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ANALYSIS OF GENETIC DIVERSITY AMONG THE KIWIFRUIT (*Actinidia* spp.) GENOTYPES USING MOLECULAR MARKERS

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ABSTRACT

To reveal genetic diversity and provide information to conserve valuable kiwifruit genotypes, 42 kiwifruit genotypes collected from Ordu province of Northern Turkey were analyzed using a set of 11 sequence-related amplified polymorphism (SRAP) and 7 inter-primer binding site (iPBS) retrotransposons primers. Out of 85 amplified bands, a total of 40 polymorphic bands were obtained with SRAP primers. iPBS primers produced a total of 71 bands 24 of which were polymorphic. An average 5 and 3.42 polymorphic bands were obtained for SRAP and iPBS markers respectively. The similarity coefficients were calculated, and cluster analysis was performed with (NTSYSpc 2.2). Dendrograms were constructed via UPGMA based on SRAP and iPBS markers. The dendrograms constructed using the SRAP and iPBS data exhibited two clusters. The Mantel test for comparison of the SRAP and iPBS-based similarity matrices showed moderate but significant correlation (r=0.55). Most of the genotypes showed a low range of genetic diversity and more than half of the genotypes found genetically closely related with similarity coefficient ranged from 0.90 to 1.00. The result of current study showed that there is need to increase the genetic diversity of kiwifruit using different breeding approach such as mutations, crossing and germplasm introduction.

Keywords: Kiwifruit, genetic diversity, SRAP, iPBS, molecular characterization

INTRODUCTION

Kiwifruit belongs to family *Actinidiaceae* and genus *Actinidia* which includes 76 species and 125 known taxa [7, 8]. The commercial cultivars are obtained by selections of two closely related species *Actinidia chinensis* and *A. deliciosa*. Most of *Actinidia species* are diploid, with 2X=58 chromosomes while the cultivated kiwifruit, *Actinidia deliciosa* is hexaploid (2n=6x=174) [3, 19]. Kiwifruit is native to China and majority of the species are cultured in the southwest of China [18]. The first commercial orchards were established in New Zealand 1930 and the fruit exportation started in the 1970s and 1980s [6]. China is the largest producer of kiwifruit with 2.024.603 (2017) tons and followed by Italy and New Zealand. In Turkey, kiwi production started in 1988 with seedlings obtained by the Ministry of Agriculture and Rural Affairs from Italy [12]. Turkey produced 4.000 tons kiwifruit in 2004 but kiwi production increased to 56.164 tons in 2017 [17]. Kiwifruit can be grown in Marmara region, Black Sea coastline (especially Yalova, Rize and Ordu), Aegean and Mediterranean regions of Turkey. It can be accounted among the most important crops of Black Sea region after tea, hazelnut and corn [17]. The most widely planted kiwifruit cultivar is the fuzzy kiwifruit *A. deliciosa* 'Hayward' and corresponds to about half of kiwifruit cultivation throughout the world [6]. The other best-known varieties are 'Allison', 'Abbott', 'Bruno', 'Gracie', 'Montgomery' and 'Elmwood' [1]. In Turkey the first domestic kiwi registered

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with the name 'İlkaltın' in 2018 [17]. Kiwi fruit is rich in vitamin C and contains potassium and flavonoid antioxidants. The fruits, stems and roots have diuretic, febrifuge and sedative affect also used in the treatment of urinary tract, rheumatoid arthralgia, cancers of the liver and oesophagus [10]. Antitumor effect of *Actinia deliciosa* was also reported [14, 20].

Today, there are many genetic studies related to kiwi. Some of these studies are molecular-based studies, most of which were conducted with the aim of genotyping. Genotype analyses can determine the richness of hereditary information in a species gene pool, i.e. genetic diversity which is important to develop new varieties and lines with high efficiency and quality, to increase resistance to biotic and abiotic stress conditions.

Molecular markers help to determine the DNA diversity of different genotypes. SRAP markers are dominant markers with 17-18 nucleotides in length that target the open reading frames (ORFs) in genome [13]. Because it is a highly polymorphic method, they are highly preferred in molecular studies. IPBS markers are based on amplification of the primary binding site (PBS) of the reverse transcriptase (tRNA complement) in LTR retrotransposons and present a high copy number in the plant genome [10, 11].

