Identification of Loquat Cultivars by Fruit, Leaf and Endosperm Isozymes

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Abstract

In this experiment, genetic differences of 18 cultivars and/or ecotypes were detected by isozymes. Mature leaves, fruits and endosperms of loquat cultivars and some of their ecotypes were obtained from Citrus and Greenhouse Crops Research Institute in Antalya, Turkey. All samples were taken early in the morning and analysed in the same day.

By using vertical polyacrylamid slab gel electrophoresis (PAGE), isozymes banding patterns for alcohol dehydrogenase (ADH), catechol oxidase (CO), glutamate oxaloacetate transaminase (GOT), acide phosphatase (HP), and peroxidase (PER) in fruit and endosperms were analysed. All enzyme systems were also analysed in the leaves but ADH was not detected in this plant tissue.

As a result of this experiment, for ADH, all cultivars had the same banding patterns in fruits. Some biotypes within the same cultivars such as Yuvarlak Çukurgöbek and Tanaka had different banding patterns of endosperm. In CO enzyme system extracted from leaves, fruits and endosperms, had different banding patterns. All cultivars had the same banding of GOT in each of the plant tissue. But band patterns changed among tissues. In HP, three patterns were observed in the endosperm. On the other hand, leaves and fruits of loquat cultivars had only one isozyme pattern with five bands. There were one banding pattern with two bands for PER in loquat fruits, while three distinct banding patterns were observed in endosperms and leaves. Akko 1 and Akko 13 can be easily distinguished with PER isozymes of leaves, whereas Tanaka and Yuvarlak Çukurgöbek had the same banding patterns for PER in the each of three plant tissues.

Isozymic differences between cultivars suggested that isozymes may provide useful markers for cultivars identification in loquat.

INTRODUCTION

Loquat is a subtropical fruit and native to far east. It is introduced to Mediterranean basin in century. Loquat has a large number and volume of seeds and fruit flesh (periderm) is rather small. But its production is rather feasible because it is harvested when the supply of fresh fruits at the lowest in markets (Demir, 1983).

Identification of the cultivars are mostly based on the morphological traits (Uzun, 1986). They are under the effect of environmental factors. And also there is great variability in number and size of seeds which may affect the fruit shape. For this reason some morphological traits can not be a good indicator for distinguishing cultivars (Degani and Blumenfeld, 1986).

Isozymes are direct product of genes and are not mostly affected from the environmental conditions when compared at the same development stage of tissues or organs (Uzun, 1986; Arulsekar and Parfit, 1986). Isozyme polimorphism of loquat has not extensively been studied like other crops such as grape, apple etc. because of its minor importance and it is mostly grown in limited areas in subtropical regions. Loquat cultivars can easily be distinguished by SDH, PER and PGI isozymes (Degani, 1986). It was stated that purified S6PD enzyme which was extracted from mature leaf of loquats showed single banding in SDS-Page electrophoresis (Hirai, 1983).

The main objective of this study was to identify the loquat cultivars and /or ecotypes by isozymes.

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MATERIALS AND METHODS

Plant Material: Mature leaves, fruits and endosperms of loquat cultivars and some their ecotypes were obtained from Citrus and Greenhouse Crops Research Institute in Antalya, Turkey. All samples were taken early in the morning and analysed in the same day. Isozyme analysed was done in the consecutive years in 1995 and 1996.

1. Yuvarlak Çukurgöbek-1 (Y.Ç-1)
2. Yuvarlak Çukurgöbek-2 (Y.Ç-2)
3. Yuvarlak Çukurgöbek-3 (Y.Ç-3)
4. Yuvarlak Çukurgöbek-4 (Y.Ç-4)
5. Uzun Çukurgöbek-1 (U.Ç-1)
6. Uzun Çukurgöbek-2 (U.Ç-2)
7. Armudi Şekil-1 (Armudi-1)
8. Armudi Şekil-2 (Armudi-2)
9. Söbü Oval
10. Yuvarlak Armudi (Y. Armudi)
11. Hafif Uzun
12. Hafif Çukurgöbek (H.Ç)
13. Sayda
14. Gold Nugget (G. Nugget)
15. Akko-1
16. Akko-13
17. Tanaka (Isp.)
18. Tanaka (FAO)

Enzyme Extraction: Fresh leaves, fruits and endosperms were homogenized with 10 ml Tris-HCl cold extraction buffer (Arulsekar and Parfitt, 1986). The crude homogenate was centrifuged at 20000 x g at 4°C for 10 minute. Supernetants were used as enzyme sources.

