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Characterization of pistachio cultivars using isozymes

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SUMMARY - Leaf samples of 55 pistachio (*Pistacia vera* L.) cultivars from 13 countries were analysed using horizontal starch gel electrophoresis to study isoenzymatic variability for seven isoenzyme systems: aconitase (ACO), esterase (EST), glutamate oxalacetate transaminase (GOT), glucose phosphate isomerase (GPI), malate dehydrogenase (MDH), phosphoglucomutase (PGM) and shikimate dehydrogenase (SKD). Polymorphism was observed in five of them (ACO, EST, GPI, PGM and SKD). GPI showing 8 different phenotypes was the most useful for the characterization of cultivars. Twelve different combinations for female cultivars and 2 for male cultivars were found. Two female cultivars ('Bianca' and 'Red Jalap') and 4 male types ('A', 'C-Especial', 'M-P3' and 'M-P9') were identified as being of hybrid origin. No pattern of geographic distribution of isozyme variation in this species was observed.

Key words: *Pistacia vera* L., pistachio, electrophoresis, isoenzymes, cultivar, characterization.

RESUME - "Caractérisation de cultivars de pistachier en utilisant des isozymes". Des feuilles de 55 variétés de pistachier (*Pistacia vera* L.), provenant de 13 pays, ont été analysées par électrophorèse avec la finalité d'étudier la variabilité isoenzymatique de cette espèce. Sept systèmes ont été utilisés : aconitase (ACO), estérase (EST), glutamate oxalo-acétate transaminase (GOT), glucose phosphate isomérase (GPI), malate déshydrogénase (MDH), phosphoglucomutase (PGM) et shikimate déshydrogénase (SDH). Cinq systèmes (ACO, EST, GPI, PGM et SDH) ont montré du polymorphisme. La GPI, avec 8 phénotypes différents, s'est avérée le système enzymatique le plus discriminant et le plus utile pour la caractérisation variétale. Les variétés femelles se sont groupées en 12 combinaisons différentes, et les mâles en 2. Deux cultivars femelles ('Bianca' et 'Red Jalap') et 4 types mâles ('A', 'C-Especial', 'M-P3' et 'M-P9'), ont été identifiés comme étant d'origine hybride. Aucune distribution géographique isoenzymatique de cette espèce n'a été observée.

Mots-clés : *Pistacia vera* L., pistachier, électrophorèse, isoenzymes, cultivar, caractérisation.

Introduction

Pistachio (*Pistacia vera* L.) is a dioecious wind pollinated tree, female cultivars have been traditionally interplanted with males. *P. vera* is native of north-east Iran, north Afghanistan and middle Asian republics (Zohary and Hopf, 1994). This species grows under harsh climatic conditions of high temperatures and no rainfall during summer and low temperatures during winter. However, in several producing countries, orchards are under irrigated conditions. It is grown commercially mainly in Iran, Turkey, United States, Syria, Greece and Italy. In Spain, it was reintroduced in the 1980's and there are now some 800 ha, distributed mainly in Catalonia (Batlle *et al.*, 1996). Less than 100 cultivars have been reported worldwide (Maggs, 1973). IRTA's cultivars pistachio collection located in Reus (North-East of Spain) with 75 cultivars (50 female and 25 male) from 13 countries is one of the largest of this xerophytic tree in the Mediterranean basin (Vargas *et al.*, 1995).

Studies on the identification of *P. vera* cultivars using isozymes have been carried out (Richarte, 1989; Barone *et al.*, 1996), both showing polymorphism in male and female types. Studies based on different *Pistacia spp.* (Loukas and Pontikis, 1979; Parfitt and Badenes, 1993; Rovira *et al.*, 1995; Dollo, 1996) are also known. Rovira *et al.* (1995) identified different *Pistacia spp.* by the zymogram phenotypes of aconitase (ACO), esterase (EST), glutamate oxalacetate transaminase (GOT), glucose phosphate isomerase (GPI), malate dehydrogenase (MDH), phosphoglucomutase (PGM) and shikimate dehydrogenase (SKD). This work showed that 2 enzymatic systems, GOT and MDH, were invariable in *P. vera*, and that bands on the gel were localized in a different position from other *Pistacia spp.* This paper accounts the first characterization of 55 cultivars of *P. vera*.

