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Araştırma Makalesi (Research Article)

**Assessment of Genetic Diversity in Safflower (*Carthamus tinctorius* L.) Using RAPD Markers**

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**Abstract:** This research was carried out to determine the genetic distances and variability between some safflower landraces and registered varieties. As plant material, four safflower landraces (TR 49119, TR 42630, TR 42670 and TR 64702) and three registered varieties (Yenice 5-38, Remzibey 05 and Dinçer 5-118) obtained from the Aegean Agricultural Research Institute, Turkey were used. The safflower varieties were analyzed at the molecular level using RAPD markers. The polymorphic band ratios of ten RAPD primers varied from 33.3% to (OPK-11) 80% (OPS-04). The average polymorphic band ratio was found to be 63.9%. The polymorphism information content values of the RAPD primers ranged from 0.24 for OPC-03 to 0.46 for OPA-19. The mean PIC value was determined as 0.38. The mean resolution power value was found to be 3.37, the effective multiplex ratio value was 4.14, and the marker index value was 1.57. The genetic distances were obtained using NTSYS-pc 2.20j statistic package program according to Jaccard's similarity coefficient. The genetic similarity values of the safflower genotypes varied between 0.61 and 0.85. The average similarity was calculated as 0.69. The cluster analyses of the RAPD markers grouped the genotypes into two major clusters (UPGMA dendrogram). With slight differences, the landraces and registered varieties were included in separate groups. The TR 64702 line and Remzibey 05 registered variety were genetically most similar genotypes with a value of 0.85.

**Aspir (*Carthamus tinctorius* L.) 'de Genetik Çeşitliliğin RAPD Markörleri Kullanarak Değerlendirilmesi**

**Makale Bilgileri**

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**Anahtar kelimeler**

Aspir,  
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RAPD,  
UPGMA

**Öz:** Çalışmada, Ege Tarımsal Araştırma Enstitüsü Müdürlüğü'nden temin edilmiş olan 4 yerel çeşit (TR 49119, TR 42630, TR 42670 ve TR 64702) ve üç adet tescilli aspir çeşidi (Yenice 5-38, Remzibey 05 ve Dinçer 5-118) materyal olarak kullanılmıştır. Aspir çeşitleri, RAPD markörleri kullanılarak moleküler düzeyde analiz edilmiştir. Kullanılan on adet RAPD primerinin polimorfik bant oranı % 33.3 (OPK-11) ile % 80 (OPS-04) arasında değişim göstermiştir. Ortalama polimorfik bant oranı % 63.9 olarak belirlenmiştir. RAPD primerlerinin polimorfik bilgi içeriği (PIC) değerleri incelendiğinde, 0.24 (OPC-03) ile 0.46 (OPA-19) arasında değişim gösterdiği gözlenmiştir. Ortalama PIC değeri 0.38 olarak bulunmuştur. Ortalama çözümleme gücü değeri 3.37, etkili multipleks oran değeri 4.14 ve markör endeks değeri ise 1.57 olarak belirlenmiştir. NTSYS-pc 2.20j istatistik paket programında Jaccard'ın benzerlik katsayısına göre genotipler arasındaki genetik uzaklık değerleri elde edilmiştir. Aspir genotiplerine ait genetik benzerlik değerleri 0.61 ile 0.85 arasında değişim göstermiştir. Ortalama benzerlik oranı 0.69 olarak

bulunmuştur. Aspir çeşitleri UPGMA gruplandırmasına göre iki gruba ayrılmışlardır. Küçük bir nüansla yerli ve tescilli çeşitler ayrı gruplarda yer almıştır. TR 64702 yerel çeşidi ile Remzibey 05 tescilli çeşidi 0.85 değeriyle genetik olarak en benzer genotipler olarak gözlenmiştir.

## 1. Introduction

Safflower (*Carthamus tinctorius* L.) is a valuable oil crop of the Compositae family used for multiple purposes. In addition to the high quality of the oil extracted from the seeds, it is also very important for the paint industry due to its semi-drying properties. Safflower is usually utilized in the production of oil, soap, paint varnish, and polish. It can also be used as animal feed by extracting the seed oil or by converting it to silage while green. Furthermore, the stems provide heating and shelter. Pigment from the flowers can also be used in paint production (Weiss, 2000).

