.29)	Number of petals (0.23)	number of sepals (0.23)	Taste (-0.23)

The ordination of principal component axes 1 by 2 (Figure 1) displayed variation in dispersion and pattern of divergence for morpho-agronomic traits, variation was large and adequate to identify species and geographic trends. Dispersion of accessions in the first quadrant was associated with discriminatory power of leaf, petiole and receptacle pubescence with moderate on the first PC. Eleven accessions were ordered in the second quadrant, and showed discriminatory ability of number of petals and sepals. Fruit taste at commercial ripeness was responsible for dispersion of accessions in the third quadrant with negative coefficients on both PCs 1 and 2. The PCA plot provided a measure of diversity among accessions belonging to the seven *Solanum* species, as glabrous and prickled free accessions of *S. anguivi* and *S. aethiopicum* Shum group (subgenus Oliganthes) with high fruits per plant and fruits per cluster, usually small red fruits colour at physiological maturity spread out in an elongated constellation with *S macrocarpon*, characterized by big flabby, orange or yellow fruits at physiological maturity. Similarly prickled free and glabrous accessions of *S. anguivi* and *S. macrocarpon* are in constellation with highly pubescent and prickled accessions of *S. viarum and S. dasyphyllum*. Further glabrous and prickled free accessions of *S. macrocarpon, S. aethiopicum* Gilo are in elongated constellation with highly pubescent of *S. melongena*. Cluster formation based on squared Euclidean distance grouped the 45 accession into five clusters at 15% distance; clusters were formed in relation to the genetic status and are largely specie specific (Figure 2). Factors responsible for differentiation of the accessions into clusters had high percentage contribution toward genetic distance. The first cluster accommodated 6 accessions and showed specie and geographic heterogeneity. Cluster members are separated into sub clusters ‘a’ and ‘b’. MM 1158 and MM 148 are related and linked to SOS 2 (*S. integrifolium*) at the lower end, MM 457 and MM 1102 (*S. aethiopicum* Aculeatum) showed proximity to MM 12123 (*S. dasyphyllum*).

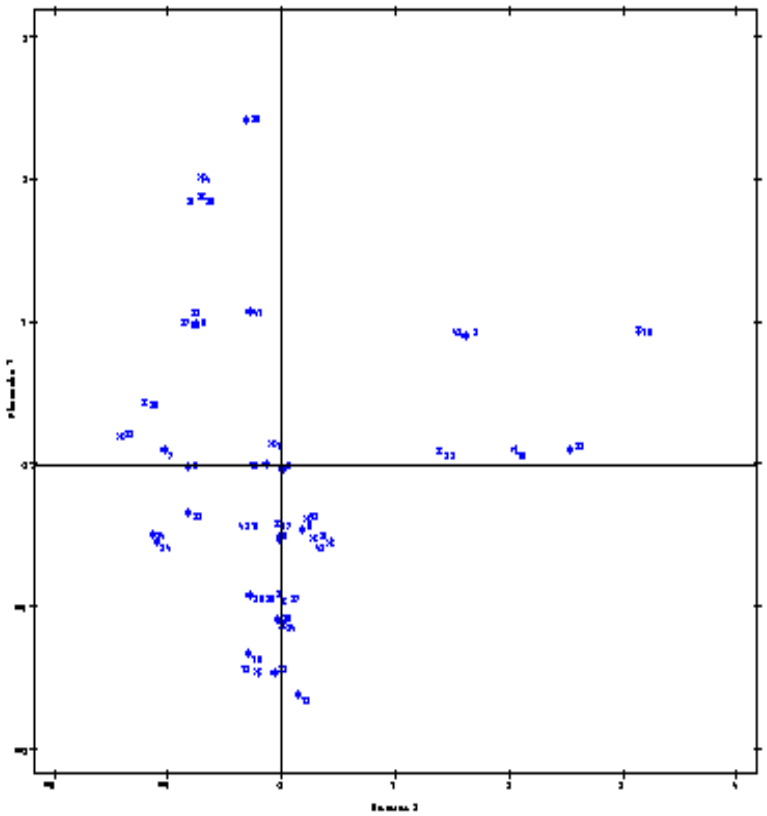

Fig. 1: Plot of the first two principal components axes showing spatial distribution of 43 accessions from seven Solanum (Eggplants) species

