KSÜ Tarım ve Doğa Derg 26 (6), 1325-1336, 2023
KSU J. Agric Nat 26 (6), 1325-1336, 2023
https://doi.org/10.18016/ksutarimdoga.vi.1168608

Evaluation of Some Local and Registered Safflower (*Carthamus tinctorius* L.) Varieties Based on SRAP Markers

R. Refika AKÇALI GIACHINO[1]
[1]Department of Field Crops, Faculty of Agriculture, Ege University, 35100 Izmir, Türkiye
[1]https://orcid.org/ 0000-0002-6473-7250
✉: refika.giachino@ege.edu.tr

ABSTRACT

Safflower (*Carthamus tinctorius* L.), a member of the Asteraceae family, is an important plant grown in the world as a source of vegetable oil. In addition, it is a versatile crop that is also used as biodiesel, animal feed, spice, dye, and medicinal plant. In this study, SRAP markers were used to determine the genetic diversity and relationships between four local and three registered safflower cultivars for use in cross-breeding programs. The twelve primer combinations yielded a total of 101 bands, including 33 polymorphic bands. The level of polymorphism of SRAP markers which were represented by the average number of total bands (NTB) (8.4), the average number of polymorphic bands (NPB) (2.8), polymorphic band ratios (PBR%) (34.5%), resolving power (RP) (1.48), effective multiplex ratio (EMR) (1.17), and marker index (MI) (0.43) was low. Conversely, polymorphism information content (PIC) (0.35), Nei's gene diversity (h) (0.36) and Shannon's information index (I) (0.55) showed a significant genetic variation in the safflower genotypes studied. The polymorphism information content of the SRAP primer combinations used in the study ranged from 0.24 to 0.46, with an average of 0.35. Genetic similarity was calculated according to Dice similarity and varied from 0.12 to 0.92, with a mean genetic similarity (GS) of 0.58. The cophenetic correlation between the Dice similarity matrix and corresponding dendrogram obtained by SRAP (r = 0.95) revealed very good compliance. The genetically close genotypes were Remzibey05 - TR64702 and TR49119 - TR42630 (GS=0.91). Also, Dinçer5-118 and Yenice5-38 were the most genetically distant varieties (GS=0.12). Dinçer5-118 was very different from other genotypes (GS=0.29).

Field Crops

Research Article

Article History
Received : 30.08.2022
Accepted : 09.05.2023

Keywords
Safflower
Carthamus tinctorius
SRAP markers
Genetic diversity
DNA fingerprint

Bazı Yerel ve Tescilli Aspir Çeşitlerinin (*Carthamus tinctorius* L.) SRAP Markörleri ile Değerlendirilmesi

ÖZET

Asteraceae familyasının bir üyesi olan aspir (*Carthamus tinctorius* L.), bitkisel yağ kaynağı olarak dünyada yetiştirilen önemli bir bitkidir. Ayrıca biyodizel, hayvan yemi, baharat, boya ve tıbbi bitki olarak da kullanılan çok yönlü bir bitkidir. Bu çalışmada, melezleme programlarında kullanılmak üzere dört yerel ve üç tescilli aspir çeşidi arasındaki genetik çeşitliliği ve ilişkileri belirlemek için SRAP markörleri kullanılmıştır. On iki primer kombinasyonu, 33 polimorfik bant olmak üzere toplam 101 bant vermiştir. Primer başına düşen ortalama bant sayısı (NTB) (8.4), ortalama polimorfik bant sayısı (NPB) (2.8), polimorfik bant oranı (%PBR) (%34.5), çözümleme gücü (RP) (1.48), efektif multipleks oranı (EMR) (1.17) ve marker indeksi (MI) (0.43) ile incelenen SRAP markörlerinin polimorfizm seviyesi düşük bulunmuştur. Aksine, polimorfizm bilgi içeriği (PIC) (0.35), Nei'nin gen çeşitliliği (h) (0.36) ve Shannon'ın bilgi indeksi (I) (0.55), çalışılan aspir genotiplerinde önemli bir genetik varyasyon göstermiştir. Çalışmada kullanılan SRAP primer kombinasyonlarının polimorfizm bilgi içeriği (PIC) 0.24 ile 0.46 arasında değişmiş olup, ortalama 0.35 olarak tespit edilmiştir. Dice'ın benzerlik katsayısına göre hesaplanan genetik benzerlik, 0.12 ile 0.92

Tarla Bitkileri

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 30.08.2022
Kabul Tarihi : 09.05.2023

Anahtar Kelimeler
Aspir
Carthamus tinctorius
SRAP markörleri
Genetik çeşitlilik
DNA parmak izi

arasında değişmiş ve ortalama genetik benzerlik (GS) 0.58 olarak belirlenmiştir. Dice benzerlik matrisi ile SRAP tarafından elde edilen dendrogram arasındaki kofenetik korelasyon (r = 0.95) çok iyi bir uyumu ortaya çıkarmıştır. Genetik olarak en yakın çeşitler Remzibey05-TR64702 ve TR49119- TR42630 (GS=0.91) olmuştur. Dinçer5-118 ve Yenice5-38 tescilli çeşitler genetik olarak en uzak çeşitler olarak bulunmuştur. (GS=0.12). Ayrıca Dinçer5-118 diğer çeşitlerden genetik olarak çok farklı olarak belirlenmiştir. (GS=0.29).

To Cite : Giachino, R.R.A., (2023). Evaluation of Some Local and Registered Safflower (Carthamus tinctorius L.) Varieties Based on SRAP Markers. *KSU J. Agric Nat 26*(6), 1325-1336. https://doi.org/10.18016/ksutarimdoga.vi.1168608

Atıf Şekli: Giachino, R.R.A., (2023). Bazı Yerel ve Tescilli Aspir Çeşitlerinin (Carthamus tinctorius L.) SRAP Markörleri ile Değerlendirilmesi. *KSÜ Tarım ve Doğa Derg 26* (6), 1325-1336. https://doi.org/10.18016/ksutarimdoga.vi.1168608

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is a member of the Asteraceae family with yellow, red, orange, white, and cream-coloured flowers (Knowles, 1989). It has a taproot system that can go about 2-3m deep and secondary roots that can grow up to 60-90 cm. Therefore, it is more suitable to grow in arid conditions than other oilseed plants. Safflower has been used as oil, spice, tea, medicinal, and dye plant. Safflower seeds contain 25-45% oil, 90% of which is made up of unsaturated fatty acids (Weiss, 2000). Its oil is highly rich in tocopherol (vitamin E) (Weiss, 1971). *Carthamin* and *Carthamidin* pigments in their contents are used for food and textile applications (Yue et al., 2013; Golkar, 2018). Moreover, because of its chemical composition, it has pharmacological functions that include antioxidant, anti-inflammatory, analgesic, antidiabetic, hepatoprotective, and antihyperlipidemic activities (Asgarpanah and Kazemivash, 2013; Delshad et al., 2018). In recent years, it has also been heavily favoured as a raw material in biodiesel production (Yesilyurt et al., 2020; Nogales-Delgado et al., 2019).

Safflower is one of humanity's oldest plants. It has been cultivated in the Mesopotamian plains and the Eastern Anatolian Region and according to archaeological findings, its domestication is likely to date back to about 2500 B.C.E. (Prance and Nesbitt 2005). Commercial production began only in the 1940s. Currently, safflower is grown in different geographical regions of the world, primarily in Kazakhstan, USA, Russian Federation, Mexico, China, India, Argentina, and Turkey. These countries account for about 90% of the world's safflower production. Turkey is one of the World's ten largest safflower producers, it ranked 8th in 2020 with 21.325 tons of safflower seed production in approximately 15,114 hectares of land (FAOSTAT, 2022). The average safflower seed yield in Turkey is 14.10 hg/ha, which is over a global average of 9.05 hg/ha. Safflower cultivation in Turkey fluctuates from year to year. In the 1960s, 900-1000 ha plantation was made, while in 2000 it fell by as much as 30 ha, and in 2009 it was planted at 21.500 ha, increasing by about 16.000 ha compared to the previous year. During the 2014-2015 cultivation seasons, safflower plantation reached a record high with approximately 45.000 ha of plantation and 70,000 tons of production (FAOSTAT, 2022; TUIK, 2022). This plant was brought to Turkey via the Balkans by Bulgarian migrants in the 1940s and was first cultivated around Balıkesir, Bursa, and Kütahya (Baydar, 2021). Today, safflower agriculture is mostly done in 37 provinces including Ankara, Muş, Aksaray, Konya, Gümüşhane, Nevşehir, Kayseri, Afyon and Uşak (TÜİK, 2022).

