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GENETIC DIVERSITY ANALYSIS OF EGGPLANT (*Solanum melongena* L.) AND RELATED WILD SPECIES IN THE PHILIPPINES USING MORPHOLOGICAL AND SSR MARKERS

X.G.I. CAGUIAT and D.M. HAUTEA*

Crop Science Cluster-Institute of Plant Breeding, College of Agriculture,
University of the Philippines Los Baños, 4031, Laguna Philippines
*Corresponding author's email: dmhautea@up.edu.ph; dmh.uplb@gmail.com

SUMMARY

Results of the first genetic diversity assessment of Philippine eggplant germplasm collection consisting of accessions of *Solanum melongena* L. and related wild species using morphological traits and molecular markers is reported in this paper. Thirty-two accessions, representing 30% of the available collection of local landraces, improved cultivars and crop wild relatives (CWR) held in the national genebank were differentiated based on 39 morphological trait descriptors and 41 Simple Sequence Repeat (SSR) markers. Ten out of the 39 morphological traits accounted for high phenotypic differences among the accessions. SSR polymorphism survey revealed that 33 out of the 41 SSR primer pairs (80.48%) detected variation among the accessions and the number of alleles ranged from 2 to 8 with a mean of 4.3 alleles per marker. The morphological trait and SSR data were analyzed as separate and combined data sets using principal component analysis (PCA) and unweighted pair-group method with arithmetic averages (UPGMA) cluster analysis. Similar results were obtained from the 3 data sets. Landraces, cultivars and crop wild relatives (CWR) were clearly differentiated. However, analysis of all 3 data sets did not distinguish the *S. melongena* landraces based on geographic areas of collection. CWRs were the most diverse group, followed by the landraces, while the improved cultivars were the least diverse. This study provided significant information for the need to increase the present eggplant collection and to widen the genetic diversity of currently cultivated eggplant varieties in the Philippines.

Keywords: Eggplant, *Solanum melongena* L., Philippine germplasm, wild species, genetic diversity, SSR markers

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INTRODUCTION

Eggplant (*Solanum melongena* L.) is recognized as one of the most important members of the Solanaceae family which includes economically important species like potato, tomato, tobacco and pepper (Doganlar *et al*., 2002b; Knapp *et al*., 2013). Eggplant is grown extensively as cash crop by mostly small-scale farmers in many countries, particularly in Asia. Together with China and India, the Philippines is one of the top 10 eggplant-producing countries in the world based on area of production (FAO CropStat, 2012). For the past 10 years, eggplant has been the leading vegetable crop in the Philippines in terms of production volume and area planted (BAS CountryStat, 2012).

Eggplant is believed to have originated in Asia, in the Indo-Burmese region (Vavilov, 1928; Ishiiki *et al*., 1994) but the most recent DNA sequencing studies suggested that eggplant arose from Africa (Li *et al*., 2010; Weese and Bohs, 2010). Three origins of domestication have been postulated (India, China and Indonesia/Malaysia) but recent views tend to agree on a minimum of 2 domestication events in favor of India and China (Knapp *et al*., 2013 and cited references therein). There are 3 cultivated eggplant species: *S. aethiopicum* and *S. macrocarpon*, grown primarily in Africa, and the more familiar *S. melongena,* cultivated worldwide. Most eggplant wild relatives are from Africa. Based on crossing and biosystematics data, eggplant wild relatives are generally recognized under 2 broadly conceived species in informal classification, *S. incanum* and *S. melongena.* Recent taxonomic revision based on morphological and molecular data recognized 10 distinct species under these 2 broadly categorized eggplant relatives (Knapp *et al*. 2013).

The advent of molecular biology made possible the use of molecular genetic marker technology which led to the further understanding of the genetic diversity in various crop species. In eggplant, earlier studies included reports on few chloroplast DNA analyses (Sakata *et al*., 1991; Sakata and Lester, 1997) and isozyme marker analysis (Isshiki *et al*., 1994a, b, c; Karihaloo and Gottlieb, 1995; Kaur *et al*., 2004). These were later replaced with more abundant and polymorphic markers such as restriction fragment length polymorphism (RFLP) markers (Isshiki *et al*., 1998; Isshiki *et al*., 2003; Doganlar *et al*., 2002a) and random amplified polymorphic DNA (RAPD) markers (Karihaloo *et al*., 1995; Nunome *et al*., 2001; Kashyap *et al*., 2003). More recently, simple sequence repeats (SSR) or microsatellite markers (Nunome *et al*., 2003a, b; Stagel *et al*., 2008; Muñoz-Falcon *et al*., 2009; Nunome *et al*., 2009) and amplified fragment length polymorphism (AFLP) markers were developed and used in eggplant diversity assessment. Dominant markers such as RAPDs and AFLP have been applied to *S. melongena* and were proven to be a suitable tool for assessing genetic diversity (Mace *et al*., 1999b). However, co-dominant markers such as simple sequence repeat (SSRs) could generate more information and has high repeatability than dominant markers. Co-dominant markers are multi-allelic, highly abundant, are well-distributed in the genome, and are suitable for high throughput PCR which make them ideal for diversity studies (Powell, 1996). SSR markers have been successfully used in eggplant for analyses of diversity (Nunome *et al*., 2003a, b; 2009; Stagel *et al*., 2008; Prohens *et al*., 2008; Muñoz-Falcon *et al*., 2009; Tumbilen *et al*., 2009; Demir *et al*., 2010; Sunseri *et al*., 2010; Ge *et al*., 2011), conservation (Prohens *et al*., 2008; Muñoz-Falcon *et al*., 2009), and protection of the crop (Muñoz-Falcon *et al*. 2011; Sunseri *et al*., 2010; Singh *et al*., 2006).

Although eggplant is not native to the Philippines, eggplant landraces, farmers' varieties and crop wild relatives (CWR) are found almost all over the country. The national genebank i.e. National Plant Genetic Resources Laboratory (NPGRL) of the Institute of Plant Breeding, Crop Science Cluster at the University of the Philippines Los Baños collects and manages the eggplant germplasm collection. The NPGRL eggplant germplasm collection is predominantly composed of landraces and cultivars of *S. melongena* L., and a few accessions of *S. americanum* Henderson or *S. nigrum* L. (also locally called 'unti'), *S. torvum* Sw., *S. aethiopicum* L., *S. surratense* Burm., *S. mammosum* L. and *S. verbascifolium* Dunal (NPGRL, 1998; NPGRL Report 2011). Despite the presence of many landraces and farmers' varieties, a recent study (Chupungco *et al*., 2011) indicated that majority (75%) of the eggplant planted in most production areas consist of F_1 hybrids. Eggplant hybrid seeds are produced and distributed by private companies. The advantage of open-pollinated varieties (OPVs) as source of planting materials for succeeding planting seasons is often ignored because of the relatively higher yield, uniformity and better tolerance to pests and diseases of F_1 hybrids. The OPVs particularly the *S. melongena* landraces and the CWRs could serve as gene reserves and source of genetic diversity for improvement of cultivated eggplants.

