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Full Length Research Paper

Molecular diversity among seven *Solanum* (eggplant and relatives) species assessed by simple sequence repeats (SSRs) markers

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Seven *Solanum* species (eggplants) were investigated for molecular diversity. Besides its widespread cultivation, nutritional and economic importance, its genome has not been extensively researched. 39 *Solanum* accessions, a landrace and tomato variety (LBR 48) were molecularly analyzed by simple sequence repeat (SSR) marker technique. A dendrogram was obtained based on the Jaccard's coefficient of similarity and unweighted pair group method with arithmetic mean (UPGMA) clustering. A total of 417 alleles were amplified with the number of alleles ranging from 5 (EM 141) to 38 (EM 120 b). Polymorphism was fairly high (0.05 to 0.92) among SSR markers with high number of repeats. Findings indicate that entries originating from different parts of the world did not form a distinct cluster, and there was no association between SSR marker pattern and geographical origin. SSR markers indicated a strong genetic affinity among *S. viarum*, *S. melongena* and *S. aethiopicum* Aculeatum group. Genetic relatedness between *S. dasyphyllum* and *S. macrocarpon* and between *S. aethiopicum* and *S. macrocarpon* are important for breeding. SSR markers assayed were informative for phylogenetic analysis; and have the potential to serve as perfect markers for studying variation. For plant breeders, close genetic relationships detected provide an avenue for introgression of high yielding and resistant genes into commercial and farmers' varieties.

Key words: Simple sequence repeats (SSR) markers, genetic diversity, polymorphism, multivariate analysis, *Solanum* species.

INTRODUCTION

Eggplant comprises three closely related cultivated species that belong to subgenus Leptostemonum: *S. melongena* L. (brinjal eggplant), *S. aethiopicum* L. (scarlet eggplant), and *Solanum macrocarpon* L. (gboma eggplant) (Daunay et al., 2001). The genus *Solanum* is morphologically hypervariable and highly diverse in number of species (Levin et al., 2005) and ecogeographical distribution (Wunderlin et al., 1993). On the basis of morphological traits, four varietal groups (Lester and Niakan, 1986) within the species *S. aethiopicum* (Aculeatum, Gilo, Kumba and Shum) have been documented. In *S. aethiopicum* complex, fruits are consumed raw or stewed with other vegetables or other protein rich foods, whilst the glabrous leaves of *S. aethiopicum* (Shum and Kumba groups) and *S. macrocarpon* are boiled as green vegetables, like spinach (Shippers, 2002). Leaves, fruits and roots of *S. aethiopicum* Gilo and *S. anguivi* have a variety of medicinal importance and commonly used in treatment of many diseases. Other *Solanum* species (*S. anguivi, S. dasyphyllum, S. integrifolium* and *S. viarum*) are cultivated for medicinal purposes.

Evaluation of genetic resources is crucial for breeders to develop new cultivars or for further improvement of the existing ones in response to changes in consumer demand. Molecular markers have enormous potential to explore genetic diversity by detecting polymorphisms,

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and are useful tools for breeding, genotype identification, and determination of genome organization and evolution in plants. Microsatellites (SSR) markers are a popular source of genetic markers owing to their high reproducibility, multi-allelic nature, co-dominant inheritance, abundance, and wide genome coverage. High level of polymorphism makes SSR an ideal marker for mapping and diversity studies, fingerprinting and population genetics (Nunome et al., 2003). A number of SSR markers have been identified in Solanaceae, (eggplant inclusive) (Bindler et al., 2007). Shorter SSR motifs and longer SSRs tended to be associated with a greater number of alleles. Several workers have contributed to the characterization of the largest genus of Solanaceae family (Bohs, 1999). Few studies had been performed to determine genetic diversity of eggplant using random amplified polymorphic DNA (RAPD) (Nunome et al., 2001), amplified fragment length polymorphisms (AFLP) (Mace et al., 1999), simple sequence repeats (SSR) (Prohens et al., 2012), and inter simple sequence repeat (ISSR) (Isshiki et al., 2003). However, despite its widespread cultivation, nutritional and economic importance, the eggplant genome has not yet been extensively evaluated as for the other important solanaceous vegetables such as tomato, potato and pepper. There is no comprehensive compareson of diversity among germplasm of eggplants available from Africa, Asia, Europe and South America for purposes of improvement. The aim of this investigation was to characterize 39 eggplant accessions collected from different geographical regions (Africa, Asia, Europe and South America), though largely from Africa using SSR markers, and assess genetic diversity within species and among species for improvement purposes.

MATERIALS AND METHODS

Plant material, DNA extraction and polymerase chain reaction (PCR)

Seed of 39 accessions representing the seven *Solanum* species, a landrace (Morogoro) and a popular tomato variety (LBR 48) were selected for molecular analysis (SSR) (Table 1). Entries were selected to represent species and geographic origin. Seedlings were raised in pots, and five weeks after planting leaf tissue, they 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 of -24°C for DNA extraction. Bulked leaf samples were supposed to combine equal amounts of DNA from each individual constituting the bulk. Laboratory activities took place at the Biotechnology Laboratory, Sokoine University of Agriculture, Morogoro, Tanzania. For DNA extraction process, Wizard ® Genomic DNA Purification Kit, (catalogue number A1120, Promega, Madison, WI, USA) was utilized. The protocol was applied with modifications. Genomic DNA was extracted as bulk, instead of directly using 600 µl Nuclei lysis solution to each Eppendorf tube, the amount was added in two steps. At first step, 250 µl of solution was used for grinding (plastic pestles). The remaining 350 µl was added to each eppendorf tube and ground tissue was mixed several times for better homogeneity. Another modification was about centrifugation, instead of 3 min at 13, 000 to 16,000 *g*, samples were spun at 10.000 g for 5 min and at the 6th step in the protocol and 10,000 *g* for 2 min at the 9th step. At the 10th step, ethanol washed samples were spun again at 10,000 *g* for 2 min.

