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Genetic diversity among different genotypes of *Pistacia lentiscus* var. *chia* (mastic tree)

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Abstract. The Mastic Tree, *Pistacia Lentiscus* var. *chia*, belongs to the Anacardiaceae family and is cultivated only on the southern part of the Greek island of Chios. The male tree gives a resin product known for its therapeutic qualities. Morphological characteristics suggest the existence of different genotypes that also differ in quantity and quality of the resin production. The four major genotypes of the male mastic tree, Votomos, Maroulitis, Mavroskinos and Siderakikos, have distinct phenotypes and can be easily identified. In the present study the genetic diversity between the different genotypes among the male mastic trees and female was studied using two different molecular marker techniques, RAPD and ISSR. Based on the molecular results of the present study, the entries show genetic diversity among the genotypes and within the different individuals. The female tree was grouped separately from the four different male genotypes.

Keywords. Anacardiaceae – *Pistacia Lentiscus* var. *chia* – Molecular markers – RAPD – ISSR – Genetic diversity.

Diversité génétique parmi différents génotypes de* Pistacia lentiscus *var. Chia (lentisque)

Résumé. *Le lentisque,* Pistacia lentiscus *var. chia, appartient à la famille des Anacardiaceae et est cultivé uniquement sur la partie sud de l'île grecque de Chios. L'arbre mâle donne une résine connue pour ses vertus thérapeutiques. Les caractéristiques morphologiques suggèrent l'existence de différents génotypes qui diffèrent aussi dans la quantité et la qualité de la production de résine. Les quatre principaux génotypes du lentisque mâle, Votomos, Maroulitis, Mavroskinos et Siderakikos, présentent des phénotypes distincts et peuvent être facilement identifiés. Dans la présente étude, la diversité génétique entre les différents génotypes parmi les arbres de lentisque mâles et femelles a été étudiée en utilisant deux techniques différentes de marqueurs moléculaires, RAPD et ISSR. Sur la base des résultats moléculaires de la présente étude, les données montrent la diversité génétique entre les génotypes et entre les différents individus. L'arbre femelle a été regroupé séparément des quatre génotypes mâles différents.*

Mots-clés. *Anacardiaceae* – Pistacia lentiscus *var.* chia – *Marqueurs moléculaires – RAPD – ISSR – Diversité génétique.*

I – Introduction

The Mastic Tree, *Pistacia lentiscus* var. *chia*, belongs to the Anacardiaceae family and is cultivated only on the southern part of the Greek island of Chios (Zohary, 1952; Browicz, 1987). Fossils of mastic tree leaves found on this island shows its existence on the island six thousand years ago suggesting that it is a native plant. Herodotos, on the 5th century B.C, mentions that the resin product from the phloem of the mastic tree was used for chewing (Savidis, 2000). The use of the mastic resin was widely spread on the 10th century B.C. The mastic tree is an evergreen shrub, resistant to dryness with a slow growth and lives over a hundred years. The male tree gives the highest and best quality resin, starting on the 5th-6th year with highest production on the 15th year. Attempts to cultivate and produce the high quality resin produced from these trees in other areas of the world were unsuccessful. Morphological characteristics suggest the existence of different male genotypes that also differ in quantity and quality of the resin production. The aim of the present research was to study the genetic diversity between the different genotypes among the male mastic trees and the female using two different

molecular marker techniques, RAPD and ISSR, which have been used for studying the genetic relations in *Pistacia* species (Caruso *et al.*, 1998; Hormaza *et al*., 1998; Katsiotis *et al.*, 2003; Golan-Goldhirsh *et al.*, 2004).