Materials and methods

Fifty five cultivars (36 females and 19 males) from 13 countries, located in the *Pistacia* germplasm collection held at IRTA-Mas Bové, were analysed. Samples were collected from actively expanding leaves, either from cuttings growing in a controlled chamber or from field trees. About 0.02 g of these leaves were crushed in 100 µl of extraction buffer (Tous *et al.*, 1992). Electrophoresis procedures are described in Rovira *et al.* (1995). The isoenzyme systems studied were: ACO, EST, GOT, GPI, MDH, PGM and SKD. Staining procedures for ACO, GOT, GPI, MDH and PGM can be found in Rovira *et al.* (1993); for SKD in Arús *et al.* (1994) and for EST in Rovira *et al.* (1995). Four gel electrode buffers were used: histidine-citrate 5.7 (HC 5.7) for SKD; histidine 7.0 (H 7.0) for ACO, MDH and PGM; tris-citrate 7.0 (TC 7.0) for GPI and tris-citrate 7.8 (TC 7.8) for GOT and EST. HC 5.7 is described in Rovira *et al.* (1993); H 7.0 corresponds to buffer E described by Shields *et al.* (1983); TC 7.0 and TC 7.8 can be found in Rovira *et al.* (1995).

Results and discussion

The systems with a good resolution and intensely stained bands on the zymogrames were GOT, GPI, PGM and SKD. Nevertheless zymogrames of ACO, EST and SKD were more difficult to be interpreted due to their poor intensity of the bands. Tables 1 and 2 show the enzymatic characterization of different *P. vera* cultivars (female and male, respectively) for these 7 enzymatic systems.

Aconitase (ACO). Two zones of activity (ACO-1 and ACO-2) were clearly detected, showing different bands in each one (Fig. 1). Activity was poor in all samples, so only some cultivars were characterized by this enzymatic system. In the most cathodal zone (ACO-2) was more difficult to distinguish the different bands.

Esterase (EST). This enzymatic system showed four zones of activity, but only the most clearly stained (EST-1) was polymorphic, presenting 2 different phenotypes (Fig. 2). Not all the samples analysed showed a good activity. Female cultivars that could be scored had all the same phenotype (A). The B phenotype appeared only once in a male cultivar ('C').

Glucose-6-phosphate isomerase (GPI). This system was the most polymorphic of the seven enzymatic systems analysed in *P. vera*: 8 different phenotypes could be observed in GPI-2 zone, being GPI-1 (the most anodal one) monomorphic (Fig. 3). 'Boundoky', 'Cerasola' and 'Gialla' female cultivars, presented a unique phenotype for GPI-2 (D, F and G, respectively).

Fosfoglucomutase (PGM). Two different phenotypes appeared for this enzymatic system presenting 1 or 2 bands in one zone of activity (Fig. 4). Most cultivars analysed had the same phenotype (A). Only 2 female cultivars, 'Avidon' and 'Joley', differed from this phenotype. The male cultivar 'A' was detected as an hybrid due to the different position of the bands on the gel (Rovira *et al.*, 1995).

Shikimate dehydrogenase (SKD). Two zones of activity with 2 different phenotypes in the most anodal one could be observed (Fig. 5), but there was difficult to read them because of the poor activity of the samples analysed. There was not possible to know the SKD phenotype of most of the cultivars studied.

Glutamate oxalacetate transaminase (GOT). Three zones of activity were observed (Fig. 6) without any variation as was seen in an earlier work (Rovira *et al.*, 1995). From samples giving good activity, 2 female cultivars ('Bianca' and 'Red Jalap') and 3 males ('C-Especial', 'M-P3' and 'MP-9') could be detected as being of hybrid origin.

Malate dehydrogenase (MDH). This enzymatic system was invariable in *P. vera* as was already observed in previous studies (Rovira *et al.*, 1995). Two zones of activity were observed, MDH-1 and MDH-2 (Fig. 7). The male cultivar 'A', was detected as an hybrid due to its different phenotype.