The current NPGRL collection of eggplant and CWRs could be a source of desirable traits for sustainable improvement of existing eggplant varieties in the country and in the world but the collection has not yet been fully characterized. The objectives of this study were to assess the genetic diversity in the NPGRL eggplant collection and determine differences particularly among the *S. melongena* landraces collected from various regions of the Philippines using morphological, molecular and combined data analyses.

MATERIALS AND METHODS

Plant material

A total of 32 eggplant accessions were used in this study. These comprised ~30% of the total existing eggplant collection of NPGRL as of 2011 and included 6 commercial cultivars (*S. melongena* F_1 hybrids and improved OPVs) bred by private companies and public institutions, respectively, and 26 local landraces and CWRs. Twenty-five genotypes/accessions were used for *S. melongena*. Five other species of *Solanum* that are also commonly referred to as "eggplants" and cultivated for their edible fruits were also used: *S. americanum* (2 accessions), *S. aethiopicum* (2), *S. linnaeanum* (1), *S. mammosum* (1), and *S. torvum* (1). The 25 accessions/genotypes of *S. melongena* consisted of 6 cultivated varieties (2 F1 hybrids and 4 improved OPVs) and 19 landraces collected from different provinces in the Philippines (Table 1). All entries have 2n = 24 chromosomes except *S. mammosum* which has 2n = 22 diploid chromosome number.

Morphology-based diversity analysis

The morphological characterization data of 19 eggplant accessions and 7 wild related species were obtained from the eggplant database made available by the NPGRL. On the other hand, morphological data for 4 improved eggplant OPVs: Dumaguete Long Purple (DLP), Batangas Long Purple (BLP), Mara, Concepcion and 2 F_1 hybrids (Morena and Casino) were obtained from 30 plants of each variety or hybrid grown in the greenhouse in 2011. Mean values for all 39 descriptors developed by International Plant Genetic Resources Institute (IPGRI, 1999) and modified by NPGRL (unpublished) were used as input data using Numerical Taxonomy Systems in personal computer (NTSYS-pc) and used commonly as statistical software to measure similarity, dissimilarity and diversity of different species, taxa or any other operational taxonomic units (OTUs) (Rohlf, 1993). The traits were standardized to unit variance using NTSYS program. Numerical measures of likeliness between each pair of accessions were conducted to produce a symmetrical square matrix which is necessary for classification techniques. NTYS-pc (Rohlf, 1993) software was used to analyze these data. Similarity for Qualitative Data (SIMQUAL) was first computed to determine Jaccard's similarity coefficients. Principal components were used as input variables for cluster analysis using Unweighted Pair-Group Method with Arithmetic Averages (UPGMA). Using the 'Graphics' option, the computed UPGMA data were used to construct a dendrogram.

SSR-based diversity analysis

A total of 41 eggplant SSR markers were used in this study consisting of 13 EST-SSRs and 28 non-genic SSR primers developed by Tumbilen *et al*. (2011) and Nunome *et al*. (2009) (Table 2). Selection of these SSRs was based on their high polymorphism information content (PIC) and the high quality of bands reported by the authors. Except for 5, all SSR makers used have been mapped in the 12 linkage groups of eggplant (Fukuoka *et al*., 2012).

DNA extraction, PCR amplification, and gel electrophoresis

The youngest leaves were collected from 30 individual plants of each of the 32 accessions used except for *S. torvum* with only 14 individual plants available. Genomic DNA was isolated from approximately 3 grams of the leaf samples following the DNA extraction protocol based on CIMMYT (2005) and adapted by the

Table 1. List of eggplant accessions from the Philippines used in the genetic diversity study.

N0.	NAME/ ACC. NO.	COLLECTION SITE	REGION	TAXON	TYPE
1	6346	Bontoc, Mt. Province	CAR	SM	Landrace
2	8818	Manabo, Abra	CAR	SM	Landrace
3	2778	San Carlos, Pangasinan	I	SM	Landrace
4	2789	Batac, Ilocos Norte	I	SM	Landrace
5	5427	Bayombong, Nueva Vizcaya	II	SM	Landrace
6	5909	Echague, Isabela	II	SM	Landrace
7	5983	Diffun, Quirino	II	SM	Landrace
8	3305	Magalang, Pampanga	III	SM	Landrace
9	3296	Magalang, Pampanga	III	SM	Landrace
10	8257	Catanauan, Quezon	IVA	SM	Landrace
11	3214	Morong, San Guillermo	IVA	SM	Landrace
12	5302	Baao, Camarines Sur	V	SM	Landrace
13	4253	Barotac Nuevo, Iloilo	VI	SM	Landrace
14	4874	Canlaon, Negros Oriental	VII	SM	Landrace
15	4566	Pinabatan, Western Samar	VIII	SM	Landrace
16	4871	Katipunan, Zamboanga del Norte	IX	SM	Landrace
17	6123	Balingasag, Misamis Oriental	X	SM	Landrace
18	2805	Davao City, Davao del Sur	XI	SM	Landrace
19	2809	General Santos City, S. Cotabato	XII	SM	Landrace
20	Morena F_1	Bulacan (seed company)	III	SM	F_1hybrid
21	Casino F_1	Bulacan (seed company)	III	SM	F_1hybrid
22	DLP selection	Los Baños, Laguna	IVA	SM	cultivar**
23	Mara	Los Baños, Laguna	IVA	SM	IOPV**
24	Concepcion	Concepcion, Tarlac	III	SM	IOPV**
25	BLP	Batangas	IVA	SM	cultivar**
26	6317	Bontoc, Mt. Province	CAR	SA	wild species
27	6053	Saranay, Isabela	II	SA	wild species
28	8208	Puerto Princesa, Palawan	IVB	SL	wild species
29	5763	Solano, Nueva Vizcaya	II	SAE	wild species
30	8971	Pilar, Abra	CAR	SAE	wild species
31	SMA-1	Los Baños, Laguna	IVA	SMA	wild species
32	ST-1	Los Baños, Laguna	IVA	ST	wild species

*SM - *S.melongena* SA - *S. americanum*, SL - *S. linnaeanum*, SMA -*S .mammosum,*
SAE –*S. aethiopicum*, ST - *S. torvum*
**modern cultivated varieties, DLP-Dumaguete Long Purple; BLP-Batangas Long Purple, IOPV-improved open-pollinated varieties

Table 2. SSR marker repeat motifs, expected product and map position used in the primer polymorphism survey.

