Research Paper Agriculture

Polymorphism of Some Olive Clones in Albania

* Hairi Ismaili ** Belul Gixhari

*, ** Agricultural University of Tirana. Gene Bank. 1020 Tirana-Albania

ABSTRACT

Six clones were characterized with a morphological and molecular marker SSR. The qualitative and quantitative features of the leaf, fruit, and endocarp recorded in wide frequencies (1.454 up to 7.286) and were responsible for the morphological polymorphism. Molecular polymorphism is displayed by 46 alleles and average value of 3,29 alleles per primer. The results of the obtained data show that the average of the expected heterozygote is 0,714, whereas the observed one is 0,695. Total probability of the identity (PI) is 2,78 x10-9, whereas total exceptive probability (Pe) 0,997. Polymorphic Information Content (PIC) varies from 0.395 (DCA14) to 0.854 (DCA15), with an average value 0,695. Molecular frequencies have a wide range from 0.14 up to 0.89. SSR molecular markers in correlation with the morphological ones provide a specific genetic profile for each clone and have differentiated 2 clones(Kan-V and Kan-C), as new forms within the population of cv. Kaninjot.

Keywords : olea europaea, clone, morphological, molecular, marker

Introduction

Study on clone identification firstly started with the morphological analysis for the qualitative and quantitative features (1,2,3,5). Form and symmetry of the leaf and endocarp were called morphological marker of the identity "*marker*", (3,4). Analysis on specific statistical limits of the morphological characters has proved polymorphism and characterizes the genetic profile considerably, (1,6,7,9). The object of this research is the morphological and molecular characterization of the Kaninjot cv olive clones, evaluation of the improved characteristics and their use for further genetic improvement programs.

Material and Methods

Morphological characterization.

Six clones (*tab-1*), (*Kan-J, KO-12, Kan-V, KJ-20, Kan-C and KO-14*) which have resulted from clonal selection during e 12-year-period were analyzed through *Rezgen 96/9 (COI)* for 34 characters. Respectively; 6 leaf characters, 10 fruit characters, 10 endocarp characters and 8 physiological characters, were important to characterize the identity of clones considered in the study, (2,5,8,11). The data were processed through multivariate analysis, whereas variability of the character was tested with univariate simple statistics, F-test and Tukey-kramer. Principal component analysis by eigenvalue-eigenvectors, enabled the identification of variability possessors. Analysis of the similarity matrix with Cluster Hierarchical Average method defined genetic distances and the level of similarity. Probability of clonal heterozygoteness was tested with Orthogonal regression for life Distribution Profiler of the similarity level features studied in six clones.

SSR molecular marker

Isolation of DNA and amplification of microsatellites: Selection of SSR primers was based on their differential capacity. Extraction of genomic DNA was performed with new leaves applying the method CTAB, with Fluorometer DyNa Quant™ 200, in coloring solution Hoechst at a concentration of 100-5000 ng/ml, (2,7). Whereas, 14 loci SSR : (DCA3, DCA5, DCA9, DCA11, DCA14, DCA15, DCA16, DCA18 from (6,7), GAPU59, GAPU89, GAPU71B, GAPU101, EMO90, UDO24), *by (7,10)* were selected and analyzed for the high level of information that they offer.

Standard PCR products used 10 µM per each dNTP, 0.2 µM per each primer, 20 ng genomic ADN, 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. Amplification products were tested in agarose gel, 2 % and were electrophoresed in capillary electrophoresis ABI Prism 3100 Genetic Analyzer. Allele size per each locus was determined by Gene Mapper Software Version 4,0 (*Applied Biosystems*). This apparatus determined the size, number and concentration of the amplified bands. Genetic diversity was calculated using formula of Nei ($GD=1-\Sigma Pij^2$) (11). Genetic connection among clones was evaluated based on "proportion shared alleles with the program MSAT whereas the dendrogram was constructed using UPGMA (Phylip 3.6).

Results

Morphological polymorphism.

Results from analysis of the main components (PCA) found out that a considerable quantity of the variation (96.3%) was explained from the three first axes: axes 1, 2 and 3 which explained 69.1%, 22.1% and 5.0% of the total variation. Only 6 components in Pc1, with a value of pc>021 have 69.1% of the capacity for variability and are mainly responsible for the genetic polymorphism of clones.

Based on the Euclidean distance, polymorphism of clones displays frequencies from 1.454 to 7.286 and this space distinguishes three different genetic profiles: (i) four clones in G1: Kan-J, KO-12, KJ-20 and KO-14, are located within close distances 1.454 – 2.738, and are perfect synonyms as they have close distance and maximal similarity. These clones possess the same morphological profile, with slight differences from the data of the population. (ii) Clone *Kan-V* constitutes a group (G2), with a distance (2.122) compared to (G1) and level of similarity 21.2%. (iii) Clone *Kan-C* constitutes a special group (G3), with the greatest distance of (G1) and value of 4.554. From this point of view clone Kan-V and Kan-C result to have proved variability from the varietal standard, 21.2% and 45.5%. Probability on side effects on profiling identities as per life *Ortogonal regression, displays* R^2= 0,997, thus genuineness of the morphological profile p=0.05,

and tf>2. From this point of view, the clones represent three individual identities with proved changes.