Electrophoresis: Vertical polyacrylamid slab gel electrophoresis (PAGE) was conducted at 4°C with protean II xi Slab cell. All separations were preformed on 12 % separating gel (0.375 M Tris, pH 8.8) and 4 % stacking gel (0.125 M Tris, pH 6.8). Gel dimensions 160 x 160 x 1.5 mm. Electrode buffer consisted of 0.12 M Tris-base and 0.96 M Glycine, pH 8.3. The electrophoresis was performed at a constant currant of 35 mA. The running time was determined by the movement of blue band of bromphenol blue about 8 cm from the origin.

Staining of Gels: Gels were assayed for Acide phosphatase (HP) E.C. 3.1.3.2; Alcohol dehydrogenase (ADH) E.C. 1.1.1.1; Catechol oxidase (CO) E.C. 1.10.3.1; Peroxidase (PER) E.C. 1.11.1.7 (Wolfe, 1977); Glutamate oxaloacetate transaminase (GOT) E.C.2.6.1.1.(Soltis and Soltis, 1989). Relative mobility (Rf) value of each band was calculated based upon the migration of band relative the front band of bromphenol blue (Sugiura, 1988). All enzymes migrated anodally.

RESULTS AND DISCUSSION

Enzymes of 18 cultivars and 3 different explant were purified. The isozyme banding patterns for ADH, CO, GOT, HP and PER in fruits and endosperms are shown in Fig. 1 & 2. All enzyme systems were also analysed in leaves but ADH was not detected in this plant tissue. There were no differences in isozyme banding patterns of the plant tissue between the years, but some modification were observed in the intensity of bands.

Acid phosphatase (HP): Three patterns of HP were observed in the endosperm of loquat. There were two distinct migrating zones. The fast migrating zone had up to 2 bands and very useful for cultivar identification. Leaves and fruits of loquat cultivars had only one isozyme pattern. Some biotypes within the same cultivar such as Akko, Armudi and Tanaka had different banding patterns of endosperm.

Alcohol dehydrogenase (ADH): It was not observed in loquat leaves. All cultivars had the same banding patterns in fruits. There were three banding patterns with two bands in endosperm. Some differences were observed in endosperm. Some biotypes within the same cultivar such as Yuvarlak Çukurgöbek and Tanaka had different banding patterns of endosperm.

Catechol oxidase (CO): It produced 8 anodic migrating bands forming three different isozyme band patterns in the fruits. The faster two bands were more suitable for cultivar identification than the other bands. There were five banding patterns in endosperms and six band patterns in the leaves. Söbü Oval had the different banding patterns in leaves. So, in CO enzyme system extracted from the leaves, fruit and endosperms, had different banding patterns, which may provide useful markers for cultivars identification in loquat.

Glutamate oxaloacetate transaminase (GOT): All cultivars had the same banding pattern of GOT in each plant tissue. But band patterns changed among tissues. GOT has three bands in the leaves and five bands in the fruits. The number of bands were 7 in endosperm with two distinct zones. Slow migrating zone had slur bands and it was not useful for identification. All bands were clear in the second zone but there were no polymorphism. GOT were of no value for cultivar identification since all of them had identical pattern.
Peroxidase (PER): There was only one banding pattern with two bands for PER in loquat fruits. Whereas three distinct banding patterns were observed in endosperms and leaves. Band numbers changed from 3 to 4 in endosperms and from 1 to 3 in leaves. Akko 1 and Akko 13 can easily be distinguished with PER isozymes of leaves. Whereas Tanaka and Akko-1 had the same banding patterns for PER in leaves.