SSR markers developed for *S. melongena* by Numome (2003) were synthesized by Integrated DNA Technologies, Inc., 1A, USA (Table 2) and checked for amplification in PCR reactions with the test population. 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: 50 ng of genomic DNA was added to 5x Go-flex buffer (Promega ®, Madison, W1, USA), 10 mM dNTP, 0.5 u Taq Polymerase; 25 mM $MgCl_2$, 11.15 µl PCR H_2O (Promega ®, Madison, W1, USA), 10 pmol of Forward primer and 10 pmol of Reverse primer and 1.0 µl each genomic DNA. Amplification was performed in a Perkin Elmer Thermocycler (Gene Amp 9600, Perkin Elmer Corporation, USA). PCR cycling conditions include 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. For annealing temperature, estimation was done with ± 5°C the melting temperature of the SSR markers as indicated by Integrated DNA Technology, after series of troubleshooting annealing temperature between 63 and 65°C was applied, 5 µl of PCR product for each accession, alongside 5 µl of 100 bp DNA ladder (Lonza) at 120 vA for 40 min. After the run, the gel was stained in ethidium water for 45 min and distained in distill water and photographed under UV light (UNITEC, 500 W) using video captured Cannon camera (Power Shot A650 15). Each amplified fragment was visualized as distinct bands.

Data analysis

The amplified SSR markers were scored as present (1) or absent (0), and then recorded into a binary matrix as discrete variables. The molecular size of the PCR products was estimated by comparing the position of bands with 100 base pair DNA ladder. The binary data matrix was used to compute Jaccard's similarity coefficients; = *N*AB/(*N*AB+ *N*A+ *N*B) where, *N*AB is the number of bands shared by samples, *N*A represents amplified fragments in sample A, and *N*B represents fragments in sample B. Similarity matrices based on these indices were obtained which were utilized to construct the UPGMA (unweighted pair-group method with arithmetic average) dendogram using the NTSYS-pc software (Version 2.0) (Rohlf, 1988). The goodness of fit of the UPGMA dendrogram generated with SSR data was submitted to 2-way Mantel test (Mantel, 1967). Some SSR primer banding characteris-tics namely; total number of bands (TNB), number of polymorphic bands (NPB), number of monomorphic bands (NMB), percent polymorphism band (PP); PP = NPBs/TNB were generated by each marker.

RESULTS

Simple sequence repeats (SSRs) marker identification and characterization

The TTT, CAA, AAA, and TGC are most frequently encountered repeat motifs, while CAG, CAC, TTG, GTT, CAT, and AGA are moderately encountered repeats (Table 2). Among the SSR markers, three showed perfect repeat type, six are imperfect repeat type; another six had compound repeat. The motif length was consistent (24) among the perfect repeats; this ranged from 23 to 25 nucleotides for imperfect repeats, and between 24 and 25 nucleotides for compound repeats. A total of 417 alleles were amplified from full genotype panel with number of alleles ranging from 5 (EM 141) and 38 (EM 120b), and a mean of 26 alleles per marker (Table 3). Among *S.*

Table 1. List of 39 accessions from seven *Solanum* (eggplant) species, landrace (Morogoro) and LBR 48 (*Solanum lycopersicon)* assayed with 16 SSR markers.

Accession	Specie	Origin
MM 1161	*S. aethiopicum* Shum	Benin
MM 10213	*S. aethiopicum* Gilo	Ghana
MM 1164	*S. dasyphylium*	Togo
MM 1616	*S. aethiopicum* Shum	Unknown
MM 1144	*S. macrocarpon*	Nigeria
MM 10147	*S. aethiopicum* Kumba	Burkina Faso
MM 714	*S. macrocarpon*	Zimbabwe
S0014474A	*S. viarum*	Thailand
S0017	*S. melongena*	Malaysia
S00023	*S. melongena*	India
MM 1186	*S. aethiopicum* Gilo	Unknown
MM 1207	*S. aethiopicum* Kumba	Mali
MM 981	*S. aethiopicum* Gilo	Uganda
MM 1127	*S. macrocarpon*	Benin
MM 458	*S. aethiopicum* Gilo	Japan
Taumbot	*S. anguivi*	Cameroun
S00017	*S. melongena*	Malaysia
S00022	*S. melongena*	India
MM 12209	*S. macrocarpon*	Zaire
Acc 43	*S. aethiopicum* Gilo	Unknown
MM 01143	*S. aethiopicum*	Nigeria
LBR 48	*S. lycopersicum*	Tanzania
DB3	*S. aethiopicum* Gilo	Tanzania
S 02865	*S. viarum*	Lao Peoples Republic
MM 457	*S. aethiopicum* Aculeatum	Japan
MM 01160	*S. aethiopicum* Shum	Benin
MM 01108	*S. aethiopicum* Kumba	Burkina Faso
S 0718	*S. melongena*	Indonesia
S02223	*S. viarum*	India
MM 1158	*S. aethiopicum* Aculeatum	France
MM 1102	*S. aethiopicum* Aculeatum	Burkina Faso
SOS 2	*S. Integrifolium*	Nigeria
MM 10256	*S. macrocarpon*	Ghana
MM 12126	*S. dasyphylium*	Uganda
S.00022	*S. melongena*	India
MM 1619	*S. aethiopicum* Gilo	Ivory Coast
S00735	*S. melongena*	Indonesia
MM 905	*S. macrocarpon*	Chad
MM 1483	*S. aethiopicum* Aculeatum	Unknown
Morogoro	*Solanum* spp	Tanzania
MM 1162	*S. aethiopicum* Gilo	Uganda