Molecular polymorphism:

The used markers displayed totally 46 alleles, which varied from 2 the lowest up to 5 the highest. The average of the expected heterozygote was 0,714, whereas the observed one was 0,696.

Table-1. length of bands in bp of the alleles detected with 14 markers used in the analysis, repetitive region, polymorphic band, number of alleles, H, PIC and DP in clones of Kaninjot cv olive.

Locus	Repeat motif	Longth of bands in bp.	Na	H(0)	H(E)	PIC	DP
DCA5	(GA)15	(211-225) 219	4	0.7	0.734	0.726	0.735
DCA11	(GA)26(GGGA)4	(151-195) 195	5	0.95	0.963	0.554	0.727
DCA14	(CA)18(A)6(TAA)7	(187-204) 204	3	0.85	0.861	0.395	0.655
DCA3	(GA)19	(259-270) 261	4	0.75	0.788	0.742	0.682
DCA9	(GA)23	(187-209) 201	3	0.65	0.674	0.748	0.785
DCA16	(GT)13(GA)29	(165-192) 170	3	0.85	0.851	0.813	0.684
DCA18	(CA)4CT(CA)3(GA)19	(176-192) 184	3	0.8	0.801	0.569	0.651
DCA15	(CA)3G(AC)14	(261-284) 261	3	0.9	0.914	0.854	0.732
GAPU71B	GA(AG)6(AAG)8	(139-160) 139	4	0.6	0.612	0.754	0.675
GAPU89	(AG)16(G)3(GA)9	(175-220) 175	4	0.8	0.825	0.736	0.775
GAPU101	(GA)8(G)3(AG)3	(207-233) 209	3	0.5	0.509	0.399	0.645
UDO24	(CA)11(TA)2(CA)4	(186-209) 186	3	0.55	0.617	0.738	0.625
GAPU59	(CT)9	(223-233) 233	2	0.45	0.477	0.633	0.822
EMO90	(CA)10	(201-207) 203	2	0.4	0.411	0.725	0.654

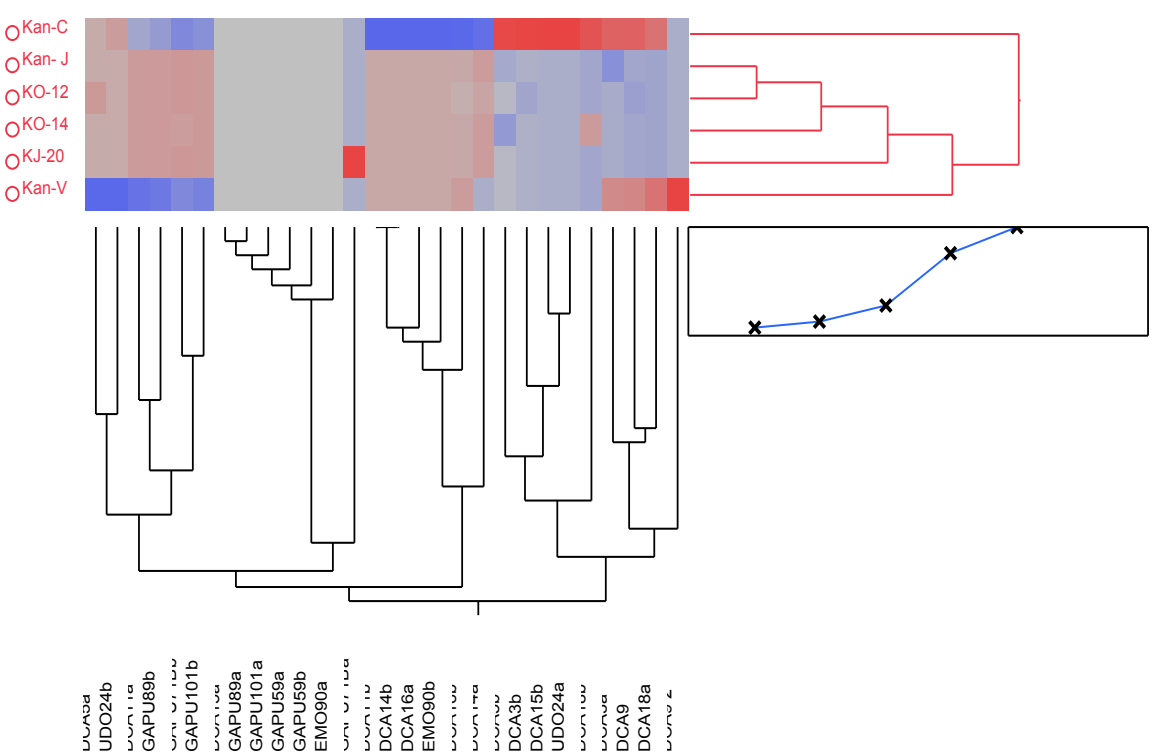

Fig. 2. Dendrogram generated by clustering UPMGA analysis based on Euclidean distance computed from pair wise comparisons of 14 markers SSR between clones of 'Kaninjot' cultivar.

