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RESEARCH ARTICLE

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Assessing diversity among traditional Greek and foreign eggplant cultivars using molecular markers and morphometrical descriptors

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Abstract

Eggplant is a widely cultivated vegetable crop of great economic importance. Its long lasting history of domestication, selection and breeding has led to the development of numerous cultivars with variable traits. In the present study, we assessed the diversity levels within and among eleven Greek and foreign cultivars, using 22 morphological descriptors and two different classes of molecular markers (retrotransposon microsatellite amplified polymorphism-REMAP markers and nuclear microsatellites). Our results, in accordance with other studies in the field showed: a) the limited levels of genetic polymorphism within the cultivars; b) the high morphological and genetic divergence existing among them as indicated by the genetic distance values calculated, which could be attributed to selection, inbreeding and bottleneck effects; and c) the lack of concordance among morphological descriptors and molecular markers. Despite these, our analysis showed that the utilization of combinations of markers is an effective method for the characterization of plant material providing also useful diagnostic tools for the identification and authentication of the selected Greek cultivars.

Additional key words: *Solanum melongena*; genetic polymorphism; phenotypic variation; morphological descriptors; REMAP; SSRs

Abbreviations used: BB (Black Beauty); G_{ST} (proportion of the total phenotypic diversity); Hs (mean within population phenotypic diversity); $\overline{H}_S$ (mean phenotypic diversity within all populations); H_T (total phenotypic diversity); HWE (Hardy-Weinberg equilibrium); ISSR (inter-simple sequence repeat); LAG (Lagada); LERWHIT (Aspri Leros); LTR (long terminal repeat); MCMC (Markov chain Monte Carlo); MELF1 (Meliton F_1); MOMA (Money Maker F_1); REMAP (retrotransposon microsatellite amplified polymorphism); SANT (Santorini); SKO (Skoutari); SSR (simple sequence repeat); TSAK (Tsakoniki); WAS (Wasesinkuro).

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Introduction

Eggplant (*Solanum melongena* L., Solanaceae) is an important vegetable crop cultivated in the tropical and temperate regions of the world. Domesticated long ago in the Indo-Burma region where the greatest diversity is found, it has been a common crop in the Middle East and around the Mediterranean basin where it was introduced by the Arabs (Daunay *et al*., 2001; Daunay & Janick, 2007). Eggplant is a fairly good source of phenolics, vitamins and minerals with antioxidant, hepatoprotective, anti-microbial, and cardio-protective properties (Kalloo, 1993; Stommel & Whitaker, 2003; Das *et al*., 2011).

Intensive selection and breeding have resulted in the development of a large number of varieties with variable morphological, physiological and qualitative

characteristics (Daunay *et al*., 2001; Kashyap *et al*., 2003; Frary *et al*., 2007). Among them, fruit color, size and shape are the most distinctive and therefore used to establish the different commercial groups, *e.g*. black, green, purple, striped and white, long, cylindrical, egg shaped etc. existing today (Kumar *et al*., 2008; Muñoz-Falcón *et al*., 2009).

Today global trade is concentrated on a limited number of eggplant elite varieties and F_1 hybrids with increased yield and stability. The progressive dominance of those genotypes results in a considerable reduction in genetic diversity posing a threat of genetic erosion and narrowing the source of useful genes available for breeding (Muñoz-Falcón *et al*., 2011; Cericola *et al*., 2013). Local cultivars on the other hand with specific and high quality characteristics represent an elite germplasm which can be used for breeding as well as in scion-rootstock combinations for increased production and tolerance to various pathogens (Bletsos *et al*., 2004; Arvanitoyannis *et al*., 2005; Rodríguez-Burruezo *et al*., 2008).

In Greece, besides the commercial F_1 hybrids and imported varieties, there are several traditional cultivars, *e.g*. Tsakoniki (short cylindrical fruits with purple stripes), Lagada (long cylindrical purple fruits), Skoutari (medium cylindrical purple fruits), EMI (large spherical purple fruits) and Santorini (large spherical white fruits), which are adapted to the local environment having an excellent texture and cooking quality. These cultivars have not been subjected to intensive breeding so far, and are available through local markets, where the fruits of the heirloom local varieties are highly appreciated (Bletsos *et al*., 1998a, 1998b).

During the last years, heirloom vegetables produced in their regions of origin, may acquire an added value in the market if protected by geographical indications (PGI) or designations of origin (PDO) (Hurtado *et al*., 2014). This approach could increase the prospects of benefits for the farmers, therefore certification of the authenticity is essential for the protection and enhancement of these cultivars (Muñoz-Falcón *et al*., 2011).

Morphological descriptors and molecular markers have been widely utilized for the characterization of eggplant material from different regions of origin, the study of diversity among different varieties and accessions and also the determination of the relationships among the cultivated eggplant with its weedy and wild forms and close relatives (Prohens *et al*., 2005; Isshiki *et al*., 2008; Demir *et al*., 2010; Ge *et al*., 2011, 2013; Muñoz-Falcón *et al*., 2011; Tümbilen *et al*., 2011a, 2011b; Hurtado *et al*., 2012; Meyer *et al*., 2012; Vilanova *et al*., 2012; Cericola *et al*., 2013; Davidar *et al*., 2015; Mutegi *et al*., 2015). Those studies have provided invaluable information regarding the management of germplasm collections, the conservation of eggplant genetic resources and the application of breeding programs. In addition, the combination of morphological descriptors with molecular markers provides a more comprehensive view about the degree of variation among different groups of cultivars since they sample different levels of diversity and thus have a complementary effect (Prohens *et al*., 2005; Muñoz-Falcón *et al*., 2009; Tümbilen *et al*., 2011b; Hurtado *et al*., 2012; Cericola *et al*., 2013).

The purpose of this study was to assess the levels of genetic and phenotypic diversity existing within and among six traditional eggplant cultivars and a F_1 hybrid from Greece using both morphological and molecular markers. We also included a commercial variety and three more cultivars from Africa (Togo) and Japan, in order to estimate the degree of variation among cultivars originating from different diversity centers and regions. Genetic variation was studied using nuclear microsatellites, as well as the retrotransposon microsatellite amplified polymorphism (REMAP) markers (Kalendar *et al.,* 1999). REMAP has been successfully applied for the determination of the genetic relationships, the germplasm identification and the assessment of genetic variation of several plant species and at various taxonomic levels (Kalia *et al*., 2010; Castro *et al*., 2012). Finally, a comparison between the genetic data and the data obtained from the morphological analysis has also been made.

Material and methods

Plant material

The eggplant cultivars studied were: Tsakoniki (TSAK), Lagada (LAG), Skoutari (SKO), Santorini (SANT), EMI, Aspri Leros (LERWHIT) and Meliton F_1 (MELF1) from Greece; RNL566 from Togo, Money Maker F_1 hybrid (MOMA) and Wasesinkuro (WAS) from Japan and finally a commercial variety named Black Beauty (BB) (Table 1). Twelve plants per cultivar were grown in a field plot at the Technological Educational Institute (TEI) of Western Greece in Messolonghi, Greece (40.6572° N, 22.8041° E) following a completely randomized block design experiment and standard horticultural practices (pest control, fertilization and pruning) for eggplant production in this area.

Morphological characterization and statistical analysis

Twelve plants per accession were characterized using 22 primary descriptors (Table 1) for plant, leaf, flower

Table 1. Cultivars studied, traits analyzed to generate the phenotypic data set, and mean for each trait and cultivar studied.