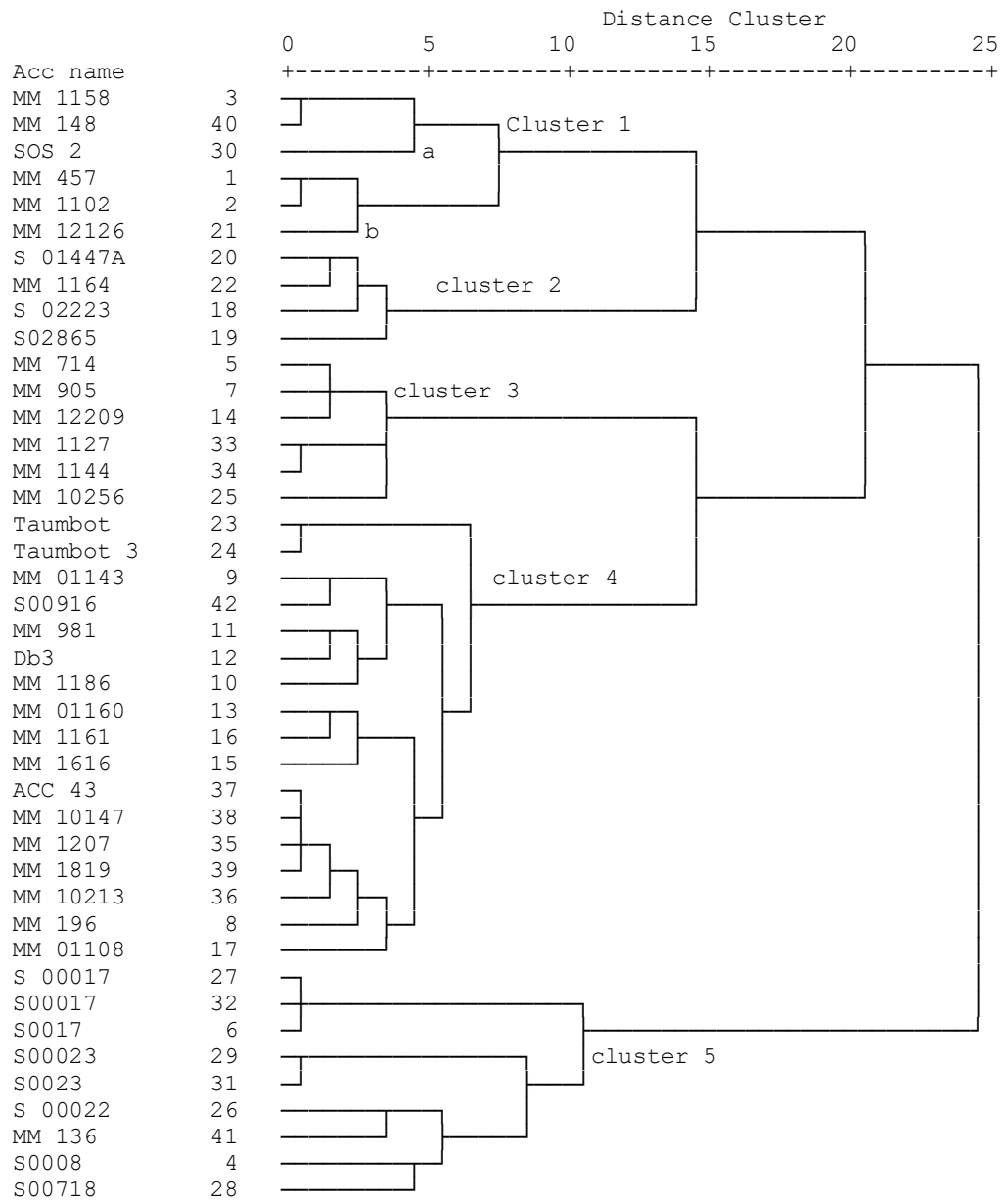

Fig. 2: Dendrogram showing clustering pattern of 42 accessions from seven Solanum species based on Euclidean coefficient values obtained from morpho agronomic data