In this study, it is aimed to determine the genetic diversity of kiwi genotypes cultivated in Ordu province of Turkey using SRAP (Sequence-Related Amplified Polymorphism) and IPBS (Inter Primer Binding Site) markers.

MATERIAL AND METHOD

Plant Material

The fresh leaves of 42 kiwifruit genotypes were collected from Ordu province of Northern Turkey. Collected samples, consisting of fresh leaf tissue, were placed in labeled plastic bags, and maintained at -20°C until use. They were analyzed using a set of 11 sequence-related amplified polymorphism (SRAP) and 7 inter-primer binding site (iPBS) retrotransposons primers.

Method

DNA extraction

DNA extraction was performed using a modified CTAB protocol with extra choloroform-isoamly precipitation and 76% ethanol washing steps [4]. Agarose gel electrophoresis (1%) was carried out for confirming the quality of the DNA samples.

Srap analysis

SRAP analysis was performed with 11 combinations (Table 1). PCR reaction was conducted with a total volume of 15 µl PCR mix containing 1.5µl 10x reaction buffer, 0.25 mM dNTPs, 1.5 mM MgCl2, 10 pmol of each primer pair, 1 unit of Taq DNA polymerase, and 20 ng DNA. The PCR program consisted of: 94°C for 3 min. initial denuration; 5 cycles of 95°C for 1 min, 35°C for 1 min, and 72°C for 1 min followed with 35 cycles for 94°C 1 min, 50°C for 1 min, 72°C for 1 min and; a final extension step of 72°C for 5 min. PCR products were separated on 2% (w/v) agarose gel at 120 V for 2 hours and visualized by UV light comparing with marker.

iPBS analysis

IPBS analysis was carried out with seven iPBS primers. PCR mix was included 20 ng DNA, 1.5µl 10x reaction buffer, 0.2 mM dNTPs, 1.5 mM MgCl2, 10 pmol of primer, 1 unit of Taq DNA polymerase and completed to 15 µl total volume. The thermocycler program started with 95°C for 3 min initial denaturation followed by 35 cycles of 95°C for 1 min, 50-55°C for 1 min, 72°C for 1 min and a final extension step 72°C for 10 min. PCR products were separated on 2% (w/v) agarose gel at 120 V for 2 hours and visualized by UV light comparing with marker.

Data analysis

For the both marker systems data was transferred into Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) version 2.1 software package (Exeter Software, Setauket, NY, USA) [16]. Similarity matrix was obtained within the SIMGEND module and the dendrogram was constructed based on the similarity matrix with the use of Unweighted Pair-Group Method (UPGMA) in the SAHN module of the NTSYS-pc software. PCA (Principal Component Analysis) was performed based on Jaccard similarity coefficient and a two and three-dimensional graph was created using the DCENTER and EIGEN procedures in NTSYS [16]. The comparison of the two marker systems was performed by the "Mantel Test" [15].

RESULT AND DISCUSSION

In the present study seven iPBS and 11 SRAP combinations were used to investigate the genetic diversity among the 42 kiwifruit genotypes. The chosen primers were successfully generated several bands with all DNA samples.

SRAP Analysis

Eight primer combinations from a total 11 primers produced polymorphic bands. The total number of bands generated by eight SRAP primers was 85 of which 40 were polymorphic (47%) and the total number of number of bands per primers combination ranged from 7 (Me13-Em11) to 15 (Me1-Em13) with an average of 10.62 bands per primers combination (Table 1). Three SRAP primer combinations produced monomorphic bands (Me9Em9, Me11Em10, Me11Em15). Pairwise genetic similarities were ranged from 0.51 to 1.00 and maximum similarity value of 1.00 was observed between 17 genotypes. The most diverse genotypes were 15 and 32 with 0.50 genetic similarity value. Cho et. al. [2] obtained a genetic similarity values ranged from 0.47-0.99 with domestic and overseas collection cultivars. Jing et.al. [9], obtained a higher polymorphism rate (100%) in a population including wild genotypes with similarity value ranged from 0.15 to 0.77 and the genotypes divided to five clusters at the genetic similarity value of 0.27 whereas in the current study the UPGMA cluster analysis produced two main groups at the similarity level of 0.56 which indicate a low level of diversity between genotypes (Figure 1) Most of the genotypes grouped on cluster I and found genetically very similar (0.90-1.00). The 16 kiwi genotypes were clustered together on dendrogram (Cluster I) with the genetic similarity value of 1.00 and found to be closely related which indicated they are probably vegetative propagated clones (Figure 1). Four genotypes (12, 32, 37, 49) placed on Cluster II. The first three Eigen values explained the 54.35% of the total variation and 2D and 3D graphs showed a similar result with dendrogram and most of the genotypes cluster together, only a few genotypes placed on separately (Figure 2–3).