Descriptors[1]	Cultivars										
	BB	LAG	TSAK	LERWHIT	SKO	EMI	SANT	MELF1	RNL566	MOMA	WAS
DSFS	51.00	53.33	54.00	52.33	48.17	42.83	49.5	49.5	53.00	45.83	47.17
FAC	8.00	5.17	1.00	1.00	3.00	7.50	1.00	4.17	9.00	7.33	3.00
FB	96.33	55.87	55.68	86.19	70.25	87.78	104.5	84.11	84.33	63.94	76.86
FC	1.00	3.67	4.17	1.00	2.00	1.00	1.00	1.67	1.00	3.33	1.33
FCP	4.33	1.67	0.00	0.50	1.67	4.00	3.33	3.00	1.00	0.67	0.00
FFC	4.67	5.00	3.00	3.00	5.00	4.67	3.00	5.00	5.00	3.67	5.00
FL	13.62	20.87	21	11.8	17.53	14.8	11.3	15.95	9.53	20.78	15.35
FLBR	2.33	7.83	7.17	4.00	6.67	5.00	1.33	5.67	1.00	7.50	6.67
FPC	8.00	7.17	5.00	1.00	7.33	7.50	1.00	6.50	9.00	7.33	6.50
FPL	3.53	7.98	4.92	4.05	5.33	6.48	5.53	5.57	6.37	4.73	4.63
FS	3.33	4.67	4.67	4.00	4.67	4.67	3.67	4.33	3.00	3.33	4.00
FW	337.38	226.97	228.62	268.85	279.47	368.4	337.62	354.02	258.83	241.58	289.92
LBLe	29.27	36.08	33.08	28.82	31.67	33.03	31.63	34.45	26.48	28.25	24.38
LBLo	5.00	3.00	3.00	5.00	5.00	3.00	5.00	5.00	5.00	5.00	5.00
LBW	21.03	23.92	22.95	19.9	22.92	22.78	27.65	24.53	17.92	17.85	15.43
LP	0.50	0.00	0.17	0.17	0.17	0.50	0.33	0.17	0.83	0.17	0.33
LPL	11.43	11.28	9.75	6.47	10.67	7.08	9.02	11.83	8.47	10.67	9.32
LSS	7.00	5.00	5.00	5.00	7.00	5.00	7.00	3.00	3.00	3.00	5.00
NOFPI	1.21	1.29	1.12	1.31	1.4	1.25	1.5	1.08	1.23	1.11	1.00
NOLFF	5.67	5.67	5.17	5.67	4.5	4.83	6.83	4.33	5.17	5.33	5.67
PGH	6.00	4.00	6.00	7.00	3.50	6.50	5.00	3.17	6.00	4.00	4.50
PH	60.33	85.00	56.83	48.67	89.33	58.00	74.00	99.33	56.50	92.33	74.67

[1] Codes of primary descriptors and their range/scale: DSFS = Days since fruit set (number); FAC = Fruit additional colour [1-9 (1 = milk white; 9 = black)]; FB = Fruit breadth (cm); FC = fruit curvature [1-9 (1 = none, fruit straight; 9 = U shaped)]; FCP = Fruit calyx prickles [0–9 (0 = none; 9 = more than 30)]; FFC = Fruit flesh color [3–7 (3 = white; 7 = green)]; FL = Fruit length (cm); FLBR = Fruit length to breadth ratio [1-9 (1 = broader than long; 9 = several times as long as broad)]; FPC = Fruit predominant color [1-9 (1 = milk white; 9 = black)]; FPL = Fruit pedicel length (cm); FS = Fruit shape [3–7 (3 = ¼ way from base to tip; 7 = ¾ way from base to tip)]; FW = Fruit weight (grams); LBLe = Leaf blade length (cm); LBLo = leaf blade lobing [1-9 (1 = very weak; 9 = very strong)]; LBW = Leaf blade width (cm); LP = Leaf prickles [0–9 (0 = none; 9 = very many >20)]; LPL = Leaf petiole length (cm); LSS = leaf surface shape [1-9 (1 = flat; 9 = very convex or bullate)]; NOLFF = Number of leaves to first flower (number); NOFPI = Number of flowers per inflorescence (number); PGH = Plant growth habit [3-7 (3 = upright; 7 = prostrate)]; PH = Plant height (cm).

and fruit characteristics available from EGGNET (Prohens *et al*., 2005; Van der Weerden & Barendse, 2007) and the International Board for Plant Genetic Resources (IBPGR, 1990).

Multivariate analysis (cluster, discriminant and principal components analysis) was applied using IBM SPSS vers. 22 software. The interpretation of the results was made using both parametric (ANOVA, t-test) and nonparametric (Kruskall-Wallis, Wilcoxon-Mann-Whitney) methods. All continuous traits were transformed in ordinal, according to Terzopoulos & Bebeli (2010). Total phenotypic diversity (H_T), phenotypic diversity within each cultivar (H_S) and its average across all of them ($\overline{H}_S$) were estimated applying Nei's genetic diversity index (Nei, 1973). The proportion of the total phenotypic diversity among cultivars (G_{ST}) was estimated by applying Eq. [2] of Terzopoulos & Bebeli (2008). The comparison of $\overline{H}_S$ of all the traits were carried out using the Tukey's mean comparison method.

DNA extraction

Total genomic DNA for 10 individual plants of each accession was extracted from 20 mg of fresh leaves using the hexadecyltrimethylammonium bromide (CTAB) method (Doyle & Doyle, 1990) with minor modifications. Quantification and quality assessment of the purified DNA was done spectrophotometrically and by gel electrophoresis. DNA samples were stored at -20 ºC until used.

DNA markers' genotyping and data analysis

REMAP markers: For the amplification of REMAP (retrotransposon microsatellite amplified polymorphism) markers, several LTR (long terminal repeat) primers (Baumel *et al*., 2002) in combination with ISSR (inter-simple sequence repeat) primers obtained from the University of British Columbia (Vancouver, Canada) primer set were initially tested. PCR reactions were

performed as described in Augustinos *et al*. (2014). Based on amplification quality and reproducibility of bands, we chose the five REMAP markers presented in Table S1 [suppl.]. Fragments were separated by agarose gel electrophoresis and visualized using a GelDocEZ imaging system (BIORAD, Germany). Fingerprints were scored to prepare binary matrices.

Microsatellite markers: Different genomic and EST-derived microsatellite markers developed for eggplant (Nunome *et al*., 2003; Stàgel *et al*., 2008; Nunome *et al*., 2009) were evaluated and those finally selected are presented in Table S2 [suppl.]. PCR reactions were performed as described in the respective references. Genotyping was carried out by radio-labeling and the PCR products were electrophoresed on 5% denaturing polyacrylamide gels and visualized by autoradiography. Scoring was performed by hand.

Data analysis: Given the low level of polymorphism detected for REMAP markers, only Nei's genetic distance after Lynch & Milligan (1994) was calculated using AFLPsurv 1.0 (Vekemans *et al*., 2002). On the contrary, several diversity indices (mean number of alleles per locus, effective number of alleles, observed and expected heterozygosity and the allelic diversity after correction for sample size) were measured using POPGENE version 1.31 (Yeh *et al*., 1999), and FSTAT (Goudet, 2001) for the SSR markers. Deviations from Hardy-Weinberg Equilibrium (HWE) were tested with the G^2 likelihood ratio test in POPGENE (Yeh *et al*., 1999). Genetic distances were measured according to Nei (1972) using POPGENE. Different packages implemented in PHYLIP 3.2 (Felsenstein, 1989) were used to construct an UPGMA dendrogram. The robustness of each node was assessed by the bootstrap method using 1000 pseudoreplicates. Visualization of the tree was made using TreeView (Page, 1996). GENALEX 6.5 software (Peakall & Smouse, 2006) was used for the analysis of molecular variance (AMOVA). To infer population structure and assign individuals to populations, we used STRUCTURE 2.3.4 software (Pritchard *et al*., 2000; Falush *et al*., 2003) with a burn-in period of 100,000 and 500,000 MCMC (Markov chain Monte Carlo) repetitions after the initial burn-in and 5 independent runs. Admixture with correlated frequencies was selected as a model, assuming K=1 to K=11 (where K stands for the assumed number of populations). The modification described by Evanno *et al*. (2005) was used to identify the 'true' number of K groups, using STRUCTURE HARVESTER software (Earl & von Holdt, 2012). A Mantel test to estimate a) the correlation between the distance matrices generated by REMAP and SSR markers respectively and b) the correlation between the SSR derived genetic data and those derived from the morphological characters was employed using PopTools version 3.2.5 (Hood, 2010) with 1000 iterations.

