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Full Length Research Paper

Genetic diversity and characterization of African eggplant germplasm collection

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Genetic diversity among 70 "scarlet eggplant" (*Solanum aethiopicum* L.) entries from different geographical origins was assessed. Entries were firstly evaluated for the main morphologic traits and chlorogenic acid content. Standard statistics and multivariate analyses were utilized to assess the phenotypic diversity and grouping the entries. In addition, amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) analyses were used to evaluate genetic relationships among entries. Differences between entries are highly significant for all descriptors. Principal component analysis (PCA) revealed that the first three components accounted for 74% of the total variance. Morphologic traits were associated with the first two components, while the third one was moderately correlated with the chlorogenic acid content. The observed similarity allowed to identify only three main groupings. The clusters obtained did not show any relationships with geographic origins and/or botanical groups. Matrices of genetic similarity from AFLP and SSR data were utilized in order to obtain a dendrogram. A large cluster included several entries from South America with limited rate of genetic variation was observed. On the contrary, higher amount of variation was observed in the cluster with entries from Africa, in which are also present in the accessions collected in Italy. These entries appeared always morphologically and genetically distinguishable from the others. These results provided additional information for the conservation, improvement and legal protection of the ecotype 'melanzana rossa di Rotonda', cultivated in Italy.

Key words: *Solanum aethiopicum L.,* scarlet eggplant, amplified fragment length polymorphism*,* simple sequence repeat, multivariate analyses, ecotype 'melanzana rossa di Rotonda'.

INTRODUCTION

The term "scarlet eggplant" (*Solanum aethiopicum* L., n = 12) describes the four cultivar groups *Gilo*, *Kumba*, *Shum* and *Aculeatum*. All groups are mainly spread and grown in Africa and South America, where they can be utilized as source of fruits (*Gilo* group) and edible leaves (*Shum* group). *S. aethiopicum* is considered interesting both for its typical production in marginal areas and for the genetic

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improvement of *Solanum melongena*. Scarlet eggplant cultivations were recently observed in South Italy, in the Regional Natural Reserve of Pollino (Laghetti et al., 1995). The common name eggplant includes three closely related cultivated species that belong to sub-genus Leptostemonum: *S. melongena* L., brinjal eggplant or aubergine, *S. aethiopicum* L., scarlet eggplant; and *Solanum macrocarpon* L., gboma eggplant (Daunay et al., 2001). The latter two species result by a domestic-cation process occurred in Africa, starting from 2 wild ancestors, *Solanum anguivi* and *Solanum dasyphyllum* respectively, (Lester, 1998). Lester and Niakan (1986), on the basis of the morphologic traits, classified four varietal groups within the species *S. aethiopicum*: *Aculeatum, Gilo, Kumba and Shum*. Each group has been selected primarily for desirable features in the parts of the plant used for food or ornament (Lester, 1986). The *Gilo* group is characterized by large and edible fruits, glabrous and edible leaves characterize the *Shum* group; both large fruit and glabrous leaf characterize the *Kumba* group. The *Aculeatum* group, used as ornamental plant is characterized by large and ribbed fruit with prickly leaf.

Scarlet eggplant is rarely spread and cultivated in Europe, despite its berries are edible such as the brinjal eggplant (*S. melongena* L.); on the contrary, it is spread in many African areas. In 2008 about 147,000 ha of egg-plants were harvested in African countries (FAO, 2009), but there is a lack of information about the scarlet eggplant.

Actually, the cultivation of this African vegetable has been decreasing, although, the berries are interesting for their nutritional and organoleptic features, such as high level of antioxidant compounds and tole-rance to the most harmfully pathogen *Fusarium* (Toppino et al., 2008). Due to the considerable phylogenetic affinity and the analogy in ploidy level with *S. melongena* the scarlet eggplant could be used to obtain benefits for eggplant genetic improvement: herbaceous grafting, interspecific cross, somatic hybridization, genetic trans-formation (Caruso, 2001; Collonier et al., 2001; Rizza et al., 2002; Toppino et al., 2008).

Recently, scarlet eggplant populations (an agro-eco-type locally named ‘melanzana rossa di Rotonda’) were found in an area close to the Regional Natural Reserve of Pollino in Basilicata region, where farmers utilized the scarlet eggplant together with the brinjal eggplant for many years (Laghetti et al., 1995). Actually, there is an increasing interest on the scarlet eggplant for safe-guarding the residual populations still cultivated in Italy and to provide useful genes to brinjal eggplant breeders. Therefore, a large collection of scarlet eggplant was collected at the University of Reggio Calabria in order to evaluate the accessions either at morphological and genetic level.

As clearly know, the genetic and morphologic analyses frequently complement each other (Patterson et al., 1993). Genetic similarity has been studied on eggplant by using chloroplast DNA molecular markers (Sakata et al., 1991; Sakata and Lester, 1994; Isshiki et al., 1998) and based on nuclear DNA, RAPD analysis (Karihaloo et al., 1995) in order to compare cultivated *S. melongena* and wild relatives, amplified fragment length polymorphism (AFLP) analysis (Mace et al., 1999; Furini and Wunder, 2004) with the aim either to distinguish different genotypes among cultivated eggplant and wild relatives and more recently to isolate marker linked to important trait (Toppino et al., 2008; Liao et al., 2009).

