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The Discovery and Identification of Varieties of Olive

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***Abstract* – This study intended to take into consideration five autochthonous varieties discovered in the territory of centennial olive groves to describe certification of identity through morphological markers and microsatellite primers.**

The chosen method and the results showed that the qualitative and quantitative features varied in a wide range (23% up to 73%) and were responsible for the morphological polymorphism. PCA expresses seven features that mastered 95.22% of the variability. Eight polymorph microsatellites are identified and used to five genotypes. Around 36 alleles have been identified and the molecular frequencies varied within 0.10 up to 0.71 a wide range. Observed values and expected to heterozygosity are 0,40 up to 0,95 and 0,51- 0,96, respectively. SSR molecular markers in correlation with the morphological ones provide a specific genetic profile for each genotype because they have large distance.

Conclusions of the study include five olive genotypes different not only among each other, but simultaneously among autochthonous varieties. The eight microsatellite predominant markers and 7 morphological features were found useful to be applied in the characterizations.

***Keywords* – Olive, Genotype, Morphological Analysis, Feature, Marker.**

I. INTRODUCTION

Identification is difficult as it should be based on analysis of morphological features. It is implied that autochthonous individuals are old, as they prove suitability with the respective environment [1]. Growth manners, outer appearance of the tree, the leaf, efflorescence, fruit, endocarp, oil content, quality etc., are important identification characteristics. It often occurs to have individuals with different characteristics within the same varietal population, which serves as an incentive to grow the interest in choosing and selecting important forms and biotypes [2] [3]. The olive in Albania has ancient phylogenies and yet needs to be studied and explored thoroughly [4] [5]. On the other hand being specifically heterozygote, it presents variability and different forms [6][7]. Identification should be taken into consideration, another thing is confusion caused by varietal homonymy and synonymy [8][9], not to skip phenotype changes caused by the relations Genotype + environment etc. [10] [11].

Morphological characters have been widely used to describe olive genotypes [12][13]. Recently, biochemical and molecular markers have been used to get better insight into the diversity of olive genetic resources. Identification using SSRs assessed differences in lengths of amplified alleles [14] [15] [16] [17].

Under these circumstances the object of study was to standardize and certify the genetic profile of the genotypes found in the old olive plantations which have been neither described, nor mentioned in Albanian on available literature [18].

Fig.1. Five new varieties: Kcarr, Lundra, Bllanic, Narta, and Ganjolla, they were discovered in olive grown century of Albania

II. MATERIALS AND METHODS

Plant materials: In situ, five representative trees more than 5 centuries old, made up of the respective populations were named as of the respective places: GPS coordinates are: Lundra (41°18'13,30"N; 19°52'27,55"E; H230)., Kcarr (42°11'32,23''N; 019°31'09,63''E; H189), Narta (40°29'04,94''N; 019°28'58,47''E; H7), Ganjolla (42°00'42,21''N; 019°35'29,00''E; H53) and Bllanic (40°20'21,01''N; 019°36'16,00''E; H379)

Morphological analysis: Different samples of leaves, flowers, fruit, endocarp were extracted from them to study their morphological characterization. Each of the morphological features was in 4 repetitions and the number of variables had a statistical limit as of UPOV 1973 and REZGEN 1987 (COI). Estimate included 34 morphological characters (18 quantitative and 16 qualitative), during 3 years 2012.- 2014, in the characteristic trees for the research. Every 100 leaves/year at the end of vegetation had the following main characteristics: height, width, surface, ratio L/l, form, symmetry etc. Every 100 fruit/tree at ripeness period: diameter (D), diameter (d), ratio D/d, average weight, form and symmetry. Every 100 endocarps the main measurements included: diameter (D), and(d), ratio D/d, average weight, numbers of grooves, form and symmetry [19] [20].

Oil extraction was carried out through the soxhlet method in high temperature through ether petroleum as an organic solvent (according to (RIO), oil percentage compared to fresh matter and olive oil was calculated using the following formula: weight of samples before Soxhlet–weight of samples after Soxhlet/weight of samples before Soxhlet × 100.