Referring to Table-1, the level of information obtained by analyzing the 14 loci is high because the average value of polymorphic Information Content (PIC) varied from 0.395 (DCA14) to 0.854 (DCA15) with an average value of 0.670. The number of alleles for locus is on average 3.29; it varies from 5 alleles for locus 2 and has the lowest number of alleles in loci 13 and 14. The observed heterozygoteness is higher in locus 2 and lower in locus 14, with values respectively 0,963 and 0,411. The group of loci used in the analysis resulted to be more informative with an average value of PIC=0,670. Total probability (PI) resulted 2,78 $x10^{-9}$, whereas total exclusive probability (Pe) is 0,997. Analysis of values for total probability has proved that the clones have three unique individual identities, as probability of being identical is 1 in 359 million individuals,(1,2,4,6,9). Discrimination Power (DP) differs from 0.625 (UDO24) up to 0.822 (GAPU59) and average value of 0.703. Dendogram-2, for the analysis of the similarity matrix with the UPGMA method, shows the formation of three distinct clusters, in cutting the dendogram at the value of (0.17). Average variability with a molecular marker SSR is about 37%. Dissimilarity coefficients go up to 0,17-0,93, based on the dissimilarity matrix which revealed a good grade of suitability with the coefficient of dissimilarity (r = 0,9851; p <0.001). Considering the data obtained by the level of polymorphism the values go from 0.17 (DCA15) up to 0.93 (GAPU59). Thus genetic variation is located between the distances 0.17 up to 0.93 Figure-3. Dendogram generated by clustering UPMGA analysis, Clone Kan-C, has a long genetic distance (60.7%) from Kan-J (representative of the standard) whereas Kan-V is part of the second group with variability level of (39%). Analysis of the information of markers on variability highlights seven markers which possess the same genetic information for the three clones, whereas DCA9 polymorphic, unique, and homogenous for the three clones. The observed heterozygoteness (Ho) results high from 0.411 (EMO90) up to 0.963 (DCA11) with an average value of 0.716. Differentiation based on morphological features and microsatellite markers showed good correlation for the level of credibility in clone differentiation, (11). Analysis revealed a positive important correlation (r = 0,923, p <0.001), among the morphological and microsatellite indices. The values of morphological polymorphism display positive linear regression with the molecular polymorphism, (r^2=0.92), by proving statistically authenticity of clonal identity. It displayed linear correlation among SSR polymorphism (H(0) and H(e) as well as life morphological probability judging on the parameters β0, β1, σ and alpha=0.05. The structure of the variation serves the clone, calculated with the formula Nei and has a strong linear correlation between DNA polymorphism and morphological polymorphism r^2=0.84-0.93, (13). Selection of 14 SSR markers and 11 morphological markers derived from (PCA) was based not only on the high number of alleles, but also on their significant heterozygoteness, from 0.411 (EMO90) up to 1 per marker (DCA11), (7,10,11,12). Discrimination possibility (DP) has varied from 0.625 (marker UDO24) to 0.822 (marker GAPU59), which were caused and correlated by the morphological components possessing variability, found within the fruit, leaf and endocarp. (r = 0,923, p <0.001).

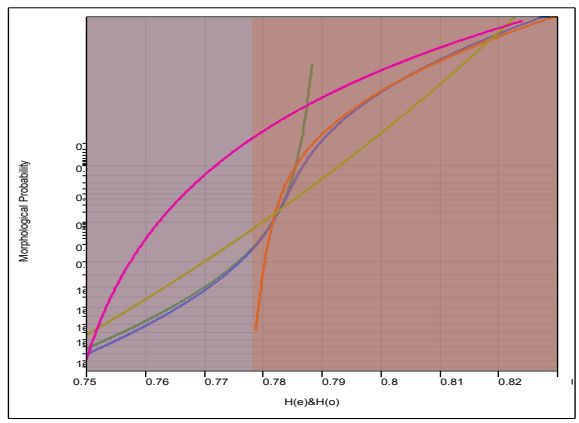

Fig-4, Dendogram for life orthogonal regression and Lognormal Results Statistics Estimates of H(0), H(e) and morphological probability.

Conclusions

Morphological indices result stable and with inter-clonal variation which have formulated three individual unique identities. Analysis of probability for the side effects on identity profiling displayed authenticity of the morphological profile.

All the double microsatellites used were successful and amplified corresponding fragments for all the clones. The data obtained by the analysis with SSR morphological markers, determined variability and produced a specific genetic profile per each clone, but with a common genetic basis. The results show that microsatellite markers were informative and differentiated 2 clones as new forms of cv. Kaninjot.

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