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Collection and Assessment of Olive Biodiversity

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A B S T R A C T

This paper gives 25-year results for olive biodiversity because its resources are of great interest for environmental protection and mainly for finding all links within each ecosystem. The method was based to the descriptive morphological and molecular characters of the tree and organs, which has enabled the characterization and identification of genotypes. As a result; the correlations of the biological constants, the elements of climate, soil and geographical position were analyzed and estimated for all resources in the territory of the country. In the composition of olive biodiversity have been identified 292 accessions which have been detected and documented. The National inventory based on the ex situ collection includes 182 accesses (country genotypes) in three local and national collections. As in the analysis of morphological and molecular marker, the results obtained have demonstrated the level of variability between each other and they have expressed the typical profile. The molecular data provided essential information for constructing the first molecular database of olive germplasm. As a conclusion, all research on resource management, study and their improvement, are priority activities and have a scientific and economic character of national importance.

Introduction

Institutions have made efforts through multi-annual programs for the identification and preservation of autochthonous germoplasms, for its assessment and sustainable use (*Blitzer, (1993)*. The olive (*Olea europaea* L. subsp. Sativa,) is an important fruit species more widespread in the western part of Albania. Through five existing ex situ collections with cultures of interest, it is realized not only the preservation and study of them but also the spread of the best ones in the local conditions (Liphschitz *et al.*, 1991). The local varieties very adapted to the environment are compared with varieties of other and foreign regions, to make precise orientation for the creation of new olive grove with the best varieties, (Caballero *et al.*, 1986). The biodiversity of the Olea family reflects the quantity, variation and variability of varieties, forms and biotypes as well as plant organisms in coexistence. It also shows the diversity in the bay of species, between species and between ecosystems. Olive has specific ecosystems wherever it is cultivated. Almost all ecosystems have been transformed considerably under the influence of human activities (Amouretti, 1986; Gixhari *et al.*, 2013).

Olive varieties are necessary to possess functions as a constituent part of the structure and ecosystem processes. About 30% of the work is positioned in the olive grove as the main activity where man benefits from their diversity. These are the reasons that people engage in selecting suitable varieties for their needs in typical ambient conditions, and thus have created a very rich diversity.

Above all, resources have a lot of interest because they protect the environment and the study of their diversity helps to find their links (Fabbri *et al.,* (1995).

This paper gives the main results achieved over 25 years in the framework of the program for managing the genetic resources of olive in Albania, particularly for genetic richness, diversity concepts for their storage and evaluation.

Materials and Methods

Exploring

For about 25 years, the bibliographic, archaeological and historical data have been provided around 120 exploration missions in areas of very old olive groves. Two groups of scientists of the Institute of Olive and the Genetic Bank have worked, coordinated and received information based on 14 " key" morphologically characters. Terms methodical basis were: centuries-old trees, big biometry and historically autochthonous. The information provided in the file was aimed at integrating the geographic, morphological and agronomic data of the autochthonous olive genetic resources detected. Here is the first characterization of identity (Hannachi *et al.*, 2007; Ismaili *et al.,* (2012).

Collecting

The program for the National Inventory based on the ex situ collection includes all the country genotypes at the local and national level.

Ex situ

Three local collections: Krekes, Valias, Pus Mezin and a national field collection in Vlora, have been realized in the last 25 years.

In them, 12 hectares have included 292 acc. of which 182 autochthonous varieties for study. Age of seedlings has been one year old and their placement has been random system by three homologues.

The primary description has been realized by the passport data of the prospected individual that has allowed identifying each genotype, (Cantini *et al.,* (1999).

In situ

Conservation is performed for all genotypes that have been explored. There is an agreement "tip" for the preservation and study, an annual protocol for technology tree and a report on the performance compiled annually.

On-farm

Conservation is implemented for accessions very old that are in need of rehabilitation, and to avoid the risk of extinction (Cimato *et al.*, (2010).

Multiplication

The data on rooting ability are studied through the mist propagation method. The protocol contains specifications for the multiplication, sanitary control, homologation, data recovery and certification.

Improvement

Selection in varietal populations with the positive "sense" method, for coefficient of periodicity, according to Pearce. Certification of genetic and sanitary clones has been for the production constants and the resistance to cycloconium. The time needed for selecting a primary source has been 10-15 years, according to methodological procedures: the selection of populations, mass selection, selection of candidates, Pomological and chemical evaluation, genetic and sanitary clones (Pearce *et al.,* 1967).