Microsatellite markers. Six markers (DCA3, DCA5, DCA11, DCA9, DCA16, DCA18) from Sefc et al. (2000) and *Two* markers (GAPU71, GAPU101) were selected for the high level of information that they offer [21] [22] [23].

DNA was extracted from 0.5 g apical leaves ground in liquid nitrogen and incubated in 2 mL CTAB buffer (tip), by extraction procedure and protocol described by Fabri and Javornik [24] [25]. Genomic DNA was stored undiluted in TE 1 X pH 8.0 (10 µM Tris, 1 µM EDTA) at -20 °C [26].

Amplification and Sequencing. Standard PCR products used 10 µM per each dNTP, 0.2 µM per each primer, 20 ng genomic DNA, 1.25 U Taq pol, and Tail 0,250 uM. Amplification was performed at Gene Amp. PCR system 9700 Thermal Cycler (*Applied Biosystems*), for 25 cycles with initially denaturation at 94 ºC for 3.0 min, 94 ºC for 45 sec, annealing at 59ºC for 45 sec and extension at 72 ºC for 1.0 min, with final extension step of 5 min at 72 ºC. Amplified bands were out of the gel and purified using the QIAquick Gel Extraction kit. The SSR analyses were checked ail alleles by sequencing ail the SSR amplification products [27] [28] [29].

Data analysis: Descriptive statistics analysis and coefficient of variation were performed for ail quantitative parameters, applies a Tukey-cramer test at a significant level of *($p<0.05$),* The traits mean values were used to perform principal component (PCA) and cluster analyses was converted into a matrix of similarity values based on Arithmetic Averages (UPGMA) for a pair of two genotypes. Allele frequencies and heterozygosities (observed and expected) and I (Shannon's information index) were calculated by GenAlex software. The power of discrimination (PD) was calculated for each SSR locus according to Brenner and Morris [30] [31]. The relationship between individuals was further analysed using JMP software [32].

III. RESULTS AND DISCUSSIONS

Morphological profile: The olive genotypes resulted to be really old and were made up of small populations. The data presented in *table 1*, *Figure 1 and 2*, show that they have variability among each other, as well as other differences among other varietal population in our country. Morphology of cumulative score (7 features in three first PC) displayed a considerable coefficient of variation which proves different varietal features. Between the morphological traits variation it is 5.4% (FR) to 27.2% (LA).

Table 1: The main data of quantitative morphometric traits evaluated for 5 olive genotypes

Genotyp	L.L	LW	LR	LA	FW	FL	FWI	FR	SW	SG	FSR	SL	SWI	SR
Kçarr	43	9.3	4.6	305	2.90	22.4	15.2	1.4	33	5.9	7.7	14.2	7.1	2
Lundra	40	8.3	4.8	258	2.24	19.8	13.3	1.5	45	5.3	4.0	15.3	6.9	2.2
Bllanic	68.3	12	5.7	506	3.60	25.3	16.1	1.6	54	5.3	5.6	18.1	8	2.2
Narta	54	12	4.5	465	4.02	22.4	15.4	1.4	64	7.1	5.3	15.3	9.2	1.6
Ganjolla	57	11	5.2	378	3.62	22.3	15.6	1.4	42	9.3	7.6	13.9	7.1	1.9
Std Dev	11.3	1.6	0.49	104	0.7	1.9	1.07	0.08	11.8	1.6	1.5	1.6	0.9	0.24
Mean	52.4	10.5	4.96	382	3.2	22.4	15.2	1.46	47.6	6.58	6.04	15.3	7.6	1.98
CV	21.5	15.2	9.8	27.2	21.8	8.4	7.0	5.4	24.7	24.3	24.8	10.4	11.8	12.1

LL: leaf length (cm), LW: leaf width (cm), LA: leaf area (cm^2), LR: leaf (length/width) ratio, FW: fruit weight (g), FL: fruit length (mm), FWI: fruit width (mm), FR: fruit (length/width) ratio, SW: stone weight (g), SL: stone length (mm), SW: stone width (mm), SR: stone (length/width) ratio, SG: number of grooves, FSR: fruit flesh to stone ratio. SD: standard deviation, CV: variation coefficient (%),