The first studies of safflower plant breeding in Turkey were initiated in the early years of the Republic. In 1931, 'Yenice 5-38' safflower variety, which is composite of 5 spiny safflower varieties, was developed by the selection method. After a long time, through the selection method, "Dinçer 5-118" and "Remzibey 05" were registered in 1977 (Köse, 2017). In 2008-2009, safflower farming gained a lot of momentum and there were large increases in both cultivation areas and production, whereas breeding studies have been limited in developing different new safflower varieties with high grain yield and oil ratio and different oil compositions. New varieties (Balcı, Linas, Olas, Zirkon, Olein, Safir) developed with selection and hybridization breeding methods have been offered to the manufacturers of safflower since 2011. Turkey has increased safflower production by more than 50.000 tons in the years 2013-2015. The government's agricultural assistance for oilseed plants has been the biggest factor in the development of safflower agriculture. However, in 2016-2019, production decreased by more than 48,000 tons. This unforeseen decline is due to the marketing problem and the fact that the producers were not able to make a profit from safflower (Ilkdoğan, 2012). Another reason is that both seed yield and oil ratio remain low compared to other oil plants such as sunflower, sesame seeds, and rapeseeds with which safflower yield competes (Baydar & Erbaş, 2020). One of the main factors necessary for increasing productivity in safflower agriculture is the use of efficient and high-quality improved seeding. For safflower production to be

stable and sustainable in Turkey, alternative cultivation and breeding methods that will increase grain and oil yield and better cope with biotic and abiotic stress factors should be implemented considering the demands of producers, industrialists, and consumers.

The variation required for breeding studies is provided from proprietary varieties, local varieties, and wild relatives. Therefore, it is important to reveal genetic states of the cultivated populations for proper design of breeding programs and successful sustainability of populations to improve both yields and oil quality. Molecular markers are an important tool for assessing the levels and structure of genetic diversity and have been used to study genetic diversity in many breeding programs. Different molecular marker system could be used to assess germplasm diversity including SRAP (Sequence Related Amplified Polymorphism) markers, which have many advantages such as simplicity, reliability, flexibility, multiple-locus detection, genome-wide scopes, and cost-effectiveness (Li & Quiros, 2001; Li et al., 2013). It is a marker system that was developed to eliminate disadvantages related to AFLP and RAPD methods and was first used in *Brassica* species. SRAP markers are dominant, simple, and effective for amplification of open reading frames (ORFs), based on the amplification of forwards and reverse primers of 17-18 nucleotides. SRAP markers have widely been used to evaluate the genetic diversity and structure population in species, such as sesame (*Sesamum indicum* L.) (Zhang et al., 2010), soybean (*Glycin max* (L.) Merr.), peanut (*Arachis hypogaea* L.) (Baloch et al., 2010), safflower (*Carthamus tinctorius*) (Peng et al., 2008; Talebi et al., 2012), oilseed rape (*Brassica napus* L.) (Ahmad et al., 2014), flax (*Linum usitatissimum* L.) (Li et al., 2009). In this research, it was aimed to determine the relationships and genetic diversity of local and registered safflower genotypes and varieties to be used in crossbreeding programs with SRAP molecular markers.

MATERIALS and METHODS

Plant Materials

The material of the study consisted of the four local safflower genotype (TR 49119, TR 42630, TR 42670, and TR 64702) and three registered varieties (Yenice 5-38, Remzibey 05 and Dinçer 5-118) which were obtained from the Aegean Agricultural Research Institute, Izmir, Turkey. General information about the plant material (Table 1) is provided in a previous report (Giachino & Inan 2019). Molecular analyses were carried out at the laboratory of Ege University Application and Research Centre of Seed Technology (TOTEM).

DNA extraction

Genomic DNA was isolated from fresh leaves using the GenElute™ Plant Genomic DNA Miniprep Kit (Sigma-Aldrich). For this purpose, samples taken from the fresh leaves of young seedlings of 10-15 cm length were powdered with a mortar and pestle in liquid nitrogen. DNA quality and quantity were measured through 260:280 nm absorbance ratios with a spectrophotometer, and electrophoresis was conducted on 0.8% agarose gel in 1x TAE buffer at 100 V for 1 hours and stained with 0.5 µg/ml ethidium bromide (EtBr) and photographed under UV light. DNA samples were diluted to 10 ng/mL and stored at −20°C.

Sequence-related amplified polymorphism (SRAP) Analysis

The SRAP analyses were performed as described by Li & Quiros (2001) with some modifications. A total of 17 primer combinations (4 forward and 7 reverse) were screened and 12 suitable primer combinations were selected for amplification (Table 2) provided by ECS (Canada). The PCR reaction was performed in a total volume of 25 µl reaction mixtures consisting of 1X PCR buffer, 0.2 mM dNTP, 0.5 µM of each forward and reverse primer, 2 mM $MgCl_2$, 50 ng template DNA, 1 Unit Taq DNA Polymerase, and ddH_2O. DNA amplification reactions were performed in a Techne thermal cycler (Germany) using the following program: 94 °C initial denaturation for 5 min, then 5 cycles of 94 °C denaturation for 1 min, 35 °C annealing for 1 min, 72 °C elongation for 2 min, then 30 cycles of 94 °C denaturation for 1 min, 50 °C for 1 min, 72 °C for 2 min, followed by a 72 °C final extension for 5 min. The amplification products were separated by electrophoresis in 2% agarose gels in 1x TAE buffer (40 mM Tris-Acetate, 1 mM EDTA, pH: 8.0) at 100 V for 2-3 hours and stained with 0.5 µg/ml ethidium bromide (EtBr) and photographed under UV. Also, a 100 bp plus DNA ladder (Fermentas) was used as a standard marker for estimating the size of the PCR products.

Data Analysis

The SRAP bands of 12 primer combinations were graded according to their presence (1) or absence (0) in electrophoresis, and data was converted to the binary matrix using Microsoft Excel. For each SRAP primer combination, the total number of scored bands, the number of polymorphic bands, the number of monomorphic bands, and the percentage of polymorphism were determined. In addition, parameters such as polymorphism information content (PIC), resolving power (RP), effective multiplex ratio (EMR), and marker index (MI) were calculated. The PIC was calculated according to Anderson et al. (1993) by using the following formula for all primers:

$$PIC = 1 - \Sigma\, pi^2 \qquad (1)$$

where pi^2 is the frequency of the i th allele. The resolving power (RP) was calculated according to Prevost and Wilkinson (1999) for each primer

combination as follows:

$$RP = \Sigma\ I_b \quad (2)$$

where I_b is the band informativeness calculated with the following formula

$$I_b = 1 - [2 \times (0.5-p)] \quad (3)$$

and p is the proportion of seven genotypes containing the band. The effective multiplex ratio is the number of polymorphic bands detected per electrophoresis. The marker index was calculated as noted by Powell et al. (1996) and Milbourne et al. (1997) by multiplying PIC with the EMR (MI = EMR X PIC). Genetic diversity indicators such as Nei's gene diversity (h) and Shannon's information index (I) were calculated for each SRAP marker with POPGENE version 1.31 (Yeh et al., 1997). The dendrogram was generated by the Unweighted Pair Group Method Averages (UPGMA) with the NTSYSpc-2.02 software (Rohlf, 2000) using the Dice genetic similarity matrix (Dice, 1945). A principal coordinate analysis (PCoA) was also carried out using the same software. To determine the goodness-of-fit of the clustering compared with the basic data matrix, the cophenetic correlation coefficient was computed using the normalised Mantel's Z test (Mantel, 1967) via the COPH and MXCOMP procedures of NTSYS-pc, version 2.01e (Rohlf, 2000).