Characterization

Several molecular and morphologic markers are available and are considered useful in genetic diversity studies. The methodology used for describing the recovered olive biodiversity has considered a set of 32 morphological characters respectively: tree; leaf; inflorescence; fruit: and endocarp (AA.VV, 1997; Cantini *et al.,* 1999; Idrissi, *et al.,* 2004).

According to the molecular protocol 44 olive samples were collected from both in situ germplasm collections. The samples were analyzed by 14 microsatellite loci SSR.

Documenting and analysis

The main programs that have been implemented have been for Modeling, Analysis, and Statistical Authentication. *SAS ver. 8.3 (SAS Institute, Cary, NC, USA and Statgraphix software*, to analyze differences in the morphological traits; including the mean value, standard deviation amplitude, coefficient of variation, principal component analysis, similarity analysis, morphologically correlations for the features at the modeled statistical limits. An analysis of variance (AMOVA) was performed to divide the genetic variance among all ex situ and in situ varieties. Distance fix of the similarity of the varieties was analyzed using the Bayesian model based clustering algorithm. Genetic variation among the varieties obtained from in situ was evaluated by calculating genetic parameters using GenAlEx 6.5, also Nei's genetic distance. The pair wise genetic distance is calculated between varieties under ex situ and in situ cultivation conditions. Analysis of SSP olea are performed according to bioclim domain connections and exploration of indigenous genotypes by geographic position. A statistical office has been operating at the Olive Institute which has documented analyzed and certified genetic identity (Fabbri *et al.,* 1995; SAS, 2008).

Results and Discussion

The general data

In 2017 olive statistics have 12 million trees on the surface of about 55 thousand hectares. The annual production capacity is approximately 110.000 tons of fruit olives and 14 thousand tons of olive oil in consecutive years. The olive-growing area is about 10% of the total agricultural land or about 157.14m² of olive groves / inhabitant or four trees. But olive is also known for other uses: It's a tree of beautiful and very stable landscapes. It has an important economic, touristic and ecological role. It is secular tree longevity. It is evergreen tree.

The Albanian olives grove are type "family" cultivation and are localized in the following points (Ismaili *et al.,* 1995; Frezzoti, 1930; Hannachi *et al.,* 2007).

Localization

47% of olive is cultivated on hills with difficulty mechanization, 32% in the field and rest in the mountains.

Dimensions

76 thousand farms are under 1.5 hectares (76%), and rests on over 2 hectares and specialized crops.

Confusion varietal

Only 8 varieties (7 autochthonous) are more important. Seven main Albanian varieties are important but in the existing fund dominate the cultivars Kaninjot, with about 51.3% and Kryps Berati 17%. In the past the varietal structure is based on native varieties while these last 20 years have been inventoried by 13 foreign varieties for the new olive grove.

The olive germoplasm

Olea family diversity reflects the quantity, the variety and the diversity of varieties, forms and biotypes. Biodiversity provides the foundation for our agricultural systems.

It provides the sources of traits to improve yield, quality, resistance to pests and diseases and adapt to changing environmental conditions. There are different approaches to conserving biodiversity: on-farm management, in-situ conservation, ex-situ conservation and other conservation methods.

In optimal availability conditions hydrological and the energy it exercises an incalculable influence in the intensity of many physiological processes. Among the factors, the temperature is particularly important because it is the first parametric for regulating the geographical distribution of species of olive. By thermal and biological constants**,** these resources have been localized with a great density in two main area (i) under the influence of the Ionian sea and (ii) under the influence of the Adriatic Sea, Figure 1, (Koppen, 1923)**.** In this way; referred to table 1, the genetic bank administers 205 olive accesses, in local collections, in situ and on farm.

Inventory of diversity

Exploring and recognizing olive biodiversity has completed the genetic bank as an efficient instrument for the modernization of the country's olive-growing structures. The data obtained through the explorations were corresponded to the archaeological data in response to the requirements for the characterization of the autochthonous resources, origin and Albanian culture of the olive. The labels sources are (i) from the destination of use, (ii) the characteristics of the fruit., (Iii) the name of the country of cultivation, (iv) the botanical origin etc, (Ismaili *et al.*, 2013; Cantini *et al.*, 2000).

