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Peroxidase Gene Based Genetic Relationships Among Safflower Genotypes

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Abstract: Safflower (*Carthamus tinctorius* L.) is an oil crop species and is widely cultivated since ancient times in Near East. Fifteen peroxidase gene based markers (POGP) were used to fingerprint 39 genotypes (33 cultivars, 6 breeding lines) from 6 countries to asses genetic diversity within elite safflower germplasm. Fourteen POGP markers produced polymorphisms and one marker was monomorphic. POGP markers produced total of 71 bands of which 50 were polymorphic. Average number of bands produced by POGP markers were 4.7, 3.3 of which were polymorphic among the safflower genotypes. To reveal genetic relationships among the safflower genotypes, similarity matrix was calculated and UPGMA method was used to construct a dendrogram. Mean similarity was 0.80 among the genotypes with a range of 0.56-0.91. Genotypes clustered within 2 groups. Gene diversity of the markers ranged between 0.17-0.48. These results show POGP markers could be used to fingerprint to study genetic diversity of safflower genotypes.

***Keywords**: Carthamus tinctorius*, Gene diversity, Peroxidase, POGP markers

Aspirde Genetik İlişkilerin Peroksidaz Genleri Kullanılarak Belirlenmesi

Özet: Aspir (*Carthamus tinctorius* L.) önemli bir yağ bitkisidir ve eski çağlardan beri Yakın Doğu'da kütürü yapılmaktadır. On beş peroksidaz gen markörü (POGP) 6 farklı ülkeye ait 39 aspir genotipinde (33 çeşit ve 6 ıslah hattı) genetik çeşitliliği araştırmak için kullanılmıştır. On dört POGP markörü aspirde polimorfizimler üretirken bir markör monomorfik olarak bulunmuştur. POGP markörleri aspirde toplam 71 band üretmiş ve üretilen bantlardan 50 tanesi polimorfik olarak bulunmuştur. Aspirde POGP markörü başına üretilen ortalama band sayısı 4.7 iken, ortalama polimorfik band sayısı 3.3 olmuştur. Aspirde genetik akrabalık ilişkilerini ortaya çıkarmak için benzerlik matrisi hesaplanmış ve UPGMA metodu ile dendrogram yapılmıştır. Aspir genotiplerinin benzerlik oranları 0.59-0.91 arasında değişim göstermiş ve ortalama benzerlik 0.80 olarak bulunmuştur ve genotipler 2 grup altında toplanmıştır. Markörlerin tespit ettiği gen çeşitliliği 0.17-0.48 arasında olmuştur. Mevcut sonuçlar, aspirde genetik çeşitliliği çalışmak için parmak izi yönteminde POGP markörlerinin kullanılabileceğini göstermektedir.

***Anahtar Kelimeler**: Carthamus tinctorius*, Genetik çeşitlilik, Peroksidaz, POGP markör

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1. Introduction

Safflower is an important crop species and belongs to Asteraceae family. It has been cultivated for its flowers, seeds and oil from China to the Mediterranean region [1]. It requires minimal input to grow and suitable to be grown on marginal lands due to its salinity and drought tolerance [2, 3]. Safflower production is increasing in Turkey from 250 ha in 2003 to 39.571 ha in 2017 [4]. Currently the main producers are USA, India, Mexico, Kazakhstan, Russia, Turkey and China in the world [5]. The current oil content of safflower cultivars grown in Turkey changes between 24-30% with average yield of 100 kg/da, therefore there is a need to improve existing varieties through breeding [2].

Even though safflower has been cultivated for centuries, modern breeding efforts and selection studies started in 1950s [6]. Morphological and agronomic characters, and the molecular studies could be used to characterize genetic diversity of crop species [7]. Characterization of safflower germplasm has been based largely on agronomic characters [8-10]. Molecular marker studies were also conducted to study genetic diversity exist in safflower germplasm using different marker techniques involving RAPD (Randomly amplified polymorphic DNA) and ISSR (Inter simple sequence repeats) [11-13], AFLPs (Amplified fragment length polymorphisms) [14, 15], SSRs (Simple sequence repeats) [16, 17].