Table 1. General features of safflower genotypes used in the study*
Çizelge 1. Araştırmada kullanılan aspir genotiplerinin genel özellikleri

Local Genotypes	*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	Mersin	Anamur	850 m	360442N	0325003E
Registered Varieties	*Registration Year*	*Breeding Method*	*Colour of Flower*	*Plant Length*	*Structure*	*Breeding Institution*
5-Yenice 5-38	1931	Selection	Red	100-120 cm	non-spiny	GKTAEM
6-Remzibey 05	2005	Selection	Yellow-orange	60-80 cm	spiny	GKTAEM
7-Dinçer 5-118	1977	Selection	Orange-red	90-110 cm	non-spiny	GKTAEM

(GKTAEM): Transitional Zone Agricultural Research Institute
*Taken from previous work by Giachino & Inan (2019)

Table 2. Sequence information of the SRAP primers used in this study
Çizelge 2. Çalışmada kullanılan SRAP primerinin dizi bilgisi

Forward primers	*5'→3' sequence*	*Reverse primers*	*5'→3' sequence*
Me1	TGAGTCCAAACCGGATA	Em1	GACTGCGTACGAATTAAT
Me3	TGAGTCCAAACCGGAAT	Em2	GACTGCGTACGAATTTGC
Me4	TGAGTCCAAACCGGACC	Em3	GACTGCGTACGAATTGAC
Me5	TGAGTCCAAACCGGAAG	Em4	GACTGCGTACGAATTTGA
		Em5	GACTGCGTACGAATTAAC
		Em7	GACTGCGTACGAATTATG
		Em8	GACTGCGTACGAATTAGC

RESULTS

Safflower genotypes were evaluated for determining genetic diversity using SRAP primer combinations. A total of 19 primer combinations were screened, of which 12 primer combinations yielded evaluable bands. The 12 primer combinations generated a total of 101 bands, including 33 polymorphic bands. The polymorphic band ratio is 32.6%. The number of total bands (NTB), number of polymorphic bands (NPB), polymorphic bands ratios (PBR %), PIC, RP, EMR, and MI values are presented in Table 3. The NTB amplified by each primer combination ranged from 2 (Me4xEm3) to 11 (Me3xEm2, Me4xEm1, Me5xEm5, and Me5xEm8) with an average of 8.4, and their molecular weights were between 80 and 1.600 bp. The NPB with each primer combination ranged from 1 (Me1xEm2, Me3xEm3, and Me4xEm3) to 6 (Me3xEm1) with an average of 2.8. The PBR of the primer combinations ranged from 13% (Me3xEm3) to 67% (Me3xEm1). The average percentage of the polymorphic bands was calculated as 34.5%. Only in 4 SRAP primer combinations (Me3xEm1, Me3xEm4, Me4xEm3, Me5xEm4), PBRs were found to be 50%, as well as, greater than 50%. The PBR of the other eight primer combinations was observed to be below 50%.

The PIC values for the 12 primer combinations varied from 0.24 to 0.46 with an average of 0.35. The same results were obtained for RAPD primers reported by Giachino and Inan (2019), PIC ranged from 0.24 to 0.46 with an average of 0.38. The lowest value was observed in three primer combinations, including Me1xEm2, Me3xEm4, and Me4xEm3, and the highest PIC value

was seen in Me3xEm1. Half of the SRAP primer combinations exceeded the average PIC values (0.35) (Table 3).

Table 3. Diversity parameters evaluated using SRAP markers to investigate the genetic diversity of safflower genotypes

Çizelge 3. Aspir genotiplerinin genetik çeşitliliğini araştırmak için SRAP belirteçleri kullanılarak değerlendirilen çeşitlilik parametreleri

Primer combination	*NTB*	*NPB*	*PBR%*	*PIC*	*RP*	*EMR*	*MI*	*h*	*I*
Me1xEm2	7	1	14	0.24	0.29	0.14	0.03	0.25	0.41
Me3xEm1	9	6	67	0.46	4.57	4.00	1.85	0.46	0.65
Me3xEm2	11	2	18	0.41	1.14	0.36	0.15	0.41	0.60
Me3xEm3	8	1	13	0.41	0.57	0.13	0.05	0.41	0.60
Me3xEm4	6	4	67	0.24	1.14	2.67	0.65	0.24	0.41
Me3xEm5	7	2	29	0.33	0.86	0.57	0.19	0.33	0.50
Me4xEm1	11	3	27	0.44	2.00	0.82	0.36	0.44	0.63
Me4xEm3	2	1	50	0.24	0.29	0.50	0.12	0.24	0.41
Me4xEm7	8	2	25	0.33	0.86	0.50	0.16	0.33	0.50
Me5xEm4	10	5	50	0.41	3.14	2.50	1.02	0.41	0.59
Me5xEm5	11	4	36	0.29	1.43	1.45	0.42	0.29	0.46
Me5xEm8	11	2	18	0.45	1.43	0.36	0.16	0.45	0.64
AV	**8.4**	**2.8**	**34.5**	**0.35**	**1.48**	**1.17**	**0.43**	**0.36**	**0.55**
Min.	**2**	**1**	**13**	**0.24**	**0.29**	**0.13**	**0.03**	**0.24**	**0.41**
Max.	**11**	**6**	**67**	**0.46**	**4.57**	**4.00**	**1.85**	**0.46**	**0.65**
Total	**101**	**33**	**32.6**	**-**	**-**	**-**	**-**	**-**	**-**

NTB: Number of total bands, NPB: Number of polymorphic bands, PBR%: Polymorphic band ratios, PIC: Polymorphism information content, RP: Resolving power, EMR: Effective multiplex ratio, MI: Marker index, h: Nei's gene diversity, I: Shannon's information index

The RP ranged from 0.29 (for primer combinations of Me1xEm2 and Me4xEm3) to 4.57 (for primer combinations of Me3xEm1) with an overall average of 1.48. Only three primer combinations (Me3xEm1, Me4xEm1, and Me5xEm4) exceeded the mean RP value. The highest EMR was 4.0, observed in Me3xEm1, but the lowest EMR was 0.13, obtained from Me3xEm3, and the average value was 1.17 per primer combination. The MI values ranged from 0.03 to 1.85, with an average of 0.43. Maximum MI was observed in the Me3xEm1 primer combination, and the minimum MI was obtained with SRAP primer combinations of Me1xEm2 (0.03) and Me3xEm3 (0.05).

The Nei's gene diversity values ranged from 0.24 to 0.46, with a mean of 0.36 (Table 3). The higher gene diversity was found in Me3xEm1 primer combination while the lower in Me3xEm4 and Me4xEm3 as 0.24. The Shannon's information index ranged from 0.41 (for primer combinations of Me1xEm2, Me3xEm4 and Me4xEm3) to 0.65 (for primer combination of Me3xEm1) with an overall average of 0.55.

When evaluated on the basis of SRAP primer pairs; the Me3xEm1 primer pair was the most informative by giving highest mean values of number of polymorphic bands (6), polymorphic band ratios (67), polymorphism information content (0.46), resolving power (4.57), effective multiplex ratio (4.00), marker index (1.85), Nei's gene diversity (0.46), and Shannon's information index (0.65), while the Me1xEm2 primer pair was least informative by reproducing low values of number of polymorphic bands (1), polymorphic band ratios (14), polymorphism information content (0.24), resolving power (0.29), effective multiplex ratio (0.14), marker index (0.03), Nei's gene diversity (0.25), and Shannon's information index (0.41).