The safflower plant is South Asian origin and was first cultivated in the Asian continent, and in countries in the Middle East and Mediterranean, from which it is believed that it spread all over the world. This plant was brought to Turkey by Bulgarian immigrants through the Balkans and first cultivated around Balıkesir, Bursa and Kütahya. Today, it is most commonly cultivated in the Turkish provinces of Isparta, Burdur, Eskişehir and Kütahya (Er et al., 2010).

Turkey is one of the world's top 20 producers of safflower. In 2018, 35 000 tons of safflower seeds were produced on 24 700 hectares (ha) of land. In Turkey, the mean safflower seed yield is 182.64 kg/ha, which is much higher than the global average of 82.16 kg/ha. In recent years, safflower cultivation in the country has gained great importance, and the plantation area increased from 1 700 ha in 2007 to 24 700 ha in 2018. In the 2014-2015 growing season, the safflower plantation area reached a record level of approximately 45 000 ha (FAO, 2017; TÜİK, 2018). The safflower plant has the potential to tackle oil deficit that is increasing year by year in Turkey (Baydar and Kara, 2010).

Extensive research on molecular markers based on DNA continues throughout the world. Molecular markers are widely used to detect genetic relationships between different varieties, identify plant species and varieties, compare molecular marker analysis of morphological and chemical properties of plants, control F1 hybrid seeds, and determine genetic variability between and within populations.

Most DNA markers are based on polymerase chain reaction (PCR) amplification and need little amounts of DNA for analysis. The PCR procedure was first realized by Mullis et al. (1986), who, several years later in 1993, received the Nobel Prize in medicine and physiology for this invention. One of the most commonly used methods based on PCR amplification is random amplified polymorphic DNA (RAPD) markers developed by Williams et al. (1990). They are easy to apply and cost-effective. Despite the disadvantage of the relatively low repeatability, their higher polymorphism rates and requirement of no radioactive material have resulted in their successful application in many varieties, such as basil (Giachino et al., 2014) and hypericum (Tonk et al., 2011).

Although research on safflower has previously been conducted in certain geographical regions of Turkey, such as Eastern Anatolia, Central Anatolia, and The Black Sea, as well as Tekirdağ province, there is a need for further breeding activities for the registration of varieties. Resistance of safflower to increasing temperature in recent years due to global warming makes it a valuable plant to be cultivated in rotation. This study aimed to determine the genetic distances and variability between safflower landraces and registered varieties using the RAPD molecular marker system.

## 2. Materials and Methods

### 2.1. Plant Materials

Four safflower landraces (TR 49119, TR 42630, TR 42670 and TR 64702) and three registered varieties (Yenice 5-38, Remzibey 05 and Dinçer 5-118) were obtained from the Aegean Agricultural Research Institute, Izmir, Turkey. Table 1 presents the characteristics of the landraces and registered varieties. Molecular analyzes were carried out at the central laboratory of Ege University Faculty of Agriculture (EGE AGROLAB).

## 2.2. DNA extraction and PCR amplification

The seeds of the safflower samples constituting the study material were planting in separate pots and germinated. Samples taken from fresh leaves of young seedlings of 10-15 cm in length were used for DNA isolation. The fresh leaves were crushed to powder in liquid nitrogen containing porcelain mortars, and DNA isolation was performed using the GenElute™ Plant Genomic DNA Miniprep (Sigma-Aldrich) isolation kit.

The amount of DNA for the PCR reaction was measured by a spectrophotometer and diluted to 10 ng in µl. To check the DNA quality, the samples were run on 1% agarose gel using electrophoresis. Out of 20 random 10-mer oligonucleotide primers obtained from Operon Technologies Inc. (USA), 10 primers that amplified clear and reproducible band profiles were selected.