iPBS Analysis

iPBS primers produced a total of 71 bands 24 of which were polymorphic (33%) and total number of bands ranged from 6 (2376) to 13 (2249) with average 10.14 total bands for per primer and 3.42 polymorphic band for each primer. Pairwise genetic similarities were ranged from 0.34 to 1.00 and maximum similarity value of 1.00 was observed between 17 genotypes. Genotypes 1 and 32 were found to be genetically diverse with minimum similarity value of 0.34.

The UPGMA cluster analysis produced two main groups (Figure 4). Similar to SRAP results, four genotypes (18, 32, 37, 49) placed on Cluster II. The remaining genotypes grouped on Cluster I. Seventeen genotypes in Cluster I were found closely related with the genetic similarity value of 1.00 like results obtained with SRAP markers. The first three Eigen values explained the 62.33% of the total variation and most of the genotypes cluster together only a few genotypes placed on separately in 2D and 3D graphs (Figure 5 and 6).

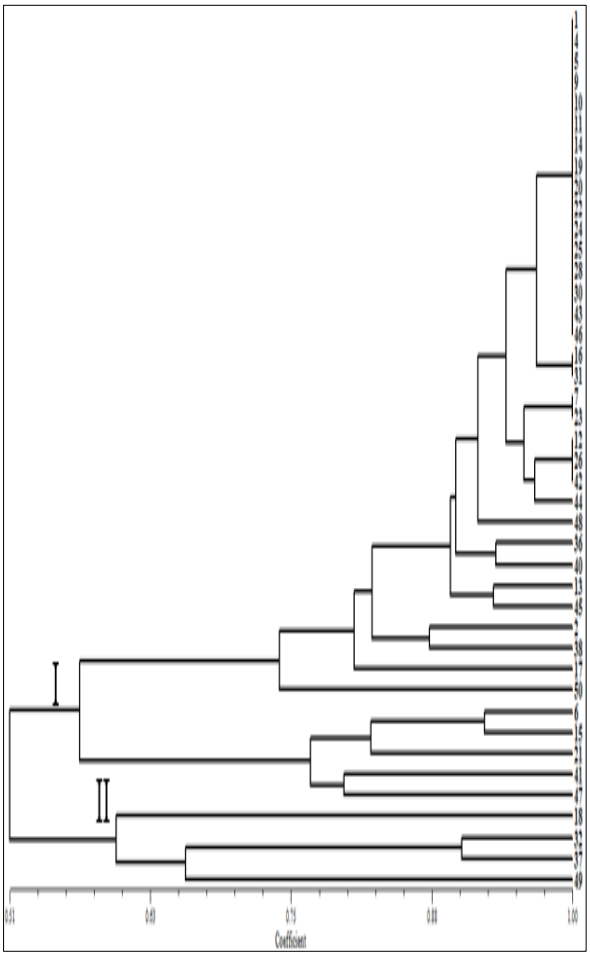

Figure 1. Dendrogram of 42 kiwifruit genotypes constructed by UPGMA based on SRAP markers

Şekil 1. SRAP analizi verileri kullanılarak 42 kivi genotipi için yapılan cluster analizi (UPGMA) ile elde edilen dendrogram