LOCUS	REPEAT TYPE AND LENGTH	EXPECTED PRODUCT (bp)	POSITION AND LG NO. (cM)	SOURCE
Genomic SSRs				
emi03L23	$(TG)_6$	160-162	35.1 (1)	N
eme01B01	$(AG)_{21}$	236-245	56.6 (1)	N
eme01D03	$(AG)_{15}$	273-275	84.8 (1)	N
emd01B12	$(TG)_{16}$	258-266	64.1 (2)	N
ema0060	$(AT)_5(GT)_{11}$	380-397	47.2 (4)	N
emb01O20	$(AG)_{11}$	300-307	69.6 (4)	N
ema0008	$(GT)_{12}$	287-290	43.9 (5)	N
emf11D18	$(TA)_{10}(TG)_{51}$	286-289	24.8 (6)	N
emg11D22	$(TA)_6(TG)_{12}$	298-291	74.0 (6)	N
emf21A23	$(TC)_4(CT)_3...(TC)_{23}$	273-277	34.0 (7)	N
emd07A07	$(AC)_{13}$	292-296	34.0 (7)	N
emi03K06	$(TA)_{19}(TG)_{23}$	280-285	3.9 (8)	N
emb01N07	$(AG)_{14}$	325-330	57.4 (9)	N
emh01F12	$(TA)_5...(AG)_{13}$	233-235	32.1 (10)	N
emi06F08	$(TA)_3...(TA)_4...(AC)_{12}A(TA)_{15}T(AG)_{13}$	256-261	28.8 (11)	N
emh05H12	$(TC)_{27}(TG)_{11}$	187-203	0.0 (12)	N
emi02K11	$(AC)_3...(AC)_{12}A(TA)_3$	297-299	13.3 (12)	N
em135	$(CA)_{11}(GA)_{20}$	256-260	17.6 (1)	N
em4_1	$(CA)_{14}T(AC)_6(AT)_6$	172-180	56.3 (2)	N
em155	$(CT)_{10}...(CT)_{38}$	271-285	105.1 (3)	N
em119	$(GGAGG)_5...(AT)_8(GT)_3AT(GT)_{14}$	198-201	62.0 (4)	N
em117	$(AC)_{19}(AT)_{11}$	134-142	42.0 (5)	N
em134	$(GT)_2GC(GT)_6$	184-186	1.6 (6)	N
em120	$(AC)_{16}$	160-164	37.7 (8)	N
em114	$(AC)_{13}$	234-236	0.0 (8)	N
em206	$(ATG)_{10}$	183-187	59.5 (9)	N
emd05F08	$(TA)_5(CA)_{10}$	264-272	30.5 (10)	N
em140	$(CA)_{4..}(AC)_4AT(AC)_6(AT)_5G(TA)_{13}$	284-320	24.1 (11)	N
eme03F04	$(TC)_{31}$	215-260	0.0 (12)	N
EST-SSRs				
ecm001	$(TC)_{17}$	224-229	37.5(1)	N
ecm090	$(TG)_3...(TG)_5$	162-164	101.2(1)	N
ecm070	$(TG)_8$	222-224	66.1 (5)	N
ecm023	$(AT)_3...(TA)_6$	253-257	56.5 (7)	N
ecm009	$(TAT)_{13}...(CA)_3$	240-245	58.2 (7)	N
ecm032	$(AC)_8...(AG)_4$	276-281	14.0 (9)	N
smSSR24	(TCA)5	220-229	*	T

smSSR36	(CTG)5	240-310	*	T
smSSR45	(TTC)5	172-182	*	T
smSSR46	(CAC)5	258-264	*	T
smSSR47	(AGA)5	180-186	*	T

N- Nunome *et al*. (2003) T-Tumbilen *et al*. (2011) * Unmapped

IPB Genetics Laboratory with minor modifications. DNA concentration was estimated by visual comparison with known λ DNA concentration standards in 1% agarose gel. PCR reaction was carried out in a volume of 10 µl containing 10 ng genomic DNA, 1.5mM $MgCl_2$, 0.2 µM dNTP mix, 0.2 µM of each primer, 1 Unit of *Taq* DNA polymerase and 1x PCR buffer. Reactions were performed in a G-Storm thermocyler (GMI, Inc. UK) using the following PCR profile: initial denaturation for 5 minutes at 94 °C followed by 35 cycles of (1) denaturation at 94 °C for 1 minute, (2) annealing at recommended SSR primer temperature for 1 minute, and (3) extension at 72 °C for 1 minute; and final extension at 72 °C for 5 minutes. PCR conditions for all the SSR primers used differed only in their annealing temperature. PCR products were analyzed by gel electrophoresis in a 5% denaturing polyacrylamide gel. A silver staining procedure was used to resolve bands after electrophoresis. Molecular weight was estimated using MVIII ladder (Roche Applied Science, Germany).

Molecular data analysis

Polymorphism survey was done using 41 SSR primers on 7 representative genotypes (2 commercial, 3 landrace and 2 related wild species) with equal amounts of pooled DNA from 14-30 individual plants per accession/sample. Polymorphic primers were then selected for subsequent diversity analysis of the complete set of 32 accessions/genotypes used. The polymorphic SSR bands for each individual were scored individually for the presence or absence of the expected bands. This resulted in a binary data of 1's and 0's. The polymorphism information content (PIC) was determined. The number of polymorphic alleles for each SSR marker was calculated using the PowerMarker program (Liu and Muse, 2005). The Jaccard's similarity coefficients were used as input for a cluster analysis using UPGMA in NTSYS-pc 2.1 software package (Applied Biostatistics, Port Jefferson, New Jersey, USA). In order to verify the accuracy of the groupings, phenograms were tested by bootstrap analysis with 1000 replications, using the GenAIEx Version 6.5 (Peakal and Smouse, 2012).

Combined Data Analysis

Combined data set contained 9 morphological marker data and the 12 SSR allelic data of 714 individuals from 32 populations. This data set was then used as input for a cluster analysis using UPGMA to generate a dendrogram using NTSYSpc 2.1 software packages (Applied Biostatistics, Port Jefferson, New Jersey, USA).

RESULTS AND DISCUSSION

Morphology-based Analysis

Morphological Trait Variation

The range of variation observed in quantitative and qualitative traits among the 32 accessions/genotypes used is presented in Table 3. Leaf prickles (or spines) showed the least amount of variation (90% of all accessions have no spines), which reflected the local preference for eggplant varieties without spines. Out of the 39 morphological traits measured, 28 traits were included in the output of the GenAIEx (Peakal and Smouse, 2012). Principal component analysis (PCA) was performed in order to determine which of the eggplant morphological descriptors used accounted for the most variation observed. Sneath and Sokal (1973) indicated that higher Eigen vector coefficients of the traits could be associated to respective Principal Component (PC) axes. From the principal x

Table 3. Range of variation in quantitative characters and predominance of qualitative descriptors in Philippine eggplant (*S. melongena* L.) and wild relatives.