Results

Morphological data

The total phenotypic diversity (H_T) for each trait ranged from 0.19 (fruit curvature) to 0.64 (plant height, leaf surface shape), with an average of 0.51 (Table 2). The proportion of the total phenotypic diversity (G_{ST}) among all cultivars ranged from 0.14 (days since fruit set) to 1 (leaf blade lobing and leaf surface shape). Mean phenotypic diversity within populations ($\overline{H}_S$) ranged from 0 (leaf blade lobing and leaf surface shape) to 0.51 with an average value of 0.24 (Table 2). Using Tukey's mean comparison method, we detected a significant difference in the within populations diversity for the characters studied. The trait which had the highest $\overline{H}_S$ values is plant growth habit (in 6 cultivars) and the traits with the lowest value of phenotypic diversity within individual landraces were leaf blade lobbing and leaf surface shape.

Cluster analysis was made with hierarchical methods (Ward's method). The dendrogram obtained (Fig. 1), clearly separated in two main clusters, but if we want to dissociate the cultivars from the fruit color we could use three, as we did. In the first cluster (BB, EMI, RNL566) the cultivars had less plant height, but similar plant growth habit with more prickliness, small fruit length, with dark fruit predominant and additional color, dark fruit flesh color, less fruit curvature and fruit length to breadth ratio. In the second cluster (SKO, MELF1, MOMA, WAS, LAG, TSAK) the cultivars had high plant height, but a slight plant growth habit with more prickliness, large fruit length with less dark fruit predominant and additional color, dark fruit flesh color, large fruit curvature and fruit length to breadth ratio. In the third cluster (LERWHIT, SANT) the cultivars had shorter plant height, but a more prostate plant growth habit with less prickliness, small fruit length with subtle fruit predominant and additional color, subtle fruit flesh color, less fruit curvature and fruit length to breadth ratio.

To determine the diversity of multiple descriptors for the morphological traits, a principal components analysis was carried out based on the correlation matrix, and varimax rotation method with Kaiser normalization was employed. The first three principal components cumulatively explained 66.8% of the total variance (Table 3, Fig. 2). The first component (30.4% of the total variance) was highly positive correlated with the fruit breadth, the fruit weight, the leaf prickles

Table 2. Values of H_T, G_{ST}, $\bar{H}_S$ for each morphological trait across all cultivars and also the number of occurrences of the traits with the highest and lowest H_S in the cultivars studied.

Traits	H_T	G_{ST}	$\bar{H}_S$[1]	No. of occurrences in 11 populations	
				Highest	Lower
DSFS	0.59	0.14	0.51 (0.28-0.67)	1	1
FAC	0.51	0.82	0.09 (0.00-0.50)	2	9
FB	0.62	0.72	0.17 (0.00-0.50)	1	5
FC	0.19	0.37	0.12 (0.00-0.44)	3	8
FCP	0.50	0.62	0.19 (0.00-0.50)	1	6
FFC	0.46	0.80	0.09 (0.00-0.44)	1	8
FL	0.46	0.58	0.19 (0.00-0.61)	1	6
FLBR	0.59	0.63	0.22 (0.00-0.61)	1	5
FPC	0.49	0.82	0.09 (0.00-0.50)	2	9
FPL	0.62	0.36	0.40 (0.00-0.61)	1	1
FS	0.50	0.35	0.32 (0.00-0.50)	2	1
FW	0.51	0.33	0.34 (0.00-0.61)	1	2
LBLe	0.63	0.30	0.44 (0.28-0.61)	1	2
LBLo	0.40	1.00	0.00	-	11
LBW	0.62	0.32	0.42 (0.28-0.61)	3	5
LP	0.42	0.15	0.35 (0.00-0.61)	1	1
LPL	0.55	0.46	0.30 (0.00-0.61)	1	3
LSS	0.64	1.00	0.00	-	11
NOFPI	0.26	0.25	0.20 (0.00-0.50)	2	6
NOLFF	0.35	0.36	0.22 (0.00-0.50)	1	3
PGH	0.58	0.49	0.30 (0.00-0.50)	6	4
PH	0.64	0.63	0.24 (0.00-0.50)	1	3
HSD[2]			0.29		
Mean	0.51	0.52	0.24		

[1] Range of H_S in each morphological trait is in parenthesis; [2] HSD (Tukey-Kramer Honestly Significant Difference) at $p \leq 0.0$.

and the fruit calyx prickles, and highly negative with the fruit curvature, the fruit length and the fruit length to breadth ratio. The second component (19.2% of the total variance) was highly positively correlated with the leaf blade width, the number of flowers per inflorescence and the leaf blade length and moderate negatively with the leaf prickles and the fruit additional color. The third component (17.2% of the total variance) was highly positive correlated with the fruit predominant color and the fruit flesh color and negatively correlated with the number of leaves to first flower and the plant growth habit.

Genetic variability of *S. melongena* cultivars

REMAP markers: The five primer combinations selected revealed a total of 87 bands, most of which were polymorphic as shown in Table S1 [suppl.]. Nevertheless, in most cases polymorphism was due to the presence or absence of specific bands in very few samples. Therefore, no further genetic variability analysis was performed, with the exception of Nei's genetic distance values among the cultivars studied. Still, the combined use of these markers could provide an easily applied diagnostic tool for some of these cultivars (data not shown).

Microsatellite markers: For the genotyping of 10 individuals from each eggplant cultivar, nine *S. melongena* specific microsatellite markers were chosen (Table S2 [suppl.]). The overall variability detected was very low since only 2-6 alleles (3.67 per locus) were revealed for each locus. This low variability is also evident by the genetic diversity measures calculated (Table 4). As expected, the two EST-derived markers were the less polymorphic, presenting only two alleles each and null heterozygosity.

The eggplant cultivars studied presented a low degree of polymorphism, as evident by all measures (Table 5). Three of them were monomorphic for all microsatellite markers tested. Among the most polymorphic were the two cultivars from Japan (WAS and MOMA) and one from Greece (LAG). As showed by AMOVA the large proportion of the variability is due to among populations differentiation (84%) and only a small fraction is due to within population differentiation (16%).

Genetic distances (Nei, 1972) ranged from 0.0679 (WAS-MOMA) to 1.7628 (LAG-BB). Except the two

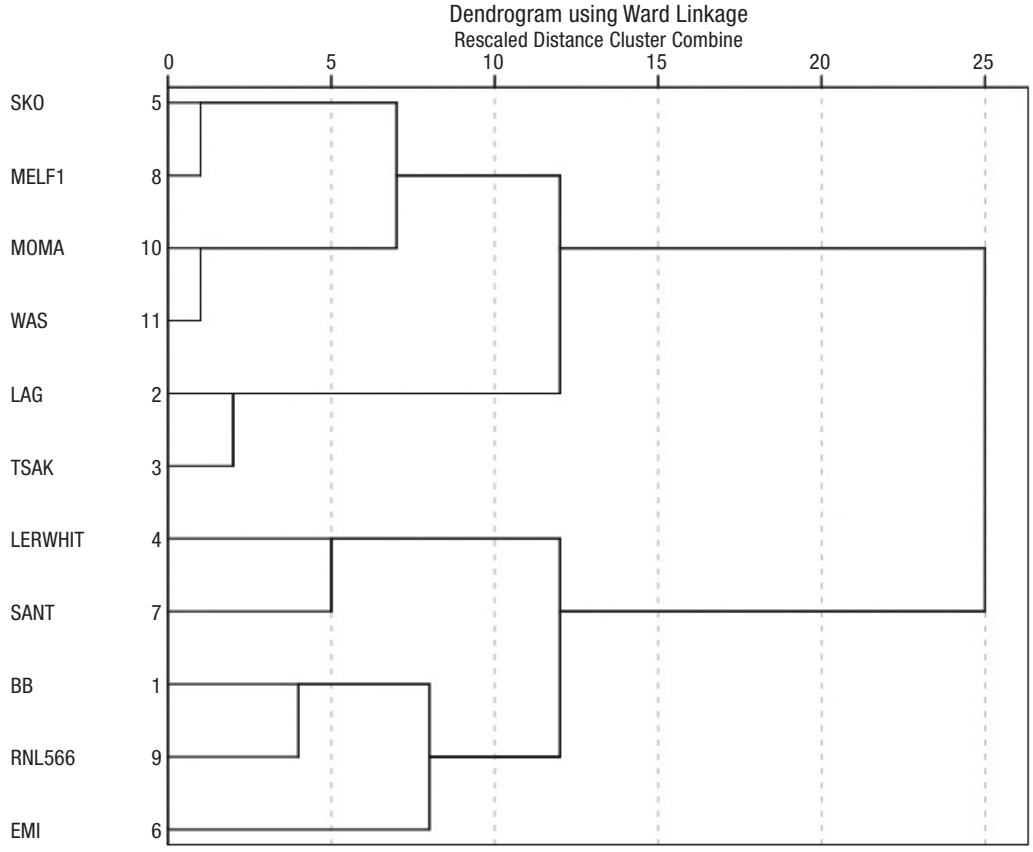

Figure 1. Clustering corresponding to the eleven cultivars studied using Ward's cluster method.