melongena, it was evident that all the SSR markers were polymorphic, and the allele number ranged from 2 (EM 141) to 7 (EM 120 b). Further, 61 alleles were amplified from genotypic panel from six accessions of *S. melongena* assayed, and on the average 4.0 alleles per locus was detected (Table 3). 32 accessions of *S. aethiopicum* and six accessions of *S. macrocarpon* assayed showed considerable polymorphism (Plate 1). Among *S. aethiopicum*, 191 alleles were recorded with 12 alleles per locus per SSR marker. A total of 87 alleles with six alleles per SSR marker per locus for *S. macrocarpon* were amplified. The number of alleles per SSR marker for *S. viarum* was approximately 1.6 alleles; on the average S. dasyphyllum recorded 1.7 alleles per SSR marker. Only one accession each was assayed for the landrace and for tomato variety LBR 48, and the result showed 4 and 8

Table 2. List of SSR markers used in this investigation.

Primer name	Primer sequence 5'-3'	Repeat motif (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 R- AGG GGA GAG GAA ACT TGA TTG GAC	$(AC)_{19}$ $(AT)_{11}$	24 24	65	160	120-220	45.8 50	Imperfect
EM 120a	F-GGA TCA ACT GAA GAG CTG GTG GTT R-CAG AGC TTC AAT GTT CCA TTT CAC A	$(AC)_{16}$	24 25	65	160	100-218	50 40	Perfect
EM120b	F- CAA AAG ATA AAA AGC TGC CGG ATG R-CAT GCG TGA GTT TTG GAG AGA GAG	$AC)_{16}$	24 24	65	248	80-240	41.6 50	Perfect
EM 131	F- TCT GGG ACA CCA AGT GAA AAA TCA R- TGC GTT TTT GGC TCC TCT ATG AAT	(AT)5(AC)3A(AC)14(AT)7GTA(TG)5(TA)3	24 24	65	213	120-220	41.6 41.6	Compound
EM 119	F-CCC CAC CCC ATT TGT GTT ATG TT R-ACC CGA GAG CTA TGG AGT GTT CTG	$(GGAGG)_5$ $(AT)_8$	23 24	65	210	100-210	47.8 54.1	Imperfect
EM 141	F- TCT GCA TCG AAT GTC TAC ACC AAA R-AAA AGC GCT TGC ACT ACA CCT GAA T	$(AT)_{16}$ $(GT)_{19}$	24 25	65	228	100-260	41.6 44	Imperfect
EM 114	F-AGC CTA AAC TTG GTT GGT TTT TGC R-GAA GCT TTA AGA GCC TTC TAT GCA G	$(AC)_{13}$	24 25	65	221	159-250	41.6 44	Perfect
EM 127	F-CAG ACA CAA TGC TGA GCC AAA AT R-CGG TTT AAT CAT AGC GGT GAC CTT	$(AC)_{13}(AT)_{13}$	23 24	65	200	150-230	43.4 45.8	Imperfect
EM 107	F-GGC CCT AGA CTG AGC CTG AAA TGT T R-TGC TAC AAC CAA CAC AAC CCT CAA	$(AC)_{13}(AT)_7$	25 24	65	214	100-240	52 45.8	Imperfect
EM 116	F-TTA GAA ATT TCG GAA CAA AGA GA R-CCA CAT GAA ACT TGG ACC AAT GAG	$(AC)_{12}(AT)_8$	23 24	62	246	150-230	30.4 45.8	Imperfect
EM 128	F- TAG CGG TGC TAG GTC CAT CAT CTC A R-TTC TCA AGA AGT TGC TCC AAA GGA	(CA)26(TA)19	25 24	60	295	100-231	52 41.6	Imperfect

Table 2. Contd.

EM 133	F- GCG GAT CAC CTG CAG TTA CAT TAC R- TCC TTT GAC CTA TAG TGG CAC GTA GT	(AC)13(AT)4	24 26	65	177	120-220	50 46.1	Imperfect
EM 134	F-AGT AAG GGA AAG TGC TGA CGA AGG R-CAG AGT CAT CGT TAT GGG GAG GTT	$(GT)_2GC(GT)_6$	24 24	65	168	120-300	50 58.3	Imperfect
EM 140	F-CCA AAA CAA TTT CCA GTG ACT GTG C R-GAC CAG AAT GCC CCT CAA ATT AAA	$(AC)_4GC(AC)_5T(AC)_3ATGC(AC)_4$ $AT(AC)_6(AT)_5G(TA)_{13}$	25 24	65	268	150-300	44 41.6	Compound
EM 145	F-TGA TTT GGC CCT TAA GCC TAA GTA TG R-GAC TCC TCA AGC CTT TAC CTC CAA	$(TG)_3TA(TG)_8$ $(TA)_6$	26 24	65	165	145-220	42.3 50	Compound
EM 146	F-GGA CCA AAG CGA AAT TTT CAC AAC R-TTG CAC CAA TTG GGA AGT AAC ACA	$(AC)_{19}(AT)_{11}AC(AT)_2$	24 24	63	288	120-350	41.6 41.6	Compound
EM 104a	F- TGG ATC TGC AAA GAA AAG GAG AAA G R- CGC AAA TCG GGT AGA CTT TCG AT	(TC)9(AC)38(AT)19	25 23	60	246	200-350	40 47.8	Compound
EM 139	F-TGC TAA GTC GTC ATC CAA CAA GAA R-GAT TTT GGC TCC TTG ACC ATT TTG	(AC)6AT(AC)11(AT)10	24 24	65	258	130-340	45.8 41.6	Compound

Table 3. Polymorphism of the SSRs in 39 accessions from seven *Solanum* (eggplants and relatives) species, landrace and LBR 48 (*Solanum lycopersicon*) (n = 41).