Olive genetic fund consisting of *Olea europaea* L. subsp. Sativa 164 genotypes, *Olea europaea L. subsp. sylvestris* 8 genotypes and *Olea europaea* L. subsp. Oleaster 31 genotypes. Have local designations in composition of two spots.

Wild olive or "argelidhe" *olea europaea L. subsp. Sylvestris*, which are characterized in the form of barbed bushes and the fruit, is very small. Have small leaves, small fruit with big endocarp, very short inter-junction (Cantini *et al.*, 2000).

Olivastra, *Olea europaea l. Subsp.oleaster*, which differ for many features with wild olive, because fruits resemble the cultivated forms, have higher weight, have sprig with longer inter-junction, usually do not have thorns.

Correlates of biological constants subspecies, elements of climate, soil and geographical position were analyzed and is estimated the coefficient of diversity, genetic wealth and regionalization of the subspecies on the territory (Figure 1; Baldini *et al.*, 1955).

Indigenous genetic fund has good value use and in general the genotypes derive: (i) from the species *Olea europaea* sativa and (ii) genotypes with small and sporadic population of continuous improvement of the Olea *Europaea oleaster*, which are present in Albania since 12 thousand years (Ismaili, 2012; Breton *et al.*, 2006).

Collecting

Through the collecting process have been made possible: the multiplication and creation of the ex situ field collection. We have created the ex situ databases (National Inventory) and have accumulated the most important genotypes at risk of extinction phenomenon. The essential descriptions of collected varieties have been the second characterization (AA.VV., 1997).

In-situ

Conservation and use refers to the maintenance and use of plant populations in the habitats where they naturally. We have carried out the preservation and evaluation of 205 cultivars, forms, in the places of origin (table-1).

Because, they have been the product of natural selection of genetic material, that have stimulated this process. In situ conservation is considered as the optimal form of preserving genetic diversity in olive species. In situ conservation objectives are physical inventory, pomology assessment and database, type AlbGenBank. In general, we have made the basic description, only in 11 properties of variability in PC_1, PC_2 and PC_3 in situ.

We have realized the pomology catalog for each genotype based respectively on the features: 1 fruit, 4 endocarp, 3 leaves, oil, production index, cycloconium resistance and molecular profile based on 14 SSR loci.

Ex-situ

Conservation of germplasm takes place outside of the natural habitat, which contains the number of native genotypes that represent the entire germplasm diversity. The conservation of cultivated plants in *ex situ* collections is essential for the optimal management and use of their genetic resources. At the local level, three field collections were created in 1972, 1984, and 1995, in Shamogjin 109 native and foreign acc, Krekez 56 native and foreign acc, and Pus mezini 63 native and foreign acc. Meanwhile in 2011 was conducted field collection in Tirana which provides 63 native varieties. In these local collections were studied and evaluated cultivars with good adaptability. *According to* Table -1 *and* Figures 1, during the 25 years, 272 total accessions were studied but repeated in three repetitions and homologues. This means that the number of individuals in ex situ conservation consists of 84 native cultivars while 91 others are foreign cultivars. Some cultivars are important and have been repeated to study in all field collections.

In each local collection, conclusions are formulated for the main agronomic and technological indices of cultivated genotypes and the level of suitability in their natural environment. Meanwhile, conclusions are drawn about the spread of the most suitable cultivars in the respective provinces. As a result, ex situ represent the only option for conserving the biodiversity, being the tool through which the development of new olive grove is correlated with the studies and innovations of these collections. All information on the behavior of autochthonous genotypes in a cultivation site has been disclosed through various publications**.** This information will be used as a test for the research program of assistance to the new orientation olive grove.

Table.1 Olive germoplasm is represented by 3 basic populations (wild and domesticated form). In Albania, 4 sub species were identified

Ssp.	On Farm	in situ	ex situ
Olea europeae ssp.sativa	47	164	177
Olea europeae ssp.oleaster	17	31	5
Olea europeae ssp.sylvestris	-	8	0
Olea europeae ssp.cuspidata	-	1	0
gjithesej…………	64	205	182

Note: ex situ conservation consists of 84 native and 91 foreign cultivars

Fig.1 Analysis of olea according bioclim connections and exploration of indigenous genotypes by geographic position

