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Evaluating the Biodiversity of Almond Cultivars From a Germplasm Collection Field in Southern Italy

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ABSTRACT

During the past 30 years many constraints combine to limit the production and to reduce the growing areas of almond. Actually, some of these marginal areas are subjected to a negative trend of land degradation. Public policies to achieve sustainable land use advise the use of native species to contribute as an excellent opportunity to bring together two objectives the native germplasm safeguard and the land conservation.

After many years dedicated to the activity of protecting the almond (*Prunus amygdalus* Batsch) germplasm, a large collection of great interest and usefulness for the genetic improvement has been made available in the farm of the Experimental Agronomic Institute of Bari in Apulia region. Our objective was to describe the phenotypic diversity present in the Italian collection that could be used to devise a core collection that would contain much of the diversity found in the whole collection. Based on twenty quantitative plant, kernel and nut traits 88 almond Italian entries were grouped into seven clusters by disjoint cluster analysis. To determine the importance of the traits and levels of similarity among groups, discriminate analysis was applied. According to these analyzes double kernels, nut and kernels size, flowering date, kernel yield and shelling percentage, were important traits discriminating among different groups. The main trait discriminating among phenotypical groups was the double kernels. This analysis can help germplasm curators and plant breeders in choosing the most favorable entries to build a core subset of the almond collection for breeding purpose. Also, preserving small areas where almond varieties can be safeguarded using new cultural techniques could be a valid and practical alternative for the farmers to maintaining genetic resources in less productive agroecosystems under low-input conditions.

INTRODUCTION

Prunus amygdalus Batsch, the almond tree, is one of the oldest and traditional crops widely present in southern Italy and in several countries around the Mediterranean basin. Due to its particular pedoclimatic and geographical conditions it has been the center of origin of several native cultivars (Bultadhakis, 1923; Fanelli, 1939). During the past 30 years many constraints combine to limit the production and to reduce the growing areas of almond: the yield potentials of existing varieties are poor; the yields are notoriously variable; depredation by pest, diseases and parasites are severe; inputs are limited and production techniques are inefficient (Moleas, 1990; Godini, 1996). All these constraints along with the unfavorable import-export conditions have caused a negative trend in maintaining the numerous old varieties present in different almond growing areas. This has determined a strong genetic decline of the local gene pool with the real risk of loosing useful genetic resources for future breeding programs and variety orientation. To safeguard the remaining almond germplasm and to develop new improved varieties well adapted to the environmental conditions of the different growing areas, it was suggested that a wide germplasm collection with a large genetic diversity be set up (Damigella and Fatta del Bosco, 1979; Alberghina, 1992; De Giorgio and Stelluti, 1995).

With this in mind, since the 1960's, the Agronomic Experimental Institute of Bari has made efforts to safeguard the almond germplasm by collecting and growing many typical varieties, foreign varieties, and new clones from different provenances in an experimental farm located in Bitetto, near Bari. The collection has been assembled using many different sources and entries have been added without discrimination between origin, provenance, or other phenotypic traits. The Bari collection is large and represents many distinct environments, however, better access to and use of the genetic resources has became important issues (Brown, 1989; Crossa et al., 1994; Jana and Addala, 1998). To maintain and exploit crop germplasm efficiently, an understanding of the diversity, its assortment among and within entries, and the population structure of the collection is required (Erskine and Muhlbauer, 1991). Also, multivariate techniques can help in evaluating large data sets, resolving several phenotypic and genotypic measurements into fewer, more interpretable, and more easily visualized groups (Souza and Sorrells, 1991; Brown, 1991; Suso et al., 1993; Polignano et al.; 1993; Clements and Cowling, 1994; De Giorgio and Stelluti, 1995; Russo and Polignano, 1996; Ahmad et al., 1997; Azar et al., 1997; Polignano et al., 1999)

In previous works phenological, carpological and productive traits in the whole collection have been already reported (De Giorgio and Stelluti, 1995, De Giorgio et al., 1996; De Giorgio et al., 1996, Ferri et al. 1996). However, the extent and patterns of diversity that exist within the Italian almond collection have been not sufficiently described.

The objectives of the present work can be summarized as following: a) – to obtain a synthetic descriptions of the Italian entries of almond; b) – to group together similar

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Table 1 Code, cultivar name, and provenance of 88 Italian almond entries tested.