Members of cluster 1 are glabrous and non-prickly (group a) or pubescent and prickled (group 'b'), fruit length ranged from 3 to 6 cm, and fruit width ranged from 3.2 to 7 cm. (Figure 2). The second cluster comprised 4 accessions starting from S01447A to S02865, two accessions each to *S. dasyphyllum* and *S. viarum*; cluster members are highly pubescent with prickled spines on vegetative parts. Cluster 3 showed specie-specific cluster (*S. macrocarpon*) cluster members are characterized by glabrous leaves, stems and good tasty fruits. MM 1127 and MM 1144 are most related probably due to purple petal pigmentation. Accessions of *S. aethiopicum* groups and *S. anguivi* predominates the fourth cluster. The Shum group displayed intermediate phenotypic performance and

were ordered into a grouped flanked on both sides by the Gilo and Kumba groups. Cluster members are either glabrous or moderately pubescent, characterized by moderate to high fruits per plant. Nine accessions of *S. melongena* were ordered into the fifth cluster, S 00017, S0017, S 0017 and S 00023 and S 0023 are most related, they marked lowest phenotypic distance in this cluster. Cluster members showed pubescence on plant parts, with presence and absence of prickles.

Table 4 Intra-class averages and range of genetic divergence in morph-agronomic traits among seven Solanum (Eggplant) species

	Cluster 1		Cluster 2		Cluster 3		Cluster 4		Cluster 5	
Traits	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Fruit length	3 – 6	5.0	3 – 6	5	3 – 5	4	4 – 11	7.5	1 – 6	3.5
Fruit width	3 – 7	5.0	3 – 7	5	7 – 10	9	4 – 17	10	1.3 -8.0	4.7
Fruits per plant	46 - 55	42	56 - -89	73	12 – 32	22	23-478	251	13 – 65	39
Fruit yield per hectare	34 – 49	42	25 – 33	2 9	55 – 69	62	28 – 75	52	44 – 76	60
Fruits per infructescence	2 – 4	3	1 – 2	1.5	2	2	2 - 14	7	2 – 8	5
Fruit pH at commercial ripeness stage	5.1 - 6.2	6	5.6 - 5.9	5.8	5.5 – 5.9	5.7	5.0 - 6.1	5.6	5.1 – 5.8	5.5
Time to fruit browning	4 – 20	11	9 – 17	13	3 – 5	4	1 – 10	5	2 – 5	3.5
Fruit cluster per plant	9 – 18	14	10 – 24	17	1-3	2	2 – 14	13	8 – 24	16
Seed yield per hectare	0.25 - 0.89	0.57	0.5 – 1.2	0.7	0.5 – 1.2	0.9	0.55-1.12	0.84	0.44 - 1.63	1.04
Fruit taste	1 – 2	2	2	2	2	2	1 – 2	1.5	1 – 2	1
Days to first flowering	64 – 85	75	56 – 82	69	55 - 83	69	52 – 82	67	63 – 65	64
Flower size	2	2	1 – 3	2	3	3	2	2	3	3
Total Soluble solid	3.4 – 9.0	6.2	4.0 - 5.4	4.7	4.6 – 6.0	5.3	4 - 7.7	5.85	5 – 7.1	6.05

Molecular (SSR) diversity

A total of 459 alleles were amplified from full genotype panel with number of alleles varied widely (Table 5), and a number of amplified alleles per marker ranged between 10 (PR 141) and 40 (PR 120b). The mean allele per locus per marker was 5.3 for *S. melongena*, 12.0 for *S. aethiopicum*, and 5.0 for *S. macrocarpon*. Among *S. melongena and S. aethiopicum*, all the SSR markers were polymorphic, allele number ranged from 3 and 18. Further, 86 alleles were amplified from genotypic panel from 10 accessions of *S. melongena* assayed, on the average 5.3 alleles per locus was detected. Thirty-two accessions of *S. aethiopicum* and six accessions of *S. macrocarpon* assayed showed considerable polymorphism. Among *S. aethiopicum* 191 alleles were recorded with 12 alleles per locus per SSR marker, a total of 87 alleles with 5.0 alleles per SSR marker per locus for *S. macrocarpon* were amplified. Only one accession each was assayed for Morogoro landrace, Arusha landrace, LBR 48 (Solanum *lycopersicum)* and Tanya (*Solanum lycopersicum*). The result showed 3, 7, 8 and 5 alleles respectively. The amplicon size was in agreement with expectation.

Table 5. Number of alleles, number of monomorphic loci, polymorphic loci, percent polymorphic and proportion of polymorphism generated by each SSR primer.