After forming zymograms for each of all enzyme systems, matrix was constructed by using similarity index and from these data, dendograms were obtained by the UPGMA method. Fruits analyses showed that there was 100 % similarity in isosymes investigated among Y.Ç-1, Y.Ç-3, Y.Ç-4, U.Ç-1, U.Ç-2, Armudi-1, Hafif Uzun, Y. Armudi and Y.Ç-2, Söbü Oval, Tanaka (FAO), Akko –13, Gold Nugget, Sayda, H.Ç, Tanaka (Isp.), The similarity levels among Akko-1 and Y.Ç-1, Y.Ç-3, Y.Ç-4, U.Ç-1, U.Ç-2, Armudi-1, Armudi-2, Y. Armudi, Hafif Uzun were the farthest by being 95 % similar (Fig. 3 & 6).

The similarity level reached 100 % in the isosyme patterns between Y.Ç-1 and Y.Ç-3; among Y.Ç-2, U.Ç-1 and Armudi-2; between Yuvarlak Armudi and Hafif Uzun; between U.Ç-2 and Armudi-2; between Sayda and Akko-13, while the similarities between Tanaka (Isp.) and Gold Nugget were the farthest by being 65 % similar (Fig. 4 & 7).

On the other hand, leaves analyses showed that there was 100 % similarity in terms of isosymes investigated among Y.Ç-1, Y.Ç-2, Y.Ç-3, U.Ç-1, Akko –13, Armudi-1, Hafif Uzun, Y. Armudi. However, the similarities among Y.Ç-4, Söbü Oval, Akko-1, Sayda, Tanaka (FAO), Tanaka (Isp.), were the farthest by being 72 % similar to each other (Fig. 5 & 8).

The isozymic differences between cultivars suggested that isozymes may provide useful markers for cultivar identification in loquat.

Literature Cited

Arulsekar, S. and Parfitt, D.E. 1986. Isozyme analysis procedures for stone fruits, almonds, grape, walnut, pistachio and fig. HortScience, 21, 4, 928-933.

Degani, C. and Blumenfeld, A. 1986. The use of isozyme analysis for differentiation between loquat cultivars. HortScience, 21, 6, 1457-1458.

Demir, Ş. 1983. Antalya yöresi için ümitvar yenidünya (*Eribotrya japonica* (Thumb). Lindl.) çeşitleri. Bahçe, 12, 1: 5-16.

Hirai, M. 1983. Seasonal changes in sorbitol phosphate dehydrogenase in Loquat leaf. Plant&Cell. Physiol. 24, 5, 925-931.

Uzun, H.İ. 1986. Bazı üzüm çeşitlerinin ampelografik özellikleri, kateşol oksidaz izoenzim bantlarından teşhisleri ve sıcaklık toplamları üzerinde araştırmalar. Ege Üniversitesi, Ziraat Fakültesi, Ph. D. Thessis.

Soltis, D.E. and Soltis, P.S. 1989. Polyploidy, breeding systems and genetic differentitation in homosporous pteridophytes. In isozymes in plant biology, 241-259.

Suguira, A., Tao, R. and Tomama, T. 1988. Distinguishing between Japanase persimmon cultivars (*Diospyros kaki* L.) by means of pollen isozymes. Scientia Hortic. 36: 67-77.

Wolfe, W.H. 1977. Application of isozyme banding patterns to the identification of cultivars and species of grapevines (Vitis). Ph.D.Thessis.

Figures

Fig. 1. Zymogram representation of ADH, CO, GOT, HP and PER enzyme systems extracted from leaves (A), fruits (B) and endosperm (C) in loquat. Rf value are indicated near the isozyme bands.