The Dice similarity matrix was generated using NT-SYS software to analyse the SRAP data of safflower genotypes. Genetic similarity was calculated by making pairwise comparisons between all local and registered safflower genotypes by this matrix. Genetic similarity (GS) showed a wide distribution and varied from 0.12 to 0.92, with a mean similarity of 0.58 (Table 4). Genetically, the closest genotypes were Remzibey 05 and TR64702 with a value of 0.92, followed by TR49119 and TR42630 local genotypes with a value of 0.91, indicating a very close relationship. Dinçer 5-118 and Yenice 5-38 were the most genetically distant varieties with a value of 0.12. The cophenetic correlation coefficient, which is a measure of the correlation between the dendrogram, and similarity matrix calculated using the Z test (Mantel, 1967), was found to be 0.95, indicating that the clustering result shows very good compliance with the genetic similarity matrix.

Figure 1 shows the dendrogram based on the SRAP data. In the UPGMA dendrogram, safflower genotypes clustered into two main groups based on the Dice coefficient. Cluster I was further divided into three

subclusters: Subcluster 1 includes 3 local genotypes: TR 49119, TR 42630, and TR 42670. Of these, the TR49119 and TR42630 local genotypes were observed to be extremely similar with a coefficient of 0.91. Subcluster 2 includes TR 64702 landrace and Remzibey 05 cultivar which are the closest genotypes, with Dice values of 0.92. (Table 4). Subcluster 3 comprised a single variety, Yenice5-38. Cluster II also consisted of a single variety, Dinçer5-118. The distribution determined by UPGMA analysis also revealed that Dinçer5-118 registered variety was genetically very different from Yenice5-38 (0.12 GS) and from all other genotypes with 0.29 GS.

Table 4. The genetic similarity matrix based on the Dice coefficient calculated from SRAP data of safflower genotypes

Çizelge 4. Aspir çeşitlerinin SRAP verilerinden hesaplanan Dice katsayısına dayalı genetik benzerlik matrisi

	TR49119	*TR42630*	*TR42670*	*TR64702*	*Yenice5-38*	*Remzibey05*	*Dinçer5-118*
TR49119	1.00						
TR42630	**0.91**	1.00					
TR42670	0.79	0.78	1.00				
TR64702	0.67	0.63	0.79	1.00			
Yenice5-38	0.56	0.56	0.65	0.58	1.00		
Remzibey05	0.64	0.60	0.76	**0.92**	0.67	1.00	
Dinçer5-118	0.32	0.24	0.28	0.39	**0.12**	0.40	1.00

The bold values indicate the maximum and minimum genetic similarity values among the landraces

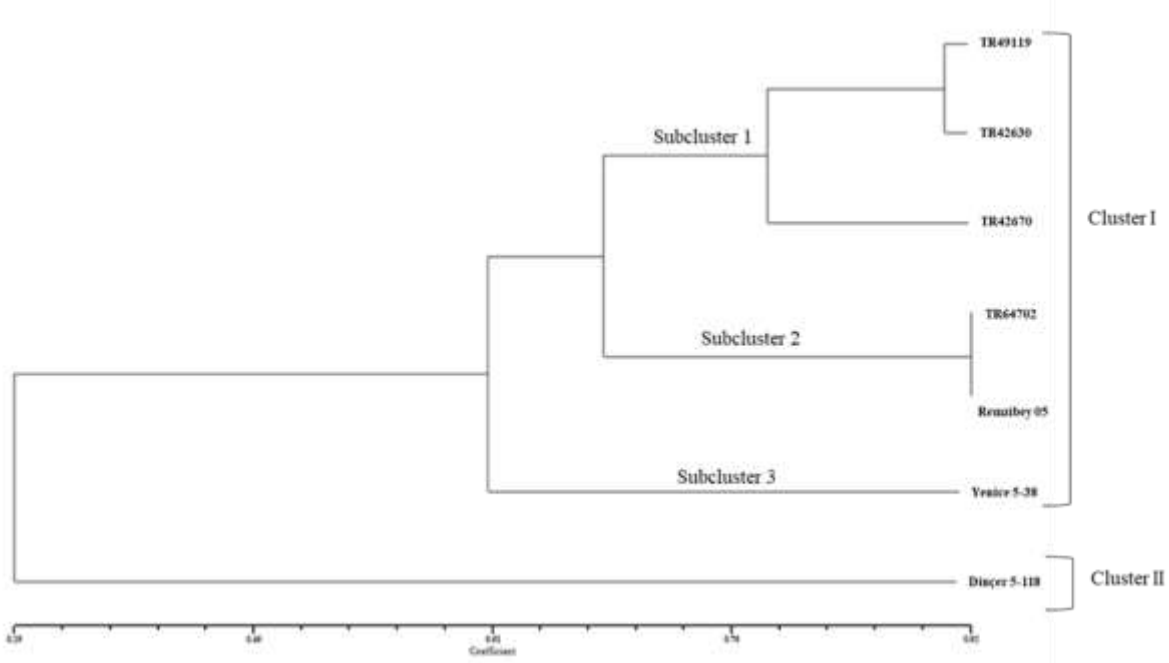

Figure 1. The UPGMA dendrogram of the safflower genotypes generated from SRAP data.
Şekil 1. Aspir çeşitlerinin SRAP verilerinden oluşturulan UPGMA dendrogramı

The results of PCoA for the SRAP data are presented in Fig. 2. The first two principal coordinates explained 66.5% and 13.5% of the total molecular variation, respectively. This corresponds to 80% of the total variation. The results of the PCoA analysis were in good agreement with the cluster analysis. The PCoA plot of SRAP clearly showed the main clusters of safflower genotypes.

DISCUSSION and CONCLUSIONS

In this research, the relationships and genetic diversity between local and registered safflower genotypes were determined by SRAP markers that are easy to apply and reliable. Twelve primer combinations yielded a total of 101 bands, including 33 polymorphic bands. The polymorphic band ratio is 34.5%. The level of polymorphism of SRAP markers, represented by the average number of total bands (8.4), average number of polymorphic bands (2.8), percentage of polymorphism (34.5 %), resolving power (1.48), effective multiplex ratio (1.17), and marker index (0.43), was low. Especially, the number of polymorphic bands detected with each primer combination is considerably lower than Peng et al.

(2008) (30), Talebi et al. (2012) (18.7), Mokhtari et al. (2013) (20.3) and slightly less than Golkar & Mokhtari (2018) (7.3). The possible explanation for the relatively low number of polymorphic bands may be associated with the investigated local and registered genotypes and different combinations of loci in the present research. The level of polymorphism is influenced by the number of markers, population size, and the type of plant material used in the study (Kiran et al., 2017).