II – Materials and methods

Plant material from four different male genotypes and a female tree were collected from the island of Chios (Table 1). A total of 11 subsamples were used, from which one was female. DNA from young leaves was extracted using the Invisorb Spin Plant Mini Kit, Invitek. Ten RAPD Primers were tested and six of them were used (Table 2). PCR-RAPD mixture contained: 0.27 mM dNTPS, 1xBuffer, 2.0 mM $MgCl_2$, 0.3 μM RAPD Primer, 1 U Taq and 50-70 ngr DNA. The final volume of the reaction was 25 μl. PCR-RAPD program contained: 94°C for 2 minutes and 30 cycles of 94°C for 45 sec, 40°C for 1 min and 72°C for 2 min. Final extension was at 72°C for 7 min. Additionally, six ISSR primers were tested and three of them were used (Table 2). PCR-ISSR mixture contained: 0.3 mM dNTPS, 1xBuffer, 2.0 mM $MgCl_2$, 0,1μM ISSR primer, 1 U Taq and 15-30 ngr DNA. The final volume of the reaction was 25 μl. PCR-ISSR program contained: 94°C for 7 minutes and 45 cycles of 94°C for 45 sec, 52°C for 45 sec, 72°C for 2 min. Final extension was at 72°C for 7 min. PCR products were separated in 1,5% and 2,5% agarose gels for RAPDs and ISSR, respectively, and digitally photographed under UV light (Fig. 1). Data were analysed using the NTSYSpc 2.02i software (Rolf, 1998). The genetic similarities were calculated using the Jaccard algorithm and the phenogram was constructed using UPGMA (Unweighted Pair Group Method with Arithmetic means) and PCOORDA (Principal Coordinate Analysis).

Table 1. Different genotypes and subsamples used in the study

	Genotypes	Subsamples	
1.	Votomos	Votomos1	Male
2.		Votomos2	
3.	Maroulitis	Maroulitis1	
4.		Maroulitis2	
5.		Maroulitis3	
6.	Mavroskinos	Mavroskinos1	
7.		Mavroskinos2	
8.		Mavroskinos3	
9.	Siderakikos	Siderakikos1	
10.		Siderakikos2	
11.	Female	Female	Female

III – Results and discussion

Ten RAPD and six ISSR markers were tested and six RAPD and tree ISSR markers were finally used giving 115 polymorphic bands out of a total of 121 (Table 2). Genetic similarities among the entries ranged from 0.682 (Siderakikos-1 & Votomos-2) to 0.125 (Female & Maroulitis-1) (Fig. 1). RAPD primers produce 50 bands with three of them monomorphic (6%). The number of produced bands per RAPD primer ranged from 5 (RI-4 and OPG-2) to 13 (OPI-4) (Fig. 1). Genetic similarities among the entries using RAPD ranged from 0.999 (Siderakikos-1 & Siderakikos-2) to 0.251 (Female & Votomos-2). ISSR primers produce 71 bands with two of them monomorphic (3%). The number of produced bands per ISSR primer ranged from 20 (842 UBC) to 26 (856 UBC). Genetic similarities among the entries using ISSR ranged from 0.682

(Votomos-2 & Mavroskinos-3) to 0.125 (Female & Maroulitis-1). The four major genotypes of the mastic tree, Votomos, Maroulitis, Mavroskinos and Siderakikos, have distinct phenotypes and can be easily identified. Based on the molecular results of the present study, the entries show genetic diversity among the genotypes (Figs 2 and 3). The female tree was grouped separately from the four different male genotypes. RAPD primers group the male entries according to their genotypes, while ISSR differentiate individual entries belonging to the same genotype. The tree first dimensions of PCOORDA explain 0.625 of total variability, which means that the entries are separated very well with RAPD and ISSR techniques, and they show genetic diversity. All analysis methods (UPGMA-PCOORDA) group individuals in similar groups.