CHARACTERS	MIN.	MAX.	RANGE	MEAN ± S.D.	C.V.
Average No of Flower	1.00	9.00	1-9	2.28 ± 2.2	1.21
Fruit length	0.67	27.43	0.67-27.43	11.43 ± 7.8	15.64
Fruit breadth	0.77	8.29	0.77-8.29	4.80 ± 1.9	0.94
Number of locules	0.00	9.00	0-9	4.07 ± 2.26	1.29
Fruit density	1.00	9.00	1-9	4.88 ± 2.47	1.73
Fruit inflorescence	1.00	7.80	1-7.80	2.40 ± 2.4	1.7
100 seed weight	0.50	19.80	0.50-19.80	4.93 ± 4.37	5.41
Plant growth habit	Upright (80%) and Intermediate (20%)				
Plant height	Intermediate (57 %), Short (37%) and Small (7%)				
Plant branching	Intermediate (70%), Weak (20%) and Strong (10%)				
Petiole color	Green (53%), Greenish violet (43%) and Dark violet (3%)				
Petiole length	Long (30%), Very long (30%), Short (20%), Intermediate (13%), and Very short (7%)				
Leaf blade length	Intermediate (77%), Short (13%) and Long (10%)				
Leaf blade width	Wide (77%), Intermediate (20%) and Narrow (7%)				
Leaf blade lobing	Intermediate (47%), Strong (43%), Weak (7%) and Very strong (3%)				
Leaf blade tip angle	Intermediate (47%), Acute (20%), Obtuse (20%), Very acute (7%) and Very obtuse (7%)				
Leaf blade color	Dark green (40%), Green (30%), Greenish violet (27%) and Light green (3%)				
Leaf prickles (or spines)	None (90%) and Few (10%)				
Leaf hair	Few (27%), Very few (17%), Intermediate (13%), and Many (7%)				
Corolla color	Light violet (63%), Bluish violet (17%), White (10%) and Pale violet (10%)				
Relative style length	Long (57%), Intermediate (27%), and Short (10%)				
Fruit shape ratio	1/2 base to tip (60%), 1/4 base to tip (23%) and 3/4 base to tip (10%)				
Fruit curvature	Slightly curved (50%), No curvatures (43%), and Curved (17%)				
Fruit pedicel length	Long (43%), Intermediate (23%), Short (13%), and Very short (7%)				
Fruit color at commercial ripeness	Purple (50%), Green (13%), Lilac green (10%), Purple black (10%), Deep yellow (7%), Fire red (7%), and Scarlet red (3%)				
Fruit position	Pendant (80%), Semi-pendant (13%), Semi-erect (3%) and Horizontal (3%)				
Fruit flavor	Bitter (33%), Intermediate (33%), Sweet (20%) and Not determined (3%)				
Fruit length and breadth ratio	Broader than long (80%), Long as broad (10%), 3x as long as broad (7%) and Slightly longer than broad (3%)				

component analysis (PCA), the first 9 PCs gave Eigen values >1.0 and cumulatively accounted for 83.52% of the total variation observed indicating a high degree of variation for these characters. The most important descriptors accounted for more than 50% of the morphological variation in the first 4 principal components (PC1-PC4). These descriptors include: average number of flowers, corolla color and petiole length observed in PC1; calyx length, plant growth habit, pedicel thickness, seed number and fruit color in observed in PC2; leaf color, petiole color, and leaf blade width observed in PC3; and fruit cross section (or diameter) and fruit color distribution under PC4. The results indicate that there is sufficient variation for the morphological traits observed in the first 4 principal components in the current NPGRL eggplant germplasm collection which could be used to improve eggplant cultivars for these traits.

Genetic relationships based on morphological traits

The relationships among the 32 eggplant and CWR were revealed by principal component analysis (PCA) performed with simple-matching coefficient calculated from the 39 morphological traits. The projections of the 32 accessions/genotypes in a 2-dimensional graph were plotted and presented in Figure 1. The first (PC1) and second (PC2) coordinates of the PCA performed using morphological data accounted for 32.01% of the variation observed. Based on the 2D graph analysis, 3 major groups were formed but there were some accessions that formed separate groups from the rest of the members of their expected group. The first quadrant contained most of the eggplant landraces except for accessions 5302 and 4253 (quadrant 2); 6346 and 4871 (quadrant 3). Quadrant 2 contains most of the CWR except for the 2 accessions of *S. americanum* and *S. torvum* (quadrant 4).

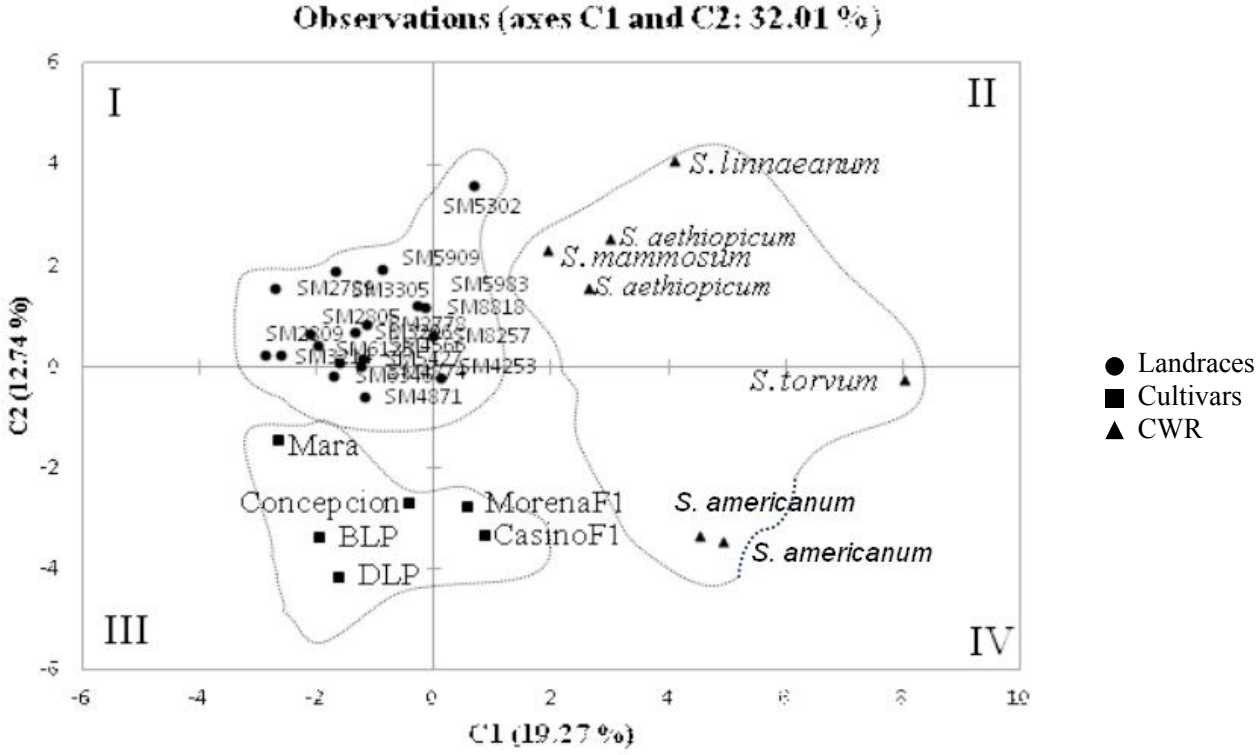
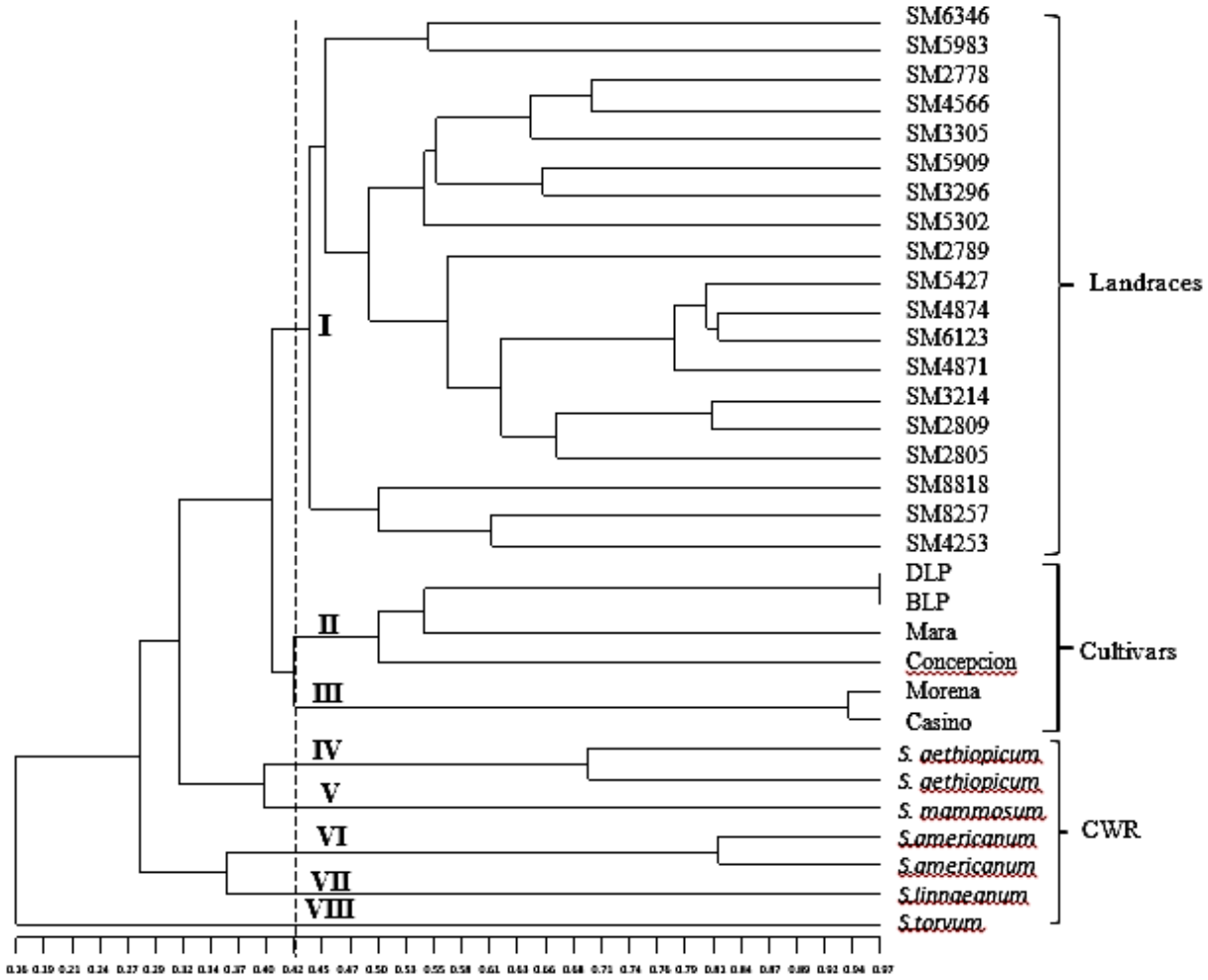