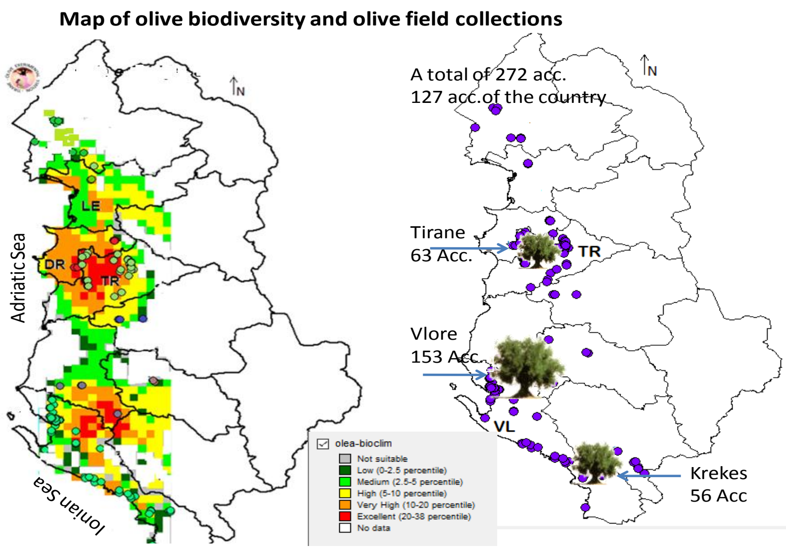


Fig.2 The dimensional relationships between the most important morphological traits of accessions of Albanian olive

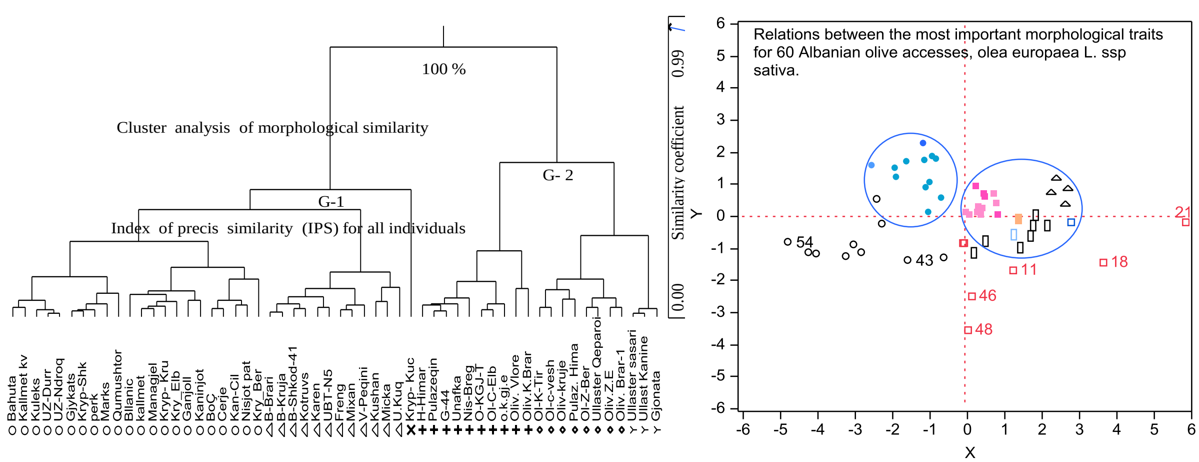
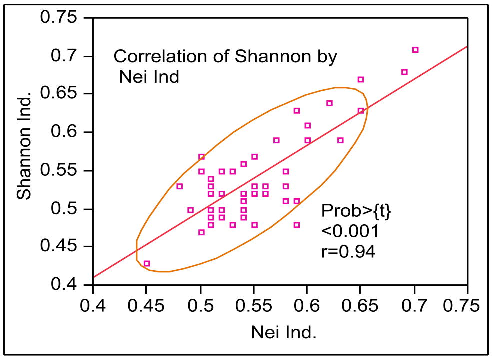


Fig.3 and 4 Correlation between morphological and molecular indexes for analyzed Albanian olive genotypes and Shannon and Nei index



