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Molecular characterization of an ancient Olea europaea tree located on the Brijuni islands of (Croatia) by SSR markers analysis

Molekularna karakterizacija starog stabla masline Olea europea na Brijunima analizom SSR markera

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SAŽETAK

Istraživanja obuhvaćaju molekularnu karakterizaciju 1600 godina starog stabla masline Olea europea na Brijunima analizom SSR markera. Provedene su izmjere svojstava ploda i lišća starog stabla masline Brijunke i standardne istarske sorte Buga (Buža). Rezultati istraživanja obrađeni su varijacijsko-statistički.

Ključne riječi: maslina, molekularna karakterizacija, SSR markeri

ABSTRACT

Investigations were carried out on molecular characterization of a 1600 years old olive tree located on the Brijuni islands (Croatia) by SSR markers analysis. Measurements of fruits and leaves features were carried out on the old tree Brijunka and on the standard Istrian cultivar Buga (Buža). Measurement data on fruits and leaves were calculated statistically.

Key words: olive tree, molecular characterisation, SSR markers

INTRODUCTION

The aim of the work was to achieve the molecular characterization of an olive tree of historical interest found on the Brijuni islands, Istrian archipelago (Adriatic Sea, Croatia).

From the available nature guide it is possible to deduce that the tree, the subject of this study is about 1600 years old. For the characterization and identification of the olive samples, the microsatellite markers (also called SSR, Simple sequence repeats) were used. The SSR represent, at this point, the most efficient and confident method for molecular characterization, being highly

polymorphic and reproducible (Baldoni et al. 2009). They allow obtaining simple profiles with one or two allelic forms for each cultivar.

The SSR analysis consists of the PCR amplification of fragments containing repeated dimers (known as microsatellites) by means of primers built on their flanking regions. On the basis of the number of repeats, fragments of varying lengths are amplified, which then can be separated and observed. The obtained fingerprint is characteristic and unique to each variety and constitutes its "bar-code". Those varieties to related each other would have similar, but not identical, fingerprints, whilst different trees of the same variety will have the equal fingerprints.

MATERIAL AND METHODS

Investigations were carried out on a 1600 years old olive tree located on the Brijuni islands (Croatia). The Brijuni are the climatic part of north Mediterranean with mild Mediterranean climate, lots of sun and warm weather, with plenty of humidity creating rich vegetation. The average air temperature in winter is 6.3°C, in spring 12.2°C, in summer 22.2°C and in autumn 14.8°C, The average annual precipitation is 817 millimetres with relatively high percent age of moisture in the air (76%). The soil is *terra rossa*, typical, luvic, anthropogenized, and medium deep. Ecological conditions for olive culture are very favourable (Miljković 1976; Miljković and Žužić 1987). Two samples collected from the foliage and from the trunk respectively of the olive tree investigated were analyzed. The DNA was extracted from the leaves using the kit GenElute (Sigma). Each sample was subjected to fingerprinting using the microsatellite markers. Sixteen highly polymorphic SSR loci, as resulting from previous investigations (Baldoni et al., 2009), were analyzed. It has to be taken into account that the use of only three loci (DCA9, DCA 16 and DCA17) is reported to be sufficient to discriminate between more than 100 varieties (Sarrie et al., 2006).

Name of locus	Authors
DCA3, DCA4, DCA5, DCA7, DCA9, DCA13, DCA15, DCA16, DCA17, DCA18	Sefc et al., 2000
GAPU45, GAPU71B, GAPU103A, GAPU101	Carriero et al., 2002
UDO43	Cipriani et al., 2002
EMO-90	de La Rosa et al., 2002

In order to verify possible and to examine the similarity level between the studied genotype and those present in neighboring areas or those of other ancient olive trees, the data were compared with those already available at the CNR Institute of Plant Genetics, Perugia. in particular the comparison was made with 28 samples from Albania and more than 80 varieties from Italy and other Mediterranean Countries.

Figure 1. Old olive tree on the Brijuni

RESULTS

Results of investigation of elaiographic features of fruits and leaves of the old olive tree Brijunka and the standard Istrian cultivar Buga (Buža) are presented in tables 1, 2 and 3 and illustrated in Fig. 2, 3, 4, 5, 6 and 7.