Figure 1. Two-dimensional graph showing the relationship among eggplant and wild relatives in the Philippines using morphology-based similarity coefficients.

Commercial varieties grouped into quadrant 3 except for Morena and Casino which are both commercial F1 hybrids released by the same company. Generally, the graph indicated a clear separation between the cultivated species and the crop wild relatives. A dendrogram with scale from 0.16 to 0.97 based on Jaccard's similarity coefficient was also constructed and clearly separated the 32 accessions into 4 main clusters (*S. melongena* and 3 small CWR clusters) and 8 sub-clusters (I-VIII) when a line was drawn at similarity coefficient of 0.42 (Figure 2). The largest sub-cluster within the *S. melongena* cluster contained all 19 landraces (I). The improved cultivars separated into 2 sub-groups: OPVs (group II - BLP, DLP, Mara and Concepcion) and F1 hybrids (group III - Morena and Casino). The 5 remaining sub-clusters contained the species-specific crop wild relatives (CWR). The CWR clusters corresponded to their known species classification. Two sub-clusters (*S. aethiopicum* (IV) and *S. americanum* (V) contained 2 accessions each while the other 3 clusters formed single-accession groups (*S. mammosum*, (VI), *S. linnaeanum* (VII) and *S. torvum* (VIII).



SM- *S. melongena* cultivars (Mara, Concepcion, Batangas Long Purple, Dumaguete Long Purple, Casino F_1 and Morena F_1) and landrace

Figure 2. A dendrogram based on Jaccard' similarity coefficients of 32 *S. melongena* accessions/genotypes in the Philippines based on 39 morphological trait descriptors.

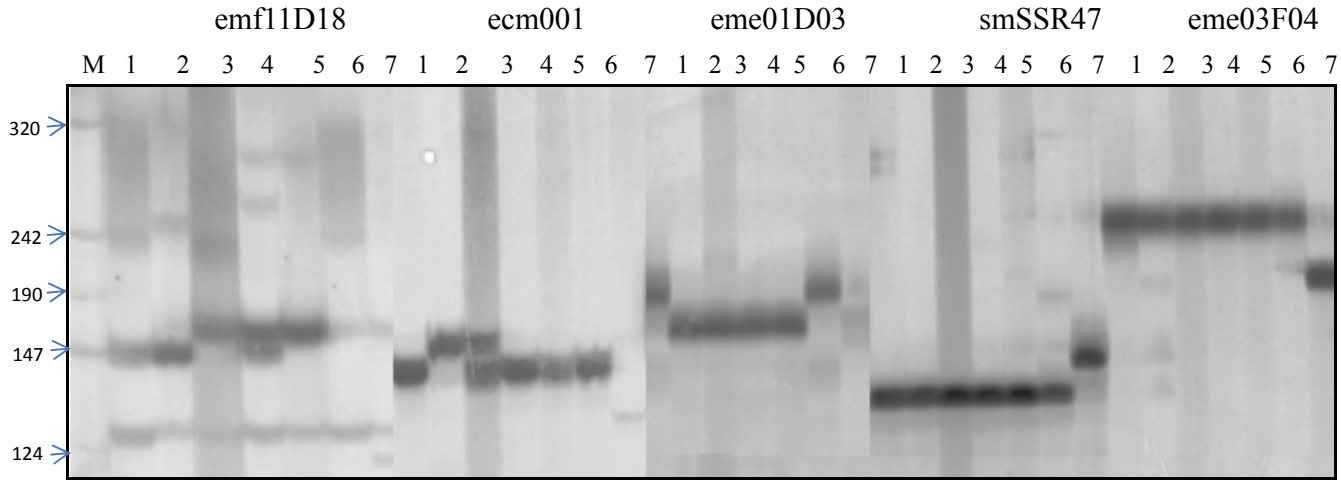
The results also indicated that *S. melongena* landraces did not show any clustering pattern based on the regions where they were collected. The analysis detected higher similarity coefficients among the *S. melongena* cultivars (0.42-0.97) and the landraces (0.43-0.94) compared to the CWR (0.16-0.81). The highest similarity coefficient (0.97) was observed between BLP and DLP, both commercial open-pollinated varieties. The high similarity coefficient between BLP and DLP suggests that they could be the same variety, or line selection that came from the same variety but were given different local names associated with the provinces where they were grown. In the same manner, it was not surprising that Morena and Casino also exhibited high similarity coefficient (0.94) because they were produced by the same seed company, hence the hybrids are likely to have related parents and common germplasm. As expected, *S. torvum* has the least similarity to the rest of the group. Although *S. torvum* belongs to the genus *Solanum*, but it does not belong under the 2 broadly categorized closely related eggplant wild relatives *S. incanum* and *S. melongena* (Knapp et al. 2013). While most of the eggplant wild species originated from Africa, *S. torvum* is native to West Indies, India, Myanmar, Thailand, Philippines, Malaysia, China and tropical America and widely naturalized in South and Southeast Asia (Nasir, 1985). It possesses a number of desirable horticultural traits such as disease resistance (Kumchai *et al*. 2013) and has medicinal uses (Yousaf *et al*., 2013).