Table 1. Phenotypes of female cultivars of *P. vera* for different enzymatic systems

Cultivar	Origin [†]	Enzymatic systems							
		ACO-1	ACO-2	EST	GPI	PGM	SKD	GOT ^{††}	MDH ^{††}
'Aegina'	(GR)	A	-	A	A	A	A	t	t
'Ashoury'	(SY)	-	-	A	A	A	B	t	t
'Avdat'	(IS)	A	-	A	A	A	B	t	t
'Avidon'	(IS)	A	-	A	C	B	A	t	t
'Bianca'	(IT)	-	-	A	B	A	-	h	t
'Bianca Regina'	(IT)	-	-	A	C	A	-	t	t
'Boundoky'	(SY)	-	-	A	D	A	-	t	t
'Bronte'	(IT)	-	A	A	B	A	-	t	t
'Capuccia'	(IT)	-	-	-	E	A	-	t	t
'Cavaillon'	(FR)	-	-	-	A	A	-	-	t
'Cerasola'	(IT)	-	-	-	F	A	-	-	t
'El Guetar'	(TUN)	-	-	-	-	A	-	t	-
'Ghiandolara'	(IT)	B	-	A	H	A	B	t	t
'Gialla'	(IT)	-	-	-	G	A	B	-	-
'Insolia'	(IT)	B	-	-	E	A	-	t	t
'Irak-1'	(IR)	A	-	-	-	A	-	t	t
'Irak-2'	(IR)	-	-	A	B	A	B	t	t
'Joley'	(US)	B	B	A	A	B	B	t	t
'Kastel'	(IS)	-	-	-	A	A	-	-	t
'Kerman'	(US)	B	-	A	A	A	B	t	t
'Larnaka'	(CY)	A	-	A	A	A	-	t	t
'Lassen'	(US)	-	-	-	A	A	-	t	t
'Lathwardy'	(SY)	A	-	A	B	A	-	t	t
'Marawhy'	(SY)	-	-	A	H	A	-	t	t
'Mateur'	(TUN)	A	-	A	A	A	-	t	t
'Muntaz'	(IRA)	-	-	A	B	A	-	t	t
'Ouleymy'	(SY)	-	A	A	B	A	-	t	t
'Pignatone'	(IT)	A	-	A	C	A	B	-	t
'Red Aleppo'	(US)	A	-	A	A	A	B	t	t
'Red Jalap'	(SY)	-	-	-	A	A	B	h	t
'Safeed'	(IRA)	-	-	-	A	A	B	-	t
'Sirora'	(AUS)	B	-	-	A	A	-	t	t
'Sfax'	(US)	-	-	A	A	A	-	t	t
'Silvana'	(IT)	B	-	-	E	A	-	t	-
'Tardiva di Serradifalco'	(IT)	-	-	-	E	A	-	-	-
'White Ouleymy'	(SY)	A	-	A	A	A	A	t	t

[†](AUS): Australia; (CY): Cyprus; (FR): France; (GR): Greece; (IR): Irak; (IRA): Iran; (IS): Israel; (IT): Italy; (SY): Syria; (TU): Turkey; (TUN): Tunisia; (US): United States

^{††}GOT and MDH systems were invariable in *P. vera*

t: true to type; h: hybrid; - no score

In this work, the hybrid origin of some female and male cultivars held in IRTA's collection could be clarified. Two female cultivars presented different phenotype for GOT system (invariable for the other cultivars of *P. vera*): 'Bianca' from Italy and 'Red Jalap' from Syria (Table 1). Four male cultivars were also detected as hybrids (Table 2): 'A' (from Greece) due to the phenotype presented for PGM and MDH systems, 'C-Especial' (from Cyprus), 'MP-3' and 'MP-9' (selected at IRTA from Sicilian seeds) by their phenotype for GOT system. These 4 males presented also differences in some morphological traits from *P. vera* (leaves, shoots, etc.). Tree habit and leaves of 'MP-3' and 'MP-9' resemble *P. terebinthus*.