SSR Primer	TNB	NMB	NPB	NAB	PP	PPB	No. of amplified bands
EM 133	47	16	8	23	0.51	0.33	23
EM 114	47	8	25	14	0.70	0.76	39
EM 107	47	34	7	06	0.87	0.17	23
EM 104	47	21	2	24	0.49	0.08	40
EM 120a	47	15	20	12	0.74	0.57	37
EM 120b	47	31	7	09	0.81	0.18	27
EM 145	47	6	23	12	0.62	0.79	29
EM 128	47	24	6	18	0.64	0.20	33
EM 119	47	18	15	14	0.70	0.45	22
EM 134	47	14	7	26	0.45	0.33	30
EM 139	47	19	6	22	0.53	0.24	29
EM 117	47	19	13	05	0.68	0.41	27
EM 116	47	20	6	21	0.55	0.30	32
EM 146	47	28	5	14	0.70	0.15	28
EM 140	47	15	12	20	0.57	0.44	30
EM 145	47	11	18	18	0.62	0.62	10
EM 141	47	2	11	34	0.38	0.85	12

TNB= Total number of bands, NPB= Number of polymorphic bands, NAB= Number of absent bands,

NMB= Number of monomorphic bands, PP= Percent polymorphism, PPB=Proportion of polymorphic bands

Dendrogram constructed based on microsatellites (SSR primers) data using Jaccards coefficient and UPGMA clustering method grouped the 47 accessions into 5 clusters, on a scale between 0.60 and 1.00, with a mean similarity of 0.82, in contrast to dendrogram based on morpho-agronomic traits specie specific clusters were not evident (Figure 3). At 73% similarity entries were restricted into 5 clusters, the first cluster comprised of 2 accessions (MM 198 and Morogoro local) at 86% similarity. The second cluster comprised six accessions starting from MM 1616 to Fovembot-2, and was divided into two groups, S00916 (*S. aethiopicum*) and Tanya *(solanum lycopersicum),* both are related at 0.91. Arusha local (a landrace) was linked to this group at 0.85 similarity coefficient (upper end), further Fovembot 2 showed similarity to this group at 0.84 relatedness value. MM 1616 and S0017 are most related (0.91), both are linked to other group members at 0.75 relatedness value. The third cluster accommodated 17 accessions divided into groups 'a' and 'b'. Eight accessions are restricted to group 'a' starting from MM 714 to S 000719 and group 'b' from LBR 48 to MM 1102. The groups displayed specie and geographic heterogeneity and showed preponderance of *S. aethiopicum* compared to *S. melongena*, *S. macrocarpon* and *S. viarum*. MM 714 (*S. macrocarpon*) and Db3, MM 01160 (Shum group) and MM 12126 (*S. dasyphylium*), and S00718 (*S. melongena*) and S 02223 (*S. viarum*) marked highest similarity coefficient (1.00), S00718 (*S. melongena*) and S 02223 (*S. viarum*) are related and similar to S 02865 at the upper end at approx 0.95 similarity. *S. macrocarpon* and *S. melongena* belong to the same sub genus Leptostenomum, but section *Melongena*. MM 1158 at the lower end was related to this group at 0.93 similarity value, MM 1102 represents an outlier in this group. Still in group 'a' LBR 48 and S 0008 (*S.*

melongena) are related at 0.91. This group was linked to MM 148 (Aculeatum) at 0.86. MM 714 (*S. macrocarpon*) and Db3 (Gilo), MM 01160 (Shum group) and MM 12126 (*S. dasyphylium*) are most similar; they are related at 0.94 similarity coefficient. MM 457 (Aculeatum group) showed genetic relatedness to this group at 0.89 similarity coefficient. Two entries S 0017 and S 00719 (*S. melongena*) displayed proximity with low genetic similarity in cluster 3.