The microsatellites simple sequence repeat (SSR) or sequence tagged microsatellite site (STMS) are poly-merase chain reaction (PCR)-based, highly polymorphic, multi-allelic, frequently codominant, highly reproducible, randomly and widely distributed in the genome (Powell et al., 1996). STMS are being used in several plant species for genome mapping and marker-assisted selection as well as for germplasm analysis. In eggplant the micro-satellites are widely studied and iso-lated (Nunome et al., 2003; Stagel et al., 2008) and used to assess genetic relationship among the wild, weedy species and cultivated eggplant (Behera et al., 2005). Despite its economic importance, in contrast to those of the other cultivated solanaceous crops, eggplant genome is not widely studied. Nowadays, only a genetic map con-structed with AFLP, RAPD and SSR markers (Nunome et al., 2001; 2009) and a comparative genetic map, based on tomato sequences (Doganlar et al., 2002; Wu et al., 2009) are available for this Solanaceous species. The support of molecular markers to a morphologic charac-terization appears to be crucial, taking into account that phenotypic traits are frequently not sufficient to identify different entries. Recently, a clear model for identifying several entries of cultivated eggplant together with some eggplant wild species is reported (Muñoz-Falcón et al., 2008).

In the present paper, a characterization based on morphologic, genetic and chemical data on 70 scarlet eggplant accessions were reported. In particular, the germplasm collection, included entries collected in Basilicata region and several from South American and African, was evaluated for morphologic traits and the genetic distances among genotypes were estimated by using AFLP and SSR markers. Furthermore, phenolic compounds content of berries, also identified as hydroxycinnamic acid (HCA) derivatives (with potential antioxidant activity), was checked by using HPLC analy-sis. The main aims were to describe the genetic variation, to identify superior genotypes useful for eggplant improvement and finally to fingerprint the agro-ecotype ‘melanzana rossa di Rotonda’ in order to pro-mote the expansion of this crop in the marginal areas of South Italy.

Table 1. Code, accession number, cluster, group and origin of *S.aethiopicum* L. entries.

Code	Accession No.	Cluster*	Group	Origin	Code	Accession No.	Cluster	Group	Origin
1	PI441881[1]	III	Gilo	Brazil	36	PI441890	II	Gilo	Brazil
2	PI441841	II	Gilo	Brazil	37	PI441902	III	Gilo	Brazil
3	PI441882	II	Gilo	Brazil	38	PI441895	II	Gilo	Brazil
4	PI441909	III	Gilo	Brazil	39	974750086[2]	III	Gilo	Brazil
5	PI441903	II	Gilo	Brazil	40	994750021	II	n. c.	Cameroon
6	PI441912	II	Gilo	Brazil	41	924750173	III	n. c.	Africa
7	PI420226	I	Gilo	W. Africa	42	924750114	III	Gilo	Uganda
8	PI420230	I	Gilo	W. Africa	43	4904750126	III	n. c.	Africa
9	PI424860	II	Gilo	Brazil	44	974750105	II	n. c.	Africa
10	PI441884	II	Gilo	Brazil	45	814750050	II	Gilo	Africa
11	PI441887	II	Gilo	Brazil	46	804750136	II	Aculeatum	Africa
12	PI441905	II	Gilo	Brazil	47	90475166	I	n. c.	Africa
13	PI441891	II	Gilo	Brazil	48	924750116	II	Shum	Uganda
14	PI441847	II	Gilo	Brazil	49	814750089	II	n. c.	Africa
15	PI441838	II	n. c.	Brazil	50	964750043	II	n. c.	Africa
16	PI441896	II	Gilo	Brazil	51	964750021	II	n. c.	Brazil
17	PI441878	III	Gilo	Brazil	52	914750093	III	Aculeatum	Africa
18	PI441886	I	Gilo	Brazil	53	974750118	II	n. c.	Africa
19	PI441898	II	Gilo	Brazil	54	A04750069	I	n. c.	Africa
20	PI441893	II	Gilo	Brazil	55	934750034	II	Kumba	Africa
21	PI441859	II	Gilo	Brazil	56	974750109	II	Gilo	Africa
22	PI441862	III	Gilo	Brazil	57	904750224	I	Aculeatum	Uganda
23	PI441839	II	Gilo	Brazil	58	964750076	III	Gilo	Africa
24	PI441879	II	Gilo	Brazil	59	984750003	III	Gilo	Africa
25	PI441901	III	Gilo	Brazil	60	964750120	III	n. c.	Africa
26	PI441904	II	Gilo	Brazil	61	994750017	I	Shum	Cameroon
27	PI441883	II	Gilo	Brazil	62	904750190	III	Gilo	Burkina Faso
28	PI441894	II	Gilo	Brazil	63	994750018	I	Gilo	Cameroon
29	PI441900	II	Gilo	Brazil	64	SOL70/75[3]	III	n. c.	Africa
30	PI441876	III	Gilo	Brazil	65	SOL124/80[3]	III	n. c.	Italy
31	PI441865	II	Gilo	Brazil	66	UB1[4]	II	Gilo	Italy
32	PI441897	II	Gilo	Brazil	67	UB2	II	Gilo	Italy
33	PI441861	II	Gilo	Brazil	68	UB3	II	Gilo	Italy
34	PI441885	II	Gilo	Brazil	69	RNL 216[5]	II	Kumba	Africa
35	PI194166	II	Gilo	Croatia	70	BIRM 0344[5]	II	Aculeatum	Africa

*This cluster classification is referred to the PCA analysis [1]USDA-ARS GRIN collection,[2]ECP/GR Eggplant Database, Radboud University Nijmegen (NL),[3]IPK - GIBIS collection, Gatersleben (D),[4]University of Basilicata collection, Potenza (I),[5]Entries of EGGNET collection, Montfavet, Avignon (F), n. c. = not classified.