Based on the results shown above, it can be concluded that morphology-based analysis was effective in differentiating the eggplant accessions in the current germplasm collection in accordance with their known species and gene pool delineation. However, one must bear in mind that morphological traits are known to be largely influenced by the environment (Hillis, 1987) which could result in variation without associated changes at the DNA sequence level.

Molecular analysis

SSR polymorphism purvey and characterization

A total of 41 SSR primers were surveyed initially for polymorphism in 7 representative genotypes consisting of 2 commercial cultivars (*S. melongena* cv. DLP and F1 hybrid (Morena); 3 landraces (*S. melongena* accessions 8257, 4253, and 3305); and 2wild related species (*S. americanum*). Figure 3 shows representative gels of the 7 accessions/genotypes analyzed using 5 polymorphic SSR markers.



Legend: 1= *S. melongena* accession no. 8257; 2 = *S. melongena* accession no. 3305; 3= *S.melongena* var. Morena; 4= *S.melongena* cv. Dumaguete Long Purple; 5= *S. melongena* L. accession no. 4253; 6 = *S. americanum*; and *S. torvum;* M = Marker VIII with known sizes in base pairs (bp).

Figure 3. Representative gels of 7 accessions/genotypes analyzed using 5 polymorphic SSR markers in 5% denaturing polyacrylamide gel visualized in silver stain

Results revealed that 33 out of the 41 SSR primer pairs (80.48%) generated the expected PCR products (Table 4). These 33 SSR markers consisted of 28 mapped and 5 unmapped SSR markers loci. Twenty-eight of the 33 SSR primer pairs amplified a polymorphic locus. Twelve of the 28 polymorphic SSR markers were detected in both cultivated eggplant and wild species used. Nine out of the 12 primers were genomic SSRs (75%) while only 3 out of the 12 (25%) were EST-SSRs. Seven SSR markers (ecm001, emf11D18, em117, ecm023, eme01D03, ecm009 and emf21A23) were detected only among *S. melongena* genotypes. Two SSR markers (em155 and emd07A07) were monomorphic among the *S. melongena* genotypes. The number of alleles ranged from 1-5 per SSR marker locus. More alleles were detected in the cultivated eggplant (37-40) than in the wild related species *S. americanum* and *S. torvum* (32-34). The calculated polymorphism information content (PIC) values among the 7 accessions varied between 0.0263 and 0.6785 (mean 0.1114). Genomic SSR marker em117 had the highest PIC, while EST-SSR markers, smSSR24, smSSR45 and ecm032 have lower PIC values.

The results of the SSR marker survey obtained in this study were consistent with earlier reports by several groups working on eggplants (Nunome *et al*., 2003; 2009; Tumbilen *et al*., 2011; Muñoz-Falcon *et al.*, 2011). The 75 alleles amplified from the 7 accessions/genotypes using 33 SSR markers all fall within the range of the expected product sizes published (Nunome *et al*., 2003; 2009; Tumbilen *et al*., 2011). Null alleles (absence of amplification product) detected by the 2 groups in several *Solanum* wild relatives were also detected in this study. Earlier reports that genomic SSRs were more polymorphic compared with EST-SSRs (Nunome *et al*., 2009 and Muñoz-Falcon *et al.*, 2011) were confirmed by the results of this study which showed higher PIC value for genomic SSR compared with EST-SSR markers.. Finally, this study also confirmed the results of earlier reports which observed the amplification of EST-SSR across different *Solanum* species (Prohens *et al*., 2005; Demir *et al*., 2009; Stagel *et al*., 2008) indicating that EST-SSRs is highly transferable across species while genomic SSRs are more appropriate to detect intra-species variation. Thus, it is more advantageous to use genomic SSR markers if the materials being studied consist mostly of *S. melongena*, while it is more advantageous to include EST-SSRs when the materials contain CWRs as in the case of genetic diversity of a germplasm collection containing landraces and wild relatives.