The reaction volume was 15 µl, consisting of 50 ng genomic DNA, 200 µM of each dNTP (dATP, dTTP, dCTP, and dGTP), 0.5 µM primer (Operon Technologies), 1xTaq DNA polymerase buffer (100 mM Tris-HCL, pH: 8.3, 500 mM KCL and 0.01% gelatin), 3 mM MgCl<sub>2</sub>, and 0.5 unit Taq DNA polymerase enzyme (Sigma). The PCR process was carried out in a thermal cycler (Thermo Scientific Arctic with Gradient) according to the PCR program of 5 min at 94 °C for one cycle; 1 min at 94 °C, 1.30 min at 42 °C and 72°C for 1 min for 40 cycles, and finally 10 min at 72 °C (Mahasi et al., 2009).

Table 1. Various characteristics of safflower landraces and registered varieties used in the study

Landrace	Collection Year	Province	District	Altitude	Latitude	Longitude
1-TR49119	1988	Isparta	Gelendost	860 m	380715N	0310055E
2-TR42630	1980	Edirne	Havsa	40 m	412054N	0265523E
3-TR42670	1980	Tekirdağ	Saray	240 m	412626N	0275519E
4-TR64702	1996	İçel	Anamur	850 m	360442N	0325003E
Registered Variety	Registration Year	Breeding Method	Color of Flower	Plant Length (cm)	Structure	Breeding Institution
Y-Yenice 5-38	1931	Selection	Red	100-120	Spineless	GKTAEM
R-Remzibey 05	2005	Selection	Yellow	60-80	Spined	GKTAEM
D-Dinçer 5-118	1977	Selection	Orange	90-110	Spineless	GKTAEM

(GKTAEM): Transitional Zone Agricultural Research Institute

After PCR, the amplified DNAs were run on 2% agarose gel using electrophoresis. The gel was stained with 2 µg/ml ethidium bromide to visualize and evaluate DNA. The gels were visualized by the Vilbert Lourmat UV imaging system to evaluate the bands.

## 2.3. Data analysis

The presence (1) or absence (0) of RAPD bands was scored across seven safflower lines and registered varieties. For each primer used in RAPD analyses, the total number of bands scored, number of polymorphic bands, number of monomorphic bands and percentage of polymorphism were determined. By conducting a pairwise comparison between all genotypes using the Simqual module of NTSYS-pc software version 2.20j (Rohlf, 2000), the genetic distances based on the Jaccard coefficient (Jaccard, 1908) were calculated. The distance coefficients obtained were used to construct dendrograms using the unweighted pair group method with arithmetic averages (UPGMA) employing the sequential, agglomerative, hierarchical, and nested clustering (SAHN) algorithm included in the same software package. To determine the goodness of fit of clustering to the basic data matrix, the cophenetic correlation coefficient was calculated using the normalized Mantel statistics Z test (Mantel, 1967) via the COPH and MXCOMP procedures of NTSYS-pc version 2.20j (Rohlf, 2000). The binary data was also subjected to a principal coordinate analysis (PCoA) using the same software.

The polymorphism information content (PIC) values were calculated according to Anderson et al. (1993), using the following formula for all primers:  $PIC = 1 - \sum p_i^2$ , where  $p_i^2$  is the frequency of the  $i$ th allele. The resolving power (RP) according to Prevost and Wilkinson (1999) of each primer was calculated as:  $R_p = \sum I_b$ , where  $I_b$  is the band informativeness with  $I_b = 1 - [2 \times (0.5-p)]$  and  $p$  is the proportion of seven genotypes containing the band. The marker index (MI) was calculated as described by Powell et al. (1996) and Milbourne et al. (1997) by multiplying PIC with the effective multiplex ratio (EMR), defined as the product of the fraction of polymorphic loci and number of polymorphic loci.

### 3. Results and Discussion

Ten of the 20 primers used in the study (OPA-03, OPA-10, OPA-19, OPA-20, OPAA-02, OPAA-07, OPAA-10, OPC-03, OPK-11, and OPS-04) produced bands that were suitable for an evaluation. Table 2 presents the names, base sequences, total number of bands (TB), number of polymorphic bands (PBs), number of monomorphic bands (MBs), polymorphic band ratio (PBR%), PIC, RP, EMR and MI of the 10 primers evaluated in this study.