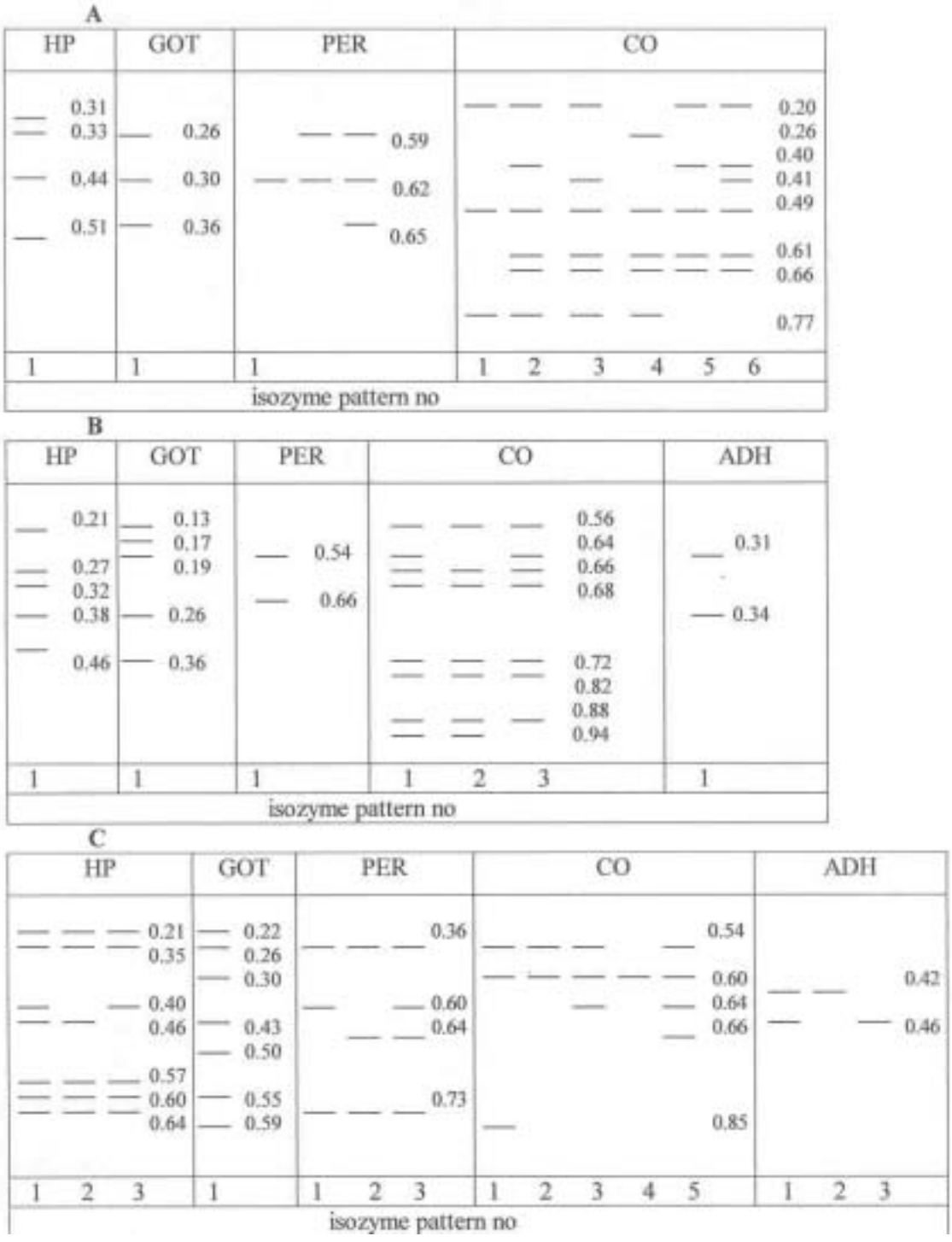

Fig. 2. Classification of loquat cultivars using isozyme banding patterns of ADH, CO, GOT, HP and PER enzyme systems extracted from leaves, fruits and endosperms.