Table 2: Genotypic profiles for eight simple sequence repeat markers which have resulted in five new olive genotypes

Name of new genotypes	DCA5	DCA11	DCA3	DCA9	DCA16	DCA18	GAPU71	GAPU101
Kçarr	195-195	128-235	130-261	145-182	139-196	176-210	122-161	192-209
Lundra	195-204	161-161	261-271	187-209	139-165	186-190	139-159	207-213
Bllanic	175-211	144-160	246-246	145-206	155-155	182-182	148-148	205-205
Narta	219-225	157-177	191-262	204-204	165-165	184-192	139-139	207-213
Ganjolla	195-218	144-188	145-240	138-231	135-182	176-187	118-168	190-208

According to the data analysed and reported in Table 1 and figure 2, for the quantitative traits, 18 PC possess 100% of the genetic variability. But the first three PCs have more important because with seven characters have possessed 95.2% of the variability. In the PC[1], length of leaf, fruit weight, forms the base of the fruit, diameter (d), and number of groove of stone. These are powerful characters and constitute the first axis *Figure 2 and 3.*

These 7 predominant morphological characters analysed through PC_OA (*figure 2*) showed distances among each other in coordinate space. Distances of similarity were calculated with these predominant features and their variability was displayed in a short summary. *Figure 2*, shows a two-dimensional PCA based on the matrix of variability showing different positions in four spaces of coordinate axis. The morphological characteristics constitute important markers for the identity and pursuant to thorough analysis the genotypes are grouped as of the closest distances and in pairs via the weighted method [33].

In figure 3, for the clustering history distance ; the similarity degree between the five new olive is evaluated based on 7 traits (*Table-1*). Cluster of similarity in *figure-3*, has presented the genotypes arranged in 2 groups, (G1) including one group (Lundra), whereas the others are within the second group; they are different from each other and not synonyms. Morphological similarity range from 23% to 73%, with an average value respectively of 48% and have proved the high degree of morphological diversity.

The smallest similarity value was observed between 'Kcarr' and 'Bllanic' It is a consequence of changes in the form and symmetry of the fruit, leaf and endocarp. The maximum morphological similarity (MS) between varieties (MS = 0.732) was found between 'Kcarr and Ganjoll », *figura-3*. These two genotypes have the same origin, to the north of the country.

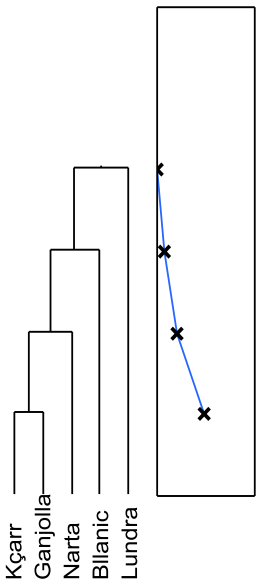

Fig.3. Clustering for the similarity of 5 genotypes based on distance computed of morphological trait

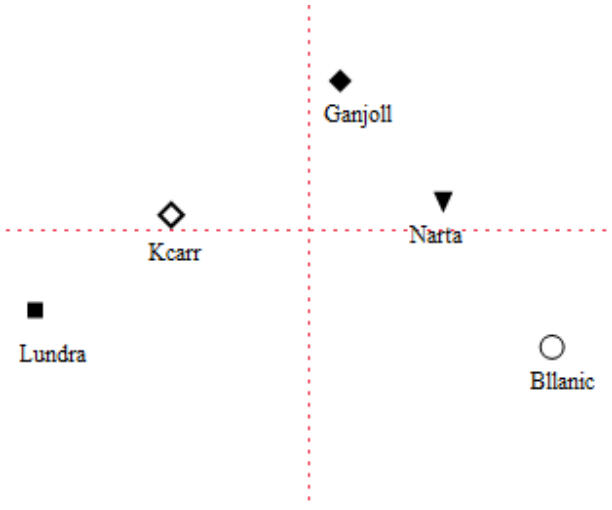

Fig.2. Distribution of olive genotypes on the first and second canonical components of morphological traits