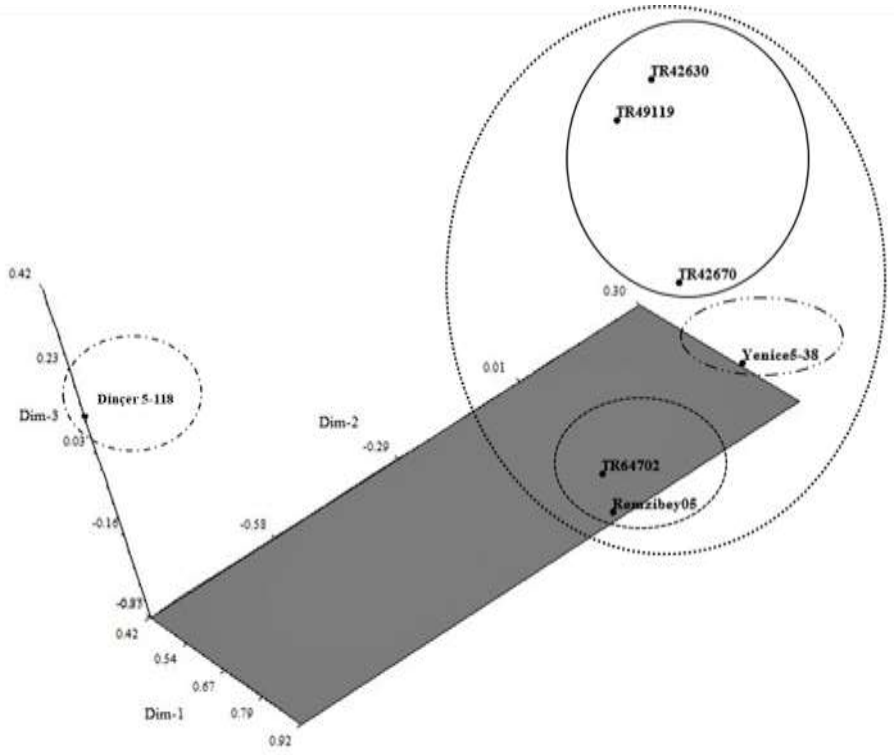

Figure 2. Principal coordinate analysis of safflower varieties based on the genetic similarity matrix generated from SRAP data.

Şekil 2. SRAP verilerinden oluşturulan genetik benzerlik matrisine dayalı olarak aspir çeşitlerinin temel koordinat analizi

A low level of polymorphism in safflower is also evident in previous studies. In our previous research with RAPD markers conducted on the same material of the current study, the number of polymorphic bands was similarly low (ranged from 2 to 9 with an average of 6) (Giachino & Inan, 2019). In addition, the number of polymorphic bands produced by the Me3xEm3, Me1xEm2, Me3xEm2, and Me5xEm8 primer combinations is considerably lower than that produced by the others, which may partly contribute to the low polymorphic fragments. Lee et al. (2014) observed an average of 2.8 alleles per SSR locus in a collection of 100 safflower accessions from different centres of similarity. Kiran et al. (2017) reported SSR alleles per locus ranged from 2 to 15 with an average of 3.6 in a collection of 148 safflower accessions representing 15 countries. Small polymorphic band numbers resulted in a relatively low average polymorphism percentage (34.5%). Similar results were also revealed by Lei et al. (2013) (35%). Several safflower genetic diversity studies with SRAP markers reported a greater result than the polymorphism ratio found in this study, including Peng et al. (2008) (57%), Talebi et al. (2012) (62.2%), Mokhtari et al. (2013) (82%), and Golkar & Mokhtari (2018) (76.3%). Also, in a previous RAPD study of Giachino & Inan (2019), the polymorphism ratio of the same varieties was observed as 63.9%. Indeed, Sehgal et al. (2009) reported a low average of polymorphic genes in the Turkish population (0.15 ratio) in a collection of 85 safflower accessions from different regional gene pools (originating from 24 countries). Furthermore, Tonguç et al. (2011) employed AFLP markers in 38 varieties and lines including three registered varieties used in the present study and reported that the average polymorphic percentage was 27.5%. Also, in the same research, AFLP analysis performed with 61 safflower ecotypes, 41 of which originating from Turkey and 13 from different countries, exhibited that the polymorphism ratio of the ecotypes was lesser than the polymorphism ratio of the cultivars and lines of studies and this paper (between 5.4-22.7% and average 14%).

The measure of PIC is an important component and one of the key information and statistical indicators in

the implementation of the planning of breeding programs (Chesnokov & Artemyeva, 2015). PIC reflects a discriminating ability of the marker and depends on the number of known alleles and their distribution frequency, thus representing genetic diversity (Giachino, 2020). A classification of the informativeness of dominant markers was proposed by Serrote et al. (2020) based on PIC values: low (0 to 0.10), medium (0.10 to 0.25), high (0.30 to 0.40), and very high (0.40 to 0.50). Accordingly, it is evident that the SRAP markers used in this study have high informative power with an average of 0.35. In addition, half of 12 primer pairs (Me3xEm1-0.46, Me5xEm8-0.45, Me4xEm1-0.44, Me3xEm2-0.41, Me3xEm3-0.41, Me5xEm4-0.41) have a very high distinguishing capacity, which may be more useful for genetic characterization in safflower as well as in other plants. The mean PIC was consistent with that of Golkar & Mokhtari (2018) (0.35). The findings of Talebi et al. (2012), Mokhtari et al. (2013), and Tonguç et al. (2011) revealed results of 0.33, 0.28, 0.29, respectively.

The RP ranged from 0.29 to 4.57 with an overall average of 1.48. Only three primer combinations (Me3xEm1, Me4xEm1, and Me5xEm4) exceeded the mean RP value. Different RP values were reported for RAPD (2.07), ISSR (2.44) (Safavi et al., 2010), ISSR (8.72) (Majidi & Zadhoush 2014), and RAPD (3.37) markers (Giachino & Inan 2019) which were used on safflower genotypes. The MI values ranged from 0.03 to 1.85, with an average of 0.43. Maximum MI was observed in the Me3xEm1 primer combination, and the minimum MI was obtained with SRAP primer combinations of Me1xEm2 (0.03) and Me3xEm3 (0.05). In safflower, different mean MI values have been reported for various RAPD, ISSR, AFLP markers (1.41, 0.70, 18.2, respectively) (Seghal and Raina 2005).

Genetic diversity of safflower genotypes generated by SRAP primers was measured by calculating indicators of genetic diversity such as Nei's gene diversity (h) and Shannon's information index (I). The average Nei's gene diversity values ranged from 0.24 to 0.46, with an average value of 0.36, which compatible with previous SRAP analysis results were reported by Mokhtari et al. (2013) for Sixty-two safflower accessions (0.26-0.44, av. h=0.36). Furthermore, obtained average Nei's gene diversity value was greater than the values found by Talebi et al. (2012) for SRAP markers (h=0.30), Ali et al. (2019) for İPBS- retrotransposon markers (h=0.21) and Yıldız et al. (2022) for POGP markers (h=0.27). Whereas Ali et al. (2020) studied 131 safflower accessions using ISSR markers and found Nei's gene diversity as 0.38, which is slightly higher than the value (0.36) obtained in this study. Shannon's information index values ranged between 0.41 and 0.65, with an average of 0.55. Similar results were reported by Ali et al. (2020) for ISSR markers (0.44–0.65, av. I=0.557). The mean Shannon's information index was found higher than previously reported studies of Hassani et al. (2020) and Talebi et al. (2012) revealed 0.43 and 0.17 respectively for SRAP markers in safflower. Golkar and Mokhtari (2018) for SRAP and SCoT markers, Ali et al. (2019) for IPBS markers, reported a lower Shannon information index mean (0.35, 0.33, respectively). Nei's gene diversity and Shannon's information index values are measures of genetic diversity, which explain the evolutionary pressure on alleles and the mutation rate (Bonneuil et al. 2012). Nei's genetic diversity and Shannon's information index values for all primers were 0.36 and 0.55 respectively, this indicates a prominent genetic diversity at the level of local and registered genotypes.