SSR Primer	Number of alleles									Number of amplified band
	S. melongena	***S. aethiopicum***	***S. macrocarpon***	***S. viarum***	***S. integrifolium***	***S. anguivi***	***S. dasyphylium***	**Landrace**	**LBR 48**	
EM 133	2	10	3	1	1	1	2	0	0	20
EM 107	5	17	7	3	1	1	2	0	1	37
EM 104	4	8	5	1	0	1	1	0	1	21
EM 120b	7	17	7	2	1	1	2	1	1	38
EM 120a	5	18	6	2	1	1	2	1	1	37
EM 145	6	9	5	2	0	1	1	0	1	25
EM 128	6	15	4	0	0	1	2	0	0	28
EM 119	4	14	7	3	1	1	0	0	1	31
EM 134	2	10	4	2	1	0	2	0	0	20

Table 3. Contd.

EM 139	2	13	6	0	1	1	1	1	0	22
EM 117	4	11	6	1	1	1	2	0	0	27
EM 116	3	11	8	1	1	1	1	0	0	26
EM 146	5	15	6	1	0	0	2	0	1	29
EM 140	3	8	6	3	1	1	2	0	1	25
EM 114	2	12	7	1	1	1	2	0	0	26
EM 141	1	3	0	0	0	0	1	0	0	05

alleles, respectively.

This investigation showed that 16 of the 18 SSR markers were informative at interspecific and intraspecific diversity among *Solanum* species. In *S. aethiopicum*, positive and significant correlation between allele number and total SSR length (r = 0.91, P = 0.001) was observed. *S. anguivi* showed a positive and significant correlation with SSR length (r = 0.50 P = 0.05). The association between allele number and SSR length was positive and had significant coefficient (r = 0.76 P = 0.001) in *S. macrocarpon*. Similarly, in *S. melongena*, the association between allele number and SSR length was positive and had significant estimates (r = 0.78, P < 0.001). The percent polymorphism (0.03 to 0.93) and proportion of polymorphic bands (0.12 to 0.82) were quite high and varied (Table 4). The SSR markers investigated showed monomorphic, polymorphic bands (heterozygous), and double banding pattern was absent.

The dendogram produced by Jaccard's coefficient and UPGMA clustering method resulted in similarities from 0.45 and 1.00, with mean similarity of 0.73 (Figure 1). When using a similarity value of 0.59, 41 accessions were restricted to four clusters, while seven clusters were evident at 0.71 similarities. Interestingly, 70% of the accessions assayed had genetic similarities higher than 0.90. The first cluster comprised two accessions (MM136 and S00023) from *S. melongena* and one accession of *S. viarum* (S 01447A); both MM 136 and S 00023 showed resemblance value of 0.83, this represents maximum relatedness in this cluster. This group is related to S 01447A (*S. viarum*) sourced from Thailand at 0.70 similarity and represents lowest similarity value in cluster 1. The second cluster accommodated two accessions namely: landrace (Morogoro) and MM 196 (Kumba group), they recorded similarity value of 0.83. Interestingly, similarity values for clusters 1 and 2 were consistent (0.83). The third cluster starting from MM 1164 to MM 01108, showed species and geographic heterogeneity. At 0.72 similarity value, the cluster members were separated into two groups; 'a' and 'b'. Tomato variety LBR 48 (*Solanum lycopersicum*) and MM 148 are genetically related with 0.77 resemblance value, and restricted to group 'a' at the upper end.

While S 02865 (*S. viarum*), S 00718 (*S. melongena*), S 02223 (*S. viarum*), MM 1158 (*S. aethiopicum* Aculeatum) and MM 1108 (*S aethiopicum* Shum) were placed in group 'b'. S 000718 and S 02223 marked highest similarity value in the third cluster, and were related to S 02865 (*S. viarum*) at 0.93 resemblance value (at the upper end of the cluster), and to MM 1158 at similarity value of 0.72. The group is further related to MM 01108 (Kumba) at 0.73 similarity coefficient. Of the nine accessions placed in the fourth cluster, seven accessions were from Africa and were interspersed among others sourced from Asia (Malaysia and Japan). At the upper end of this cluster was MM 1164 (*S. dasyphyllum*) and MM 1144 (*S. macrocarpon*); they were related at 0.85 similarity value, both of which were placed in the series *Macrocarpa* of section *Melongena*. Similarly, MM 12126 (*S. dasyphyllum*) and MM 1819 (*S. aethiopicum* Gilo) were restricted to another group at 0.79 similarity; both groups were similar at 0.72. At the lower end of the fourth cluster, MM 714 (*S. macrocarpon*) and MM 457 (*S. aethiopicum* Aculeatum) were related. Db_3 (*S. aethiopicum* Gilo) and MM 01160 (*S. aethiopicum* Shum) were genetically related; they had 0.93 similarity coefficient. S 00017 (*S. melongena*) was related to cluster 4 members at 0.78 similarity value. The fifth cluster comprised MM 1616 (*S. aethiopicum* Shum), MM 1102 (*S. aethiopicum* Aculeatum) and MM 1207 (*S. aethiopicum* Kumba) all from West Africa; they formed a specie-specific group, and were related at 0.72 similarity.

Additionally, S 00017 and S 00719 (*S. melongena*) from Asia showed genetic proximity and were related at 0.78 similarity value in cluster 6. The seventh cluster had the largest member starting from MM 1161 to S 00023, taking in together accessions from *S. aethiopicum* Gilo,

Table 4. Number of alleles, number of monomorphic loci, polymorphic loci, percent polymorphic and proportion of polymorphism generated by each SSR primer.