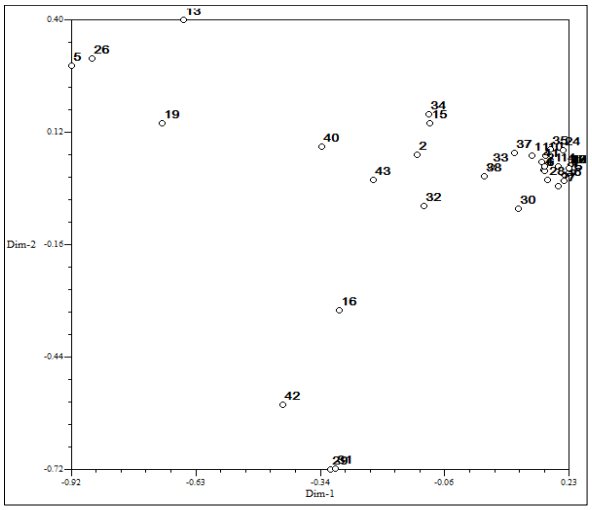

Figure 2. Two-dimensional plot of the principal component analysis of 42 kiwifruit genotypes based on SRAP markers

Şekil 2. SRAP analizi verileri kullanılarak 42 kivi genotipi için oluşturulan iki boyutlu kümeleme analizi grafiği (PCA)

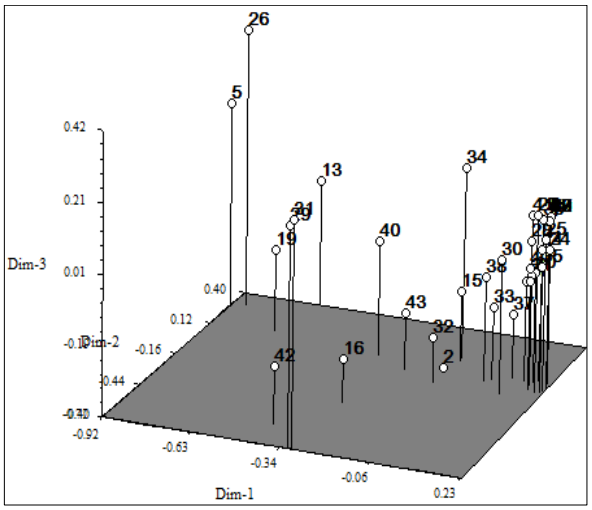

Figure 3. Three-dimensional plot of the principal component analysis of 42 kiwifruit genotypes based on SRAP markers

Şekil 3. SRAP markör analizi verileri kullanılarak 42 kivi genotipi için oluşturulan üç boyutlu kümeleme analizi grafiği (PCA)

Table 1. Selected sequence-related amplified polymorphism (SRAP) and inter-primer binding site (iPBS) primers, with their respective sequences and number of amplified and polymorphic fragments

Primer	Primer codes	Sequence 5'-3'	Number of total bands	Number of polymorphic bands	Fragment size (bp)
IPBS	2079	AGGTGGGCGCCA	12	4	300-780
	2376	TAGATGGCACCA	6	1	400
	2232	AGAGAGGCTCGGATACCA	11	4	280-480
	2228	CATTGGCTCTTGATACCA	11	6	255-950
	2249	AACCGACCTCTGATACCA	13	2	500-750
	2230	TCTAGGCGTCTGATACCA	9	4	400-560
	2251	GAACAGGCGATGATACCA	9	3	300-550
	TOTAL		71	24	
SRAP	ME13-EM11	(F)TGAGTCCAAACCGGAAG (R)GACTGCGTACGAATTCTA	7	2	750-1250
	ME1-EM13	(F)TGAGTCCAAACCGGATA (R)GACTGCGTACGAATTGGT	15	8	200-1200
	ME2-EM8	(F)TGAGTCCAAACCGGAGC (R)GACTGCGTACGAATTAGC	8	3	400-1250
	ME4-EM6	(F)TGAGTCCAAACCGGACC (R)GACTGCGTACGAATTGCA	10	9	210-1250
	ME3-EM1	(F)TGAGTCCAAACCGGAGT (R)GACTGCGTACGAATTCAA	13	7	230-1700
	ME3-EM3	(F)TGAGTCCAAACCGGAGT (R)GACTGCGTACGAATTGAC	13	5	100-600
	ME5-EM5	(F)TGAGTCCAAACCGGAAG (R)GACTGCGTACGAATTCAA	10	4	180-600
	ME9-EM9	(F)TGAGTCCAAACCGGACA (R)GACTGCGTACGAATTTCA	Monomorphic	-	-
	ME11-EM10	(F)TGAGTCCAAACCGGAAC (R)GACTGCGTACGAATTTAG	Monomorphic	-	-
	ME9-EM 12	(F)TGA GTC CAA ACC GGAGG (R)GAC TGC GTA CGA ATT CTC	9	2	300-800
	ME11 EM15	(F)TGAGTCCAAACCGGAAC (R)GACTGCGTACGAATTCTG	Monomorphic	-	-
	TOTAL		85	40	