Genetic relationships based on SSR data

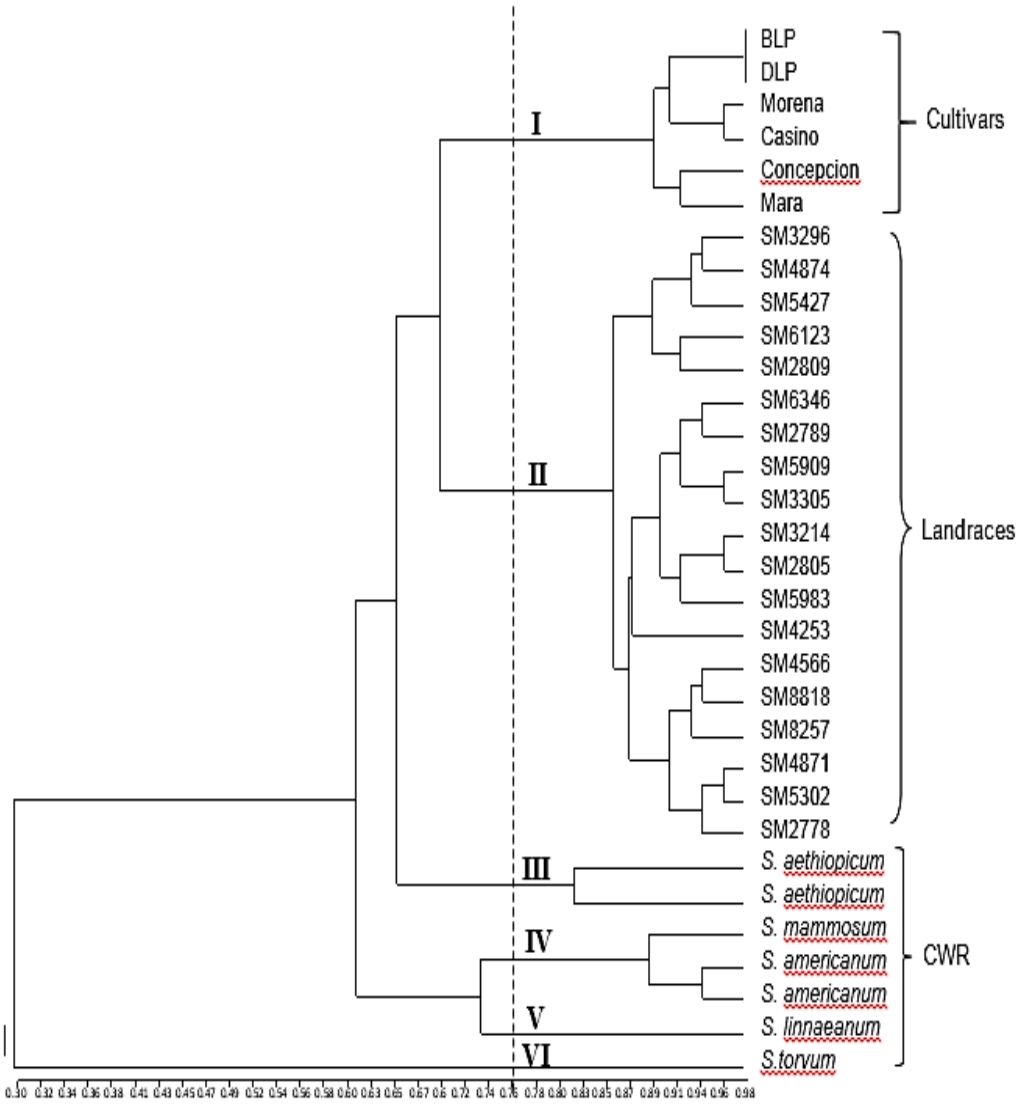
A dendrogram based on similarity coefficients of 32 accessions/genotypes using 12 SSR loci was also constructed (Figure 4). The results were similar compared with the morphology-based analysis except for some differences observed in the number of sub-clusters and the association between a few accessions within the sub-clusters. The dendrogram consistently separated the *S. melongena* landraces, improved cultivars and the crop wild relatives (CWR). The dendrogram revealed 6 sub-clusters at similarity coefficient of 0.76. The largest sub-cluster also contained all 19 landraces followed by the sub-cluster containing the cultivars. The separation of the OPVs from the F1 hybrids was not as clear compared to the dendrogram from the morphological analysis. Mara and Concepcion formed separate groups from the other 2 OPVs (BLP and DLP), with the F_1 hybrids in between the 2 sub-groups of OPVs indicating that the F_1 hybrids share the same genetic background with the OPVs The CWR formed the remaining sub-clusters: 1 sub-cluster with the 2 *S. aethiopicum* accessions; 2 sub-clusters formed single-accession groups (*S. torvum* and *S. linnaeanum);* and 1 sub-cluster containing the 2 *S. americanum* accessions and *S. mammosum*.

The SSR analysis likewise did not detect regional groupings associated with areas of collection among accessions of *S. melongena* landraces. Similarity coefficients detected by SSR markers between accessions were higher (0.30 to 0.98) compared with the range observed based on morphological data (0.16-0.97). The difference could be attributed to the greater influence of environment on morphological traits than on molecular traits (Hillis, 1987). Using SSR marker data, the highest similarity coefficient (0.98) was also detected between BLP and DLP, both commercial open-pollinated varieties (OPV).

Table 4. Summary of polymorphism survey of SSR with repeat motif observed in 7 representative genotypes of eggplant cultivars and landraces and crop wild related species.

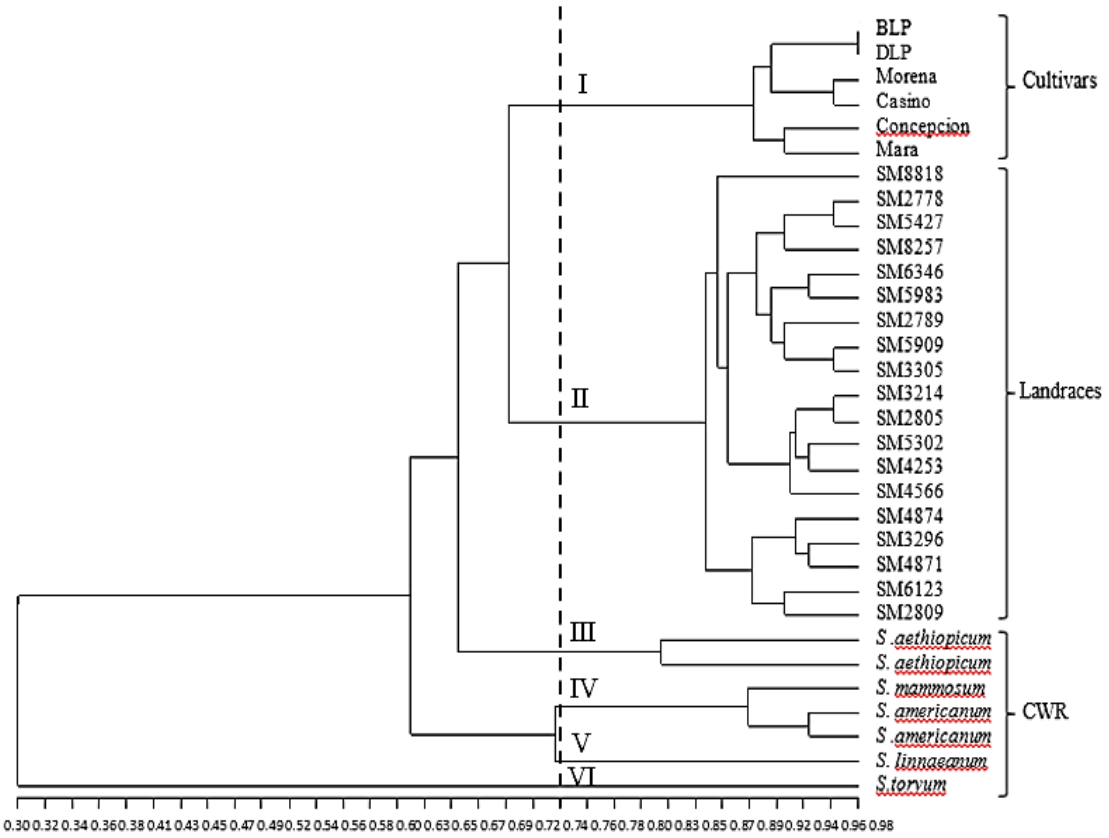
Primer	LG	Expected Product Size Range (bp)	Samples and Observed Alleles (bp)							POLY-MORPHIC (YES/NO)	No. of alleles
			S.melongena L.					*S. americanum*	*S. torvum*		
			3305	4253	Morena	DLP	8257				
ecm001	1	224-229	224	229	224,229	224	224	224	Null	Y	3
ecm090	1	162-164	162 164	162 164	162 164	162 164	162 164	162	162 190	Y	5
eme01D03	1	273-275	275	273	273	273	273	275	Null	Y	3
em135	1	256-260	256 260	256 260	258 330	256 260	258 330	Null	Null	Y	2
eme01B01	1	236-245	242	243	243	243	243	243	245	Y	3
emi03L23	1	160-162	160	160	162	162	162	162	Null	Y	3
em4_1	2	172-180	101	101	101	101	101	101	101	N	1
emd01B12	2	258-266	258	258	258	258	258	258	Null	Y	2
em155	3	271-285	285	271	285	271	271	Null	Null	Y	3
emb01O20	4	300-307	307	307	307	307	307	307	300	Y	2
em119	4	198-201	201	201	201	201	201	201	Null	Y	2
ema0060	4	380-397	397	395 397	397	395 397	397	Null	Null	Y	3
ecm070	5	222-224	198	198	198	198	198	198	198	N	1
em117	5	134-142	136	142	136	136	142	136	134	Y	3
ema0008	5	287-290	287	287	287	287	287	287	Null	Y	2
emf11D18	6	286-289	286,289	286,289	286	286,289	289	Null	Null	Y	3
em134	6	184-186	185	185	185	185	185	185	186	Y	2