cultivars from Japan, all samples were significantly differentiated from each other, as shown using FSTAT (α=0.05) (Table 6). In the UPGMA dendrogram (Fig. 3), LERWHIT, SANT and BB were clustering apart from the other cultivars. A second clustering was formed by the non-Greek, non-European cultivars (RNL566, WAS and MOMA), although the two varieties from Japan seemed to be more closely related to the remaining Greek varieties. The STRUCTURE analysis supported the presence of three main clusters. Graphic representation of structuring at K=3 (Fig. 4) is well in accordance with the UPGMA dendrogram. The LERWHIT, SANT and BB cultivars are forming the first cluster, the non-European are forming a second, while the remaining five Greek cultivars are forming the third.

A stepwise discriminant analysis was performed using the clusters of the UPGMA dendrogram as a grouping variable and the morphometrical descriptors as independent variables in order to compare the morphometrical descriptors with the molecular genetic data.

As it seems, from our analysis, discrimination between the clusters of the UPGMA dendrogram could be made if we use the morphometrical descriptors: plant height (PH), number of leaves to first flower (NOLFF), leaf blade length (LBLe), leaf blade lobing (LBLo), leaf surface shape (LSS) and fruit shape (FS).

The standardized canonical discriminant functions were:

$$Dis\_1 = -2.99 * PH - 3.03 * NoLFF + 5.27 * LBLe + 1.67 * LBLo + 2.43 * LSS + 2.44 * FS, \text{ and}$$
$$Dis\_2 = -2.14 * PH + 0.99 * NoLFF + 1.22 * LBLe + 2.31 * LBLo + 0.51 * LSS + 0.05 * FS$$

In Fig. S1 [suppl.], an ASCII territorial map plot shows the relative location of the boundaries for the different clusters of the UPGMA dendrogram. Finally, the Mantel test showed no correlation between the genetic and the morphological data (r=0.044, p=0.61), as well as between the SSR and REMAP data (r=0.291, p=0.933).

Discussion

In this study the morphological and genetic diversity of the most promising Greek eggplant traditional cultivars with high quality and reputation at least at the local markets were examined. Some of those cultivars could achieve an advanced market status as candidates

Table 3. Coefficients for morphological traits contributing to the three leading principal components and proportion of total variance explained by the principal components analysis.

Traits	Principal components coefficients		
	PCA1	PCA2	PCA3
Days since fruit set			-0.387
Fruit additional color	0.331	-0.424	0.641
Fruit breadth	0.948		
Fruit calyx prickles	0.702	0.520	
Fruit curvature	-0.918		
Fruit flesh color			0.839
Fruit length	-0.854		0.321
Fruit length to breadth ratio	-0.847		0.324
Fruit pedicel length		0.362	0.384
Fruit predominant color		-0.345	0.847
Fruit shape	-0.469	0.644	
Fruit weight	0.793	0.357	
Leaf blade length		0.828	
Leaf blade lobing	0.432	-0.378	
Leaf blade width		0.929	
Leaf petiole length			0.606
Leaf prickles	0.723	-0.476	
Leaf surface shape		0.522	-0.327
Number of flowers per inflorescence		0.851	
Number of leaves to first flower			-0.637
Plant growth habit	0.326		-0.633
Plant height		0.304	0.646
Variability %	30.421	19.208	17.293

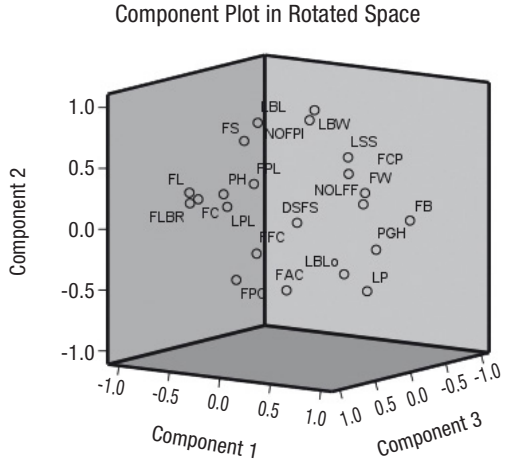

Figure 2. Principal components analysis relationships among the 22 morphological traits.

for a Protected Designation of Origin (PDO) or a Protected Geographical Indication (PGI) label (EC, 2006). Usually, products with a PDO or PGI label are perceived as unique having an added value in the market and getting a higher price (Gracia & Albisu, 2001; McLaughlin, 2004). Therefore it is necessary to devise tools for the certification of the uniqueness and authenticity of those products in order to protect and enhance them (Muñoz-Falcón *et al*., 2011; Hurtado *et al*., 2014).

Morphological diversity: The eggplant cultivars displayed a relatively high level of diversity for most of the morphological traits studied (total phenotypic diversity (H_T = 0.51). In addition, the mean phenotypic diversity among cultivars was relatively high (G_{ST} = 0.52), while the mean within population diversity was low ($\overline{H}_S$ = 0.24), as expected for cultivars.

The morphological differentiation and the proportion of phenotypic variation found among cultivars as indicated by the high G_{ST} values is more likely due to the artificial selection performed by the farmers, the mating system, the management of the genetic material, and also to the environmental conditions (Prohens *et al*., 2005; Terzopoulos & Bebeli, 2010; Hurtado *et al*., 2012). Some of the traits for which significant differences exist *e.g.* fruit size, shape and color, growth habit, prickliness among others, are controlled by a few genes which are presenting a high degree of penetration and expression. As a consequence the effect on phenotype is rapid and probably linked with response to selection and the different origin of those cultivars (Daunay, 2008; Hurtado *et al*., 2012).

Understanding which traits contribute the most to the phenotypic variation observed among and within cultivars is very important for breeding purposes as well as for conservation procedures (Terzopoulos & Bebeli, 2008). In the present study traits such as plant

Table 4. Genetic variability measures for the SSR markers used.

Marker	Sample size	n_a	n_e	AR	Ho	He
Emf21C11[a]	110	5	2.54	3.911	0.0092	0.6093
Emf11D18[a]	110	5	3.22	3.862	0.1651	0.6928
Emg11A06[a]	110	2	1.10	1.538	0.0183	0.0879
Emg21A08[a]	107	3	2.08	2.800	0.0094	0.5225
Em133[b]	108	6	3.72	5.101	0.1215	0.7346
Em135[b]	109	3	1.88	2.780	0.0278	0.4704
Emg11M09[b]	109	5	3.65	4.272	0.0278	0.7298
EEMS49[c]	110	2	1.87	1.999	0.0000	0.4667
EEMS15[c]	110	2	1.36	1.949	0.0000	0.2645
Mean	109	3.67	2.38	3.135	0.0421	0.5087

n_a: actual allele number; n_e: effective allele number; AR: allelic richness; Ho: observed heterozygosity; He: expected heterozygosity. [a]Nunome *et al*., 2009; [b]Nunome *et al*., 2003; [c]Stàgel *et al*., 2008.

Table 5. Genetic variability measures and markers that are out of equilibrium (HWE) for the eggplant cultivars studied.