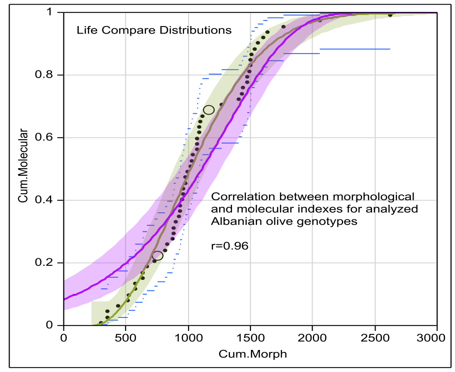
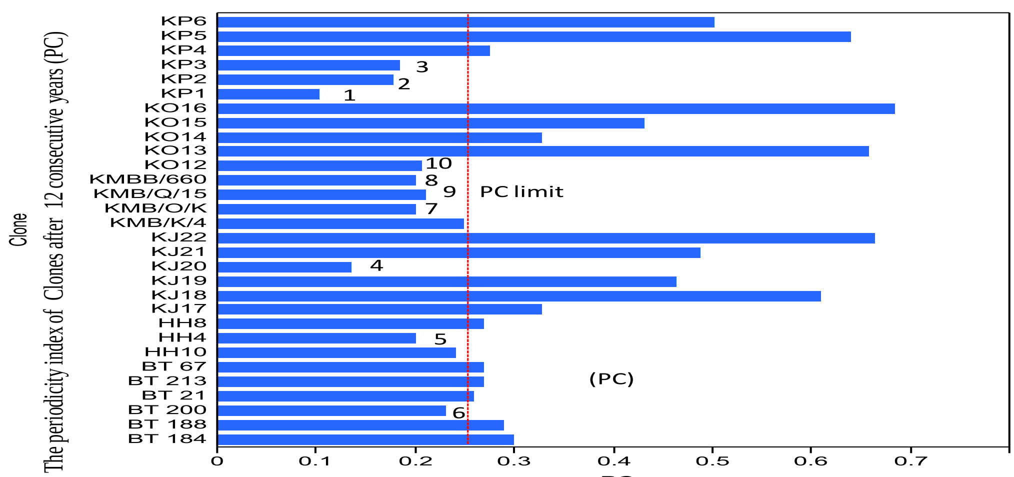


Fig.5 The periodicity index of olive clones after 12 consecutive years (PC)



On farm conservation

This type of conservation involves the maintenance of individuals on farm or in home gardens. The preservation through the use of the tree, we have in conservation 64 trees over 2 thousand years old, on farm or garden. These trees have the code of the genetic bank and all the research according to the in situ protocol of the individuals is performed. Each individual has passports with pomology, morph metric, radiometric and molecular characteristics. On the other hand they have genetic, touristic, historical and cultural values.

Morphological evaluation

The primary description has been supplemented by the passport data of the prospected cultivars which has permitted to identify and locate unequivocally all olive genotypes. The morphological characters used were evaluated consistency between homolog's and between years for the same genotype. Variations and dev.std are found within the sample and between the samples. Also, discriminatory capacity between different genotypes and the level of varietal performance has been different in any ex situ environment. For example, the growth index of olive germplasm has been influenced by the environmental factors and the development stage of the plant, since the vegetative growth of varieties has been different: in the first 10 years 1.34 m^3 / tree / year and variation between varieties 28.3%, in the second 10th anniversary 0.79 m^3 / tree / year and variation between varieties 33.4%.

The secondary characterization of the varieties held in the ex situ collections has been confirmed by providing reliable data on the agronomic and technological value for 80 varieties of subspecies sativa and oleaster. In general, the estimated 39 morphological characters have profiled the identity of each genotype. But between all the characters, the features of the leaf and the endocarp have resulted in two morphological "key" markers that together possessed over 87% of the variability, positioned on the coordinate axis (Figure 2). These two markers in general for the form and symmetry are not influenced by environmental factors. Only seven features in the two PC analyses, possessed over 87% of the variation listed in PC_1 and PC_2, and for the future focus only on these characters to reduce cost. According to clustering analysis, the country's germoplasm has distinctly classified profiles conforming to morphological fix distances (*cluster 3.1*). The genetic similarity among the genotypes, based on 14 microsatellite loci, ranged from 0.17 to 1.00, with a mean of 0.503, while based on morphologic markers ranged from 0.011 to 0.99.

According to Figure 2, genetic relationships between native genotypes were visualized in 2 groups and 12 subgroups. Each of the main groups subdivides into several small clusters consisting of related genotypes with great similarity.

The overall proportions of the membership of the sample to each of the two clusters were 62.5% and 37.5% for cluster 1 and 2, respectively. Average distances between individuals within a cluster were generally 0.0163 and two clusters were 0.027 and 0.046 for clusters 1 and 2, respectively.