Cultivars	Leaf				Fruit					Endosperm				
	CO	GOT	HP	PER	ADH	CO	GOT	HP	PER	ADH	CO	GOT	HP	PER
Y.Ç-1	1	1	1	1	1	2	1	1	1	2	1	1	1	3
Y.Ç-2	2	1	1	1	1	1	1	1	1	1	2	1	2	3
Y.Ç-3	2	1	1	1	1	2	1	1	1	2	1	1	1	3
Y.Ç-4	2	1	1	1	1	2	1	1	1	2	2	1	1	3
H.Ç	2	1	1	2	1	1	1	1	1	1	3	1	1	2
U.Ç-1	2	1	1	1	1	2	1	1	1	1	3	1	1	3
U.Ç-2	2	1	1	1	1	2	1	1	1	1	4	1	1	3
Armudi-1	3	1	1	1	1	2	1	1	1	1	4	1	1	3
Armudi-2	3	1	1	1	1	2	1	1	1	1	2	1	3	3
Söbü Oval	4	1	1	1	1	1	1	1	1	1	2	1	2	2
Y.Armudi	3	1	1	1	1	2	1	1	1	1	3	1	2	1
Hafif Uzun	3	1	1	1	1	2	1	1	1	1	3	1	1	1
Sayda	5	1	1	1	1	1	1	1	1	1	3	1	1	3
G.Nugget	2	1	1	2	1	1	1	1	1	1	3	1	2	1
Akko-1	2	1	1	3	1	3	1	1	1	1	3	1	3	3
Akko-13	2	1	1	1	1	1	1	1	1	1	3	1	1	3
Tanaka (I)	6	1	1	3	1	1	1	1	1	3	5	1	3	2
Tanaka (F)	6	1	1	3	1	1	1	1	1	1	5	1	1	2

Fig. 3. Similarity index of loquat cultivars using 5 enzyme systems extracted from fruits (%)

Cultivars	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. Y.Ç-1	100																	
2. Y.Ç-2	97	-																
3. Y.Ç-3	100	97	-															
4. Y.Ç-4	100	97	100	-														
5. U.Ç-1	100	97	100	100	-													
6. U.Ç-2	100	97	100	100	100	-												
7. Armudi-1	100	97	100	100	100	100	-											
8. Armudi-2	100	97	100	100	100	100	100	-										
9. Söbü Oval	97	100	97	97	97	97	97	97	-									
10. Y.Armudi	100	97	100	100	100	100	100	100	97	-								
11. Hafif Uzun	100	97	100	100	100	100	100	100	97	100	-							
12. H.Ç.	97	100	97	97	97	97	97	97	100	97	97	-						
13. Sayda	97	100	97	97	97	97	97	97	100	97	97	100	-					
14. G.Nugget	97	100	97	97	97	97	97	97	100	97	97	100	100	-				
15. Akko-1	95	97	95	95	95	95	95	95	97	95	95	97	97	97	-			
16. Akko-13	97	100	97	97	97	97	97	97	100	97	97	100	100	100	97	-		
17. Tanaka (I)	97	100	97	97	97	97	97	97	100	97	97	100	100	100	97	100	-	
18. Tanaka (F)	97	100	97	97	97	97	97	97	100	97	97	100	100	100	97	100	100	-

Fig. 4. Similarity index of loquat cultivars using 5 enzyme systems extracted from endosperms (%).

Cultivars	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. Y.Ç-1	100																	
2. Y.Ç-2	90	-																
3. Y.Ç-3	100	90	-															
4. Y.Ç-4	100	90	100	-														
5. U.Ç-1	90	100	90	90	-													
6. U.Ç-2	80	90	80	80	90	-												
7. Armudi-1	80	90	80	80	90	100	-											
8. Armudi-2	90	100	90	90	100	90	90	-										
9. Söbü Oval	79	89	79	79	89	79	79	89	-									
10. Y.Armudi	83	93	83	83	93	83	83	93	83	-								
11. Hafif Uzun	83	93	83	83	93	83	83	93	83	100	-							
12. H.Ç.	76	86	76	76	86	79	79	86	82	79	79	-						
13. Sayda	83	93	83	83	93	86	86	86	89	86	86	93	-					
14. G.Nugget	79	89	79	79	89	79	79	89	78	92	96	75	82	-				
15. Akko-1	90	100	90	90	100	90	90	100	89	93	93	86	93	89	-			
16. Akko-13	83	93	83	83	93	86	86	93	82	86	86	86	100	82	93	-		
17. Tanaka (I)	66	76	66	66	76	69	69	76	79	69	69	76	83	65	76	83	-	
18. Tanaka (F)	76	86	76	76	86	79	79	86	89	79	79	89	93	75	86	93	90	-

Fig. 5. Similarity index of loquat cultivars using 4 enzyme systems extracted from leaves (%).