The RAPD analysis revealed that the 10 primers provided a total of 90 bands, 60 polymorphic and 30 monomorphic. The mean number of PBs per primer was calculated as 6, and the mean number of MBs per primer as 3. Sixty-four (57.6%) PBs and 25 (28.0%) MBs were produced by the other 18 primers in 85 accessions (Seghal et al., 2009). In their study investigating 193 safflower accessions, Khan et al. (2009) reported 78 PBs produced by 15 RAPD primers. Mahasi et al. (2009) evaluated a total of 61 amplification products with an average of 4.4 band frequency per primer.

Table 2. The RAPD primers and various marker parameters evaluated in safflower genotypes

Primer	Sequence	TB	PB	MB	(PBR%)	PIC	RP	EMR	MI
OPA-03	5'-AGTCAGCCAC-3'	12	9	3	75	0.39	5.14	6.75	2.63
OPA-10	5'-GTGATCGCAG-3'	9	6	3	66.7	0.41	3.71	4	1.63
OPA-19	5'-GTGATCGCAG-3'	6	3	3	50	0.46	2.29	1.5	0.69
OPA-20	5'-CAAACGTCGG-3'	11	7	4	63.6	0.41	4.29	4.45	1.82
OPAA-02	5'-GTTGCGATCC-3'	10	7	3	70	0.40	4.29	4.9	1.94
OPAA-07	5'-GAGACCAGAC-3'	9	7	2	77.7	0.36	3.71	5.44	1.97
OPAA-10	5'-TGGTCGGGTG-3'	11	8	3	72.7	0.39	4.57	5.82	2.26
OPC-03	5'-CTACGCTCAC-3'	6	3	3	50	0.24	0.86	1.5	0.37
OPK-11	5'-AATGCCCCAG-3'	6	2	4	33.3	0.37	1.14	0.67	0.24
OPS-04	5'-CACCCCCTTG-3'	10	8	2	80	0.34	3.71	6.4	2.16
Average		9	6	3	63.9 <sup>a</sup>	0.38 <sup>a</sup>	3.37 <sup>a</sup>	4.14 <sup>a</sup>	1.57 <sup>a</sup>
Minimum		6	2	2	33.3	0.24	0.86	0.67	0.24
Maximum		12	9	4	80	0.46	5.14	6.75	2.63
Total		90	60	30	-	-	-	-	-

<sup>a</sup> Average of the column

When the primers were further evaluated, it was determined that the highest number of bands was 12, obtained from the OPA-03 primer, and the lowest was 6 bands found using OPA-19, OPC-03 and OPK-11. The highest number of PBs was observed in OPA-03 and MBs in OPA-20 and OPK-11. The PBR of the primers ranged from 33.3% (OPK-11) to 80% (OPS-04), with the mean PBR being calculated as 63.9%. OPK-11 with the minimum number of bands was determined as the only primer with a PBR of less than 50% (33.3%). The PBR of all the remaining nine primers was observed to be 50% or higher. Examples of RAPD profiles produced using OPS-04 and OPAA-02 are presented in Figure 1. Safavi et al. (2010), who evaluated 20 safflower accessions, reported a PBR of 80.08% yielded by 13 RAPD primers. Seghal et al. (2009) obtained a total of 111 amplification products from 22 RAPD primers in 85 *C. tinctorius* accessions, with the number of bands ranging from 3 to 9. In a study of 14 safflower genotypes using RAPD, ISSR and AFLP methods, the number of PBs produced by the RAPD method was 1-8, and PBR was 12.5%-88.8% (Seghal and Raina, 2005).