PCoA multivariate approach was used to complement the information obtained from the cluster analysis (Naik et al., 2017). The results of the PCoA analysis were in good agreement with the cluster analysis. The first two principal coordinates explained correspond 80% of the total variation. Accordingly, this result indicates the appropriate distribution of SRAP markers across the entire genome and confirms the results of the cluster analysis. Genetic similarity values showed a wide distribution ranging from 0.12 to 0.92, with a mean similarity of 0.58. Different genetic similarities have been reported previously among safflower. Peng et al. (2008) reported a mean similarity of 0.57 among 23 safflower populations. Golkar & Mokhtari (2018) reported that the similarity coefficient ranged from 0.53 to 1 with an average of 0.76 among one hundred safflower genotypes. The similarity matrix of genotypes used in the SRAP analysis by Talebi et al. (2012) ranged from 0.33 to 0.91, with an average of 0.51. In the UPGMA analysis which shows very good compliance with the genetic similarity matrix (r=0.95), local and registered genotypes were almost distinctly clustered. Safflower genotypes clustered into two main groups. Cluster I was further divided into three subclusters. Subcluster 1 includes 3 local genotypes: TR 49119, TR 42630, and TR 42670. Of these, the TR49119 and TR42630 local genotypes were observed to be extremely similar with a coefficient of 0.91. TR49119 is a local genotype collected from the 'Gelendost' district of 'Isparta' province. It is located in the transition zone between the Mediterranean climate and the continental climate that is dominant in Central Anatolia. It is situated on the coast of 'Lake Eğirdir' at an altitude of 860 m. TR42630 was collected from the 'Havsa' district of Edirne province at the altitude of 40 m (Table 1) in the Trakya Region of Turkey. It demonstrates a hybrid climate between the Mediterranean and Black Sea climates, which can be considered a mild oceanic climate (Akgün et al., 2013). There is an altitude difference of about 800 m and 800 km between these two varieties. Interestingly, although they have such

different geographical conditions, they show high similarities. The common point here is that both varieties are in the transitional zone. The other local genotype TR 42670 in subcluster 1 was collected from 'Saray' district of 'Tekirdağ' province, located in the Thrace region, at an altitude of 240 m. It shows high similarity with other local genotypes, with 0.78-0.79 GS. Consequently, varieties from different locations are grouped in the same cluster. A possible explanation for this may be the exchange of seeds materials among producers at different periods in different provinces of Turkey (Giachino, 2020). Moreover, these high similarity coefficients may be due to the narrowing of the genetic base by long-term selection. This is in accordance with some of the previous studies which concluded that local genotypes from different geographic regions may be genetically similar (Naik et al., 2017; Kiran et al., 2017). Subcluster 2 includes TR 64702 landrace and Remzibey05 cultivar which are the closest genotypes, with Dice values of 0.92. That was confirmed by RAPD markers (GS=0.85) similarly (Giachino and Inan 2019). TR 64702 landrace was collected from 'Anamur' district of 'Mersin' province at 850 m altitudes. Remzibey05 is a spiny variety with yellow flowers, relatively short height, and 25-35% oil content (with 2.5-3 times more oleic acid content than the other two varieties (Cosge et al., 2007)). It was improved by the selection method as a composite of landraces (Köse, 2017). Remzibey05 cultivar is probably from a common genetic origin with the TR 64702 local genotype. In other words, the very high similarity between the Remzibey05 cultivar and the TR 64702 landrace with a value of 0.92 can be explained by the fact that these two genotypes are descended from the same ancestor. Each of subcluster 3 and Cluster II comprised a single variety, Yenice5-38, and Dinçer5-118, respectively. The distribution determined by UPGMA analysis also revealed that Dinçer5-118 registered cultivar is very different genetically between themselves and from other genotypes, with 0.29 GS. Furthermore, an extreme variation was observed between these two cultivars, with a similarity of 0.12 (Table 4). This is predictable for two varieties with different characteristics, which were registered approximately 45 years apart. These two ancient cultivars were developed via selection as a composite of 5 non-spiny safflower cultivars. Dinçer5-118 stands out in terms of grain yield, especially in regions where the distribution of precipitation is regular throughout the growing season (Köse, 2017).

In this research, the relationships and genetic diversity between local genotypes and registered safflower varieties were determined by SRAP molecular markers that are easy to apply and reliable. Different marker parameters, viz number of total bands, number of polymorphic bands, polymorphic band ratios, resolving power, effective multiplex ratio and marker index of the SRAP markers were found to be low. In contrast, genetic diversity indicators such as polymorphism information content (0.35), Nei's gene diversity (0.36) and Shannon's information index (0.55) showed a significant genetic variation of the studied safflower genotypes. Genetic similarity values showed a wide distribution, and local genotypes originating from different geographical regions were genetically close. In addition, a very high genetic distance was determined between the old, registered cultivars Dinçer 5-118 and Yenice 5-38. Moreover, Dinçer 5-118 was classified as quite distant from all other genotypes. Dinçer 5-118 and Yenice 5-38 can be used as genitors in breeding studies. In future studies, safflower breeding programs can be planned by using more plant materials with various characteristics and advanced molecular breeding techniques.

ACKNOWLEDGEMENTS

Laboratory analysis of this research was conducted at Ege University Application and Research Centre of Seed Technology (TOTEM). I am grateful to Prof. Dr. Hulya İLBİ for giving me this opportunity. I also thank Dr. Dilşat Yeğenoğlu for her assistance in statistical calculations.

Conflict of interest

The author declares that they have no conflict of interest.

REFERENCES

Ahmad, R. Quiros, C.F., Rahman, H., & Swati, Z.A. (2014). Genetic diversity analyses of *Brassica napus* accessions using SRAP molecular markers. *Plant Genetic Resources: Characterization and Utilisation, 12*,14-21. https://doi.org/10.1017/S147926211300021X

Akgün, F., Akkiraz, M.S., Üçbaş, S.D., Bozcu, M., Kapan Yeşilyurt, S., & Bozcu, A. (2013). Oligocene vegetation and climate characteristics in north-west Turkey: Data from the South-western part of the Thrace Basin. *Turkish Journal of Earth Science, 22*, 277-303. https://journals.tubitak.gov.tr/earth/vol22/iss2/8

Ali, F., Yılmaz, A., Nadeem, M. A., Habyarimana, E., Subaşı, I., Nawaz, M. A., Chaudhary, H.J., Shahid, M.Q., Ercişli, S., Zia, M.A., Chung, G. & Baloch, F. S. (2019). Mobile genomic element diversity in world collection of safflowers (*Carthamus tinctorius* L.) panel using İPBS-retrotransposon markers. *PloS one*, 14(2), e0211985.

Ali, F., Nadeem, M. A., Habyarimana, E., Yılmaz, A., Nawaz, M. A., Khalil, I. H., Ercişli, S., Chung, G., Chaudhary, H.J.& Baloch, F. S. (2020). Molecular characterization of genetic diversity and similarity centers of safflower accessions with ISSR markers. *Brazilian Journal of Botany*, 43, 109-121.

Anderson, J.A., Churchill, G.A., Autrique, J.E., Tanksley, S.D., & Sorrells, M.E. (1993). Optimizing parental selection for genetic linkage maps. *Genome, 36*,181-186. https://doi.org/10.1139/g93-024

Asgarpanah, J., & Kazemivash, N. (2013). Photochemistry, pharmacology, and medicinal properties of *Carthamus tinctorius* L. *Chinese Journal of Integrative Medicine, 19*,153-159. https://doi.org/10.1007/s11655-013-1354-5

Amini, F., Saeidi, G., Arzani, A. (2008). Study of genetic diversity in safflower genotypes using agro-morphological traits and RAPD markers. *Euphytica, 163*,21-30. DOI: https://doi.org/10.1007/s10681-007-9556-6

Baloch, F.S., Kurt, C., Arıoğlu, H., & Ozkan, H. (2010). Assaying of diversity among soybean (*Glycin max* (L.) Merr.) and peanut (*Arachis hypogaea* L.) genotypes at DNA level. *Turkish Journal of Agriculture and Forestry,* 34, 285-301. https://doi.org/10.3906/tar-0907-281

Barati, M., & Arzani, A. (2012). Genetic diversity revealed by EST-SSR markers in cultivated and wild safflower. *Biochemical Systematics and Ecology, 44*,117-123. https://doi.org/10.1016/j.bse.2012.04.013

Baydar, H. (2021). Tarla Bitkileri Tarımı ve Endüstrisi (Ders Notu) Isparta Uygulamalı Bilimler Üniversitesi. Tbtf-tab- Isparta. p.293 (In Turkish).