Table 2. RAPD and ISSR primers used in the study, number of produced bands and number of monomorphic bands per primer

	Primer	Number of bands	Number of monomorphic bands
RAPD			
1.	OPRI– 04	5	1
2.	OPRI– 14	7	0
3.	OPRI– 16	9	1
4.	OPI– 04	13	0
5.	OPG– 02	5	0
6.	RAPD-08	11	1
RAPD total		50	3
ISSR			
1.	842	20	1
2.	850	25	1
3.	856	26	
ISSR total		71	2
Total of RAPD and ISSR		121	5

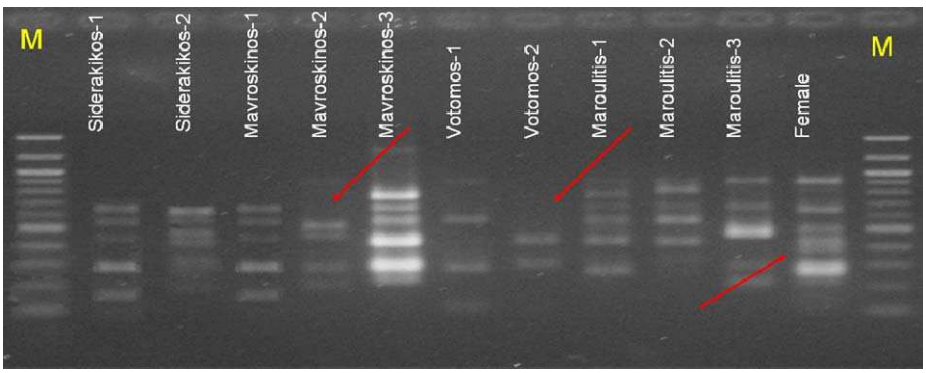

Fig. 1. RAPD primer RI-16. Letter "M" marks the 100bp DNA ladder. Red arrows mark differences between individuals.

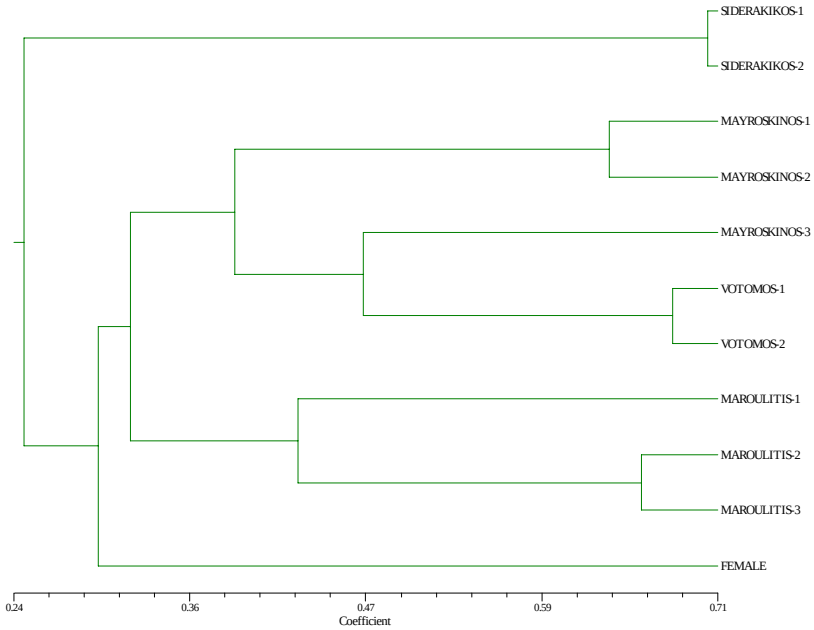

Fig. 2. UPGMA dendrogram using both RAPD and ISSR primers data.