Three accessions (S00023, MM 135 and S 01447A) are placed in cluster 4, S 00023 (*S. melongena*) and MM 136 (*S. macrocarpon*) are grouped (0.90) and further related to S01447A (*S. viarum*) at 0.80 similarity. The fifth cluster accommodated 19 accessions starting from MM 1164 to S 0023, all entries belong to *S. aethiopicum* Gilo, Kumba and Shum, with exception of four entries of *S. macrocarpon*, two entries to *S. melongena* and one to *S. anguivi*. This cluster was divided into two groups 'a' and 'b'. Group 'a' comprised 12 accessions starting from Acc 43 to MM 10256 and group 'b' from MM 10147 to S0023. MM 01143 (Gilo) and MM 905 (*S. macrocarpon*) returned highest similarity coefficient, and is related to MM 981 (Gilo) at 0.95 similarity value. Interestingly MM 1164 (*S. dasyphylium*), MM 1144 and MM 12209 (*S. macrocarpon*) all of which are placed in the series Macrocarpa of the section Melongena formed a group in cluster 4. Similarly MM 1161 (Shum), MM 10213 (Gilo) and SOS-2 (S. *integrifolium*) and Fovembot (*S. anguivi*) all of the same section Oliganthes are related at 0.88 similarity coefficient. In Group 'b' MM 10147, S0017, MM 1188 and MM 1127 are related at 0.91 similarity coefficient, while MM 1207, S00022, and S0023 paired at 0.90 similarity value. S 0017 (*S. melongena*) and MM 1186 are high similarity value (0.96) and further linked to MM 10147 at 0.91 similarity coefficient. Further MM 1207 (Kumba) and S 00022 (*S. melongena*) are similar (0.96) and related to S 0023 (*S. melongena*) at 0.91.