Table 1. Biometric characteristics of fruit of old olive tree Brijunka

Feature	$\bar{x}$ (mm)	Range of variation (mm)		St. dev. s	Variance s^2	cv
		Min.	Max.			
Length of fruit	15,80	14	17	0,913	0,833	5,78
Width of fruit	12,28	11	14	0,614	0,377	5,00
L/W	1,29	1,17	1,42	0,068	0,005	5,27
Weight of fruit	1,147	0,868	1,411	0,124	0,015	10,8
Weight of stone	0,322	0,219	0,399	0,039	0,002	12,1
Weight of flesh	0,825	0,649	1,018	0,089	0,008	10,8
Length of stone	12	11	13	0,640	0,410	5,33
Width of stone	8	7	9	0,5	0,25	6,25
L/W	1,51	1,33	1,71	0,087	0,008	5,76

Table 2. Biometric characteristics of fruit of old Istran olive cultivar Buga (Buža)

Feature	$\bar{x}$ (mm)	Range of variation (mm)		St. dev. s	Variance s^2	cv
		Min.	Max.			
Length of fruit	22,64	21	25	0,952	0,906	4,21
Width of fruit	20,60	20	22	0,614	0,037	2,81
L/W of fruit	1,10	1,05	1,20	0,041	0,002	3,73
Weight of fruit	4,897	4,261	5,794	0,408	0,167	8,33
Weight of stone	0,523	0,420	0,640	0,053	0,003	10,13
Weight of flesh	4,374	3,774	5,158	0,371	0,138	8,48
Length of stone	14,36	13	17	1,113	1,240	7,75
Width of stone	9,60	9	10	0,5	0,25	5,20
L/W of stone	1,50	1,30	1,78	0,129	0,17	8,60

Table 3. Biometric characteristics of leaves

Cultivar	Feature	$\bar{x}$ (mm)	Range of variation (mm)		St. dev. s	Variance s^2	cv
			Min.	Max.			
Old olive tree Brijunka	Length of leaf	50,76	46	56	3,004	9,023	5,92
	Width of leaf	13,4	12	16	1,190	1,417	8,88
Istrian cultivar Buga (Buža)	Length of leaf	57,20	48	76	5,923	35,083	10,35
	Width of leaf	13,9	9	21	3,807	14,490	27,35

Figure 2 Old olive tree Brijunka

Figure 3 Fruit and the stone of Brijunka

Figure 4 Leaves of Brijunka

Figure 5 Cultivar Buga (Buža)

Figure 6 Fruit and stone of Buga (Buža)

Figure 7 Leaves of Buga (Buža)

Greater variability properties of fruits were found in an old olive Brijunka than in the standard Istrian cultivar Buga (Buža). Buga has a much higher variability of length and width of the leaf than the old olive Brijunka as is evident from the values of *s*, s^2 and *cv*. The microsatellite alleles obtained from the SSR analysis are reported in Table 4. The profiles of the two parts of the plant were identical, showing that the plant was propagated using shoots or cuttings, and not by grafting (the rootstock would be different from the scion). This method of propagation brings the studied tree close to the ones from central/Northern Italy, that are usually never grafted, whilst those from Southern Italy and from the Islands are commonly grafted onto wild olive trees (Baldoni et al., 2006).

Table 4. Fingerprints (allelic length of the 16 SSR markers) of the two samples from the ancient olive tree located on the Brijuni (Croatia)

	DCA3	DCA4	DCA5	DCA7	DCA9	DCA13	DCA15	DCA16
Samples from crown	244-244	167-189	204-206	149-149	175-187	118-134	242-242	144-150
Samples from suckers	244-244	167-189	204-206	149-149	175-187	118-134	242-242	144-150
	DCA17	**DCA18**	**GAPU45**	**GAPU71b**	**GA101**	**GA103**	**UDO43**	**EMO-90**
Samples from crown	111-125	176-178	181-195	121-127	192-198	175-175	176-178	195-195
Samples from suckers	111-125	176-178	181-195	121-127	192-198	175-175	176-178	195-195

The comparison of the SSR profiles of the analyzed plant with those of the above indicated samples, has demonstrated that this genotype does not correspond with any of them. On the other hand, the sample has shown a significant similarity with the following sample:

- cv Dobrovnica (Slovenia)
- cv Tendellone (Italy, minor variety of Umbria)
- some ancient trees from Albania

Nevertheless, for some alleles it is possible to find differences in these samples as well, therefore it is possible to exclude identity with these cultivars and confirm only a significant relationship with them.

These results prove the uniqueness of the genotype analyzed and advise recovering and propagation of the old tree, aiming its conservation.

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