Comparison of SRAP and iPBS Marker Systems

SRAP and iPBS markers produced 10.62 and 10.14 total band for each primer respectively. The number of polymorphic bands for each primer were 5 for SRAP and 3.42 for iPBS markers. The polymorphism rate was 47% and 33% for SRAP and iPBS respectively. The genetic similarity value ranged from 0.44 to 1.00 on the dendrogram obtained with combined data of SRAP and iPBS markers. The genotypes divided into two group and the four genotypes (18, 32, 37 and 49) were classified separately from the rest of the genotypes. Many of the genotypes showed a narrow genetic diversity with combined data also (Figure 7). The Mantel test for comparison of the SRAP and iPBS based similarity matrices showed moderate but significant correlation (r=0.55).

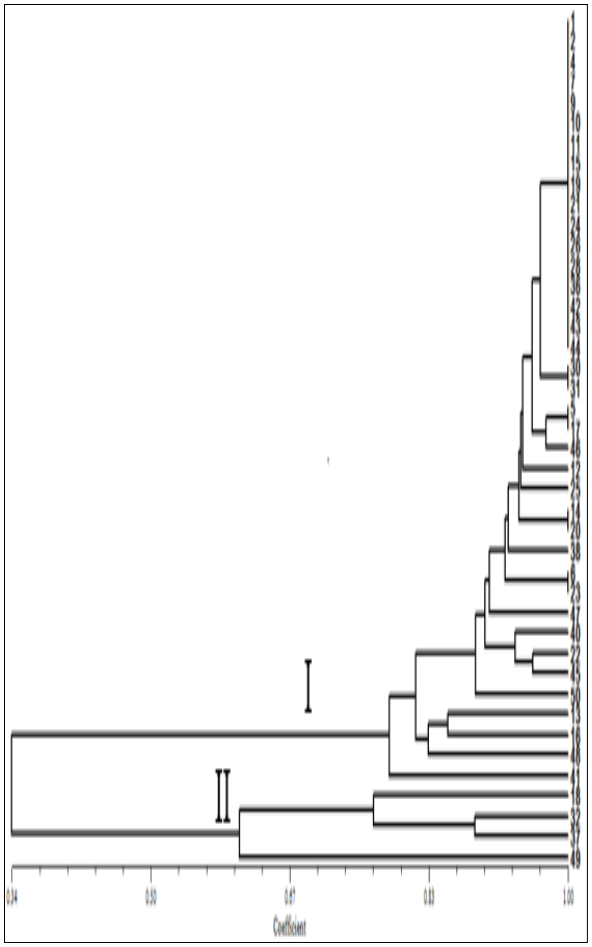

Figure 4. Dendrogram of 42 kiwifruit genotypes constructed by UPGMA based on iPBS markers

Şekil 4. iPBS markör analizi verileri kullanılarak 42 kivi genotipi için yapılan cluster analizi (UPGMA) ile elde edilen dendrogram

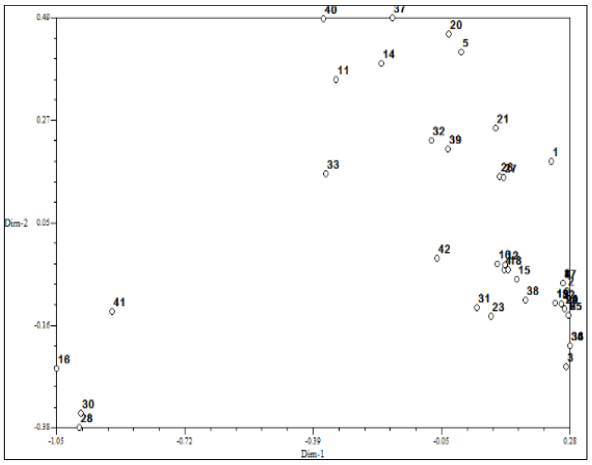

Figure 5. Two-dimensional plot of the principal component analysis of 42 kiwifruit genotypes based on iPBS markers