The PIC values of the RAPD primers were observed to vary between 0.24 (OPC-03) and 0.46 (OPA-19). The average PIC value was determined as 0.38. A higher PIC value indicates that the RAPD marker is more informative. Six of 10 primers (OPA-03, OPA-10, OPA-19, OPA-20, OPAA-

02, and OPAA-10) produced an above-average PIC value. In previous studies on safflower genotypes, the PIC value was reported to range from 0.26 to 0.71 by Amini et al. (2008) and from 0.16 to 0.45 by Safavi et al. (2010). The mean PIC value of the current study was 0.26, which is lower than reported by the latter authors.

The RP of the primers ranged from 0.86 to 5.14, with the highest value being obtained from the OPA-03 primer and the lowest from OPC-03. The mean RP was calculated as 3.37. Unlike the current study, Seghal et al. (2009) found the average RP value for RAPD primers as 14.36. Similarly, Majidi and Zadhoush (2014) determined the average RP value of 20 ISSR primers as 8.72 in 102 safflower accessions. In this study, the highest EMR was 6.75, which was observed in OPA-03, the lowest EMR was 0.67, obtained from OPK-11, and the average value was 4.14 per primer. Concerning MI, the highest (2.63) and lowest (0.24) values were detected in OPA-03 and OPK-11 primers, respectively, and the average MI of the RAPD primers was calculated as 1.57. For safflower, a higher average MI (6.0) has previously been reported (Seghal et al., 2009).

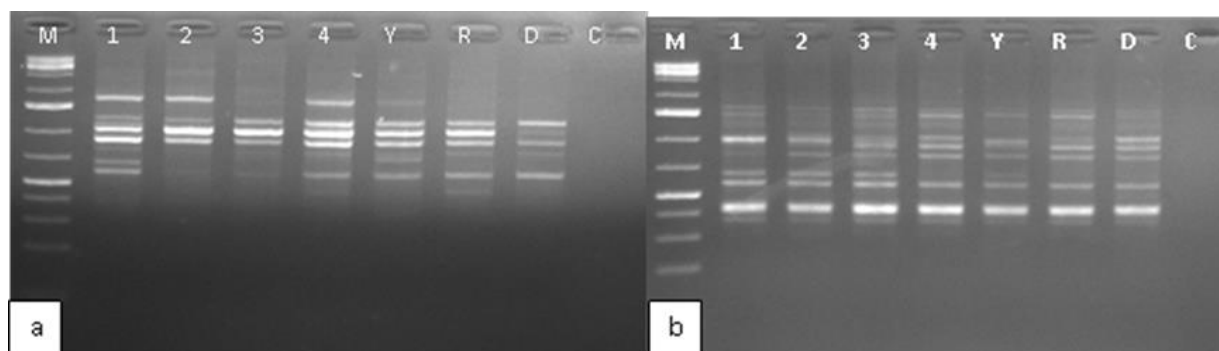


Figure 1. RAPD marker profiles of safflower varieties produced with primers OPS04 (a) and OPAA02 (b). M: 1 kb plus size ladder, C: PCR-control, and numbers show the genotypic numbers given in Table 1.

Figure 2 presents the UPGMA dendrogram of the safflower genotypes based on Jaccard's coefficient. According to this dendrogram, the safflower genotypes were clustered under two groups. In the first group were TR 49119, TR 42630 and TR 42670 landraces, whereas the second group contained the TR 64702 landrace and Remzibey 05, Dinçer 5-118 and Yenice 5-38 registered varieties. Again, the dendrogram revealed that TR 64702 and Remzibey 05 were genetically the closest genotypes with a value of 0.85. In their study investigating genetic differences based on agro-morphological characteristics of safflower, Amini et al. (2008) used 15 polymorphic RAPD primers. Their cluster analysis based on RAPD markers and 54% similarity coefficient divided the genotypes into five groups. The presence of high genetic variation and polymorphism in agro-morphological characteristics and DNA level suggests that selection programs can be developed using these agronomic characteristics.