The enzymatic characterization allow to group female cultivars in 12 different combinations and male cultivars in 2 (Tables 3 and 5, respectively). Female and male cultivars which can belong to different groups are shown in Tables 4 and 6, respectively. Five female cultivars ('Avidon' from Israel,

'Boundoky' from Syria, 'Cerasola' and 'Gialla' from Italy and 'Joley' from US), were identified as having a unique phenotype.

Table 2. Phenotypes of male cultivars of *P. vera* for different enzymatic systems

Cultivar	Origin [†]	Enzymatic systems						
		ACO-1	EST	GPI	PGM	SKD	GOT ^{††}	MDH ^{††}
'A'	(GR)	-	-	-	h	A	-	h
'Ask'	(IS)	-	-	-	A	-	-	-
'C'	(GR)	B	B	A	A	B	-	t
'C-Especial'	(CY)	-	-	A	A	-	h	-
'Enk'	(IS)	-	-	A	A	A	-	t
'M-P3'	(SP)	-	-	B	A	-	h	-
'M-P9'	(SP)	-	-	A	A	-	h	-
'M-3'	(IT)	B	-	A	A	-	-	-
'M-8'	(IT)	-	-	-	A	-	-	-
'M-11'	(SY)	-	-	-	A	-	-	-
'M-36'	(SY)	-	-	A	-	-	-	-
'M-38'	(SY)	B	-	-	A	-	-	-
'M-57'	(SY)	A	-	-	A	A	-	-
'M-502'	(IT)	-	-	A	A	-	-	t
'Naz'	(IS)	-	-	-	A	-	-	t
'PetersT.41'	(US)	-	-	A	A	-	-	-
'PetersT.71'	(US)	-	-	-	A	-	-	t
'25A'	(TUN)	B	-	A	A	-	-	-
'40A'	(TUN)	-	-	A	A	-	-	-

[†](CY): Cyprus; (GR): Greece; (IS): Israel; (IT): Italy; (SY): Syria; (SP): Spain; (TUN): Tunisia; (US): United States

^{††}GOT and MDH systems were invariable in *P. vera*

t: true to type; h: hybrid; - no score

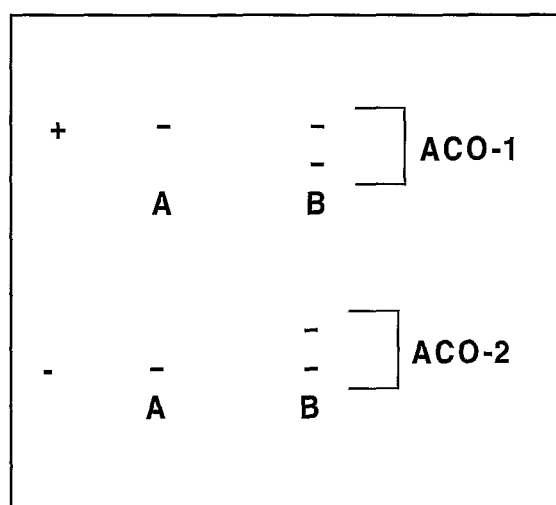


Fig. 1. Schematic zymogram for ACO.

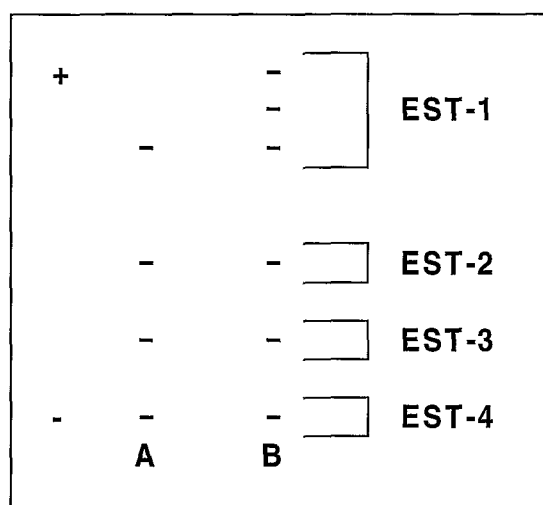


Fig. 2. Schematic zymogram for EST.