emg11D22	6	298-291	296	295	296	296	296	296	296	Y	2
ecm023	7	253-257	253, 257	253, 257	253, 257	253, 257	253, 257	Null	Null	Y	3
ecm009	7	240-245	240,242	240,242	240,242	240,242	240,242	Null	Null	Y	3
emd07A07	7	292-296	296	296	296	296	296	Null	Null	Y	2
emf21A23	7	273-277	273	273	277	277	273	273	Null	Y	3
em114	8	234-236	234	234	234	234	234	234	210	Y	2
em120	8	160-164	164	164	164	164	164	164	160	Y	2
ecm032	9	276-281	281	281	281	281	281	281	281	N	1
emb01N07	9	325-330	329	329	329	329	329	230	230	Y	2
emd05F08	10	264-272	264	264	264	264	264	264	Null	Y	2
eme03F04	12	215-260	260	260	260	260	260	260	245	Y	2
smSSR24	**	220-229	220	220	220	220	220	220	220	N	1
smSSR45	**	172-182	172	172	172	172	172	172	172	N	1
smSSR46	**	258-264	258	258	258	258	258	258	Null	Y	2
smSSR47	**	180-186	186	186	186	186	186	186	200	Y	2
smSSR36	**	240-310	310	310	310	310	310	350	310	Y	2
Total			40	39	39		37	27:7 (null)	8:16 (null)	16Y:5N	75



SM- *S. melongena* cultivars (Mara, Concepcion, Batangas Long Purple, Dumaguete Long Purple, Casino F_1 and Morena F_1) and landraces

Figure 4. Dendrogram showing the genetic relationship of 32 eggplant accessions/genotypes (landraces and cultivars) with its crop wild relatives (CWR) in the Philippines based on UPGMA cluster analysis 12 SSR marker data.



SM- *S. melongena* landraces; BLP-Batangas Long Purple; DLP- Dumaguete Long Purple
CWR- Crop Wild Relatives

Figure 5. Dendrogram showing the genetic relationships of 32 eggplant accessions/genotypes (landraces and cultivars) with its crop wild relatives (CWR) in the Philippines based on UPGMA cluster analysis of 21 principal components i.e. Nine morphological trait data and 12 SSR marker data.

SSR analysis also detected high similarity levels between several landrace accessions, namely: accessions 5909 and 3305; 3214 and 2805; 4871 and 5302 and the commercial hybrids Morena and Casino. SSR-based analysis also detected the least similarity (0.30) between *S. torvum* and the rest of the accessions/genotypes used.

Genetic Relationships Based on Combined Data Analysis

In general, similar results were obtained using morphological, SSR and combined data sets. The dendrogram constructed from combined data set (Figure 5) consistently showed the separation of the 32 accessions into 3 major groups i.e. landraces, cultivars and CWR. The land races formed the largest sub-cluster. *S. torvum* again formed a separate group from the rest of the accessions/genotypes. Accessions of *S americanum* and *S. mammosum* formed 1 cluster while *S. aethiopicum* accessions formed a single cluster in the combined analysis, which is more consistent with the result obtained from molecular data than from morphological data. Among the CWR, all 3 data sets indicated that the species closest to *S. melongena* is *S. aethiopicum*, the eggplant species cultivated in Africa instead of *S. linnaeanum,* the possible ancestor of *S. melongena* (Li et al., 2010) and

the only species used in the present study recognized under the *S. incanum* and *S. melongena* eggplant relatives (Knapp *et al*. 2013). It should be noted that the result obtained in this study was based only on 2 accessions of *S. aethiopicum* and 1 accession of *S. linnaeanum*. Therefore, further analysis should be done with more accessions and the identity of the accessions used should be re-examined based on Knapp *et al.,* (2013) classification before a definitive conclusion can be made.

Combined data analyses also did not group the eggplant landraces according to the region where they were collected. Prohens and co-workers (2005) who studied 28 Spanish traditional varieties also found that there was no clear regional differentiation between accessions using both morphological and molecular analyses. Except for minor changes in the grouping of landraces, combined data analysis showed more congruence with the results of SSR data analysis. Higher similarity coefficients among landrace accessions were also detected in the combined data analyses. The pairs of accessions which showed higher similarity coefficients (0.96) in the combined data analysis (Acc. 2778 and Acc. 5427; Acc. 5909 and Acc. 3305; Acc. 3214 and Acc. 2805) were the same based on SSR data except for Acc. 4871 and 5302. These results indicate that although the accessions showed more morphological differences, they could have originated from much related sources with similar DNA sequences. Thus, morphology-based analysis alone is not sufficient due to environmental effects on phenotypic characters (Nunome *et al.,* 2003a; Stagel *et al.,* 2008; Tumbilen *et al*., 2011; Sunseri *et al.,* 2010; Muñoz-Falcon, 2011). It was strongly suggested that combination of 2 marker systems in studying genetic diversity is highly recommended than using only 1 analysis (Cortese *et al.,* 2011).

CONCLUSION

This study has successfully used morphological, SSR and combined analyses to characterize the genetic diversity and relationships among the eggplant accessions currently held in a Philippine genebank. Combining morphological and molecular characters in the analysis allowed for more accurate assessment of variation. Morphological, molecular and combined trait analyses consistently recognized the main groups of eggplants (cultivars, land races and CWR) in the collection. CWR exhibited higher variation in all 3 analyses compared to the landraces and cultivars. Among the landraces, morphological variability was moderately high but low diversity was observed based on SSR and combined data analyses. The current commercial eggplant hybrids and improved OPVs that are widely planted in the country have very high similarity coefficients. All 3 data sets did not detect any association between regional area of collection among the *S. melogena* landraces and cultivars. The information provided by this study would be of great relevance in designing future collection and management of the country's eggplant germplasm collection. Since eggplant is an introduced crop in the Philippines, it is therefore an advantage to add more foreign accessions for both *S. melongena* and CWR especially those coming from centers of origin and diversity for eggplant. Introduction of diverse foreign cultivars will increase the level of diversity in the current collection which can be used by plant breeders to develop cultivars with wider genetic base that can better respond to the challenges posed by environmental change.

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