Baydar, H., & Erbaş, S. (2020). Yerli ve Milli Aspir Çeşitlerimiz: Olein, Zirkon ve Safir. *Ziraat Fakültesi Dergisi Türkiye 13. Ulusal, I. Uluslararası Tarla Bitkileri Kongresi Özel Sayısı*: 233-237(In Turkish).

Bonneuil, C., Goffaux, R., Bonnin, I., Montalent, P., Hamon, C., Balfourier, F., & Goldringer, I. (2012). A new integrative indicator to assess crop genetic diversity. *Ecological Indicators*, 23, 280-289.

Chapman, M.A., Hvala, J., Strever, J., & Burke, J.M. (2010). Population genetic analysis of safflower (*Carthamus tinctorius*; Asteraceae) reveals a near eastern origin and five centres of diversity. *American Journal of Botany, 97*, 831-840. https://doi.org/10.3732/ajb.0900137

Chesnokov, Y.V., & Artemyeva, A.M. (2015). Evaluation of the Measure of Polymorphism Information of Genetic Diversity *Agricultural Biology, 50*, 571-578. https://doi.org/10.15389/agrobiology.2015.5.571

Cosge, B., Gurbuz, B., & Kiralan, M. (2007). Oil content and fatty acid composition of some safflower (*Carthamus tinctorius* L.) varieties sown in spring and winter. *International Journal of Natural and Engineering Sciences, 1*,11-15.

Delshad, E., Yousefi, M., Sasannezhad, P., Rakhshandeh, H., & Ayati, Z. (2018). Medical uses of *Carthamus tinctorius* L. (Safflower): a comprehensive review from Traditional Medicine to Modern Medicine. *Electron Physician, 10*, 6672-6681. http://dx.doi.org/10.19082/6672

Dice, L.R. (1945). Measures of the amount of ecologic association between species. *Ecology, 26*,297–302.

Ebrahimi, F., Majidi, M.M., Arzani, A., & Mohammadi-Nejad, G. (2017). Association analysis of molecular markers with traits under drought stress in safflower. *Crop and Pasture Science, 68*,167-175. https://doi.org/10.1071/CP16252

FAOSTAT, (2022). *Food and Agriculture Organization of the United Nations Statistical Databases*. http://www.fao.org/faostat/en/ [Erişim Tarihi: 15. 08.2022]

Giachino, R.R.A. (2020). Investigation of the genetic variation of anise (*Pimpinella anisum* L.) using RAPD and ISSR markers. *Genetic Resources and Crop Evolution, 67*, 763-780. https://doi.org/10.1007/s10722-019-00861-y

Giachino, R.R.A., & Inan, D. (2019). Assessment of genetic diversity in Safflower (*Carthamus tinctorius* L.) using RAPD markers. *Yuzuncu Yıl University Journal of Agricultural Sciences, 29*(2), 300-308. https://doi.org/10.29133/yyutbd.560936

Golkar, P., Arzani, A., & Rezaei, A.M. (2011). Genetic variation in safflower (*Carthamus tinctorious* L.) for seed quality-related traits and inter simple sequence repeat (ISSR) markers. *International Journal of Molecular Sciences, 12*, 2664-2677. https://doi.org/10.3390/ijms12042664

Golkar, P. (2018). Inheritance of carthamin and carthamidin in safflower (*Carthamus tinctorius* L.). *Journal of genetics, 97*, 331-336. https://doi.org/10.1007/s12041-018-0909-9

Golkar, P., & Mokhtari, N. (2018). Molecular diversity assessment of a world collection of safflower genotypes by SRAP and SCoT molecular markers. *Physiology and Molecular Biology of Plants, 24*,1261-1271. https://doi.org/10.1007/s12298-018-0545-0

Hamdan, Y.A.S., García-Moreno, M.J., Redondo-Nevado, J., Velasco, L., & Pérez-Vich, B. (2011). Development and characterization of genomic microsatellite markers in safflower (*Carthamus tinctorius* L.). *Plant Breeding, 130*,237-241. https://doi.org/10.1111/j.1439-0523.2010.01826.x

Hassani, S.M.R., Talebi, R., Pourdad, S.S., Naji, A.M., & Fayaz, F. (2020). Morphological description, genetic diversity and population structure of safflower (*Carthamus tinctorius* L.) mini core collection using SRAP and SSR markers. *Biotechnology & Biotechnological Equipment, 34*,1043-1055. https://doi.org/10.1080/13102818.2020.1818620

Ilkdoğan, U. (2012). *Türkiye'de Aspir Üretimi İçin Gerekli Koşullar ve Oluşturulacak Politikalar* (Tez no: 978-605-4672-04-2). [Doktora Tezi, Ankara Üniversitesi Fen Bilimleri Enstitüsü Tarım Ekonomisi Ana Bilim Dalı]. (In Turkish).

Johnson, R.C., Kisha, T.J., & Evans, M.A. (2007). Characterizing safflower germplasm with AFLP molecular markers. *Crop Science, 47*,1728-1736. https://doi.org/10.2135/cropsci2006.12.0757

Kiran, B.U., Mukta, N., Kadirvel, P., Alivelu, K., Kishore, P., Senthilvel, S., & Varaprasad, K.S. (2017). Genetic diversity in safflower (*Carthamus tinctorius* L.) germplasm as revealed by SSR markers. *Plant Genetic Resources: Characterization and Utilization, 15*,1-11. https://doi.org/10.1017/S1479262115000295

Knowles, P.F. (1989). Safflower. In: Downey RK, Robbelen G, Ashri A (eds) *Oil crops of the world*. New York 363-374.

Kobuk, M., Ekinci, K., & Erbaş, S. (2019). Aspir (*Carthamus tinctorius* L.) genotiplerinin fiziksel ve kimyasal özelliklerinin belirlenmesi. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 22(1), 89-96. DOI:10.18016/ ksutarimdoga.vi.455408

Köse, A. (2017). Eskişehir Koşulları Altında Bazı Aspir (*Carthamus tinctorius* L.) Çeşitlerinin Tarımsal Performanslarının Belirlenmesi. *Selcuk Journal of Agriculture and Food Science*, 31, 1-7 (In Turkish).

Kumar, S., Ambreen, H., Murali, T.V., Bali, S., Agarwal, M., Kumar, A., Goel, S., & Jagannath, A. (2014). Assessment of genetic diversity and population structure in a global reference collection of 531 accessions of *Carthamus tinctorius* (safflower) using AFLP markers. *Plant Molecular Biology Reporter*, 33,1299-1313. https://doi.org/ 10.1007/s11105-014-0828-8

Lee, G.A., Sung, J.S., Lee, S.Y., Chung, J.W., Yi, J.Y., Kim, Y.Y., & Lee, M.C. (2014). Genetic assessment of safflower (*Carthamus tinctorius* L.) collection with microsatellite markers acquired via pyrosequencing method. *Molecular Ecology Resources, 14*, 69-78. https://doi.org/10.1111/1755-0998.12146.

Lei, J., Li, Y., Liu, B., Tang, T., Liu, H., Yan, X., Qin, R., & Li, G. (2013). Establishment and Optimization of SRAP-PCR and Preliminarily Analysis of Genomes in *Carthamus tinctorius* L. *Botanical Research, 2*, 62-66. http://dx.doi.org/ 10.12677/br.2013.22011

Li, G., & Quiros, C.F. (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. *Theoretical and Applied Genetics, 103*, 455-461. https://doi.org/10.1007/s001220100570

Li, D., Yadong, Z., Chao, L., Tu, S., & Li, M. (2009). Optimization of SRAP-PCR reaction system in flax. *Journal of Northeast Agricultural University, 16*, 17-22.

Li, G., McVetty, P.B.E., Quiros, C.F. (2013). SRAP molecular marker technology in plant science. In: Andersen SB (ed.) Plant Breeding from Laboratories to Fields. Rijeka, Croatia: InTech, pp.23-43.

