CHARACTERIZATION OF A PRIVATE GREEK OLIVE VARIETY COLLECTION USING MOLECULAR MARKERS

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Introduction

One of the most ancient cultivated fruit tree species in the Mediterranean Basin is olive. Its cultivation has a great socioeconomic impact for all countries present in the area. During the last decade non-Mediterranean countries are also trying to establish olive cultivation, mainly due to the unique nutritional value of the olive oil. The oldest evidence for olive tree cultivation in Greece was in Crete during the middle- Minoan period, 2.160-2000 BC, where olive seeds were found to be crashed. According to Tavanti, in ancient Greece, at least 15 cultivars have been described based on morphological appearance (Prevost and Mostardini, 1999). Nowadays Greece is considered as a secondary centre of diversity (Damania 1995). In a recent study, a high level of polymorphism was revealed among 19 major Greek cultivars along with eight cultivars with regional or ornamental importance (Hagidimitriou et al. 2005). However, at least 80 more less known cultivars are sporadically cultivated in different parts of Greece, some of which might be synonyms or clonal selections

In the present study we investigate the genetic diversity of a private collection with more than 100 entries collected all around Greece, including olive seedlings and species *Olea chrysophylla* and *O. cuspidata*, using three different molecular techniques, the randomly amplified polymorphic DNA (RAPD), the simple sequence repeats (SSRs) and the inter-simple sequence repeats (SSRs).

Materials and Methods

1.Plant material and DNA extraction.

A total of 101 *O. sativa* entries along with *O. chrysophylla* and *O. cuspidata* were included in the present study. DNA was extracted from healthy young leaves using the Invisorb Spin Plant Mini Kit (Invitek). DNA quality was tested in a 1% agarose gel electrophoresis and its concentration was determined spectrophotometrically.

2.PCR primers

From the 30 RAPD primers tested 10 were selected, along with seven SSR primer pairs and three ISSR.

3.Data analysis

The similarity matrix was calculated using the Jaccard's algorithm. The phenogram was constructed using the neighbour joining method. The analysis was performed using the NTSYS-pc v2.11x.

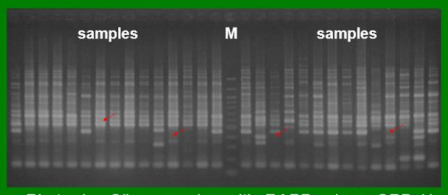

Photo 1. Olive samples with RAPD primer OPB-11, analyzing in 2,2%w/v agaroze gel, staining with ethidium bromide

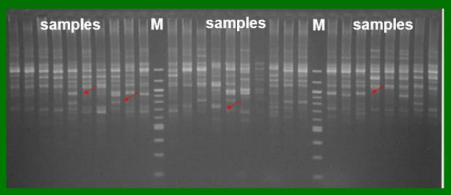

Photo 2. Olive samples with ISSR primer UBC-856, analyzing in 2,6%w/v agaroze gel, staining with ethidium bromide

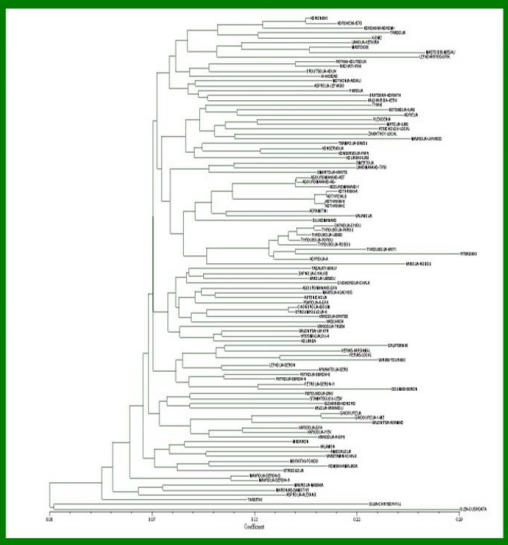

Figure 1. Cultivar relationships among 103 olive entries of Greek origin using RAPDs, SSRs and ISSRs data.

Table 1. Olive variety entries and species (numbers correspond to gel lanes).