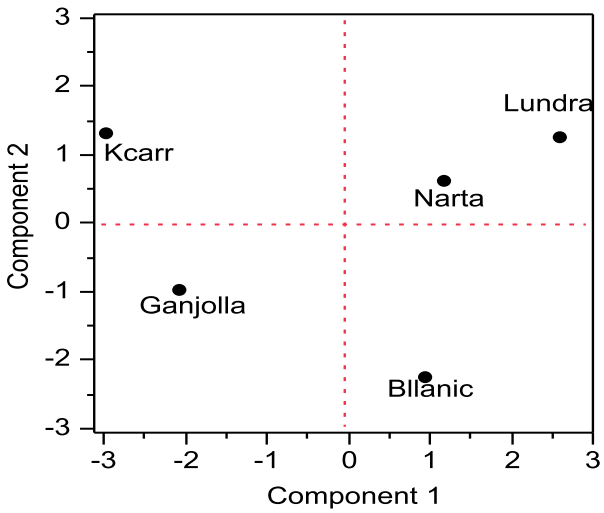

Fig.4. Distribution of olive genotypes on the first and second canonical components of molecular markers.

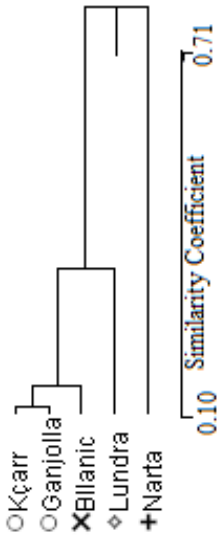

Fig.5. Cluster analysis of olive genotypes using Ward's method based on Euclidean distance similarity coefficients using molecular markers.

The genotypic profiles: Polymorphism of satellites has always shown the presence and lack of amplified bands and has calculated coefficient of similarity among the five genotypes.

In *table-2*, are presented the Genetic profiles for each locus. In general, have resulted significant differences between the locus of the base pair and they have expressed different frequencies. The shortest allele among these eight loci was 118 base pairs (bp) for GAPU71b, whereas the longest allele was 271 bp for DCA3. The lowest allelic frequency was observed for alleles 213 bp of GAPU101, 210 bp of DCA18, and 196 bp at DCA16. Alleles 235 bp of DCA11 and 196 bp of DCA16 showed the relatively highest frequency (0.575).

As of *table-2*, and PCoA in *figure-4*, which presents analysis of molecular profile the results show that the genotypes are individual characters, are specific and present variability among each other considering the molecular aspect.

This information is because; a total of 36 alleles were found for the eight loci with an average of 4.5 alleles per locus ranging from 3 for GAPU101 to 7 for DCA11. Around 36 alleles have been identified and the molecular frequencies varied within 0.10-0.71 a wide range. These occur as a result of the wide amplitude and the number of alleles per locus which varies at its maximum 7 alleles per locus 2 up to the lowest number of alleles in loci 6 and 8 and their average of 4,5. The observed heterozygosity has average 0.69 and ranged from 0.4 to 0.95. Whereas the average PD was 0.72, while values ranged from 0.64 (GAPU101) to 0.84 (DCA3) . In *table-3*; Observed heterozigozity is higher in locus 2 and lower in 8 of values respectively 0,95 and 0,4. The whole number of loci used in analysis was informative as it had average value of *PIC =0,70* which confirms a high degree of variability. The eight predominant SSR markers of variability showed and gave a specific profile of the identity per each genotype almost identical to morphological markers.

In *figure-4 and 5*, the similarity degree between the 5 new olive genotypes is evaluated based on SSR markers, range from 28% to 64% with an average value respectively of 46%. These variables have proved the high degree of inter varietal genetic diversity. The smallest similarity value was observed between 'Kcarr' and 'Lundra'. It is a consequence of changes in the form and symmetry of the fruit, leaf and endocarp. The maximum genetic similarity (GS) between varieties (GS = 0.61) was found between 'Kcarr and Ganjoll », which are very similar morphological characteristics. These were discovered in the same geographical area.