Şekil 5. iPBS markör analizi verileri kullanılarak 42 kivi genotipi için oluşturulan iki boyutlu kümeleme analizi grafiği (PCA)

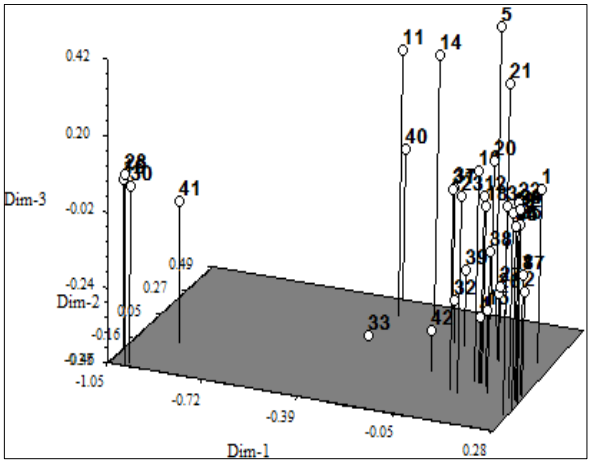

Figure 6. Three-dimensional plot of the principal component analysis of 42 kiwifruit genotypes based on iPBS markers

Şekil 6. iPBS markör analizi verileri kullanılarak 42 kivi genotipi için oluşturulan üç boyutlu kümeleme analizi grafiği (PCA)

CONCLUSION

In this study the genetic diversity between the kiwifruit genotypes collected from Ordu province were analyzed using SRAP and iPBS markers. To the best of our knowledge this is the first time to investigate the genetic diversity among kiwifruit genotypes using iPBS marker and can be recommended for future genetic studies of kiwifruit. The results of the current study showed there is a narrow genetic diversity in the region for kiwifruit. The new kiwifruit cultivars should be

introduced to the region by adaptation, crossbreeding and mutation breeding to increase the genetic diversity and market share.

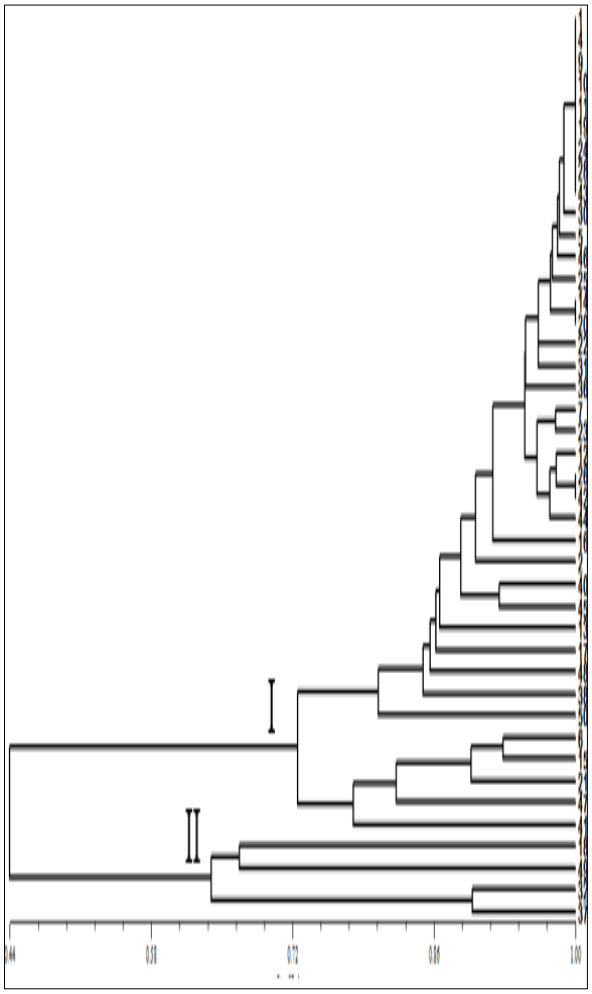

Figure 7. Dendrogram of 42 kiwifruit genotypes constructed by UPGMA based on SRAP and iPBS markers

Şekil 7. SRAP ve iPBS markör analizi verileri kullanılarak 42 kivi genotipi için yapılan cluster analizi (UPGMA) ile elde edilen dendrogram

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