Code	Cultivar name	Provenance	Code	Cultivar name	Provenance
1	A Grappolo	San Vito dei Normanni (BR)	45	Mincone	Casamassima (BA)
2	Albanese	Ceglie Messapico (BR)	46	Mollese di Canneto	"
3	Antonio De Vito	Toritto (BA)	47	Monaca	Turi (BA)
4	Banchiere	Monte Sant'Angelo (FG)	48	Montrone	Andria (BA)
5	Barese	Monopoli (BA)	49	Naturale di M. Vella	Acquaviva delle Fonti (BA)
6	Barlettana	Cellammare (BA)	50	Nocella	San Vito dei Normanni (BR)
7	Cacciola	Valenzano (BA)	51	Occhio D'Argento	Santeramo (BA)
8	Caporusso	Acquaviva delle Fonti (BA)	52	Occhio Rosso di Trani	Cerignola (FG)
9	Caputo	Conversano (BA)	53	Pappamucco	Monte Sant'Angelo (FG)
10	Catalini	Bitetto (BA)	54	Pastanella	Bitonto (BA)
11	Catuccia	Massafra (TA)	55	Pavone	Acquaviva delle Fonti (BA)
12	Catucedda	Alberobello (BA)	56	Pendulo	Manfredonia (FG)
13	Centopezze	Monopoli (BA)	57	Pepparuddo	Adelfia (BA)
14	Chino	Taranto (TA)	58	Pettolecchia	Putignano (BA)
15	Ciavea	Massafra (TA)	59	Piangente	Gioia del Colle (BA)
16	Cicerchia Amara	Andria (BA)	60	Pidocchioso	Bitetto (BA)
17	Cinquanta Vignali	Bisceglie (BA)	61	Pignatidde	Toritto (BA)
18	Cosimo di Bari	Toritto (BA)	62	Piscalze	Palo del Colle (BA)
19	Cristomorto	Spinazzola (BA)	63	Pizzutella	Manfredonia (FG)
20	Del Lago	Molfetta (BA)	64	Primicerio	Adelfia (BA)
21	Della Madonna	Molfetta (BA)	65	Pulita	Trani (BA)
22	Della Madonna	San Giovanni Rotondo (FG)	66	Putignano	Molfetta (BA)
23	D'Aloia	Valenzano (BA)	67	Rachele	Massafra (TA)
24	Falsa Barese	Palo del Colle (BA)	68	Rachele Tenera	Crispiano (TA)
25	Falsa Catuccia	Ostuni (BR)	69	Rachelina	Bitetto (BA)
26	Ferrante	Bitonto (BA)	70	Rana	Corato (BA)
27	Ficarazza	Ruvo di Puglia (BA)	71	Rana Gentile	Ruvo di Puglia (BA)
28	Fico D'India	Putignano (BA)	72	Reale	Gravina di Puglia ((BA)
29	Filippo Ceo	Taranto (TA)	73	Riviezzo	Ostuni (BR)
30	Fragiulietta	Andria (BA)	74	Rossa	Altamura (BA)
31	Fragiulio	Bisceglie (BA)	75	Santeramo	Andria (BA)
32	Franciscudda	Acquaviva delle Fonti (BA)	76	Santoro	Cerignola (FG)
33	Galgano	Ginosa (TA)	77	Scorza Verde	Conversano (BA)
34	Genco	Taranto (TA)	78	Senz'arte	Minervino Murge (BA)
35	Gioia	Putignano (BA)	79	Tenente	Corato (BA)
36	Giunco di Cozze	Alberobello (BA)	80	Tondina	Ostuni (BR)
37	Giunco di Cozze	Ostuni (BR)	81	Trianella	Corato (BA)
38	Irene Lanzolla	Cassano Murge (BA)	82	Tribuzio	Acquaviva delle Fonti (BA)
39	Lorenza Tribuzio	Acquaviva delle Fonti (BA)	83	Tuono	Taranto (TA)
40	Mancina	Bitetto (BA)	84	Viscarda	Massafra (TA)
41	Marchione	Conversano (BA)	85	Vuoi o non Vuoi	Casamassima (BA)
42	M. Carolina Tribuzio	Acquaviva delle Fonti (BA)	86	Zanzanidde	Conversano (BA)
43	Maria Tribuzio	"	87	Zia Comara	Giovinazzo (BA)
44	Mincaccetta	Palo del Colle (BA)	88	Zin Zin	Capurso (BA)

entries; c) – to assess the relative contribution of different traits to the total divergence; d) – pointed out the importance of the preservation and the utilization of almond germplasm and the habitats where it is still present.