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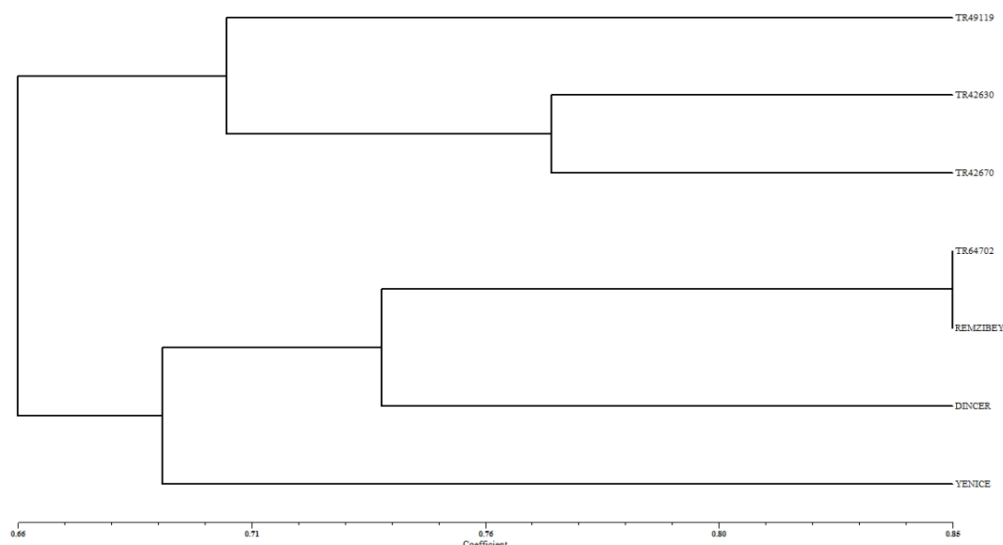


Figure 2. The UPGMA dendrogram based on the similarity index of the safflower landraces and registered varieties from RAPD markers

The genetic similarity matrix of safflower genotypes are given in Table 3. The genetic similarity values ranged from 0.61 to 0.85, with the mean value being calculated as 0.69. The TR 64702 landrace and Remzibey 05 registered variety were genetically the most similar genotypes with a value of 0.85, whereas the most distant were Dinçer 5-118 and Yenice 5-38 registered varieties and TR 64702 and TR 49119 landraces with a genetic similarity value of 0.61. This was followed by Dinçer 5-118 and TR 42630 having a value of 0.62. In a study using the RAPD method to determine the degree of polymorphism in safflower accessions (Mahasi et al., 2009), 14 randomized primers produced 61 amplification products. Evaluation in NTSYS program divided them into eight groups with a similarity coefficient of 0.79. Other researchers reported the genetic similarity rate between safflower accessions as 0.92 (Seghal and Raina, 2005) and 0.83 (Seghal et al. 2009).

Table 3. The genetic similarity matrix of the safflower genotypes by Jaccard's coefficient based on RAPD bands.

Genotype	TR 49119	TR 42630	TR 42670	TR 64702	Yenice 5-38	Remzibey 05	Dinçer 5-118
TR 49119	1.00						
TR 42630	0.75	1.00					
TR 42670	0.65	0.77	1.00				
TR 64702	0.61	0.67	0.65	1.00			
Yenice 5-38	0.65	0.73	0.64	0.72	1.00		
Remzibey 05	0.67	0.70	0.69	0.85	0.75	1.00	
Dinçer 5-118	0.68	0.62	0.63	0.73	0.61	0.74	1.00

The cophenetic correlation coefficient between the dendrogram and the similarity matrix for the RAPD markers data was significant ( $r = 0.78$ ), indicating that the result of the cluster was a good fit to the genetic similarity matrix. The three-dimensional plot of PCoA derived based on the RAPD data showed that the first three basic coordinate components were 73.51%, 7.64 and 6.04%, respectively. The results of the PCoA analysis were in good accord with the cluster analysis, dividing the safflower varieties into two groups (Figure 3). In a previous study using RAPD markers in 20 safflower genotypes, three factors were found to explain 78% of the total variation (Amini et al., 2008).