	NAME		NAME		NAME		NAME
1	KORONEIKI	27	K-OM2	53	BRATSERA-KORINTHIAS	79	PSAROLIA-A-GPA
2	KORONEIKI-HSTO	28	AGOUROMANAKO-GPA	54	STAMATOGLOU-LESVOS	80	DAFNOLIA-CHALKIDIKIS
3	PATRINI-KOUTSOURELIA	29	AGOUROMANAKO-ASTROUS	55	KALOLIA-KARAKOLIA	81	SALONITSA-LEFKTRON
4	RACHATI-IYEK	30	AGOUROMANAKO-AG-MAMMA	56	PETROLIA-SERON-S	82	VROSIMI-GIALOU-A
5	LIANOLIA-KERKIRAS	31	AGOUROMANAKO-1	57	PETROLIA-SERON-H-1	83	KOLIBADA
6	MASTOIDIS	32	ADRAMITINI	58	PETROLIA-SERON-H-2	84	STROGILOLIA
7	MASTOIDIS-MEGALI-PAP	33	VALANOLIA	59	LEFKOLIA-SERON	85	KONSERVOLIA
8	THIAKI	34	DAFNOLIA-CHIOU	60	MAVROLIA-SERON-S	86	KONSERVOLIA-PAPACHR
9	PLEXIDENIA	35	THROUBOLIA-PAROU	61	MAVROLIA-SERON-H	87	VRASTAMINI-CHALKIDIKIS
10	SMERTOLIA	36	THROUBOLIA-LESBOU	62	ARVANITOLIA-SERON	88	KAROLIA-LESVOU
11	SMERTOLIA-KARITSIOTI	37	THROUBOLIA-RODOU	63	GOUMES-SERON	89	KALAMON
12	LIANOMANAKO-TIROU	38	THROUBOLIA-KRITIS	64	ASPROLIA-ALEXANDR	90	CHONDROLIACHALK-AG-MAMA
13	TRAGOLIA	39	THROUBOLIA-POROU	65	ASPROLIA-LEFKADOS	91	AETONICHOLIA
14	MAVROLIA-MESINIAS	40	PITSADEIKI	66	CHONDROLIA IGOUMENITSAS	92	SALONITSA-ADAMKO
15	LEFKOKARPOS-ORN.	41	KOPROLIA-A	67	STROUMPOULOLIA-KERKIRAS	93	KARIDOLIA-IYEK
16	*OLEA CHRYSOPHYLLA*	42	GLYKOMANAKO	68	KARIDOLIA-TRIZINIAS-LOCAL	94	KALOKAIRIDA-KERKIRAS
17	ATSICHOLOU-LOCAL	43	MEGARON	69	KARIDOLIA-SPETSON-LOCAL	95	BOTSIKOLIA-ILIAS
18	ZAKINTHOY-LOCAL	44	PIKROLIA	70	MORAITIKI-FOKIDOS	96	AGRIELIA
19	MAVROLIA-LEFKADOS	45	KAROLIA-RODOU	71	ROMEIKI-AMALIADAS	97	*OLEA CUSPIDATA*
20	MATOLIA-ILIAS	46	THASITIKI	72	MAKRILIA-VLACHOS	98	KORONEIKI-AGROMILLORA
21	PISTOUNOLIA-ZAKINTHOY	47	GALATSANIKI	73	AMIGDALOLIA	99	MARONIAS-SAMOTHRAKIS
22	TSAMPOLIA-DAVOU	48	VERIAS-KARDAMI-LOCAL	74	VASILIKADA	100	KOTHREIKI-A
23	BROUTSOLIA-KOLINON	49	PIERIAS-LOCAL	75	GAIDOURELIA	101	KOTHREIKI-B
24	N-K-GIGAS	50	VERIAS-TOURKIKI	76	GAIDOURELIA-1-MEGALO	102	KOTHREIKI-C
25	MOTHONIA-AIGIALIAS	51	KLONARES-KOROPIOU	77	KARIDOLIA-GPA	103	KOTHREIKI-D
26	TAGALATI -EARLY	52	KOLIREIKI-ILIAS	78	KARIDOLIA-A-GPA		

Results and Discussion

A total of 235 bands were scored, from which 165 were RAPD, 21 SSRs and 49 ISSRs. A single phenogram was constructed since the methods used multiply different parts of the genome. The genetic similarities ranged from 1.00 for 'Kothreiki B' and 'Kothreiki C' (two clonal selections) to 0.37 for *O. cuspidata* and 'Mastoides m-pap'. A high CCC was calculated revealing a good fitness of the genetic similarity matrix to the obtained phenogram. All *O. sativa* entries were grouped together connected to an outer branch containing the two species, *O.chrysophylla* and *O. cuspidata*, as expected (Figure 1). Entries having the same name but collected from different parts of Greece tended to be clustered together. Thus, 'Throumbolia' from the islands of Paros, Lesvos, Poros and Rhodos were clustered together, along with 'Dafnolia' from the island of Chios and 'Koprolia'. By comparing genetic similarities among these entries we can conclude that the latter two should be considered synonyms with the former. Accordingly, four entries of cultivar 'Kothreiki' are found clustered together, as well as two entries of 'Koroneiki', one of which is a product from tissue culture, and three entries of 'Agouromanako'. Thus, entries with genetic similarities higher than 0.90 could be identified as belonging to the same cultivar or be synonyms. In the same context, an entry identified as 'Agouromanako GPA' that was not found to be directly clustered to the rest of the entries with the same name and having a genetic similarity lower than 0.90 to other 'Agouromanako' entries, it can be considered as a misidentified entry. Two entries with different names, i.e. 'Stroumboulolia' from the island of Corfu and 'Chondrolia' from the port of Igoumenitsa, which is just across the island of Corfu, were found tightly clustered (genetic similarity 0,95), and can be considered as the same cultivar.

Literature Cited

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➢ Prevost, G and Mostardini, S. 1999. The scholarly study and botanical classification of the olive. Olivæ 78:60-77.