Cultivars	Sample size	n_a	n_e	AR	Ho	He	Polymorphic loci	HWE
BB	10	1.000	1.000	1.000	0.000	0.000	0/12	-
EMI	10	1.111	1.012	1.089	0.011	0.011	1/12	0/1
LAG	10	1.778	1.294	1.708	0.035	0.188	5/12	5/5
LERWHIT	10	1.111	1.067	1.111	0.033	0.044	1/12	0/1
MELF1	10	1.222	1.170	1.222	0.160	0.099	2/12	1/2
MOMA	10	1.556	1.449	1.556	0.056	0.258	5/12	4/5
RNL566	10	1.000	1.000	1.000	0.000	0.000	0/12	-
SANT	10	1.000	1.000	1.000	0.000	0.000	0/12	-
SKO	10	1.333	1.270	1.333	0.099	0.155	3/12	1/3
TSAK	10	1.222	1.049	1.215	0.000	0.042	2/12	2/2
WAS	10	1.778	1.483	1.755	0.078	0.283	6/12	3/6

n_a: actual allele number; n_e: effective allele number; AR: allelic richness; Ho: observed heterozygosity; He: expected heterozygosity.

height, fruit calyx prickles, fruit length, fruit breadth, fruit length to breadth ratio, fruit predominant, additional and flesh color tend to be highly differentiated among cultivars while most of them exhibited low diversity within the cultivars. Analysis of morphological diversity showed that clustering of cultivars was strongly associated with plant height and fruit appearance such as fruit length, shape and color. Clustering of different eggplant cultivars according to fruit appearance has also been reported by other researchers (Choudhury, 1976; Tümbilen *et al*., 2011b; Cericola *et al*., 2013). This was also evident in the PCA analysis where in the first component (30.4% of the total variance) the most important parameters for separating the cultivars were those related to fruit appearance and shape such as fruit breadth, fruit weight fruit calyx prickles and fruit length. Moreover, as shown in the Ward's dendrogram two major clusters were formed based primarily on those characters. In the first cluster there were the SKO, MELF1, MOMA, WAS, LAG, TSAK cultivars which are predominantly taller with longer fruits, while in the second cluster were those with smaller fruit length and plant height. Within this cluster another sub-cluster was formed by LERWHIT and SANT based exclusively on fruit color.

Genetic diversity: Both REMAP and SSR markers showed that the overall polymorphism revealed is low. This is in agreement with the current literature, where larger collections and more markers have been independently applied (Stàgel *et al*., 2008; Muñoz-Falcón *et al*., 2009; Ge *et al*., 2011, 2013; Muñoz-Falcón *et al*., 2011; Hurtado *et al*., 2012; Cericola *et al*., 2013). Because *S. melongena* is a largely autogamous species, most heritage and commercial varieties are expected to be highly homozygous (Cericola *et al*., 2013). In addition extensive phenotypic-based selection and the use of a limited number of individuals as starting material in breeding programs have deprived this species of the bigger part of its original genetic pool, reducing thus the amount of genetic diversity available today (Muñoz-Falcón *et al*., 2009).

In our study, no significant associations were observed between REMAP and SSR markers, according to the Mantel test employed. This might be due to the type of genetic polymorphism detected and the different type of information provided by each molecular

Table 6. Genetic distances (below diagonal) and significance of population differentiation (above diagonal).

Cultivars	TSAK	EMI	MELF1	LAG	SANT	SKO	LERWHIT	BB	WAS	MOMA	RNL566
TSAK	-	*	*	*	*	*	*	*	*	*	*
EMI	0.785	-	*	*	*	*	*	*	*	*	*
MELF1	0.441	0.393	-	*	*	*	*	*	*	*	*
LAG	0.428	0.282	0.238	-	*	*	*	*	*	*	*
SANT	1.484	1.499	1.232	1.612	-	*	*	*	*	*	*
SKO	0.569	0.183	0.179	0.305	1.158	-	*	*	*	*	*
LERWHIT	1.057	1.072	0.874	1.323	0.343	0.669	-	*	*	*	*
BB	1.535	1.499	1.232	1.763	0.405	1.319	0.729	-	*	*	*
WAS	0.544	0.345	0.312	0.430	1.105	0.261	0.646	1.323	-	NS	*
MOMA	0.480	0.316	0.288	0.415	1.441	0.208	0.783	1.556	0.068	-	*
RNL566	1.112	0.592	0.707	0.695	0.811	0.651	1.077	0.588	0.693	0.553	-

Genetic distances are estimated according to Nei (1972). Significance level for population differentiation at 0.05; *significant differentiation; NS= not significant differentiation.

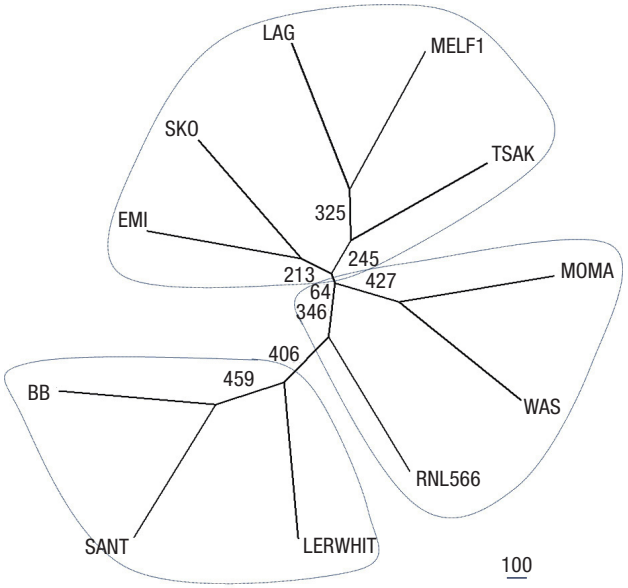

Figure 3. UPGMA dendrogram for the eggplant cultivars studied based on the microsatellite allele frequencies.

marker system used (Ghislain *et al*., 2006). Also, poor correlation between the used genetic markers most likely indicates that they refer to different subsets of loci in the genome as for example the SSR and REMAP markers in our case (Biswas *et al*., 2010; Mandoulakami *et al*., 2015). It would be surprising to obtain a better insight of the diversity existing in the *S. melongena* populations with REMAP markers than with microsatellite markers. However, the combination of the REMAP markers used here produced specific patterns of bands that can distinguish among the different cultivars.

Nuclear SSRs have been widely used to study the genetic diversity and the relationships of different eggplant groups and cultivars of so far. SSRs have the ability to detect genetic relationships based on specific traits, probably caused by its sensitivity to neutrality and/or linkage disequilibrium, and therefore are more appropriate in studying specific sets of genetically related materials (Muñoz-Falcón *et al*., 2009).

The overall variability detected in our study was low since the SSR markers produced an average of 3.67 alleles per locus. Muñoz-Falcón *et al*. (2011) using also genomic and EST-SSRs revealed low levels of polymorphism. This could be due to the intensive breeding efforts and the narrow genetic background (Ge *et al*., 2011). On the other hand Behera *et al*. (2006) found broader diversity in 92 South Asian eggplant accessions but such greater variation was expected since South Asia is a primary center of eggplant diversity. According to Tümbilen *et al*. (2011a) genetic diversity based on molecular data is highly dependent on the number and type of marker chosen and the accessions tested and this could be the case in our study.