MATERIALS AND METHODS

Plant material

Scarlet eggplant germplasm collection included 70 entries, 38 from the USDA-ARS National Germplasm Resources Laboratory, Beltsville Maryland, 25 from the Botanical Garden, Radboud University Nijmegen, the Netherlands; 2 from the plant genetics and crop plant research (IPK), Gatersleben, Germany; 2 from Eggnet collection maintained at Montfavet, Avignon, France and the other 3 were local ecotypes of 'melanzana rossa di Rotonda' collected in the Regional Natural Reserve of Pollino. Accession number, varietal group and origin of *S. aethiopicum* L. entries utilized are listed in Table 1. Entries were evaluated in field experiment during 2007 growing season at Metaponto (Basilicata region, South Italy). Sowing was done in March and the seedlings were transplanted some days later, when the plantlets reached the three true leaves stage. An experimental design with randomized blocks and two replications was adopted. The plants were grown in single row plots of variable length, distance between the rows was 1.5 m and plants were spaced 80 cm apart in each row. A drip-irrigation system provided water throughout the experimental period according to the

climatic trend. Data on the main morphologic descriptors were collected on five plants for each accession: plant height, flowering time, flowers per inflorescence, fruits per inflorescence, fruits per plant, yield per plant, while, fruit length, fruit width and fruit weight were evaluated on five fruits per plant.

Biochemical and molecular analysis

Variation of fruit chlorogenic acid content (CGA) was estimated, by using 4 fruits for each entry harvested at the same maturity stage. The analyses were performed according to Stommel and Whitaker (2003) with minor modifications. CGA was extracted and separated through Reversed Phase HPLC (RP-HPLC) starting from elution times in chromatographic analysis and ultraviolet (UV) absorbance spectrum (325 nm) relative to the sesamol internal standard and an external standard of authentic chlorogenic acid (1,3,4,5-tetra-hydroxycyclohexane carboxylic acid) purchased from Sigma-Aldrich (C3878). Means of three lectures for each sample were recorded as µmol/100 g dry weight (% of total). The genomic DNA was extracted from two grams of young leaves for each entry according to a slightly modified CTAB protocol (Doyle and Doyle, 1987). The AFLP analysis was carried out according to the protocol of Aggarwal et al. (2002) with minor modifications. The amplicons obtained by using 5' marked selective primers (three different fluorochromes) were analyzed through capillary electrophoresis technology using the automatic sequencer ABI Prism 310 (Perkin Elmer Applied Biosystems). The pre-amplification AFLP reaction was carried out using 10 primer combinations on the analysed genotypes (Table 4). Polymorphic fragments in the range 35 - 500 bps were scored as present (1) or absent (0) generating a binary matrix. The SSR analyses were carried out by using motives already set up for cultivated eggplant (Nunome et al., 2003), according to the following protocol: 10 ng of genomic DNA was added to a 25 µl mix solution containing 1.5 mM MgCl2, 0.4 U Taq polymerase, 200 µM dNTP, 1× Taq polymerase buffer, 0.16 pM of the marked primer and 0.32 pM of the not marked primer. A 1:40 dilution of the amplified products was performed and 1 µl of each dilution was added to a mix of 10 µl of deionised formamide. A GENESCAN 500 ROXs was used as internal standard. Therefore, the mix was denatured for 4 min at 94°C. All the samples were analyzed on ABI PRISM 373 genetic analyser (Applied Biosystems) and the chromatograms obtained were examined through the software GENESCAN 3.7 (Applied Biosystems). The counts and the analysis of the alleles were then performed by using the genotyper software (Applied Biosystems).

Statistical analysis

Plot means were used in the univariate analysis of variance (ANOVA procedure). Two methods of multivariate analysis (principal component and cluster analysis) were utilized in order to investigate the multi-descriptor variation, summarizing the data and grouping the entries on the basis of their similarity. PROC PRIN and CLUSTER (option Ward's) procedures of statistical analysis system (SAS Institute, 1987) were used; clustering was done in a step-wise method optimizing the various parameters provided. Results from both analyses were collected and combined in order to underline the phenotypic variability observed by a graphic clustering representation. Finally a plot was performed using the program STATISTICA (StatSoft, 1995). SSR and AFLP data were used to perform a cluster analysis by means of Dice genetic similarity coefficients (Dice, 1945). SSR and AFLP matrices were tested for correlation (5000 permutations) using the Mantel test (Mantel, 1967). A unique matrix of similarity coefficients, on the basis of the significant correlation, was extrapolated for cluster analysis (unweighted pair group method average (UPGMA) to generate a dendrogram showing genetic variation patterns among entries. All the analyses were performed by means of NTSYS vers. 2.02 software (Rolph, 1998).