Plant peroxidase genes are multigene family and have diverse functions in abiotic and biotic stress tolerance, lignification, cellular growth, fruit ripening and plant senescense [18, 19]. Peroxidase genes have low sequence similarity [20], making them suitable for peroxidase gene based polymorphism (POGP) marker studies [21]. Genetic diversity of different plant species have been assessed using POGP markers such as; buffalo grass [21], apples [22], watermelons [23], *Citrus* spp. [24] and pepper [25]. The aim of the present study was to asses genetic diversity present in safflower genotypes using POGP markers.

2. Material and Method

2.1 Plant material

Total of 39 safflower genotypes consisting of 33 cultivars and 6 breeding lines representing 6 countries were used in the study (Table 1). Plant material was obtained from Agean Agricultural Research Institute (Izmir, Turkey) and from USDA Western Regional Plant Introduction Station (Pullman, WA, USA).

2.2 DNA extraction and POGP marker amplification

DNA was extracted from young leaves with a CTAB method [26]. DNA pellets were dissolved in 100 µl TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) and stored at -20 °C until they were used.

Previously designed POGP primer pairs were used for DNA amplification. These primers were designed from rice peroxidase genes and their sequence information was given by Gulsen et al. [21]. Fifteen primer pairs were used to amplify safflower peroxidase genes in the study. PCR reactions consisted of 2 µl template DNA, 400 µM of each primer pair, 200 µM dNTP mix, 1.5 µl 10X PCR buffer, 2.5 mM $MgCl_2$, 1.2 µl bovine serum albumin (0.8 µg/µl), 0.5 unit of Taq polymerase. Reaction volume was

brought to 15 µl with ddH_2O. Polymerase chain reaction (PCR) amplifications were carried out with the following cycling parameters; one cycle of 2 min at 94 °C for initial denaturation, 35 cycles of 1 min at 94 °C, 1 min at 50-56 °C, 1 min at 72 °C, and one cycle of 5 min at 72 °C for final extension. PCR products were separated on 2.5% agarose gels in 1X TBE buffer at 90 V for 4 to 5 h and stained with ethidium bromide (10mg/ml). Gel images were taken using Kodak imaging system (Gel logic 1500) under UV light.

Table 1. Plant material used in the study and their country of origins and registration status.

	Accession number	Cultivar name	Country	Registration status
1	PI 572475	Saffire	Canada	Cultivar
2	PI 592391	AC Sunset	Canada	Cultivar
3	PI 559909	AC Sterling	Canada	Cultivar
4	PI 603206	Lesaf 414	Canada	Breeding line
5	PI 514632	Ziyang	China	Cultivar
6	PI 514631	Yuyao	China	Cultivar
7	PI 514624	Shufu	China	Cultivar
8	PI 514620	Huaxian	China	Cultivar
9	PI 506426	FO-2	China	Cultivar
10	PI 537110	Quiriego 88	Mexico	Cultivar
11	PI 537111	Sahuaripa 88	Mexico	Cultivar
12	PI 561703	San Jose 89	Mexico	Cultivar
13	PI 610263	Enana	Spain	Breeding line
14	W6 16828	Rinconada	Spain	Cultivar
15	W6 16833	CH-353	Spain	Breeding line
16	TR 69497	Dinçer 5-118	Turkey	Cultivar
17	TR 69498	Yenice 5-38	Turkey	Cultivar
18	TR 69499	Remzibey-05	Turkey	Cultivar
19	PI 538025	Montola 2000	USA	Cultivar
20	PI 601166	Oker	USA	Cultivar
21	PI 572465	4022	USA	Breeding line
22	PI 572439	PCA	USA	Breeding line
23	PI 572418	Arizona Saffl. CIII	USA	Breeding line
24	PI 572421	Frio	USA	Cultivar
25	PI 560177	Oleic Leed	USA	Cultivar
26	PI 538779	Centennial	USA	Cultivar
27	PI 601506	S-517	USA	Cultivar
28	PI 572472	Rehbein	USA	Cultivar
29	PI 525458	Finch	USA	Cultivar
30	PI 572436	Leed	USA	Cultivar
31	PI 572415	55-633	USA	Cultivar
32	PI 508098	Hartman	USA	Cultivar
33	PI 537695	Ole	USA	Cultivar
34	PI 572434	UC-1	USA	Cultivar
35	PI 572414	US-10	USA	Cultivar
36	PI 572471	Sidwill	USA	Cultivar
37	PI 537694	Royal	USA	Cultivar
38	PI 537692	Gila	USA	Cultivar
39	PI 525457	Girard	USA	Cultivar

2.3 Data analysis

Amplified bands were scored as present (1) or as absent (0), and the data matrix were analyzed with Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) v 2.1 software [27]. Similarity matrix was constructed with Dice coefficient and the dendrogram was constructed using unweighted pair group method arithmetic average (UPGMA) method to reveal genetic relationships between the genotypes. Gene diversity (GD) values of POGP markers were calculated with GDDOM software package [28].