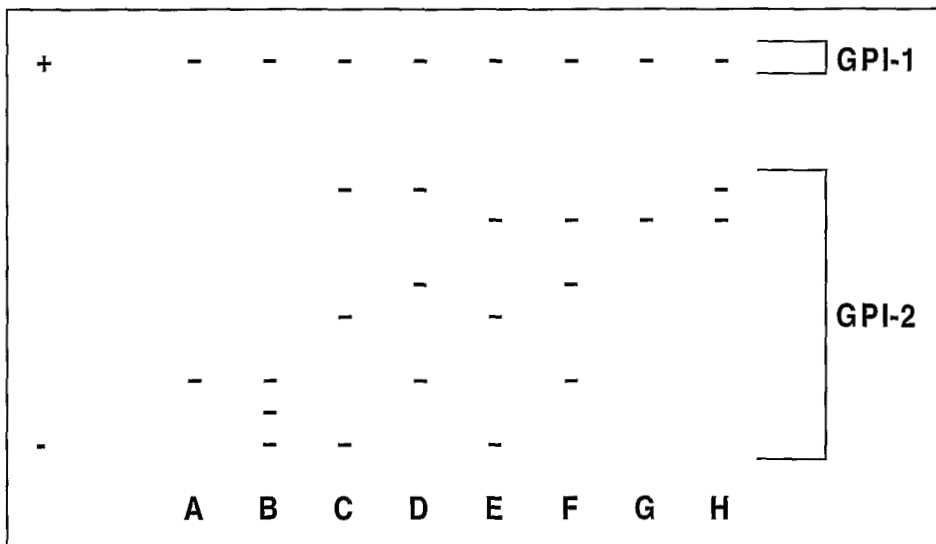


Fig. 3. Schematic zymogram for GPI.

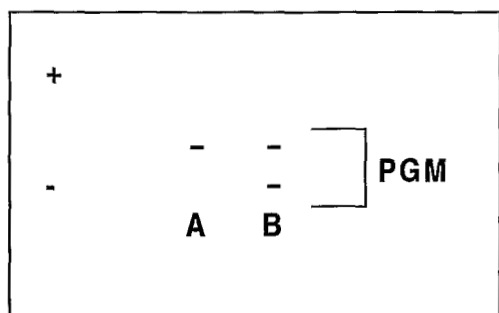


Fig. 4. Schematic zymogram for PGM.

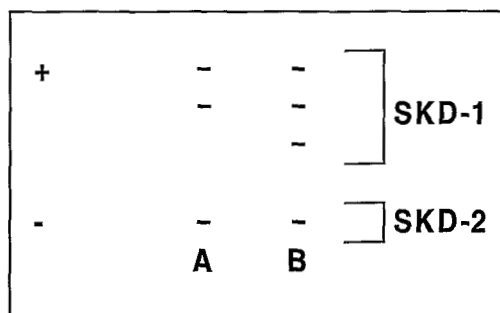


Fig. 5. Schematic zymogram for SKD.

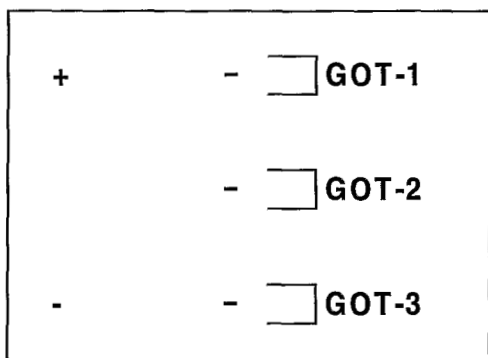


Fig. 6. Schematic zymogram for GOT.

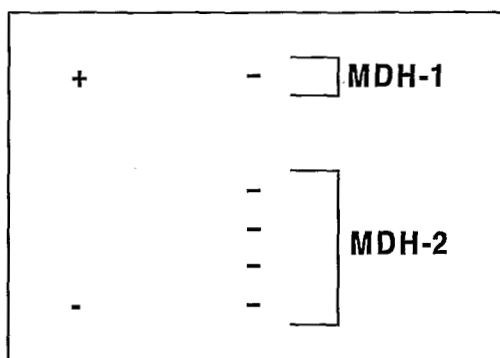


Fig. 7. Schematic zymogram for MDH.