SSR Primer	Number of monomorphic band	Number of polymorphic band	Number of absent band	% Polymorphism	Proportion of polymorphic band
EM 133	12	8	21	0.48	0.40
EM 114	5	25	10	0.76	0.82
EM 107	32	6	3	0.93	0.16
EM 104	19	2	20	0.51	0.30
EM 120a	14	20	7	0.83	0.59
EM 120b	31	7	3	0.92	0.18
EM 145	6	23	12	0.70	0.79
EM 128	23	6	12	0.71	0.21
EM 119	17	14	10	0.75	0.45
EM 134	14	7	20	0.51	0.33
EM 139	17	6	16	0.56	0.26
EM 117	14	13	14	0.65	0.48
EM 116	20	6	15	0.63	0.30
EM 146	23	5	13	0.71	0.12
EM 140	13	12	16	0.61	0.48
EM 145	7	18	16	0.72	0.72
EM 141	1	11	30	0.03	0.25

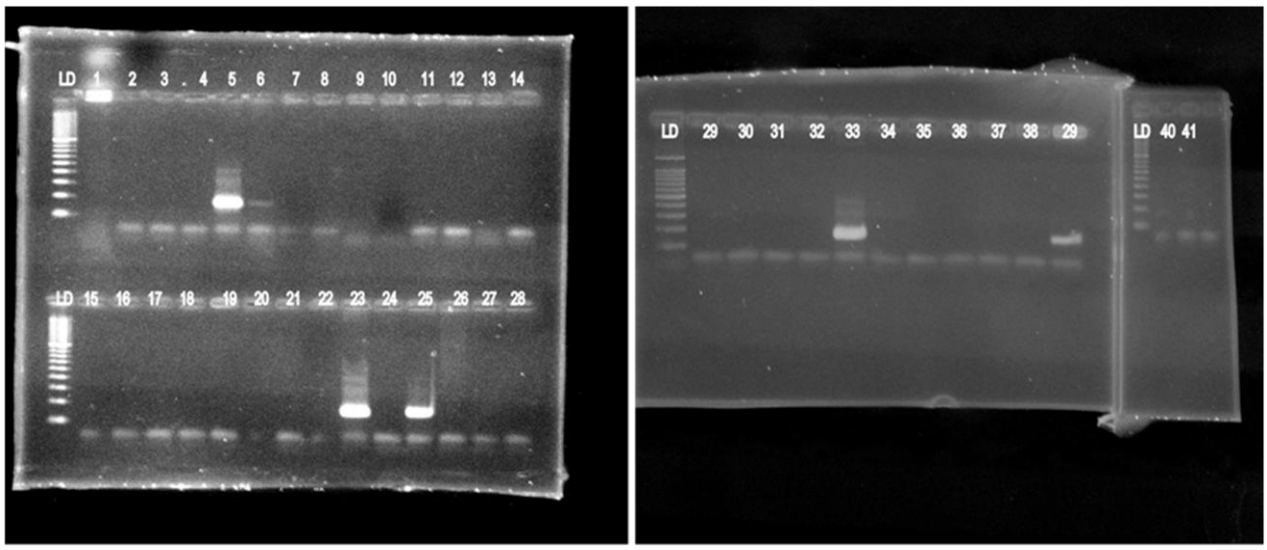

Plate 1. Gel electrophoresis analysis of polymerase chain reaction products amplified for some accessions from seven *Solanum* (eggplant) species, a landrace and LBR 48 digested with EM 107.

Kumba, Shum and Aculeatum groups, *S. integrifolium*, *S. anguivi* and *S. melongena* with S 00022 (*S. melongena*) as an outlier. MM 1161 and MM 10213 were genetically similar at the lower end of the cluster and were related to S0S 2 (*S. integrifolium*) at 0.84 similarity value. Fovembot (*S. anguivi*) was related to this group at 0.84 similarity value; these accessions were placed in the series Oliganthes of section Aethiocarpa. MM 981 (Gilo) and MM 12209 were similar at 0.93 and related to MM 905 (*S. macrocarpon*) at 0.84. Two accessions (MM 10147 and MM 1207) were dispersed among *S. aethiopicum* Gilo; the group was related to *S. melongena* (S 00022). The clustering pattern noticed in this investigation showed that accessions of *S. aethiopicum* and *S. melongena* formed strictly monophyletic group compared to other species. Moderate genetic variability was observed among three accessions of *S. aethiopicum* Aculeatum, as they were separated into different groups in cluster 3.