MATERIAL AND METHODS

A total of 88 entries of Italian almond collection maintained at farm of Agronomic Experimental Institute of Bari were examined. All entries were planted and grafted in 1967 and 1968 respectively. Code number, local or cultivar's name and provenance of each entry are reported in Table 1. Five plant randomly selected from each entry were considered to measure 20 quantitative traits: date of initial flowering (days from January 1st), date of full flowering (days), date of final flowering (days), flowering duration (days from initial to final flowering data), kernel yield (kg/plant), shelling percentage (%), nuts with kernel failed (%), length, width and thickness of nuts and kernels (mm), nut and kernel weights (g), double kernels (%). Furthermore, length/width, length/thickness and width/thickness ratios were calculated.

Nut and kernel traits were evaluated on a sample of 1 kilogram of in shell almonds for each entry. Data checked in a sixteen-year period (1977-92) were averaged over the years. Entry means were used in the multivariate analysis.

Cluster analysis and canonical discriminate analysis, were used to index the similarities and dissimilarities among the almond entries respect to the traits considered. Data analysis followed two steps:

- clustering entries into similarity groups using original traits;
- applying canonical discriminate analysis to summarizes variation among groups.

PROC FASTCLUS and PROC CANDISC procedures in Statistical Analysis System (SAS, 1987 v.6) were used to perform both analyses.

RESULTS AND DISCUSSION

Average values, standard deviation, and ranges of entry means for each trait are reported in Table 2. The trait double kernels showed the highest standard deviation, followed by shelling percentage and flowering dates. The mean values of the double kernels were not very low, varying from 9 to 61%. The shelling percentage also presented a satisfactory mean value, but with entries having very hard (20%) or soft (59.5%) shells. The mean flowering date was mid-late (54.9 d) varying from early flowering entries (flowering on the 2nd February) to late flowering entries (flowering on the 11 March). Flowering duration date showed the lowest standard deviation value among the flowering traits. The mean values for nut and kernel weight were standard as were those of length, width and thickness. All other traits related to nut and kernel shape (from spherical form to very pronounced elongated and flattened form) evidenced less variable mean values.

Entries were first clustered according to the nearest centroid sorting method (Anderberg, 1973) that is designed for disjoint clustering of very large data sets on the basis of Euclidean distances computed from one or more quantitative traits. After three passes over the standardized data the observed similarity trend enables us to identify only seven main groupings. At level of seven clusters, the proportion of variance accounted for by the clusters was 67% (R^2 or R-Squared). This is a fairly satisfying value of the variance explained by the identified clusters.

Clustering analysis established four major clusters, with cluster IV having 23 entries, cluster VI 23, cluster VII 16 and cluster II 13. Cluster I and V consist of four and seven entries respectively. Cluster III include only two entries.

Frequency and cluster memberships are reported in Table 3. Entries are indicated in alphabetical order. Cluster means are reported in Table 4. Cluster III that has lowest values for kernel yield and several nut and kernel traits; on the contrary, presents highest value for shelling percentage, kernel failed percentage and initial flowering date. Cluster I shows entries with highest weight of nuts and kernels, and the highest percent of double kernels; while, on the opposite site cluster VI included entries characterized by lowest shelling percentage, lowest kernel weight and lowest double kernels. Clusters II, IV, V and VII plotted from left to right between clusters VI and I showed intermediate values for several traits.

Canonical analysis was used to provide a reduced dimension model that would indicate measured differences among groups. Table 5 presents the standardized canonical coefficients of the quantitative traits. The first canonical variable accounted for 61% of the among groups total variation; while, the second and third canonical variable further reduced the total variation 20 and 14%, respectively.

The canonical variant coefficients show that double kernels is a major discriminating coefficient among clusters with kernel weight and kernel yield making smaller contributions. The second canonical variant is dominated by flowering traits with nut, kernel thickness, nut length/thickness, kernel width/thickness, nut length/width and kernel length/width, having smaller components. The third canonical variant revealed that shelling percentage (0.72) play a much larger role in separating the clusters.

Table 2. Means, standard deviation (SD), minimum and maximum values of twenty quantitative traits observed in 88 entries of Italian almond collection.