Table 3: Length of bands and the number of alleles in the SSR analysis, detected in five olive

Locus	Range of Sizes (bp)	Na	H_0	H_e	PIC	DP	RR
DCA5	195(175-225)	4	0.7	0.73	0.46	0.73	(GA)15
DCA11	144(128-235)	7	0.95	0.96	0.92	0.72	(GA)15
DCA3	145(130-271)	6	0.70	0.78	0.72	0.84	(GA)19
DCA9	145(138-231)	4	0.58	0.67	0.74	0.78	(GA)23
DCA16	139(135-196)	5	0.85	0.85	0.84	0.68	(GT)13(GA)29
DCA18	176(176-210)	3	0.55	0.70	0.65	0.77	(CA)4CT(CA)3(GA)19
GAPU71b	139(118-168)	4	0.85	0.76	0.81	0.67	GA(AG)6(AAG)8
GAPU101	207(190-213)	3	0.4	0.51	0.39	0.64	(GA)8(G)3(AG)3

Na-number of different alleles.He-expected heterozygosity.H_0-Observed heterozygosity.
PIC- Polymorphic information content, DP- the power of discrimination, RR- repeat region

Genetic Diversity. The markers amplified a total of 36 alleles and the total probability of identity (PI) and total probability resulted 0,98. These proved that the used markers were considerably polymorphic; the genotypes had individual identities and the information available through experimented molecular markers showed a specific genetic profile per each genotype. These dominant markers have allowed detection of a large number of alleles per locus displaying a high level of diversity among the five genotypes.

These markers, were *highly* polymorphic and at the same time very informative [34 [35] [36]. The results obtained have demonstrated the level of variability between each other, and they have expressed the typical profile of each genotype. In this manner in the *Figure-6* shows that the olive diversity displayed through the coefficient of regression calculated between

morphological and molecular cumulative score is extremely influential $r^2=0.92$ which have shown the strong connection between the two morphological and molecular markers within the dimensions of diversity. But has important because, the most of the genetic diversity coming to differences among individuals within groups. Polymorphisms of molecular markers had strong correlation with morphological variation and they had the same importance for the certification of identity. The high level of polymorphism displayed so far in the results shows a high level of genetic variability among the autochthonous Albanian olive cultivars.

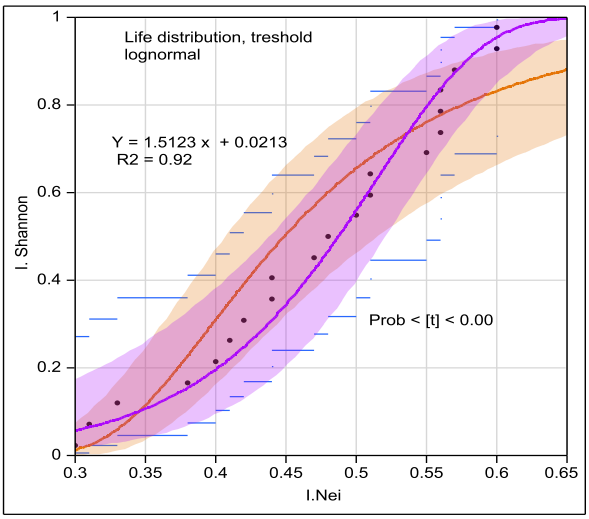

Fig.6. Plots illustrating the correlation among five olive assessed by morphological and molecular indices r2=0,929.

IV. Conclusion

Statistical analysis for morphology and molecular has defined the identity and the degree of genetic similarity. The high level of polymorphism displayed in results so far shows a high level of genetic variability among the genotypes, which make up the separate identity.

Eight microsatellite predominant markers and seven morphological markers were important, and they have had a major role for the characterizations that might be considered in the future of olive.

Sequence data from all alleles of eight loci and morphologic trait, they have been very important for the identification and are inserted into the genetic fund of Albanian Bank.

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