Cultivars	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. Y.Ç-1	100																	
2. Y.Ç-2	100	-																
3. Y.Ç-3	100	100	-															
4. Y.Ç-4	91	91	91	-														
5. U.Ç-1	100	100	100	91	-													
6. U.Ç-2	100	100	100	91	100	-												
7. Armudi-1	100	100	100	91	100	100	-											
8. Armudi-2	100	100	100	91	100	100	100	-										
9. Söbü Oval	91	91	91	100	91	91	91	91	-									
10. Y.Armudi	100	100	100	91	100	100	100	100	91	-								
11. Hafif Uzun	100	100	100	91	100	100	100	100	91	100	-							
12. H.Ç.	85	85	85	81	85	85	85	85	81	85	85	-						
13. Sayda	91	91	91	100	91	91	91	91	100	91	91	81	-					
14. G.Nugget	85	85	85	81	85	85	85	85	81	85	85	100	81	-				
15. Akko-1	74	74	74	72	74	74	74	74	72	74	74	79	72	79	-			
16. Akko-13	100	100	100	91	100	100	100	100	91	100	100	85	91	85	74	-		
17. Tanaka (I)	74	74	74	72	74	74	74	74	72	74	74	79	72	79	100	74	-	
18. Tanaka (F)	74	74	74	72	74	74	74	74	72	74	74	79	72	79	100	74	100	-

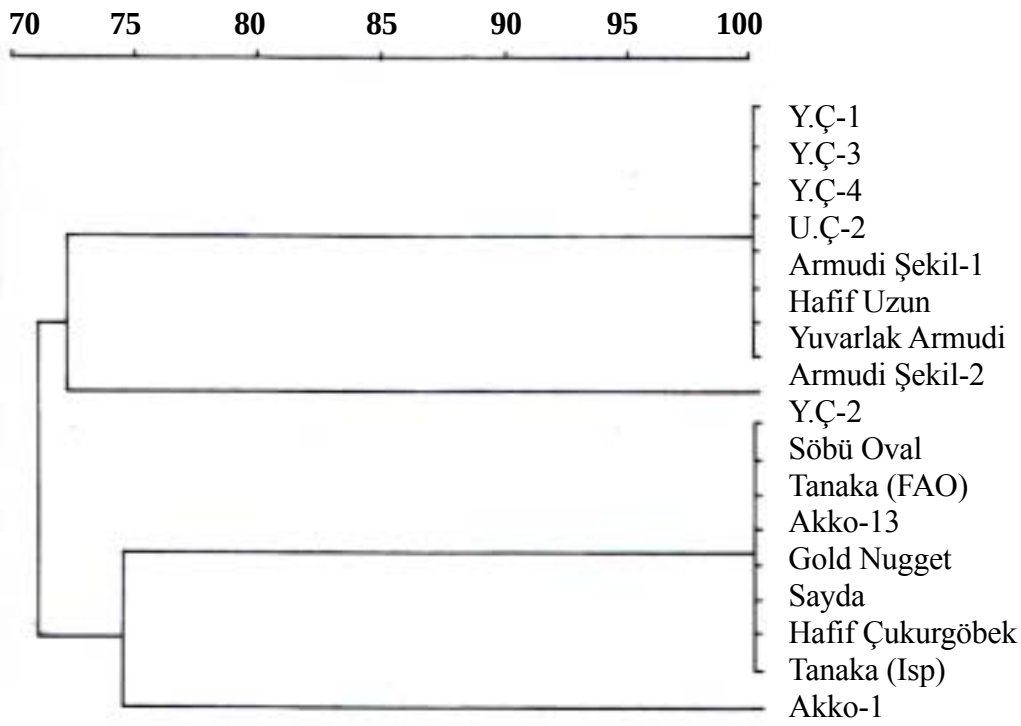

Fig. 6. Dendogram of loquat cultivars using 5 enzyme systems extracted from fruits