Trait	Mean	SD	Min	Max
1. Initial flowering date (days)	54.9	6.21	33.0	70.0
2. Full flowering date (days)	61.9	5.67	48.0	76.0
3. Final flowering date (days)	68.2	5.62	54.0	82.0
4. Flowering duration (days)	13.1	1.15	10.0	15.0
5. Kernel yield (kg)	1.7	0.96	0.2	4.5
6. Shelling percentage (%)	30.6	6.15	20.0	59.5
7. Nuts with kernel failed (%)	0.3	0.60	0	2.9
8. Nut weight (g)	5.0	0.93	2.6	7.8
9. Nut length (mm)	32.4	3.77	22.7	44.8
10. Nut width (mm)	23.8	1.79	19.4	27.9
11. Nut thickness (mm)	18.6	1.44	14.9	21.6
12. Nut length/width	1.4	0.15	0.9	1.8
13. Nut length/thickness	1.8	0.27	1.1	2.8
14. Kernel weight (g)	1.5	0.39	1.0	4.4
15. Double kernels (%)	21.4	14.85	0.9	61.1
16. Kernel length (mm)	23.1	2.28	16.2	30.9
17. Kernel width (mm)	14.8	1.04	12.2	17.6
18. Kernel thickness (mm)	8.8	0.99	6.7	12.2
19. Kernel length/width	1.6	0.16	1.1	2.1
20. Kernel width/thickness	2.7	0.50	1.6	4.5

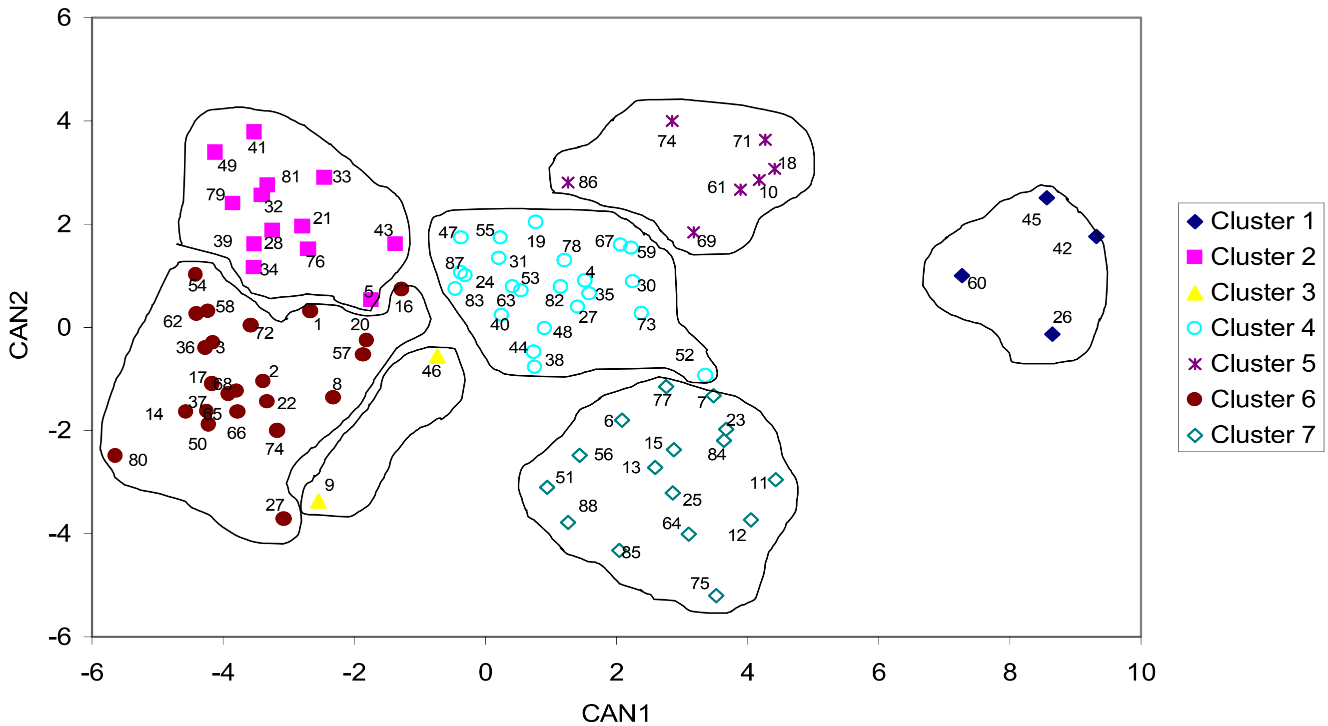

Figure 1 . Plot of 88 Italian almond entries and clusters according to the first and second canonical ***(The number is the entry code reported in Table 1).***

Table 3. Seven clusters grouping 88 entries of Italian almond collection based on twenty quantitative traits.