RESULTS

The results of the analysis of variance for all the quantitative descriptors are presented in Table 2, while range, mean value and coefficient of variation of each descriptor are presented in Table 3. The principal component analysis (PCA) was carried out in order to verify the total variation of multiple descriptors. First to third principal components loadings and their contributions to the total variation are presented in Table 3. The total contribution of the three main components was 74%; the first one is useful to distinguish 42% of total variability, the second one 20% and the third one 12%. Cluster analysis shows discrimination among entries based on different levels of similarity or dissimilarity, thus providing a hierarchical classification. The observed similarity allowed to identify three main clusters which account for 60% of the original phenotypic variation. The principal component analysis was combined with the cluster analysis in two-dimensional plots. The position of each entry on the first and second components is plotted in Figure 1a and on the first and third in Figure 1b. Each entry is represented by a code number and a symbol indicating the cluster to which it belongs. The genetic variability of *S. aethiopicum* germplasm collection was then estimated by AFLP markers, starting from 10 primer combinations (Table 4). Ten primer combinations were able to score 512 amplified fragments, of which two hundred and forty-seven were polymorphic with an efficiency of about 25 polymorphisms per primer combination and a mean percentage of 48%. These results are comparable with previously AFLP reports on related-eggplant germplasm collections (Mace et al., 1999; Furini and Wunder, 2004). The pattern of genetic variation among entries was finally estimated by means of SSR analysis at 21 loci (Table 5). Seventeen out of 23 primers amplified fragments (alleles), the number of alleles ranged from 2 to 7 with an average of 4.5. The SSR results are comparable to the first report on eggplant (Nunome et al., 2003), while Behera et al. (2005) reported only 11 out of 23 SSR as polymorphic, with an average of allele number amplified comparable to the present results (4.4). Correlations between matrices from different classes of molecular marker were performed by Mantel test showing estimated

Table 2. Analysis of variance on morphologic and agronomic descriptors.

Descriptors	Source of variation	D. of freedom	Mean square	'F'	P
Flowering time (days)	Block	1	36.01	1.58	0.214
	Entry	69	121.05	5.30	< 0.001
	Error	*69*	*22.85*		
	Total	139	71.69		
Flowers/inflorescence (n)	Block	1	0.46	0.62	0.432
	Entry	69	4.29	5.86	< 0.001
	Error	*69*	*0.73*		
	Total	139	2.50		
Plant height (cm)	Block	1	102.86	3.34	0.072
	Entry	69	475.97	15.48	< 0.001
	Error	*69*	*30.76*		
	Total	139	252.28		
Fruit length (cm)	Block	1	0.18	0.70	0.405
	Entry	69	6.79	26.66	< 0.001
	Error	*69*	*0.25*		
	Total	139	3.50		
Fruit width (cm)	Block	1	0.01	0.05	0.821
	Entry	69	3.24	16.31	< 0.001
	Error	*69*	*0.20*		
	Total	139	1.71		
Yield / plant (g)	Block	1	540082.69	11.85	0.001
	Entry	69	762944.46	8.39	< 0.001
	Error	*69*	*64357.68*		
	Total	139	305534.75		
Fruits/ plant (n)	Block	1	3177.78	5.76	0.019
	Entry	69	8120.18	14.71	< 0.001
	Error	*69*	*551.92*		
	Total	139	4327.72		
Fruit mean weight (g)	Block	1	0.01	0.00	0.985
	Entry	69	621.37	29.93	< 0.001
	Error	*69*	*20.76*		
	Total	139	318.75		
Chlorogenic acid (%)	Block	1	1700.68	8.12	0.006
	Entry	69	593162.63	2833.42	< 0.001
	Error	*69*	*209.34*		
	Total	139	294563.79		

Table 3. Ranges, means, coefficient of variation (C.V.) and derived principal components (PCs) on the morphologic and agronomic descriptors.

Descriptor	PC1	PC2	PC3	Range	Mean	C.V.
Flowering time (days)	0.05	0.58	-.29	52 - 92	72	11.75
Flowers/inflorescence (n.)	-.38	-.18	0.25	1 - 9	4	39.50
Plant height (cm)	0.04	0.60	0.27	40 - 130	86	18.46
Fruit length (cm)	0.40	0.35	-.06	0.8 – 9.1	4.1	45.61
Fruit width (cm	0.40	-.28	0.23	1.2 – 9.5	4.2	31.34
Yield / plant (g)	0.32	0.04	0.63	38.0 – 2836. 0	1321.9	41.81
Fruits/ plant (n)	-.39	0.20	0.44	2 - 485	65	67.10
Fruit weight (g)	0.45	-.11	0.18	1 - 100	31.1	57.36
Chlorogenic acid content	-.28	0.12	0.31	56.3 – 2789.8	937.2	57.91
eigenvalues	*3.75*	*1.81*	*1.08*			
Variability (%)	42	20	12			
Cumulative variability (%)	--	62	74			

Table 4. AFLP primer combinations utilized on seventy *S. aethiopicum* L. entries.