3. Results

DNA from 39 safflower genotypes were amplified with 15 POGP markers to produce polymorphism to reveal genetic relationships between the genotypes in this study. All POGP markers produced amplification products and 15 POGP markers produced a total of 71 bands across 39 safflower genotypes. Number of bands produced by POGP markers ranged from 1 (POX12a) to 9 (POX3, POX6). All markers with the exception of POX8 produced polymorphic bands whereas POX8 produced 2 bands but they were monomorphic across the 39 safflower genotypes (Table 2). Out of 71 bands produced by the POGP markers 50 were polymorphic among safflower genotypes. While average number of bands produced by POGP markers was 4.7, average number of polymorphic bands was 3.3 for POGP markers. Polymorphism content has ranged between 0-100% depending on the number of polymorphic bands produced. Average polymorphism content was 70.9% across the safflower genotypes.

Table 2. POGP markers, their amplification products and gene diversity values of the markers across 39 safflower genotypes.

Marker name	Total number of bands	Polymorphic bands	Polymorphism (%)	Gene diversity
POX1	6	5	83.3	0.18
POX2	3	3	100	0.48
POX3	9	5	55.5	0.25
POX4	7	5	71.4	0.25
POX5	6	5	83.3	0.27
POX6	9	6	66.6	0.28
POX7	4	3	75.0	0.19
POX8	2	0	0.0	0.00
POX9	5	3	60.0	0.29
POX10c	5	4	80.0	0.28
POX10d	4	2	50.0	0.17
POX11	3	2	66.6	0.28
POX12a	1	1	100	0.26
POX12b	4	3	75.0	0.19
POX12c	3	3	100	0.43

Similarity matrix based on Dice coefficient was used to perform cluster analysis with the UPGMA method. The level of similarity between the safflower genotypes was 0.56-0.91 with an average of 0.80. Based on cluster analysis two main groups were detected among the safflower genotypes (Figure 1). The first cluster contained 30 genotypes and the second cluster had 9 genotypes. Two genotypes, Frio and AC Sterling, could not be distinguished from each other with polymorphisms produced by POGP markers.

Gene diversity values have also been calculated for each marker. Since POX8 did not produce any polymorphism among the assayed genotypes it had GD value of 0. The other markers had GD values ranging from 0.17-0.48. The average GD value for the markers was 0.25.