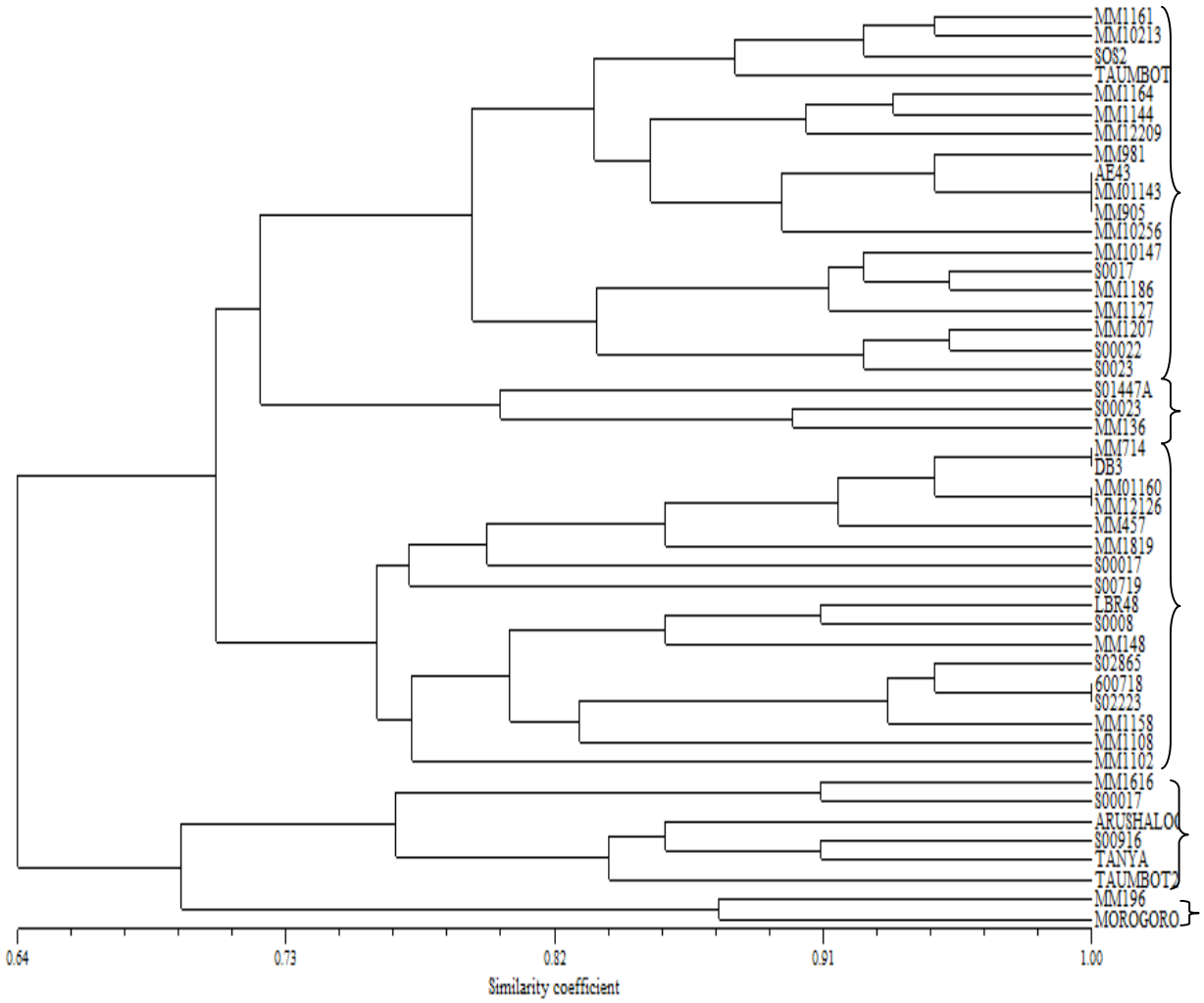

Fig. 3. A dendrogram constructed from the SSR data, using Jaccard's coefficient of similarity and UPGMA clustering among 45 accession of Solanum (eggplant)

Additionally, three accessions of *S. aethiopicum* Aculeatum group (MM 457, MM 1102 and MM 1158) are grouped in cluster 3, MM 457 grouped with MM 12126 (*S. dasyphyllum*), and are related with MM 01160 (Shum). On the other hand MM1102 and MM 1158 showed proximity, though the latter was more related to *S viarum* (S. 02223 and S 02865) and *S. melongena* (S 00718). In contrast high variability among two accessions of *S. dasyphylium* (MM 12126 and MM 1164) was evident as both were restricted into clusters 3 and 4 respectively. Two accessions of S 02223 and S 02865 (from India and Laos people republic respectively) are genetically related compared to S 01447A sourced from Thailand. Dendrogram constructed based on SSR data showed that cluster 4 and 3 had higher genetic diversity compared to other clusters, 80% of the accessions had genetic similarities of 0.80 or higher.

A comparison of dendrogram generated by morpho-agronomic traits and SSR markers indicated that three accessions (S 00017, S 0017 S 00017-1) were most related at 2% distance in Cluster 5 (Figure 3). On the other

hand dendrogram based on SSR data confirmed wide and significant variation among these accessions as they were separated and grouped with other accessions. Additionally, Fovembot and Fovembot 3 grouped tightly on the dendrogram, but showed significant variation as they were separated into clusters 2 and 5 based on SSR data. Morpho-agronomic and SSR data are consistent for high variation among *S. aethiopicum* Aculeatum and its grouping with *S. dasyphyllum* and *S. viarum*. The goodness-of-fit of the UPGMA dendrograms microsatellite data were tested by 2-way Mantel test (Mantel 1967). Moderate support for clustering patterns was observed for SSR marker (r = 0.86).

DISCUSSION

The importance of Solanum species (eggplants) in the tropics is increasing because of its nutritious food components and good market price. The PCA performed on morpho-agronomic traits showed that receptacle and petiole pubescence, prickle spines under the leaves and mid rib are adequate to discriminate among the accessions evaluated. Based on morpho-agronomic data, *S. viarum* and *S. dasyphyllum* are related for delayed flowering, prickles spines on the leaves, stem and reproductive structures, bitter tasty fruits and long time to fruit browning after cut compared to other clusters. Proximity between accessions of *S. anguivi* and *S aethiopicum* (Gilo and Shum groups) was reflective of relatedness for fruits per plant, fruit infructescence per plant and glabrous to fairly pubescent leaves. Additionally genome sharing between *S. anguivi* and *S. aethiopicum* was possible, and both belong to the section Oliganthes. High fruit number and fruits per infructescence among *S. anguivi* is in agreement with previous report by Lester and Durrands (1984), though with low variability. From plant breeder's point of view accessions of *S. anguivi* could serve as donor parent, whenever improvement is sought for increased fruit yield, high heterosis for fruit yield was possible if hybridization was carried out among *S. anguivi* and *S. aethiopicum* Gilo group. Dispersion and grouping of accessions of *S. aethiopicum* groups (Gilo and Kumba) reflect ample variability and overlap among the sub groups. This is consistent with morphological plasticity (Edmonds, 2005) and similar genomes (Agieszka, *et al.*, 2007). Specie specific clusters for *S. macrocarpon*, *S. anguivi*, *S. melongena* and *S. aethiopicum* lend weight to priori classification for these species (Daunay, 2008).