Primer combination	Primer motif	Fluorochrome utilized	Polymorphic fragments
E35/M47	E-ACA/M-CAA	FAM	28
E38/M62	E-ACT/M-CTT	FAM	34
E38/M60	E-ACT/M-CTC	FAM	22
E33/M60	E-AAG/M-CTC	JOE	29
E33/M48	E-AAG/M-CAC	JOE	15
E41/M62	E-AGG/M-CTT	JOE	16
E32/M48	E-AAC/M-CAC	NED	22
E38/M49	E-ACT/M-CAG	NED	19
E32/M49	E-AAC/M-CAG	NED	27
E35/M51	E-ACA/M-CCA	FAM	35
		Total	247

values of genetic relationship between AFLP and SSR significantly related. However, the molecular markers showed non-significant correlation with morphological traits (data not shown). A combined (AFLP and SSR) matrix of similarity coefficients were used to estimate the genetic distances among genotypes, based on the significant correlation (r = 0.506 P = 0.421) of Mantel test. The dendrogram obtained (Figure 2) showed two large main clusters and several small clusters.

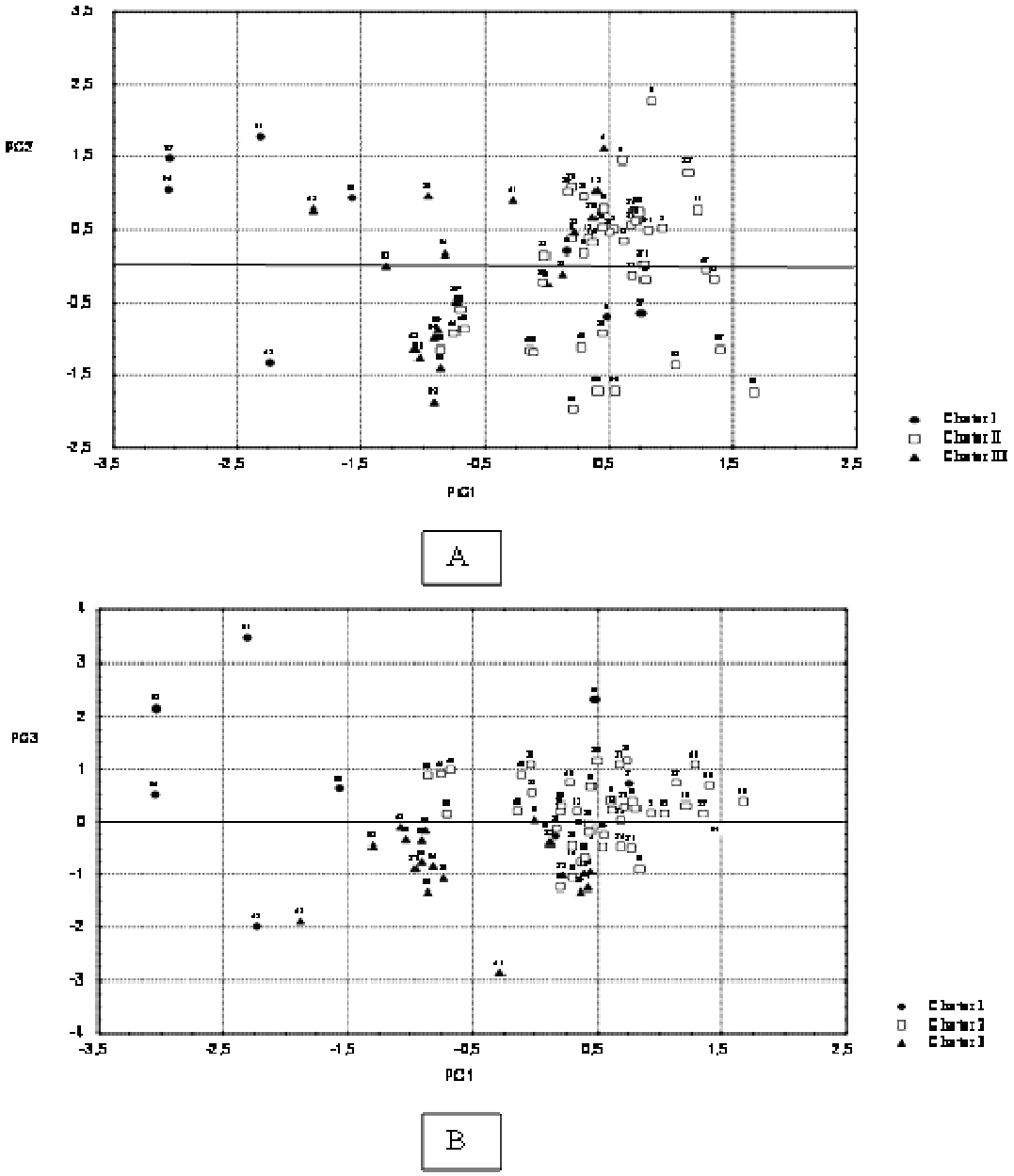
HPLC analysis showed different chlorogenic acid content in the berries of scarlet eggplant belonging to the germplasm collection (Figure 3). In particular, analyses of the berry extract from 70 scarlet eggplant entries showed differences on HCA derivatives total content (of which chlorogenic acid accounts for about 95%) among the scarlet eggplant entries. The total content of chlorogenic and iso-chlorogenic acid ranged in the s carlet eggplant from 200 to 2500 µmol/100g dry weight, lower values compared to the results reported for cultivated eggplant (Stommel and Whitaker, 2003).