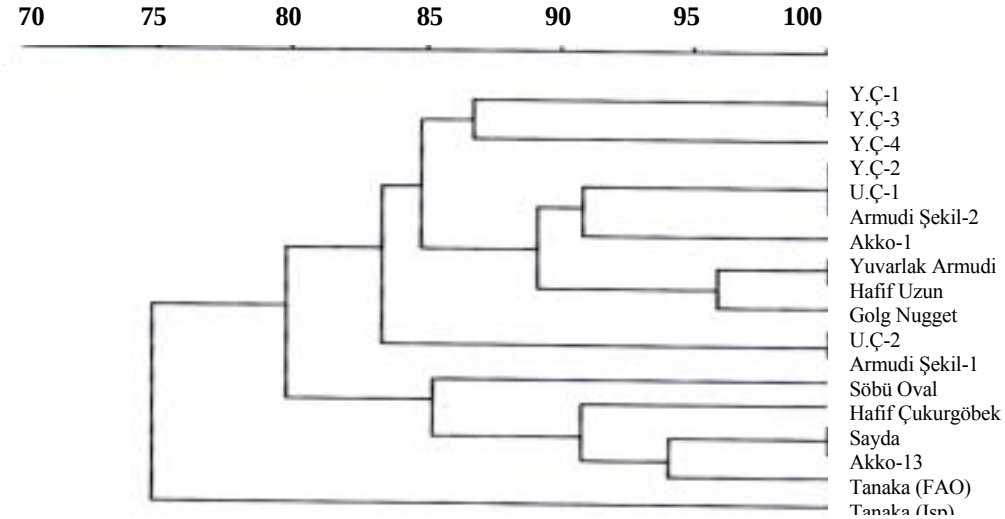

Fig. 7. Dendogram of loquat cultivars using 5 enzyme systems extracted from endosperms

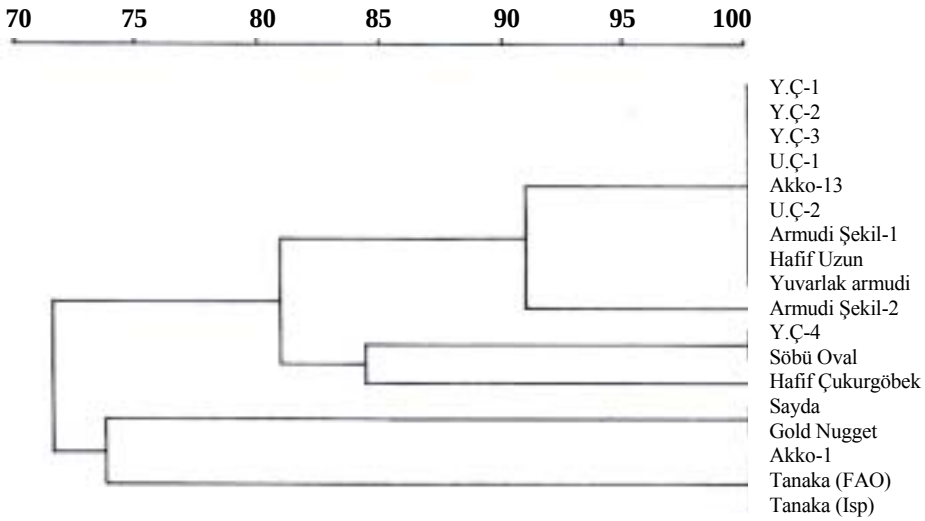

Fig. 8. Dendogram of loquat cultivars using 5 enzyme systems extracted from leaves.