Cluster	Frequency	Cluster memberships
I	4	Ferrante - M. Carolina Tribuzio –Mincone – Pidocchioso
II	13	Barese - Della Madonna (code 21) - Fico d'India – Franciscudda – Galgano – Genco – Marchione - Lorenza Tribuzio - Maria Tribuzio- Naturale di M. Vella – Santoro – Tenente - Trianella
III	2	Caputo – Mollese
IV	23	Banchiere – Cristomorto - Falsa Barese - Filippo Ceo – Fragiulietta –Fragiulio – Gioia - Irene Lanzolla – Mancina – Mincaccetta – Monaca – Montrone – Occhio Rosso di Trani – Pappamucco – Pavone –Piangente – Pizzutella – Rachele – Riviezzo - Senz'arte –Tribuzio- Tuono - Zia Comara
V	7	Catalini - Cosimo di Bari – Pignatidde – Rachelina – Rana – Rana Gentile – Zanzanidde
VI	23	A Grappolo – Albanese – Antonio de Vito – Caporusso – Chino – Cicerchia Amara – Cinquanta Vignali – Del Lago – Della Madonna (code 22) – Ficarazza – Giunco di Cozze (code 36) – Giunco di Cozze (code 37) – Nocella – Pastanella – Pepparuddo – Pettolecchia – Piscalze – Pulita – Putignano – Rachele Tenera – Reale – Rossa – Tondina
VII	16	Barlettana – Cacciola – Catuccia – Catuccedda – Centopezze – Ciavea – D'Aloia – Falsa Catuccia – Occhio D'Argento – Pendulo – Primicerio – Santeramo – Scorza Verde – Viscarda – Vuoi o non Vuoi – Zin Zin

Table 4 Cluster means for 20 quantitative traits estimated in 88 entries of Italian almond collection.

Trait	Cluster						
	I	II	III	IV	V	VI	VII
Initial flowering date *(days)*	57	60	60	56	55	51	49
Full flowering date *(days)*	64	67	66	63	71	59	56
Final flowering date *(days)*	70	73	72	69	77	66	62
Flowering duration *(days)*	13	13	13	13	12	13	13
Kernel yield *(kg)*	2.2	1.6	1.4	1.7	2.1	1.5	2.0
Shelling percentage *(%)*	30	29	55	30	32	28	34
Nuts with kernel failed *(%)*	0.6	0.2	1.0	0.6	0.1	0.2	0.3
Nut weight *(g)*	5.8	4.8	3.9	5.1	4.9	4.9	5.1
Nut length *(mm)*	34	33	28	34	31	32	30
Nut width *(mm)*	24	23	22	24	23	24	25
Nut thickness *(mm)*	19	18	19	18	18	18	20
Nut length/width	1.4	1.4	1.3	1.4	1.3	1.4	1.2
Nut length/thickness	1.8	1.9	1.5	1.9	1.7	1.8	1.5
Kernel weight *(g)*	1.7	1.6	1.4	1.5	1.5	1.3	1.7
Double kernels *(%)*	56	8	18	24	36	7	36
Kernel length *(mm)*	24	24	21	24	22	23	22
Kernel width *(mm)*	15	15	14	15	14	15	15
Kernel thickness *(mm)*	8	8	10	8	9	9	10
Kernel length/width	1.6	1.6	1.5	1.6	1.6	1.6	1.4
Kernel width/thickness	2.9	2.9	2.1	2.9	2.5	2.7	2.3

Table 5 Eigenvalues, percent of variation, cumulative variation, and correlation coefficients among original traits and canonical variables in 88 entries of the Italian almond collection.

Traits	Canonical variable		
	CAN 1	CAN 2	CAN 3
Initial flowering date	0.08	0.81	0.31
Full flowering date	0.03	0.83	0.30
Final flowering date	-0.00	0.83	0.28
Flowering duration	-0.16	-0.13	-0.15
Kernel yield	0.23	0.01	-0.03
Shelling percentage	0.24	-0.29	0.72
Nuts with kernel failed	0.14	0.04	0.18
Nut weight	0.18	0.00	-0.23
Nut length	0.01	0.26	-0.22
Nut width	0.12	-0.21	-0.16
Nut thickness	0.27	-0.54	-0.03
Nut length/width ratio	-0.08	0.38	-0.13
Nut length/thickness ratio	-0.12	0.44	-0.15
Kernel weight	0.27	-0.04	-0.01
Double kernels	0.98	-0.10	0.03
Kernel length	0.01	0.27	-0.14
Kernel width	0.10	-0.20	-0.09
Kernel thickness	0.17	-0.49	0.25
Kernel length/width ratio	-0.08	0.38	-0.07
Kernel width/thickness	-0.11	0.39	-0.22
Eigenvalue	**11.55**	**3.76**	**2.70**
Variation (%)	**61**	**20**	**14**
Variation (% Cumulative)		**81**	**96**

As the information for separating the seven clusters seems to be mainly contributed by the first two canonical variants (81%) of the quantitative traits, any obvious variation trend can be displayed with the plane defined by a two-dimensional scatter plot (Figure 1).