DISCUSSIONS

PCA performed on morphologic traits analysed showed the first component (PC1) positively related to fruit mean weight, fruit length and fruit width, while flowers per inflorescence and fruits per plant showed negative association. The second component (PC2) showed higher loadings for flowering time and plant height. Otherwise, the third component (PC3) was related to the total fruit yield (number of fruits per plant). Chlorogenic acid content seems to be related with this latter component. This observation suggested that HCA content is not related to

Table 5. Microsatellites utilized (Nunome et al., 2003) on seventy *Solanum aethiopicum* L. *entries* and obtained results in total and polymorphic fragments.

Microsatellites motif	Primer sequence (5'-3')	fragment (bp)	Annealing temp. (°C)	Allele (no.)
$(TC)_9(AC)_{38}(AT)_{19}$	TGGATCTGCAAAGAAAAGGAGAAAG CGCAAATCGGGTAGACTTTCGAT	246	60	0
$(AC)_{13}(AT)_7$	GGCCCTAGACTGAGCCTGAAATGTT TGCTACAACCAACACAACCCTCAA	214	65	6
$(AC)_{13}$	AGCCTAAACTTGGTTGGTTTTTGC GAAGCTTTAAGAGCCTTCTATGCAG	221	65	5
$(AC)_{12}(AT)_8$	TTAGAAATTTCGGAACAAAGAGA CCACATGAAACTTGGACCAATGAG	246	60	7
$(AC)_{19}(AT)_{12}$	GATCATCACTGGTTTGGGCTACAA AGGGGAGAGGAAACTTGATTGGAC	123	65	7
$(GGAGG)_5\ldots(AT)_8(GT)_3AT(GT)_{14}$	CCCCACCCCATTTGTGTTATGTT ACCCGAGAGCTATGGAGTGTTCTG	201	65	5
$(AC)_{16}$	GGATCAACTGAAGAGCTGGTGGTT CAGAGCTTCAATGTTCCATTTCACA	160	65	6
$(CA)_{26}(TA)_{19}$	TAGCGGTGCTAGGTCCATCATCTCA TTCTCAAGAAGTTGCTCCAAAGGA	295	60	0
$(AT)_5(AC)_3A(AC)_{14}(AT)_7GTA(TG)_5(TA)_3$	TCTGGGACACCAAGTGAAAAATCA TGCGTTTTTGGCTCCTCTATGAAT	213	65	5
$(AC)_{13}(AT)_4$	GCGGATCACCTGCAGTTACATTAC TCCTTTGACCTATAGTGGCACGTAGT	177	65	4
$(GT)_2GC(GT)_6$	AGTAAGGGAAAGTGCTGACGAAGG CAGAGTCATCGTTATGGGGAGGTT	168	65	5
$(CA)_{11}(GA)_20$	ATCCTGTTGCTGCTCATTTTCCTC AGGAGGATCCAAGAGGTTTGTTGA	260	65	0
$(AC)_6AT(AC)_{11}(AT)_{10}$	TGCTAAGTCGTCATCCCACAAGAA GATTTTGGCTCCTTGACCATTTTG	258	65	0
$(AC)_4GC(AC)_5T(AC)_3ATGC(AC)_4AT(AC)_6(AT)_5G(TA)_{13}$	CCAAAACAATTTCCAGTGACTGTGC GACCAGAATGCCCCTCAAATTAAA	268	65	3
$(AT)_{16}(GT)_{19}$	TCTGCATCGAATGTCTACACCAAA AAAAGCGCTTGCACTACACCTGAAT	228	65	0
$(TACA)_4TA(TACA)_4(CA)_{37}(TA)_5TG(TA)_3(TTAA)_3$	CAGTGCTACATAAATTGAGACAAGAGG GGAGGTACAACGGATTTTCATATGGT	369	65	0
$(AC)_{19}(AT)_{11}AC(AT)_2$	GGACCAAAGCGAAATTTTCACAAC TTGCACCAATTGGGAAGTAACACA	288	65	2
$(TG)_3TA(TG)_8(TA)_6$	TGATTTGGCCCTTAAGCCTAAGTATG GACTCCTCAAGCCTTTACCTCCAA	165	65	4
$(CT)_{38}$	CAAAAGATAAAAAGCTGCCGGATG CATGCGTGAGTTTTGGAGAGAGAG	248	65	5
$(AC)_{11}(AT)_8$	CTCCACGCTACTTAGGGGACTCAA AGACCACACTTGGCATGTCTTGAA	257	65	2
$(CA)_5$	TATACACCCACACGGCTTCATCAC AGCTCAAGTGAAGGGTTGAAGTGC	163	65	2



Figures 1. Principal component analysis (PCA) performed on the morphologic and bio-agronomic descriptors detected on the 70 African eggplant accessions. A) The first two component are depicted. B) The first and the third component are depicted.