Referring to the positioning in the coordinate axis, the dendrogram showed that individuals within two groups of the population of Kaninjot and White Olive cavy, were in close relationship within each other in each group. Many Sylvester's, olestra and some table cultivars have different distances with the two groups and according to the similarity distance they have their position in the space of negative and positive coordinates.

Molecular

The first conclusions were drawn on a plant material of a total of 54 olive samples (genotypes) collected from in situ germplasm collections. Identity analysis revealed 53 unique genotypes and their allelic profiles were used to calculate several genetic diversity indices. Analyzes of variance (AMOVA) has tested the level of variation between any identified genotype, degree of affinity and their distances. Based on two distinct discriminative indices, the PIC value (0.873) and the probability of identity (0.052), between the microsatellites loci, some of them and specifically the DCA9 locus were shown to be the most informative. This makes it possible in the future to analyze only the locus of information holders as well as the morphological markers.

As a conclusion, the analyzed varieties have a unique profile in addition to the few cases that have manifested the phenomenon of synonymy and homonymy. Statistical analysis as in the analysis of morphological and molecular markers, in Figures 3 and 4, have demonstrated the level of variation between each other, and they have expressed the typical profile of each genotype. In this manner the genotype diversity displayed via Coefficient of regression calculated between morphological and molecular cumulative score is extremely influential $r^2=0.93$, while according to the Nei analysis there was a correlation r = 0.94, which have shown the strong connection between the two morphological and molecular markers within the dimensions of diversity (Nei *et al.*, (1979).

Certification of genetic profiles has manifested a clear distinction of Albanian cultivars in comparison with foreign genetic material especially Greek and Italian. The same results have been published by some Spanish authors, based on their origins of native Albanian varieties based on their molecular analysis.

Apparently the polymorphisms of molecular markers had strong correlation with morphological variation and they both had the same importance for the certification of identity. The high level of polymorphism has been proven because the results show a great variety and dependable among the autochthonous olive cultivars. Genotypes in the study have low value 34.5% similarity between them, and this confirms the hypothesis of the existence of high diversity among the analyzed genotypes.

Improvement

In the populations of the three main varieties 15 clones are selected which possess constant production i.e. without periodicity, higher percentage of oil and resistant to cycloconium. Because, cycloconium is a factor of yield and the finding of resistant individuals is presumably a genetic feature. All Clones were tested through Molecular Diagnosis (dsRNA) and found to be free of the following pathogens: (ArMV) (CMV) (SLRSV) (OLYaV) (CLRV). This genetic material is valued as a primary source and is owned by seedlings production structures.

The periodicity index and the percentage of fruit oil are several percent higher compared to the varietal standards. The multiplication and spread of these clones has increased the average standard of populations, ie, it will increase the biological capacity of olive production (Ismaili *et al.*, 2013).

In the 6 chart, there are presented the clones of the Kaninjot, KB, and BT variety, which have a periodicity index below 0.25 (near zero). The clones of the variety Kryps Berati, Ulli i Bardhe and Kaninjot are administered in the primary resource fund.

Genetic erosion

We have estimated that the level of genetic erosion in the last 30 years is 11%. Those that have favored genetic erosion have been: The urbanization of the country and cutting the olive groves for construction.

Factors such as: Urbanization of countries with old olive groves, transplanting old olives for decorative purposes, Replacement with more productive but short-lived varieties (close genetic base), Planting unadapted genotypes, etc., have reduced the thousand-year-old olive germoplasm, have reduced in situ germoplasmes, olives with centuries-old history, and have stimulated genetic erosion.

The analysis of the identity of olive by morphological and molecular markers allowed the detection of unique profiles of each genotype and systematized all cases of synonyms or homonyms as an important problem in autochthonous diversity.

The Albanian olive germplasm material represents a large and very important genetic patrimony, which includes native cultivated olives, ancient and foreign cultivars and an abundant number of wild genotypes. Exploring and recognizing olive biodiversity has completed the genetic bank as an efficient instrument for the modernization of the country's olive-growing structures.

Research has proved that that the genotypes are autochthonous and their names derive from origin, fruit morphology and destination of use. Diversity has been great and distinct, based on Variety Characteristics in closely related Teritoria's culture, history, traditions and morphology of genotypes.

The present study on the molecular and morphological characterization of germplasm in some local collections has made the evaluation of the genetic relations, present and imported foreign cultivars very important for the future.

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