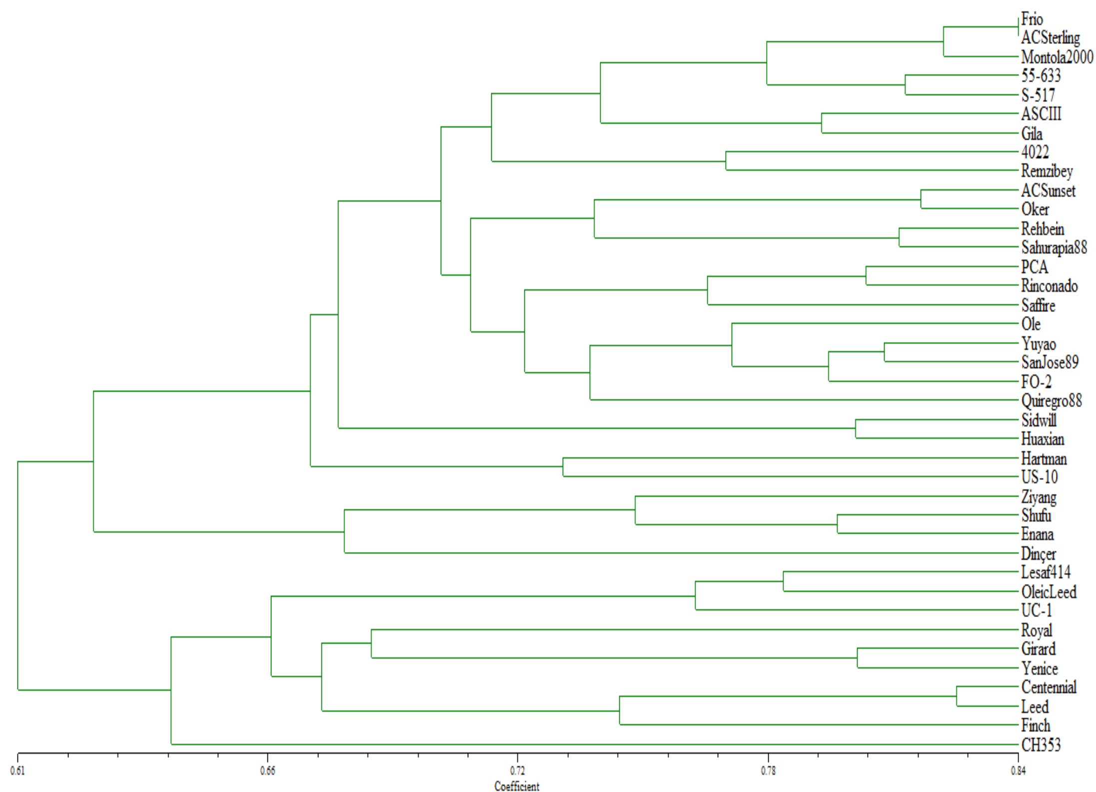

Figure1. Dendogram depicting genetic relationships beteween the safflower genotypes

4. Conclusion and Comment

Fifteen POGP markers were tested with 39 safflower genotypes and all markers yielded bands. Only POX8 did not produce polymorphism among the tested genotypes. Genetic diversity studies in different crop species were also carried out with POGP markers. In buffalo grass, POGP markers produced 52 bands with a 79% polymorphism content [21], in apples 46 bands produced and 96% of the bands were polymorphic [22], in *Citrus* spp. polymorphism percent was 99% [24], watermelons yielded 98% polymorphism among the produced bands [23] and peppers had 85.6% polymorphism rate with POGP markers [25]. In the present study, average polymorphism rate of POGP markers was 71% and was lower than the previously reported rates. Safflower genotypes yielded 4.7 bands per POGP marker and 3.3 of those were polymorphic. Apples produced on average 3.5 bands [22], *Citrus* spp. 10.6 bands [24], watermelons 12.5 bands [23] per marker. Other studies used cultivars, accessions, different species, but we only used commercial cultivars and breeding lines. Breeding causes lower genetic diversity among cultivated plant species [7] and the genetic diversity is lower in cultivars than other germplasm resources. Safflower cultivars produced polymorphisms 24% for RAPD and 18% for ISSR [12] whereas geographically diverse safflower accessions produced 57% for RAPD and 68% for ISSR polymorphism [15], which might explain lower levels of polymorphism observed in the present study.

POGP markers produced similarity range between 0.56-0.91 with an average of 0.80. Genetic diversity studies using POGP markers in other crops produced 0.80 for buffalo grass [21], 0.59 for watermelons [23], 0.82 for *Citrus* spp. [24] and 0.56 for apples [22]. Clustering analysis revealed 2 groups among the safflower genotypes. Upper group contained 30 genotypes while the lower group contained 9 genotypes (Figure 1). Genotypes from different countries did not group together. Frio and AC Sterling could not be distinguished from each other by the cluster analysis. Levels of similarity observed in the study is in agreement with other studies.

Gene diversity value corresponds to expected heterozygosity and depends on the number of alleles and their distribution among the genotypes. Its value ranges between 0-0.5 [28]. GD value was 0.17-0.48 for the polymorphic POGP markers for safflower (Table 2). POX1 had 6 bands and 5 were polymorphic with GD value of 0.18 whereas POX2 had 3 polymorphic bands with a GD value of 0.48. These results shows that for genetic diversity studies distribution of alleles among the genotypes are important as well.

Different marker types could be used to deliminate genetic relationships of plant species. In the present study, POGP markers were used to asses genetic diversity among the cultivars and breeding lines of safflower. POGP markers produced comperaple levels of polymorphism to other markers systems and were found to be suitable to study genetic relationships in safflower.

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