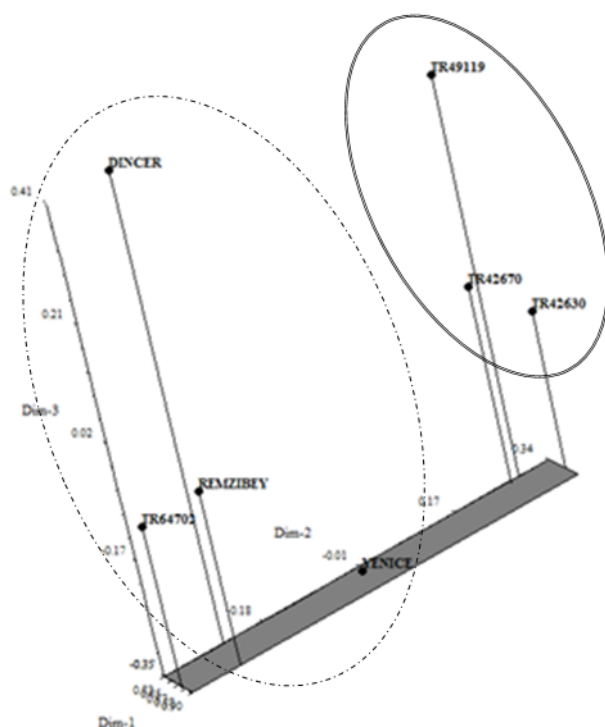


Figure 3. A 3D plot of principle coordinate analysis of safflower varieties using Jaccard's similarity coefficient of the RAPD data

Tonguç et al. (2010) examined whether a recombinant inbred lines (RIL) population developed by crossing Dinçer 5-118 and Remzibey 05 genotypes and advanced to the F6 level could be used as a mapping population. The authors employed the AFLP system and reported the total polymorphism rate as 4.5%, but observed no association between the polymorphisms detected. Dinçer 5-118 and Remzibey 05, used as parent in grouping analysis for genotypes, were included in the same group and found to be close relatives. The RIL population developed by crossing Dinçer 5-118 and Remzibey 05 was not considered suitable for use as a mapping population.

In this study, RAPD markers were used to determine the genetic distances and variability between some safflower landraces and registered varieties. Ten RAPD markers, which detected 90 different fragments, were used to characterize 7 *Carthamus tinctorius* L. landraces and registered varieties. RAPD markers revealed moderate-high degree of polymorphism (63.9%) with an average of 6 polymorphic bands per primer. The mean PIC value was determined as 0.38. The mean resolution power value was found to be 3.37, the effective multiplex ratio value was 4.14, and the marker index value was 1.57. The genetic similarity values ranged from 0.61 to 0.85 and this demonstrates that the level of genetic variation is moderate and suggests that genetic basis is relatively narrow. Similar results were reported by Giachino et al. (2014) for basil and Tonk et al. (2010) for oregano clones. The cluster analyses of the RAPD markers grouped the genotypes into two major clusters. With slight differences, the landraces and registered varieties were included in separate groups. The TR 64702 line and Remzibey 05 registered variety were genetically most similar genotypes with a value of 0.85. Consequently, RAPD analysis was successful in detecting genetic diversity and relationships among the safflower landraces and registered varieties.

#### 4. Conclusion

In Turkey, although safflower cultivation has gained great importance in the last decade and there has been a considerable augmentation in the cultivation area and production, the oil deficit remains quite high. In addition, cultivated safflower is not developed from modern varieties achieved

by breeding and it is mostly landraces containing a wide range of phenotypic variations in the population. Due to its tolerance to drought and salinity, and ability to grow in poor soil, safflower is a good candidate for the improving of new varieties with high seed and oil yield to meet the demands of producers and industrialists. Using advanced breeding methods, it is possible to obtain new varieties with high yield potential. In this study, it was found that RAPD, which is easier, faster, economic and reliable method, is a suitable method for genetic distance research in safflower landraces and registered varieties. In addition, this technique will provide great benefits for other breeding activities. Polymorphism and monomorphism values and the results of cluster analysis obtained from this research are expected to be helpful in the informed selection of parents for breeding purposes in future studies.

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