The seven clusters had varying degrees of relationships with higher values for flowering traits and lower values for nut and kernels traits. In particular, the second canonical variable aids in the resolution between VII and the II, IV, V clusters.

CONCLUSIONS

Previously works have revealed the great diversity of Bari almond collection that has been well described (De Giorgio and Stelluti, 1995; De Giorgio et al., 1996; De Giorgio et al., 1996). As regards the Italian germplasm, it was evidenced that entries come from not too different sources, but prevalently from the Apulia region. According to that in the present work the multivariate procedure has been applied exclusively to the Italian set in order to verify more precisely the phenotypic distances among entries with a common geographic origin and to identify few highly differentiated entries. The groups of entries here obtained confirm the diversity trend previously observed and could be used to set up a smaller collection on which concentrate further agronomic and genetic studies. In fact, a good representation of the nature and magnitude of the overall diversity and a synthetic description of the main traits was obtained. Detailed analysis of the entries included in the clusters is very useful for a clear interpretation of the diversity and to assess the effectiveness of the multivariate analysis. Interpretation of cluster analysis is subjective. Such a priori knowledge of entries greatly helps in interpreting cluster results.

Cluster I includes Ferrante, Maria Carolina Tribuzio, Mincone and Pidocchioso entries, which are notoriously characterized by heavy nuts and kernels and high frequency of double kernels.

The major clusters IV and VI include an high number of yielding cultivars. In particular, the cluster IV includes yielding cultivars as Falsa Barese, Gioia, Riviezzo and two yieldest cultivars Filippo Ceo and Tuono both largely cultivated in Mediterranean basin; while, cluster VI includes a remarkable number of less yielding cultivars (for example: Albanese, Antonio De Vito, Piscalze, Putignano etc.) largely cultivated and characterized by lowest frequency of double kernels which is a commercial trait quite appreciate by the market.

Cultivars characterized by tardive flowering date, low incidence of double kernels and middle value of shelling percentage (for example: Barese, Genco, Tenente among those widely cultivated) are grouped in cluster II.

Cluster V grouped yielding cultivars characterized by tardive flowering date and high incidence of double kernels as the cultivars Cosimo di Bari and Rana. Highest percent of double kernels and good productivity characterize the cultivars included in cluster VII as Barlettana, Santeramo and Catuccia among those largely diffused.

Cluster III represents a very distinct group with two to each other. The first canonical variable allows clear discrimination among the seven identified clusters. Increasing values for the first canonical variables describe clusters and entries with high percent of double kernels and heavy and thick nut; while, increasing values for the second canonical variables describe clusters and entries entries: Caputo and Mollese, both characterized by smallest kernels and latest flowering date.

In any case, the greatest part of the discrimination among entries is due to the first canonical variable, which mostly accounted for double kernel and nut thickness. Smaller differences among groups were seen according the second canonical variable. All other traits showed low diversity among groups. No relationships have been found between the different groups of almond and the locality of provenance. This is probably due to the limited and specific geographic sources of material, which could suggest the use of a common gene pool during the selection process. Unambiguous interpretation of the relationships between almond entries should be possible by biochemical and molecular procedures. In almond, however, there is a dearth of reports on molecular genetic studies.

In conclusion, all entries examined are highly adapted to the Apulia environmental conditions and could be a very interesting source of genetic diversity, which could be utilized both to develop new improved varieties and to preserve agrobiodiversity in traditional farming systems. Preserving small areas were almond varieties can be safeguarded using new cultural techniques could be a valid and practical alternative for the farmers to maintaining genetic resources in less productive agroecosystems under low-input conditions. In other words, a farm-based conservation of the almond diversity can promote the presence of the farmers on the territory. This is essential to safeguard both agricultural profits and environment. At present in Italy and especially in some southern regions, such Apulia, there is an interest return of almond tree cultivation, because of the adaptation degree and the production quality of local varieties. These are well suited to be cropped in hill areas; they provide a good income and contribute to natural conservation of landscape.

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