the important traits involved in the first two components, hence it should be possible to select genotypes with low chlorogenic acid content without affecting other important traits. Combining the principal component analysis with cluster analysis, some patterning of cultivar groups distribution related to their origins can be observed in the scatter diagram but not always (Figure 1). Therefore some clusters include different proportions of entries belonging to different botanical group and from different provenance. Considering first and second components, cluster I included 7 African entries belonging to *Gilo* (4), *Shum* (1) and unclassified groups together with 1 entry from South-America (Brasil) classified as *Shum*. Cluster II, the largestone, grouped 44 entries, including 28 from South America, 12 from Africa and 4 from Europe of which 1 from Croatia and 3 from Italy. The same cluster II

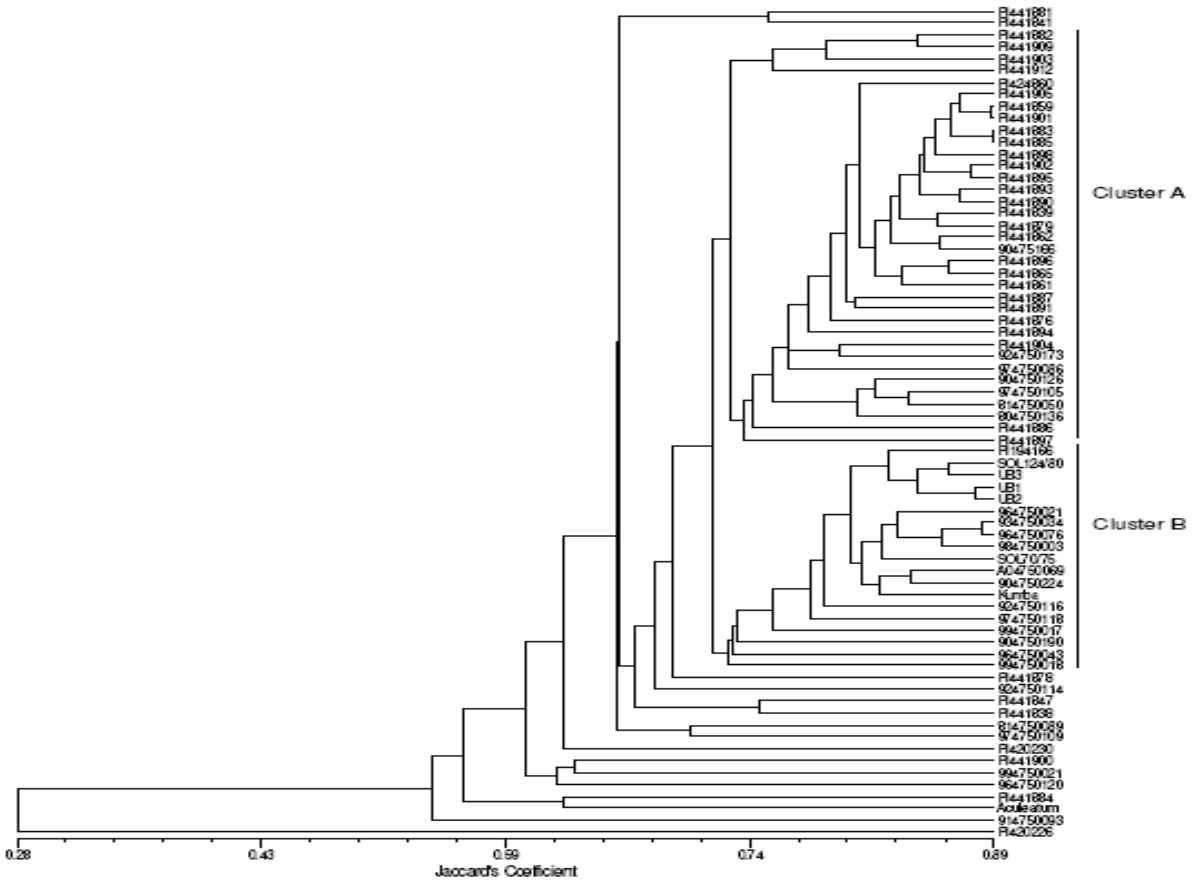


Figure 2. The dendrogram obtained from AFLP and SSR data using Jaccard's coefficients of similarity and UPGMA clustering.

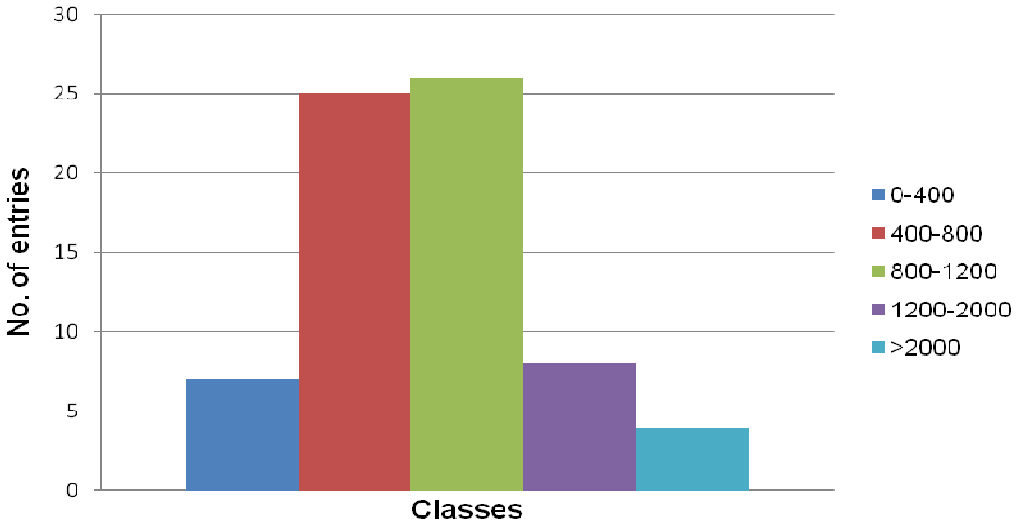


Figure 3. Histogram of frequency for the variation in chlorogenic acid content (µmol/100 g dry weight) detected on the 70 *S. aethiopicum* L. group *gilo* accessions analyzed.