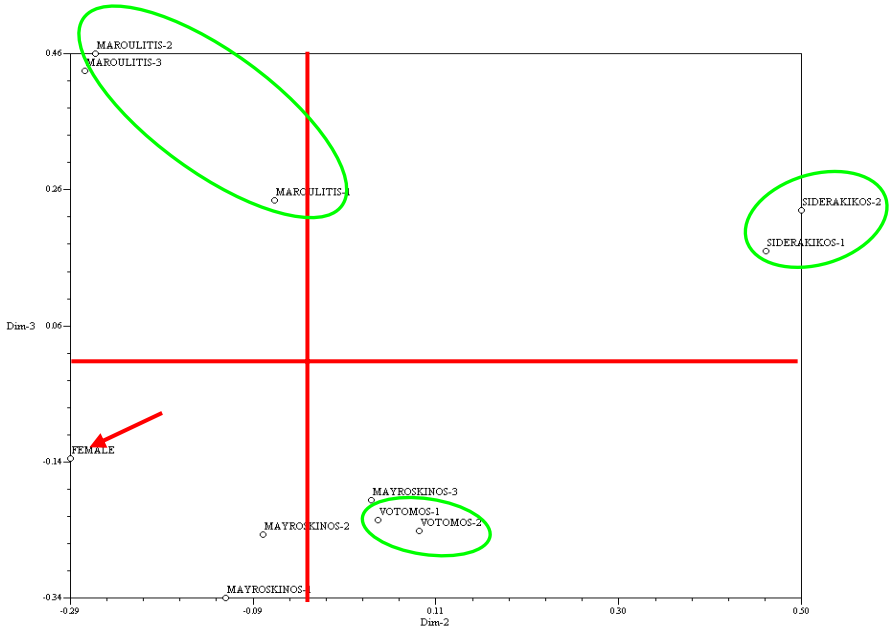

Fig. 3. 2D dimension phenogram of PCOORDA clustering method using data from both RAPD and ISSR primers, showing grouping and differences between individuals.

Conclusions

Based on the molecular results of the present study, the four major male genotypes of the mastic tree, Votomos, Maroulitis, Mavroskinos and Siderakikos, show genetic diversity. Mavroskinos and Votomos are closer genetically related while Siderakikos is more distinct from the other male genotypes (Figs 2 and 3). The female tree was grouped separately from the four different male genotypes. RAPD primers group the male entries according to their genotypes, while ISSR differentiate individual entries belonging to the same genotype. All analysis methods (UPGMA-PCOORDA) group individuals in similar groups.

References

Browicz K., 1987. *Pistacia Lentiscus* cv. *Chia (*Anacardiaceae) on Chios island. In: *Pl. Syst. Evol*., 155, p. 189-195.

Caruso T., Barone E., Zakynthinos G., Iannini C., Monastra F., Marra F.P., Batlle I., Vargas F., Romero M., Padulosi S., Greco C.I., Sabina M.R., Martelli G., Ak B.E. and Laghezali M., 1998. Genetic andphenotypic diversity in pistachio (*Pistacia vera* L.) germplasm collected in Mediterranean countries. In: *Acta Hort*., 470, p. 168-78.

Golan-Goldhirsh A., Barazani O., Wang Z.S., Khadka D.K., Saunders J.A., Kostiukovsky V. andRowland L.J., 2004. Genetic relationships among Mediterranean *Pistacia* species evaluated by RAPD and AFLP markers. In: *Plant Syst. Evol.,* 246, p. 9-18.

Hormaza J.I., Dollo L. and Polito V.S., 1998. Genetic diversity of pistachio (*Pistacia vera,* Anacardiaceae) germplasm based on Randomly Amplified Polymorphic DNA (RAPD) markers. In: *Econ. Bot*., 52, p. 78-87.

Katsiotis A., Hagidimitriou M., Drossou A., Pontikis C. and Loukas M., 2003. Genetic relationships among species and cultivars of *Pistacia* using RAPDs and AFLPs. In: *Euphytica,* 132, p. 279-286.

Rohlf M., 1998. NTSYSpc. Numerical Taxonomy and Multivariate Analysis System. Version 2.02i. Department of Ecology and Evolution. State University of New York.

Savidis T., 2000. The mastic tree of Chios (*Pistacia Lentiscus* var*. chia)*. Thessaloniki-Greece: Kyriakides Bros.

Zohary M., 1952. A monographic study of the genus *Pistacia.* In: *Palestine J. Bot. Jerusalem Ser.*, 5, p. 187-228.