Mahasi, M.J., Wachira, F.N., Pathak, R.S., & Riungu, T.C. (2009). Genetic polymorphism in exotic safflower (*Carthamus tinctorious* L.) using RAPD markers. *Journal of Plant Breeding and Crop Science, 1*, 008-012.

Majidi, M.M., & Zadhoush, S. (2014). Molecular and morphological variation in a world-wide collection of safflowers. *Crop Science, 54*, 2109-2119. https://doi.org/10.2135/cropsci2013.12.0850

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research, 27*, 175-178.

Milbourne, D., Meyer, R., Bradshaw, J.E., Baird, E., Bonar, N., Provan, J., Powell, W., & Waugh, R. (1997). Comparison of PCR-based marker systems for the analysis of genetic relationships in cultivated Potato. *Molecular Breeding, 3*, 127-136. https://doi.org/10.1023/A:1009633005390

Mokhtari, N., Rahimmalek, M., Talebi, M., & Khorrami, M. (2013). Assessment of genetic diversity among and within Carthamus species using sequence-related amplified polymorphism (SRAP) markers. *Plant Systematics and Evolution, 299*, 1285-1294. https://doi.org/10.1007/s00606-013-0796-8

Naik, A., Prajapat, P., Krishnamurthy, R., & Pathak, J.M. (2017). Assessment of genetic diversity in *Costus pictus* accessions based on RAPD and ISSR markers. *3Biotech, 7*, 70. https://doi.org/10.1007/ s13205-017-0667-z

Nogales-Delgado, S., Encinar, J.M., & González, J.F. (2019). Safflower Biodiesel: Improvement of its Oxidative Stability by using BHA and TBHQ. *Energies, 12*, 1940:1-13. https://doi.org/10.3390/ en12101940

Peng, S., Feng, N., Guo, M., Chen, Y., & Guo, Q. (2008). Genetic variation of Carthamus tinctorius L. and related species revealed by SRAP analysis. *Biochemical Systematics and Ecology, 36*, 531–538. https://doi.org/10.1016/j.bse.2008.03.010

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S., & Rafalski, A. (1996). The unity of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding, 2*, 25-238. https://doi.org/ 10.1007/BF00564200

Prance, G., & Nesbitt, M. (2005). The cultural history of plants. New York-London: Routledge. p.451.

Prevost, A., & Wilkinson, M.J. (1999). A new system of comparing PCR primers applied to ISSR fingerprinting. *Theoretical and Applied Genetics, 98*, 107-112. https://doi.org/10.1007/s001220051046

Rohlf, F.J. (2000). *NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System, Version 2.2*. Exeter Publishing, Setauket, NY, USA.

Safavi, S.A., Pourdad, S.S., Taeb, M., & Khosroshahli, M. (2010). Assessment of genetic variation among safflower (*Carthamus tinctorius* L.) accessions using agro-morphological traits and molecular markers. *Journal of Food, Agriculture & Environment, 8* (3-4), 616-625. www.world-food.net

Sehgal, D., & Raina, S.N. (2005). Genotyping safflower (*Carthamus tinctorius*) cultivars by DNA fingerprints. *Euphytica, 146*, 67-76. https://doi.org/10.1007/s10681-005-8496-2

Sehgal, D., Rajpal, V.R., Raina, S.N., Sasanuma, T., & Sasakuma, T. (2009). Assaying polymorphism at DNA level for genetic diversity diagnostics of the safflower (*Carthamus tinctorius* L.) World germplasm resources. *Genetica, 135*, 457-470. https://doi.org/10.1007/s10709-008-9292-4

Serrote, C.M.L., Reiniger, L.R.S., Silva, K.B., dos Santos Rabaiolli, S.M., & Stefanel, C.M. (2020). Determining the polymorphism information content of a molecular marker. *Gene, 726*, 144175. https://doi.org/10.1016/j.gene.2019.144175

Talebi, M., Mokhtari, N., Rahimmalek, M., & Sahhafi, S.R. (2012). Molecular characterization of *Carthamus tinctorius* and *C. oxyacanthus* germplasm using sequence related amplified polymorphism (SRAP) markers. *Plant Omics Journal* ,*5*, 136-142.

Talebi, R., Nosrati, S., Etminan, A., & Naji, A.M. (2018). Genetic diversity and population structure analysis of landrace and improved safflower (*Carthamus tinctorious* L.) germplasm using arbitrary functional gene-based molecular markers. *Biotechnology & Biotechnological Equipment, 32*, 1183-1194. https://doi.org/10.1080/13102818.2018.1499443

Tonguç, M., Erbas, S., & Baydar, H. (2011). Aspirde Geliştirilen Rekombinant Saf Hat Populasyonunun Genetik Harita Populasyonu Olarak Kullanma İmkânlarının Araştırılması. *Süleyman Demirel Üniversitesi Ziraat Fakültesi Dergisi, 6* ,1-7 (In Turkish)

TÜİK (2022). *Turkish Statistical Institute*. Türkiye İstatistik Kurumu http://www.tuik.gov.tr_[Erişim Tarihi: 15.03.2022]

Weiss, E.A. (1971). Safflower. In: Weiss EA (ed.) *Castor, Sesame and Safflower*. New York: Barnes and Noble, pp. 529-744. https://doi.org/10.1017/S0014479700005366

Weiss, E.A. (2000). Safflower. In: Weiss EA (ed.) *Oilseed Crops*. Victoria, Australia: Blackwell Science Ltd. p.109.

Yaman, H., Tarıkahya-Hacıoglu, B., Arslan, Y., & Subasi, I. (2014). Molecular characterization of the wild relatives of safflower (*Carthamus tinctorius* L.) in Turkey as revealed by ISSRs. *Genetic Resources and Crop Evolution, 61*, 595-602. https://doi.org/10.1007/s10722-013-0061-6

Yang, Y.X., Wu, W., Zheng, Y.L., Chen, L., Liu, R.J., & Huang, C.Y. (2007). Genetic diversity and relationships among safflower (*Carthamus tinctorius* L.) analysed by inter-simple sequence repeats (ISSRs). *Genetic Resources and Crop Evolution, 54*, 1043-1051. https://doi.org/10.1007/s10722-006-9192-3

Yeh, F.C., Yang, R.C., Boyle, T.B.J., Ye, Z.H., & Mao, J.X. (1997). POPGENE, the user-friendly shareware for population genetic analysis, version 1.31. *Molecular Biology and Biotechnology Centre, University of Alberta*, Canada.

Yesilyurt, M.K., Cesur, C., Aslan, V., & Yilbasi, Z. (2020). The production of biodiesel from safflower (*Carthamus tinctorius* L.) oil as a potential feedstock and its usage in compression ignition engine: a comprehensive review. *Renewable and Sustainable Energy Reviews, 119,* 1-30. https://doi.org/10.1016/j.rser.2019.109574

Yıldız, M., Altaf, M.T., Baloch, F.S., Koçak, M., Sadık, G., Kuzğun, C., Nadeem, M.A., Ali, F., Bedir, M. & Tunçtürk, M. (2022). Assessment of genetic diversity among 131 safflower (*Carthamus tinctorius* L.) accessions using peroxidase gene polymorphism (POGP) markers. *Molecular Biology Reports*, 49(7), 6531-6539.

Yue, S.J., Tang, Y.P., Li, S.J., & Duan, J.A. (2013). Chemical and biological properties of quinochalcone C-glycosides from the florets of *Carthamus tinctorius*. *Molecules, 18,* 15220-15254. https://doi.org/10.3390/molecules181215220

Zhang, Y.X., Zhang, X.R., Hua, W., Wang, L.H., & Che, Z. (2010). Analysis of genetic diversity among indigenous landraces from sesame (*Sesamum indicum* L.) core collection in China as revealed by SRAP and SSR markers. *Genes Genome, 32*(3), 207-215. https://doi.org/10.1007/s13258-009-0888-6