included the largest number of *Gilo* (32) and unclassified (7) entries. In addition this cluster included 2 *Aculeatum*, 2 *Kumba* and 1 *Shum* entries. Cluster III, in a middle position in the figure, includes 8 entries from South America (Brasil), 9 from Africa and 1 from Italy. The presence of 12, 5 and 1 entries belonging to *Gilo*, unclassified and *Aculeatum* groups, respectively characterizes cluster III. The plot of the first component against the third one shows the same clusters with a different distribution of entries based on traits related to the third component: yield per plant, number of fruits per plant and chlorogenic acid content. AFLP and SSR markers described 70 scarlet eggplant entries analyzed at least in two large clusters. Cluster A, starting from PI441882 to PI441897, include *Gilo* group entries with the exception of 804750136 entry belonging to *Aculeatum* group, the geographic origin of several entries from cluster A is South America except the entries from the Nijmegen repository. Cluster B, starting from PI194166 to 994750018, taking in together entries from *Gilo*, *Kumba, Shum* and *Aculeatum* groups, many of which were collected in Africa with the exceptions PI194166 (from Croatia) and 964750021 (from Brazil). In cluster B are also included the entries collected in Basilicata region (supporting the idea that these entries are probably introduced from Africa at the beginning of last century) and SOL124/80 that was collected in Basilicata from the IPK Institute (from the same area of cultivation). Contiguous to these main clusters, many sub-clusters or branches of couple entries are shown in the figure. Furthermore, it was possible to observe how *Aculeatum* group entries were present in different clusters. In the bottom of the figure the dendrogram is closed by the entry PI420226 classified as *Gilo* group that was collected in Western Africa and appear to be like an outgroup with large genetic differences from all entries of the germplasm collection.

Finally, HPLC analysis for HCA derivatives content underlined a wide variability in the berry of 70 scarlet eggplant entries, as showed in Figure 3. This result appears of great interest taking into account that these compounds, mainly chlorogenic acid, are considered the potential responsible of flesh browning in cultivated eggplant berries. The classes of frequency reported in the figure showed several accessions of red eggplant with a chlorogenic and iso-chlorogenic acid total amounts varying from 200 to 800 µmol/100 g of dry weight; besides, few entries overcome amounts of 2000 µmol.

Conclusions

The scarlet eggplant germplasm collection displayed large diversity for all the traits studied. PCA and cluster analysis, based on morphologic traits, allowed quantifying the similarity among entries, identifying three main groupings. Clustering pattern rarely showed relation between entries and their geographic origin and/or botanical group. AFLPs and SSRs analyses resulted in genetic similarity matrices, able to depict a dendrogram classifying the entries in few distinct groups with higher amount of variation among African entries with respect to those from South America and European countries. This observation supported the idea that these latter geographical regions could play a secondary role in the scarlet eggplant evolutionary process. The cluster analysis obtained by means of molecular markers showed a more clear entries classification in respect to the geographic origin, with some exceptions. It is interesting to note that the Italian entries, all belonging to the local ecotype 'melanzana rossa di Rotonda', appeared always to be tightly linked. In other words, they were genetically distinguishable from the other scarlet eggplant entries, with important implications for its protection and variety registration under a protected geographical indication. The developed molecular markers were able to fingerprint the typical ecotype named 'melanzana rossa di Rotonda', moreover the constitution of hybrid combination could allow to preserve a trade mark of typicality for this vegetable in Italy. Genetic relationships based on AFLPs and SSRs could be quite useful in selecting parents to be crossed for generating appropriate hybrids together with 'melanzana rossa di Rotonda' ecotype.

The Italian entries of Pollino area, most likely of African origin, can be considered of great interest for South Italy and in particular for Basilicata region, where it was probably introduced in cultivation since the beginning of last century. Therefore, a comprehensive understanding of genetic variation in *S. aethiopicum* would allow not only an efficient selection of parents for genome introgression from *S. aethiopicum* into *S. melongena*, but also the implementation of an effective genetic conservation program for this species. Interspecific hybridisation between *S. aethiopicum* and *S. melongena* offer the potential to constitute superior new cultivars of brinjal eggplant through the introduction of desirable traits as increased disease resistance. Finally, the results demonstrated that both phenotypic and molecular markers are useful for determining the genetic diversity and the relationships among *S. aethiopicum* entries. A combination of both types of data provided additional relevant information for the conservation, improvement and legal protection of the Italian ecotype 'melanzana rossa di Rotonda'.

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