The genetic differentiation as indicated by the genetic distance values estimated among the cultivars studied was high. Also all of them were significantly differentiated from each other, except the two cultivars from Japan. As shown by the UPGMA dendrogram and also by the STRUCTURE analysis our data support the existence of three main clusters, the first with the foreign cultivars, the second with the LERWHIT, SANT and BB, while the third with the remaining Greek cultivars. Interestingly the two Japanese cultivars seem to be more closely related to some of the Greek ones. Hurtado *et al*. (2012) and Cericola *et al*. (2013) failed to sufficiently discriminate eggplants from different centers of origin, although this was achieved completely by Prohens *et al*. (2005) and Muñoz-Falcón *et al*. (2009) or partly by Sunseri *et al*. (2010). A possible explanation is that the limited genetic variability of the populations of this species (shown in other studies as well) and also the extensive selection performed on a few elite cultivars for specific traits have probably lead to extensive inbreeding, hitch-hiking effects of micro-

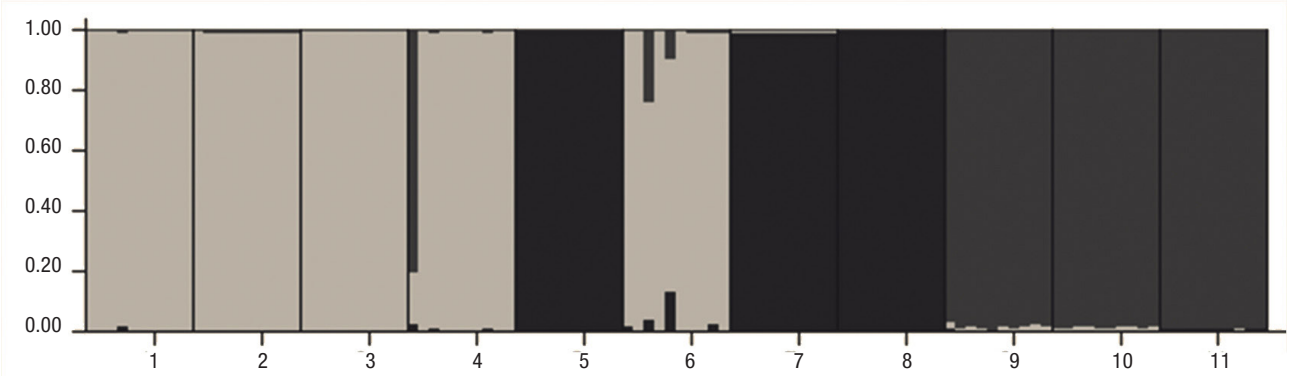

Figure 4. Structure analysis assuming K=3, according to Evanno *et al*. (2005) modification. 1=TSAK; 2=EMI; 3=MELF1; 4=LAG; 5=SANT; 6=SKO; 7=LERWHIT; 8=BB; 9=WAS; 10=MOMA; 11=RNL566.

satellite markers along with traits of choice and severe bottlenecks. All factors have probably acted together, resulting to the 'masking' of the signal of the genetic origin of eggplant varieties.

The differentiation of the LERWHIT and SANT cultivars from the remaining Greek ones may be due to the SSR markers tested which could reflect certain phenotypic differences given that these cultivars differ in fruit shape and color from the rest. Also because these two cultivars originate from small islands it is also well supported that microevolutionary forces, including natural selection under local conditions, artificial selection by farmers, genetic drift, and recombination may have favored the isolation of local variants (Portis *et al*., 2006; Muñoz-Falcón *et al*., 2011).

In a recent study by Ganopoulos *et al*. (2015), thirty-six Greek eggplant accessions, were discriminated based on High Resolution Melting (HRM) barcoding profile. In our case, although we studied fewer cultivars but with more SSR markers, we did not succeed in discriminating them using nuclear microsatellites only. This could be due to a) the utilization of different SSR markers, b) the utilization of a different resolution technology, since HRM could theoretically reveal more diversity, as discussed in Ganopoulos *et al*. (2015) and references therein. However, the interpretation of the results can be more difficult, since melting curves are not exactly 'descriptive' of the events generating these differences, c) the methodology used regarding sampling: in the present study, DNA was isolated from 10 individual plants per variety, while in Ganopoulos *et al*. (2015) DNA per variety were isolated as a bulk.

The Mantel test showed no correlation between the genetic and the morphological data. On the other hand, the stepwise discriminant analysis performed in order to compare the morphometrical descriptors with the molecular genetic data, showed that discrimination between the clusters of the UPGMA dendrogram could be made if we use the morphometrical descriptors related to plant height (PH), number of leaves to first flower (NOLFF), leaf blade length (LBLe), leaf blade lobing (LBLo), leaf surface shape (LSS) and fruit shape (FS). These descriptors and especially those related to FS have also been found to be strongly associated with the clustering of *S. melongena* cultivars in several other cases (Muñoz-Falcón *et al*., 2008; Tümbilen *et al*., 2011b; Hurtado *et al*., 2012 etc.).

The lack of correlation between morphological and molecular data has been a matter of debate since there are many studies where a similar situation has also been confirmed (Tümbilen *et al*., 2011b; Hurtado *et al*., 2012; Cericola *et al*., 2013), whereas others showed a reasonable level of phenotype/genotype correlation (Prohens *et al*., 2005; Muñoz-Falcón *et al*., 2008; Caguiat & Hautea, 2014). The correlations between morphological and molecular data usually vary depending on the plant material, the morphological descriptors and the molecular markers used (Hurtado *et al*., 2012). Morphological markers in eggplant sample traits for which variation is usually controlled by a few genes or QTLs (Doganlar *et al*., 2002; Daunay, 2008) therefore a tiny portion of the genome is responsible for the phenotypic changes observed (Cericola *et al*., 2013). On the other hand molecular markers sample variation from a larger portion of the genome, both coding and/or non-coding (Varshney *et al*., 2005; Kalia *et al*., 2010) and thus these two types of markers may follow different evolutionary paths which explains the lack of correlation observed (Hurtado *et al*., 2012). Morphological descriptors and molecular markers provide complementary information and thus characterization of germplasm with both of them allows a more accurate assessment of variation (Caguiat & Hautea, 2014).

In summary, this study has successfully used morphological descriptors and SSR markers to determine the genetic diversity levels and to characterize the most important Greek traditional cultivars together with oth-

ers from different centers of origin. Although low variability was detected within all cultivars, at least for the molecular markers used, a considerable amount of variation has been revealed among them with both the morphological descriptors and the molecular markers. Our results show that the plant material studied can be efficiently characterized for breeding and conservation purposes and also that we are able to devise tools for the effective screening of the whole Greek eggplant germplasm collection in the future. Moreover, these local varieties can be distinguished from the commercial ones and thus gain a higher market value, while the high quality standards of the final product are maintained.

References

Arvanitoyannis IS, Khah EM, Christakou EC, Bletsos FA, 2005. Effect of grafting and modified atmosphere packaging on eggplant quality parameters during storage. Int J Food Sci Tech 40: 311-322. http://dx.doi.org/10.1111/j.1365-2621.2004.00919.x.

Augustinos A, Sotirakis K, Trigas P, Kalpoutzakis E, Papasotiropoulos V, 2014. Genetic variation in three closely related *Minuartia* (Caryophyllaceae) species endemic to Greece: Implications for conservation management. Folia Geobot 49: 603-621. http://dx.doi.org/10.1007/s12224-014-9196-2.

Baumel A, Ainouche M, Kalendar R, Schulman AH, 2002. Retrotransposons and genomic stability in populations of the young allopolyploid species *Spartina anglica* C.E. Hubbard (Poaceae). Mol Bio Evol 19: 1218-1227. http://dx.doi.org/10.1093/oxfordjournals.molbev.a004182.

Behera TK, Sharma P, Singh BK, Kumar G, Kumar R, Mohapatra T, Singh NK, 2006. Assessment of genetic diversity and species relationships in eggplant (*Solanum melongena* L.) using STMS markers. Sci Hort 107: 352-357. http://dx.doi.org/10.1016/j.scienta.2005.11.004.

Biswas MK, Baig MNR, Cheng YJ, Deng XX, 2010. Retrotransposon based genetic similarity within the genus *Citrus* and its relatives. Genet Resour Crop Evol 7: 963-972. http://dx.doi.org/10.1007/s10722-010-9533-0.

Bletsos FA, Roupakias DG, Tsaktsira ML, Scaltsoyjannes AB, Thanassoulopoulos CC, 1998a. Interspecific hybrids between three eggplant (*Solanum melongena* L.) cultivars and two wild species (*Solanum torvum Sw.* and *Solanum sisymbriifolium Lam.*). Plant Breeding 117: 159-164. http://dx.doi.org/10.1111/j.1439-0523.1998.tb01471.x.