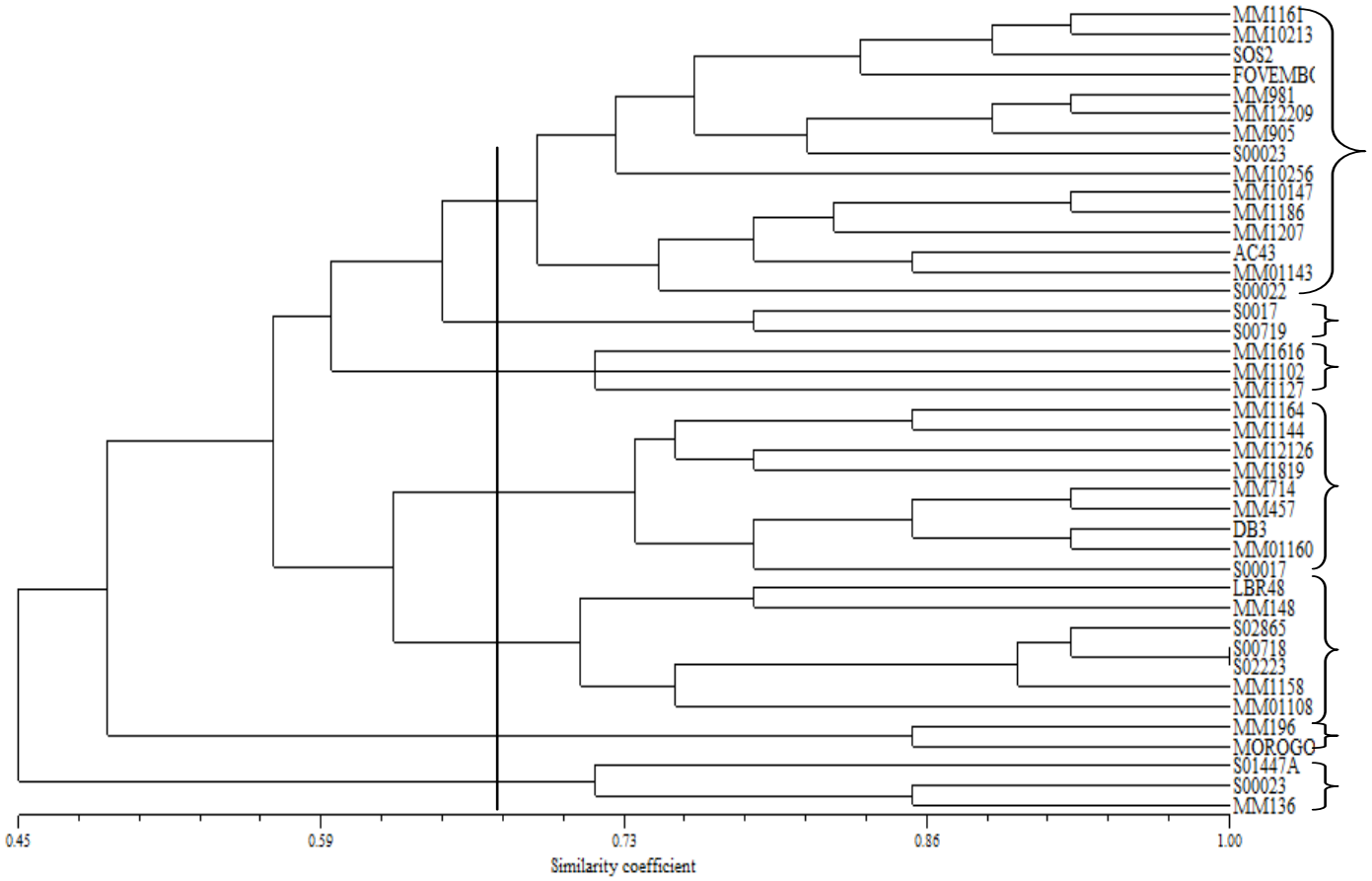

Figure 1. A dendogram constructed from the SSR data, using jaccard's coefficient of similarity and UPGMA clustering.

In addition, accessions of the Aculeatum group were dispersed among *S. aethiopicum* Gilo and Shum, *S. macrocarpon*. Distance observed among the three accessions representing Aculeatum group was moderate, and it was evident that S 02223 and S 02865 from India and Lao Peoples Republic were restricted to cluster 3 and S 01447A to cluster 1. The goodness of fit of the UPGMA dendogram generated with microsatellite data was tested by 2-way Mantel test (Mantel, 1967). Moderate support for clustering patterns was observed for SSR (r = 0.87). Figure 2 shows the dispersion of 39 accessions of Solanum (eggplant) species, landrace and tomato (LBR 48) based on SSR data; 22% of the total variation was represented on the x-axis, and 17% on the y-axis; both axes accounted for 39% of the total variation. The accessions plotted are labeled according to abbreviated species name. The PC biplot showed that the first quadrant showed species and geographic heterogeneity; it comprised accessions from *S. aethiopicum* Gilo, Kumba, Aculeatum, *S. viarum, S. dasyphyllum, S. integrifolium* and *S. macrocarpon*, with positive contribution to both PC 1 and 2. Interestingly, *S. macrocarpon* was dispersed close to *S. dasyphyllum*. Proximity between *S. viarum* and *S. melongena* was evident in the second quadrant. The landrace (Morogoro) was located close to *S. melongena* and *S. aethiopicum* Kumba in the third quadrant with negative contribution to both principal axes 1 and 2.

DISCUSSION

Diversity analysis is important for understanding genetic relationships including parentage and for the efficient management of germplasm and thereby use in breeding of improved varieties. Establishing the identity of crop variety using diversity study has assumed greater importance for protecting plant breeder's and farmer's rights. One of the main attributes of microsatellite loci is their hyper-variability, which makes them generally more informative than other molecular markers such as RFLP (Rongwen et al., 1995). Alleles per marker reported here for *S. aethiopicum, S. macrocarpon,* and *S. melongena* are larger than those indicated earlier for eggplant genome (Tumbilen et al., 2011). High mean values for number of alleles per marker was consistent with divergence among the eggplant population and number of accessions assayed for per specie. The average number of SSR alleles (4.0) detected among *S. melongena* is in