Considering morpho-agronomic traits, accessions of *S. macrocarpon* showed proximity to *S. aethiopicum*. This confirmed the conclusion reached by Furini and Wunder (2004). Aggregation of 6 accessions of *S. macrocarpon* from Africa and Asia and grouping on the dendrogram support genetic relatedness within this specie, despite cluster showing geographic heterogeneity. For selection purposes emphasis should be at the population level rather than geographic origin. In addition, phenotypic proximity between *S. macrocarpon* and *S. anguivi* Lam was reflective that *S. macrocarpon* was domesticated in Africa from its wild relatives *S. anguivi* and *S. dasyphyllum* (Lester and Niaken, 1986). For crop improvement hybridization program involving genetically diverse parents belonging to different distant clusters for example clusters 1 and 4 would provide an opportunity for aggregating gene constellations of fruit length, fruit width, fruit pH, and taste. Promising hybrid derivatives may result probably due to complementary interaction of divergent genes. Additionally, accessions in clusters 1 and 4 may evolve new population with high fruit yield and tasty fruit. Accessions grouped in clusters 4 and 5 could be used as donors in hybridization program for obtaining wide spectrum of

variation among the segregants for fruit length, width, fruits per plant and tasty fruits. Genotypes in these clusters are comparatively high yielding with higher fruit number and tasty fruits.

The level of polymorphism observed ranged between 0.05 – 0.92 indicating wide and diverse genetic base, similar to those reported by Singh *et al.,* (2006). Low amplification among species reflects narrow genetic background and probably autogamous nature of the population examined. Low polymorphism in the checks i.e. Tanya and LBR 48 (*Solanum lycopersicon*) for microsatellite markers is in line with RFLP and RAPD markers as reported previously by (Broun and Tanskley 1996). Molecular profiling with SSR marker reinforced that accessions of *S. viarum* showed affinity to *S. melongena* and *S. aethiopicum* Aculeatum group. This indicated that *S. viarum* was close to cultivated eggplants (*S. melongena*). Genetic similarity as found between *S. viarum* and *S. melongena* corroborates findings reported by Singh *et al.,* (2006) for RAPD markers. Genetic similarity among *S. viarum* and *S. melongena* may phenotypically be associated with presence of pubescence and prickles on plant parts, although these traits are more pronounced in *S. viarum* compared to *S. aethiopicum* Aculeatum and *S. melongena*. In contrast to our observation based on the morpho-agronomic traits, molecular data indicated close genetic relatedness among *S. dasyphyllum* and *S. macrocarpon* (Mace *et al.*,1999b and Levin *et al.,* 2006).

This study revealed genetic similarity among *S. aethiopicum* groups (Gilo and Shum), *S. integrifolium* (SOS-2) and *S. anguivi* (Fovembot). This may probably be that *S. aethiopicum* was domesticated from *S. anguivi* (Lester and Seck 2004) and *S. integrifolium* is synonymous to *S. aethiopicum* (Toppino, *et al*., 2008). Further, *S. anguivi*, *S. aethiopicum* and *S. integrifolium* are interfertile and could be a gene pool for genetic improvement, though reciprocal hybrids were not obtained in crosses with *S. anguivi*. Overall, it was noted that accessions from the seven Solanum (eggplant) 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 SSR marker pattern and geographical origin of the accessions. The dendrogram analyzed using SSR data revealed that accessions from the seven Solanum species had moderate to high genetic. *S. aethiopicum* (S00916) was related to Tanya and LBR 48 was similar to *S. melongena* (S0008 and S 00719). Arusha local was similar to MM 1616 (Shum) and S00017 probably due to fruit size and S00017, while Morogoro local was similar to MM 196.

CONCLUSION

Receptacle and petiole pubescence, prickle spines under the leaves and mid rib are traits of high discriminatory ability. *S. viarum* and *S. dasyphyllum* are phenotypically related. Accession of *S. anguivi* and *S. aethiopicum* (Gilo and Shum groups) are similar for fruit/plant, fruit infructescence/plant and glabrous to fairly pubescent leaves. High number of fruits and fruit/infructescence distinguished accessions of *S. anguivi* form other species. Donor parents for fruit number, fruit size and fruit yield were identified. Phenotypic similarity was found among accessions of *S. macrocarpon* irrespective of their geographic origin. Genetic similarity among accessions of *S. viarum* and *S. melongena* may phenotypically be associated with presence of pubescence and prickles on plant parts. This study revealed genetic

similarity among *S. aethiopicum* groups (Gilo and Shum), *S. integrifolium* (SOS-2) and *S. anguivi* (Fovembot). Phenotypic and genetic variability observed within and among species could be exploited for development of new varieties through hybridization and selection.

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