Bletsos FA, Roupakias DG, Thanassoulopoulos CC, 1998b. Morphological traits of the interspecific eggplant hybrid *Solanum melongena* x *Solanum torvum.* Acta Hortic 467: 155-161. http://dx.doi.org/10.17660/ActaHortic.1998.467.17.

Bletsos FA, Roupakias DG, Tsaktsira M, Scaltsoyiannis A, 2004. Production and characterization of interspecific hybrids between three eggplant (*Solanum melongena* L.) cultivars and *Solanum macrocarpum* L. Sci Hort 101: 11-21. http://dx.doi.org/10.1016/j.scienta.2003.09.011.

Caguiat XGI, Hautea DM, 2014. Genetic diversity analysis of eggplant (*Solanum melongena* L.) and related wild species in the Philippines using morphological and SSR markers. SABRAO J Breed Genet 46: 183-201.

Castro I, D'Onofrio C, Martín JP, Ortiz JM, De Lorenzis G, Ferreira V, Pinto-Carnide O, 2012. Effectiveness of AFLPs and retrotransposon-based markers for the identification of Portuguese grapevine cultivars and clones. Mol Biotechnol 52: 26-39. http://dx.doi.org/10.1007/s12033-011-9470-y.

Cericola F, Portis E, Toppino L, Barchi L, Acciarri N, Ciriaci T, Sala T, Rotino GL, Lanteri S, 2013. The population structure and diversity of eggplant from Asia and the Mediterranean Basin. PLoS One 8: e73702. http://dx.doi.org/10.1371/journal.pone.0073702.

Choudhury B, 1976. Vegetables. 4th Revise. National Book Trust, New Delhi, India, 214 pp.

Das S, Raychaudhuri U, Falchi M, Bertelli A, Braga PC, Das DK, 2011. Cardioprotective properties of raw and cooked eggplant (*Solanum melongena* L). Food & Function 2: 395-399. http://dx.doi.org/10.1039/c1fo10048c.

Daunay MC, 2008. Eggplant. In: Handbook of crop breeding, Vegetables II: Fabaceae, Liliaceae, Umbelliferae, and Solanaceae; Nuez J, Prohens F (eds). Springer, NY, pp: 163-220. http://dx.doi.org/10.1007/978-0-387-74110-9_5.

Daunay MC, Janick J, 2007. History and iconography of eggplant. Chronica Horticulturae 47: 16-22.

Daunay MC, Lester RN, Gebhardt C, Hennart JW, Jahn M, Frary A, Doganlar S, 2001. Genetic resources of eggplant (*Solanum melongena*) and allied species: a new challenge for molecular geneticists and eggplant breeders. Proc Solanaceae V, Advances in Taxonomy and Utilization, Nijmegen, NL, pp: 251-274. http://prodinra.inra.fr/record/59362.

Davidar P, Snow AA, Rajkumar M, Pasquet R, Daunay MC, Mutegi E, 2015. The potential for crop to wild hybridization in eggplant (*Solanum melongena*; Solanaceae) in southern India. Am J Bot 102: 128-138. http://dx.doi.org/10.3732/ajb.1400404.

Demir K, Bakir M, Sarikamiş G, Acunalp S, 2010. Genetic diversity of eggplant (*Solanum melongena*) germplasm from Turkey assessed by SSR and RAPD markers. Genet Mol Res 9: 1568-1576. http://dx.doi.org/10.4238/vol9-3gmr878.

Doganlar S, Frary A, Daunay MC, Lester RN, Tanksley SD, 2002. A comparative genetic linkage map of eggplant (*Solanum melongena*) and its implications for genome evolution in the Solanaceae. Genetics 161: 1697-1711.

Doyle JJ, Doyle JL, 1990. Isolation of plant DNA from fresh tissue. Focus 12: 13-15.

Earl DA, vonHoldt BM, 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Res 4: 359-361. http://dx.doi.org/10.1007/s12686-011-9548-7.

EC, 2006. Council Regulation (EC) No. 509/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. Off. L93, J. Eur. Union, pp: 12-25.

Evanno G, Regnaut S, Goudet J, 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14: 2611-20. http://dx.doi.org/10.1111/j.1365-294X.2005.02553.x.

Falush D, Stephens M, Pritchard JK, 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics 164: 1567-1587.

Felsenstein J, 1989. PHYLIP - Phylogeny Inference Package, Vers. 3.2 Cladistics 5: 164-166.

Frary A, Doganlar S, Daunay MC, 2007. Eggplant. Genome mapping and molecular breeding in plants (5). Vegetables: 231-257.

Ganopoulos I, Xanthopoulou A, Mastrogianni A, Drouzas A, Kalivas A, Bletsos F, Krommydas K, Ralli P, Tsaftaris A, Madesis P, 2015. High resolution melting (HRM) analysis in eggplant (*Solanum melongena* L.): A tool for microsatellite genotyping and molecular characterization of a Greek Genebank collection. Biochem System Ecol 58: 64-71. http://dx.doi.org/10.1016/j.bse.2014.11.003.

Ge H, Li H, Liu Y, Li X, Chen H, 2011. Characterization of novel developed expressed sequence tag (EST)-derived simple sequence repeat (SSR) markers and their application in diversity analysis of eggplant. Afr J Biotechnol 10: 9023-9031. http://dx.doi.org/10.5897/AJB11.1029.

Ge H, Liu Y, Jiang M, Zhang J, Han H, Chen H, 2013. Analysis of genetic diversity and structure of eggplant populations (*Solanum melongena* L.) in China using simple sequence repeat markers. Sci Hort 162: 71-75. http://dx.doi.org/10.1016/j.scienta.2013.08.004.

Ghislain M, Andrade D, Rodríguez F, Hijmans RJ, Spooner DM, 2006. Genetic analysis of the cultivated potato *Solanum tuberosum* L. Phureja Group using RAPDs and nuclear SSRs. Theor Appl Genet 113 (8): 1515-27. http://dx.doi.org/10.1007/s00122-006-0399-7.

Goudet J, 2001. FSTAT, version 2.9.3, A program to estimate and test gene diversities and fixation indices. Lausanne University, Lausanne, Switzerland. http://www2.unil.ch/popgen/softwares/fstat.htm.

Gracia A, Albisu LM, 2001. Food consumption in the European Union: main determinants and country differences. Agribusiness 17: 469488. http://dx.doi.org/10.1002/agr.1030.

Hood GM, 2010. PopTools vers. 3.2.5. http://www.poptools.org.

Hurtado M, Vilanova S, Plazas M, Gramazio P, Fonseka HH, Fonseka R, Prohens J, 2012. Diversity and relationships of eggplants from three geographically distant secondary centers of diversity. PLoS One 7: e41748. http://dx.doi.org/10.1371/journal.pone.0041748.

Hurtado M, Vilanova S, Plazas M, Gramazio P, Andújar I, Herraiz FJ, Castro A, Prohens J, 2014. Enhancing conservation and use of local vegetable landraces: the Almagro eggplant (*Solanum melongena* L.) case study. Genet Res Crop Evol 61: 787-795. http://dx.doi.org/10.1007/s10722-013-0073-2.

IBPGR, 1990. Descriptors for eggplant. International Board for Plant Genetic Resources, Rome.

Isshiki S, Iwata N, Khan MMR, 2008. ISSR variations in eggplant (*Solanum melongena* L.) and related Solanum species. Sci Hort 117: 186-190. http://dx.doi.org/10.1016/j.scienta.2008.04.003.

Kalendar R, Grob T, Regina M, Suoniemi A, Schulman A, 1999. IRAP and REMAP: two new retrotransposon-based DNA fingerprinting techniques. Theor Appl Genet 98: 704-711. http://dx.doi.org/10.1007/s001220051124.

Kalia RK, Rai MK, Kalia S, Singh R, Dhawan AK, 2010. Microsatellite markers: an overview of the recent progress in plants. Euphytica 177: 309-334. http://dx.doi.org/10.1007/s10681-010-0286-9.