Table 3. Groups of female cultivars of *P. vera* according their enzymatic phenotypes

Groups	Enzymatic phenotypes						Cultivars [†]
	ACO-1	ACO-2	EST	GPI	PGM	SKD	
1	A	-	A	A	A	A	'Aegina', 'White Ouleymy'
2	A	-	A	A	A	B	'Avdat', 'Red Aleppo'
3	A	-	A	C	B	A	'Avidon'
4	A	-	A	C	A	B	'Pignatone'
	-	-	A	C	A	-	'Bianca Regina'
5	-	-	A	D	A	-	'Boundoky'
6	-	A	A	B	A	-	'Bronte', 'Ouleymy'
	-	-	A	B	A	B	'Irak-2'
	A	-	A	B	A	-	'Lathwardy'
	-	-	A	B	A	-	'Muntaz'
7	B	-	-	E	A	-	'Insolia', 'Silvana'
	-	-	-	E	A	-	'Cappucia', 'Tardiva di Serradifalco'
8	-	-	-	F	A	-	'Cerasola'
9	-	-	-	G	A	B	'Gialla'
10	B	-	A	H	A	B	'Ghiandolara'
	-	-	A	H	A	-	'Marawhy'
11	B	B	A	A	B	B	'Joley'
12	B	-	A	A	A	B	'Kerman'
	B	-	-	A	A	-	'Sirora'

[†]Hybrid cultivars from Table 1 are not considered
- no score

 Table 4. Female cultivars of *P. vera* that can belong to different groups

Cultivars	Enzymatic phenotypes						Possible groups
	ACO-1	ACO-2	EST	GPI	PGM	SKD	
'Irak-1'	A	-	-	-	A	-	1,2,4,5,6,8,9
'Larnaka', 'Mateur'	A	-	A	A	A	-	1,2
'Silvana'	-	-	A	A	A	-	1,2,12
'Cavaillon', 'Kastel', 'Lassen'	-	-	-	A	A	-	1,2,12
'El Guetar'	-	-	-	-	A	-	1,2,4,5,6,7,8,9,10,12
'Ashoury'	-	-	A	A	A	B	2,12
'Safeed'	-	-	-	A	A	B	2,12

Table 5. Groups of male cultivars of *P. vera* according their enzymatic phenotypes

Groups	Enzymatic phenotypes					Cultivars [†]
	ACO-1	EST	GPI	PGM	SKD	
1	B	B	A	A	B	'C'
	B	-	A	A	-	'M-3', '25A'
	B	-	-	A	-	'M-38'
2	-	-	A	A	A	'Enk'
	A	-	-	A	A	'M-57'

[†]Hybrid cultivars from Table 2 are not considered
 - no score

Table 6. Male cultivars of *P. vera* that can belong to groups 1 or 2

Cultivars	Enzymatic phenotypes				
	ACO-1	EST	GPI	PGM	SKD
'M-502', 'Peters T.41', '40A'	-	-	A	A	-
'Ask', 'M-8', 'M-11', 'Naz', 'Peters T.71'	-	-	A	-	-
'M-36'	-	A	-	-	-

Conclusions

The isoenzymic polymorphism observed in *P. vera* was useful to characterize pistachio cultivars. Fifty five cultivars (36 females and 19 males) were analysed isoenzymatically for seven systems (ACO, EST, GPI, PGM, SKD, GOT and MDH), being the GPI the most polymorphic (8 different phenotypes) and the most useful for cultivar characterization. Within the cultivars, 12 different combinations were observed, having 5 cultivars a unique phenotype. Only 2 different groups were observed for male cultivars. In addition, 6 cultivars (2 female and 4 male), could be detected as being of hybrid origin.

Although this work allowed the enzymatic characterization of some cultivars, gel staining for all the enzyme systems was not consistently obtained. It would be useful in further studies to try with leaf samples at different growing stages to determine the best time of sampling. Further electrophoresis analysis is envisaged to complete cultivar identification.

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