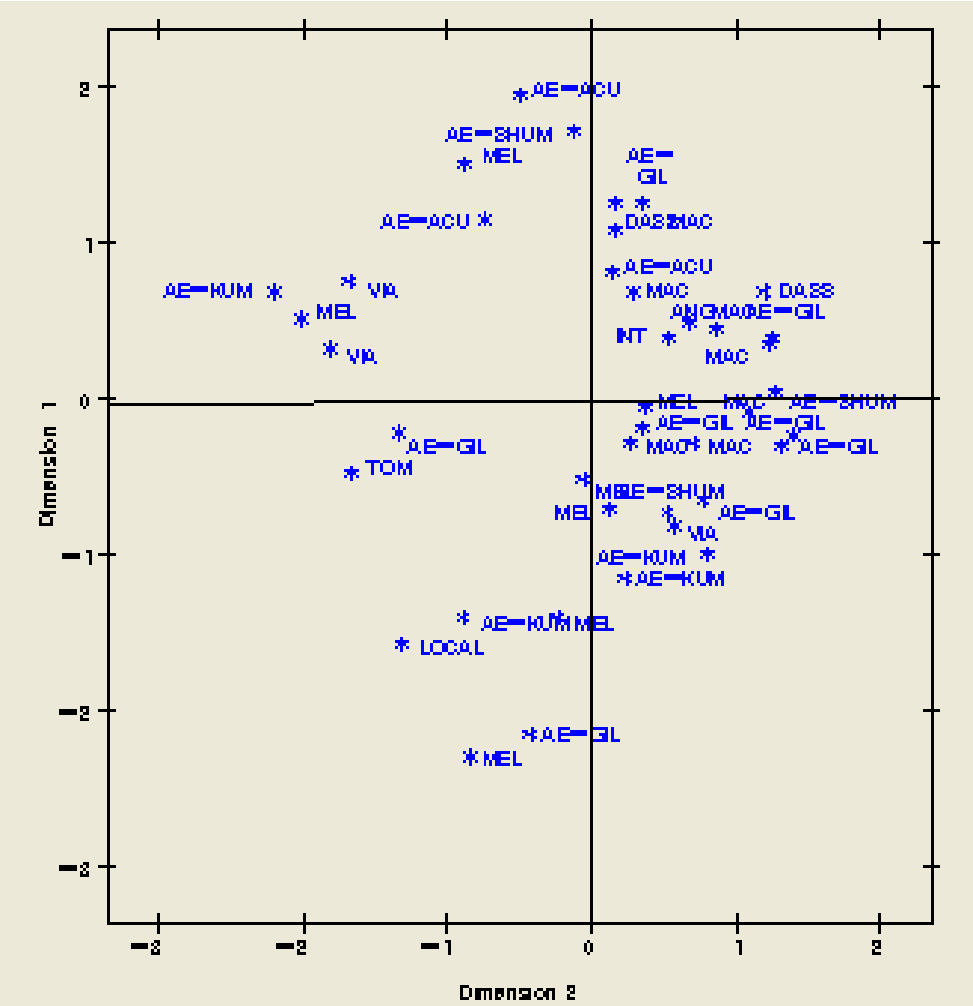

Figure 2. PC biplot of the first two principal components axes showing spatial distribution of 39 accessions from seven *Solanum* species, a landrace (Morogoro) and tomato variety LBR 48. INT = *S. integrifolium*; VIA = *S. viarum*; LOCAL = Morogoro landrace; DASS = *S. dassyphyllum*, MAC = *S. macrocarpon*; TOM = Tomato (LBR 48); AE-GIL- *S. aethiopicum* Gilo Group; AE-KUM= *S. aethiopicum* Kumba Group; AE- ACU = *S. aethiopicum* Aculeatum Group; MEL = *S. melongena*; AE-SHUM = *S. aethiopicum* Shum Group; ANG = *S. anguivi.*

agreement with those obtained in previous studies of microsatellite in *S. melongena* (Nunome et al., 2003). Polymorphism found in this study reflects a high genetic diversity among eggplant species investigated. Considerable diversity among *Solanum* species is in agreement with those reported by Prohens et al. (2005) among *S. melongena* and related species. This provides evidence that SSR markers are adequate for assessment of intraspecific and interspecific genetic variation and are informative for detecting genetic diversity and relationships in the *Solanum* genome. Similar results of SSR amplification had been consistently obtained in tomatoes (He et al., 2003). Polymorphism across eggplant assayed indicated the presence of similar protein agent among the species, and failure to amplify (EM 127 and 131) may be due to a variety of causes, including the positioning of the primers across the splicing site.

The proportion of polymorphic alleles for EM 114 and 145 suggests that these SSR markers are adequate for detecting genetic diversity among the seven *Solanum* species studied. The level of polymorphism observed was fairly high (0.05 to 0.92) indicating a wide and diverse genetic base, similar to that reported by Singh et al. (2006). Low polymorphism in tomatoes (*S. lycopersicum*) for microsatellite markers, RFLP and RAPD markers (Broun and Tanskley, 1996) is in line with observations in this study for LBR 48 (*S. lycopersicum*). Polymorphism observed for EM 120 may be associated with preponderance of AC/TG dinucleotides presumably reflecting high frequency of leucine (GAG) in polypeptides (Kantely et al., 2002). In this investigation, dinucleotide motifs were associated with high variability, and are more informative than trinucleotides motifs (Singh et al., 2006). When all SSR markers were examined, it was evi-

dent that shorter and long SSR motifs (dinucleotides) tended to be associated with a greater number of alleles; SSR markers (perfect and imperfect) detected more polymorphism compared to compound repeats. In contrast compound microsatellite marker showed high polymerphism in lettuce (van de Wiel et al., 1999). This study shows that SSR markers with more repeats detected more polymorphism in *S. aethiopicum* and *S. melongena.* Among the *Solanum* species, negative correlation coefficient between number of alleles and motif length, though not statistically significant, demonstrated a general phenomenon that was observed in previous studies in eggplant and related solanaceous species (Nunome et al., 2003). Additionally, negative correlation coefficient between motif length and number of alleles was similar to those reported for tomatoes (*S. lycopersicum*) (Fray et al., 2005). Although the SSR markers were designed based on *S. melongena* sequence, all the PCRs were successful in other species (*S. aethiopicum, S. viarum, S. integrifolium, S. dasyphyllum, S. anguivi*), presumably due to its very close relationship with *S. melongena* (Pearce and Lester, 1979). In contrast, Stàgel et al. (2008) found only 28% of microsatellite markers to be informative when 38 accessions of *S. melongena* accessions were assayed. The difference in marker polymerphism obtained in this investigation compared to AFLP in *Solanum* species indicated that both markers sampled different portion of the genome.