Kalloo G, 1993. Eggplant, *Solanum melongena*. In: Genetic improvement of vegetable crops; Kalloo G & Bergh BO (eds), Pergamon Press, Oxford, UK, pp: 587-604. http://dx.doi.org/10.1016/B978-0-08-040826-2.50047-3.

Kashyap V, Kumar SV, Collonnier C, Fusari F, Haicour R, Rotino GL, Sihachakr D, Rajam MV, 2003. Biotechnology of eggplant. Sci Hort 97: 1-25. http://dx.doi.org/10.1016/S0304-4238(02)00140-1.

Kumar G, Meena BL, Kar R, Tiwari SK, Gangopadhyay KK, Bisht IS, Mahajan RK, 2008. Morphological diversity in brinjal (*Solanum melongena* L.) germplasm accessions. Plant Genetic Resources: Characterization and Utilization 6: 232-236. http://dx.doi.org/10.1017/S1479262108994211.

Lynch M, Milligan BG, 1994. Analysis of population genetic structure with RAPD markers. Mol Ecol 3: 91-99. http://dx.doi.org/10.1111/j.1365-294X.1994.tb00109.x.

Mandoulakani BA, Sadigh P, Azizi H, Piri Y, Nasri Sh, Arzhangh S, 2015. Comparative assessment of IRAP, REMAP, ISSR, and SSR markers for evaluation of genetic diversity of Alfalfa (*Medicago sativa* L.). J Agr Sci Tech 17: 999-1010.

McLaughlin EW, 2004. The dynamics of fresh fruit and vegetable pricing in the supermarket channel. Prevent Med 39 (Suppl 2): S81-87. http://dx.doi.org/10.1016/j.ypmed.2003.12.026.

Meyer RS, Karol KG, Little DP, Nee MH, Litt A, 2012. Phylogeographic relationships among Asian eggplants and new perspectives on eggplant domestication. Mol Phyloget Evol 63: 685-701. http://dx.doi.org/10.1016/j.ympev.2012.02.006.

Muñoz-Falcón JE, Prohens J, Vilanova S, Nuez F, 2008. Characterization, diversity, and relationships of the Spanish striped (Listada) eggplants: a model for the enhancement and protection of local heirlooms. Euphytica 164: 405-419. http://dx.doi.org/10.1007/s10681-008-9688-3.

Muñoz-Falcón JE, Prohens J, Vilanova S, Nuez F, 2009. Diversity in commercial varieties and landraces of black eggplants and implications for broadening the breeders' gene pool. Ann Appl Biol 154: 453-465. http://dx.doi.org/10.1111/j.1744-7348.2009.00314.x.

Muñoz-Falcón JE, Vilanova S, Plazas M, Prohens J, 2011. Diversity, relationships, and genetic fingerprinting of the Listada de Gandía eggplant landrace using genomic SSRs and EST-SSRs. Sci Hort 129: 238-246. http://dx.doi.org/10.1016/j.scienta.2011.03.034.

Mutegi E, Snow AA, Rajkumar M, Pasquet R, Ponniah H, Daunay MC, Davidar P, 2015. Genetic diversity and

population structure of wild/weedy eggplant (*Solanum insanum* L, Solanaceae) in southern India: implications for conservation. Am J Bot 102 (1): 140-148. http://dx.doi.org/10.3732/ajb.1400403.

Nei M, 1972. Genetic distance between populations. Am Natur 106: 283-292. http://dx.doi.org/10.1086/282771.

Nei M, 1973. Analysis of gene diversity in subdivided populations. PNAS 70: 3321-3323. http://dx.doi.org/10.1073/pnas.70.12.3321.

Nunome T, Suwabe K, Iketani H, Hirai M, 2003. Identification and characterization of microsatellites in eggplant. Plant Breeding 122: 256-262. http://dx.doi.org/10.1046/j.1439-0523.2003.00816.x.

Nunome T, Negoro S, Kono I, Kanamori H, Miyatake K, Yamaguchi H, Ohyama A, Fukuoka H, 2009. Development of SSR markers derived from SSR-enriched genomic library of eggplant (*Solanum melongena* L.). Theor Appl Genet 119: 1143-1153. http://dx.doi.org/10.1007/s00122-009-1116-0.

Page RDM, 1996. TreeView: An application to display phylogenetic trees on personal computers. Comput Appl Biosci 12: 357-358.

Peakall R, Smouse PE, 2006. Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6: 288-295. http://dx.doi.org/10.1111/j.1471-8286.2005.01155.x.

Portis E, Nervo G, Cavallanti F, Barchi L, Lanteri S, 2006. Multivariate analysis of genetic relationships between Italian pepper landraces. Crop Sci 46: 2517-2525. http://dx.doi.org/10.2135/cropsci2006.04.0216.

Pritchard JK, Stephens M, Donnelly P, 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

Prohens J, Blanca JM, Nuez F, 2005. Morphological and molecular variation in a collection of eggplants from a secondary center of diversity: Implications for conservation and breeding. J Am Soc Hort Sci 130: 54-63.

Rodríguez-Burruezo A, Prohens J, Nuez F, 2008. Performance of hybrids between local varieties of eggplant (*Solanum melongena*) and its relation to the mean of parents and to morphological and genetic distances among parents. Eur J Hortic Sci 73: 76-83.

Stàgel A, Portis E, Toppino L, Rotino G, Lanteri S, 2008. Gene-based microsatellite development for mapping and phylogeny studies in eggplant. BMC Genomics 9: 357. http://dx.doi.org/10.1186/1471-2164-9-357.

Stommel JR, Whitaker BD, 2003. Phenolic acid content and composition of eggplant fruit in a germplasm core subset. J Am Soc Hort Sci 128: 704-710.

Sunseri F, Polignano GB, Alba V, Lotti C, Bisignano V, Mennella G, Alessandro AD, Bacchi M, Riccardi P, Fiore MC, Ricciardi L, 2010. Genetic diversity and characterization of African eggplant germplasm collection. Afr J Plant Sci 4: 231-241.

Terzopoulos PJ, Bebeli PJ, 2008. DNA and morphological diversity of selected Greek tomato (*Solanum lycopersicum* L.) landraces. Sci Hort 116: 354-361. http://dx.doi.org/10.1016/j.scienta.2008.02.010.

Terzopoulos PJ, Bebeli PJ, 2010. Phenotypic diversity in Greek tomato (*Solanum lycopersicum* L.) landraces. Sci Hort 126: 138-144. http://dx.doi.org/10.1016/j.scienta.2010.06.022.

Tümbilen Y, Frary A, Daunay MC, Doğanlar S, 2011a. Application of EST-SSRs to examine genetic diversity in eggplant and its close relatives. Turk J Biol 35: 125-136.

Tümbilen Y, Frary A, Mutlu S, Doganlar S, 2011b. Genetic diversity in Turkish eggplant (*Solanum melongena*) varieties as determined by morphological and molecular analyses. IRJoB 2: 16-25.

Van der Weerden GM, Barendse GWM, 2007. A web-based searchable database developed for the EGGNET project and applied to the Radboud University Solanaceae database, VI. Proc VI Int Solanaceae Conf, Genomics meet Biodiversity. Acta Hortic, Madison, WI, USA, pp: 503-506.

Varshney RK, Graner A, Sorrells ME, 2005. Genic microsatellite markers in plants: features and applications. Trends Biotechnol 23: 48-55. http://dx.doi.org/10.1016/j.tibtech.2004.11.005.

Vekemans X, Beauwens T, Lemaire M, Roldan-Ruiz I, 2002. Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. Mol Ecol 11: 139-151. http://dx.doi.org/10.1046/j.0962-1083.2001.01415.x.

Vilanova S, Manzur JP, Prohens J, 2012. Development and characterization of genomic simple sequence repeat markers in eggplant and their application to the study of diversity and relationships in a collection of different cultivar types and origins. Mol Breed 30: 647-660. http://dx.doi.org/10.1007/s11032-011-9650-2.

Yeh FC, Boyle T, Rongcai Y, Ye Z, Xiyan JM, 1999. Popgene vers. 1.31. Microsoft Window-based freeware for population genetic analysis. http://www.ualberta.ca/~fyeh/fyeh/.