The SSR marker technique showed that accessions of *S. viarum* showed genetic affinity to *S. melongena* and *S. aethiopicum* Aculeatum group; this is indicative that *S. viarum* is close to cultivated eggplants (*S. melongena*). Genetic similarity as found between *S. viarum* and *S. melongena* corroborates findings reported by Singh et al. (2006) with RAPD markers. Genetic similarity observed between *S. viarum, S. melongena* and *S. aethiopicum* Aculaetum may phenotypically be associated with the 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*. The close genetic relatedness among *S. dasyphyllum* and *S. macrocarpon* based on SSR analysis confirm earlier results obtained by Levin et al. (2005), who found that *S. macrocarpon* and *S. dasyphyllum* are sister species. However in most cases, genetic similarity infer cross compatibility. *S. dasyphyllum* and *S. aethiopicum* Gilo were genetically similar (0.78), and a plausible explanation could be that *S. macrocarpon, S. aethiopicum* and *S. dasyphyllum* originated from Africa, and could have a genome shared. Both *S. aethiopicum* and *S. macrocarpon* belong to section Oliganthes and series Aethiopica (Furini and Wunder, 2004) and could possibly have a genome in common; a phenotype common to both species is leaf lobbing. However, genetic similarity between *S. macrocarpon* and *S. dasyphyllum, S. aethiopicum* and *S. macrocarpon* on the other support the placement of *S. macrocarpon* outside Section Melongena.

Several workers are divided on the placement of *S. macrocarpon* in the section Melongena or Oliganthes (Isshiki et al., 2003; Furini and Wunder, 2004; Frary et al., 2005; Levin, 2005). Additionally genetic relatedness among *S. macrocarpon* and *S. aethiopicum* Gilo, Shum and *S. integrifolium* observed in this study suggests the placement of *S. macrocarpon* in the section Oliganthes based on SSR data. In contrast Tumbilen et al. (2011) noted that *S. aethiopicum* and *S. macrocarpon* from Turkey are dissimilar as revealed by SSR markers. In this respect, *S. anguivi*, *S. aethiopicum* and *S. integrifolium* belong to the series Athiopica and section Oliganthes.

This study reveals genetic similarity among *S. aethiopicum* subgroups (Gilo and Shum), *S. integrifolium* (SOS-2) and *S. anguivi* (Taumbot). This is consistent with the report of Lester and Seck (2004) and possibly a common genome. In addition *S. integrifolium* is synonymous to *S. aethiopicum* (Toppino et al., 2008). Tumbilen et al. (2011) found high genetic similarity between *S. aethiopicum* and *S. integrifolium* as revealed by SSR markers. *S. anguivi*, *S. aethiopicum* and *S. integrifolium* are interfertile and could be a gene pool for genetic improvement. For plant breeders close genetic relationships associated with high similarity (> 0.80) among *S. aethiopicum, S. anguivi, S. integrifolium*, and *S. anguivi* could provide an avenue for introgression of high yielding and resistant genes into commercial and farmers' varieties.

Genetic relationship between *S. aethiopicum* Aculeatum group (MM 457, MM 1102 MM 1158) and Gilo (Db_3), Shum (MM 01160, MM 1616, MM 1108) and *S. macrocarpon* (MM 714 and MM 1127) is probably because both species originated from Africa. In addition, the grouping observed among Gilo and Shum sub groups of S. *aethiopicum* is in agreement with the results reported by Sunseri et al. (2010). The landrace (Morogoro) was genetically related to *S. aethiopicum* Gilo group, and distant from other species possibly because of small to medium fruit size. 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 relationship between accessions of the full genotype panel as displayed by genetic similarity at the SSR level were in good agreement with prior taxonomic classification for *Solanum* species (Nunome et al., 2003). The dendogram obtained using SSR data revealed that accessions from seven Solanum species had moderate to high molecular genetic diversity. This was also observed in *S. melongena* accessions studied by Demir et al. (2010) using AFLP data. In the same way, Behera et al. (2006) found broader genetic diversity in 92 South Asian *S. melongena* accessions (genetic similarity bet-ween 0.37 and 0.90) using microsatellite markers. Such greater diversity was expected because South Asia is the primary

center of eggplant diversity and has shown to habour considerable morphological and molecular diver-sity (Furini and Wunder, 2004). The grouping pattern observed for *S. melongena* (Section Melongena) and *S. aethiopicum* (section Oliganthes) subgroups suggests that they may belong to the same genepool (Daunay et al*.,* 2001), and hybridization experiments between *S. melongena* and *S. aethiopicum* subgroups may evolve high yielding and disease resistant varieties (Prohens et al., 2005, 2012).

Conclusion

Microsatellite markers used in this investigation provided new information about the genetic diversity and relationships among *Solanum* (eggplant) species. The set of eggplant SSR markers used are informative for phylogenetic analysis and do have potential to serve as perfect markers for genes determining variation in phenotype. Their high level of transferability to other *Solanaceae* species can be used to provide anchoring points for the integration of genetic maps across species. Inter and intra cluster hybridization among *S. aethiopicum* sub groups, *S. melongena* and *S. macrocarpon* may contribute to crop improvement through development of new varieties.

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