ANADOLU, J. of AARI
ISSN: 1300 - 0225
27 (2) 2017, 123 - 126
MFAL

# *Molecular Characterization of Materials Selected from Some Camelina [Camelina sativa (L.) Crantz] Populations*

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*Received (Geliş tarihi): 28.07.2017 Accepted (Kabul tarihi): 17.11.2017*

***ABSTRACT: Camelina sativa*** *(L.) Crantz. is an oilseed crop which is native to Mediterranean and Central Asia. In the recent years, the* ***Camelina*** *oil became popular worldwide for human consumption, Omega-3 fatty acid content and possibility of the use as biodiesel fuel. The fact that it grows naturally in flora of Turkey, its competition power with the other plants in the growing field and the possibility of its cultivation without high amounts of nutrients make it as an alternative oil plant. Molecular characterizations of single plant, which was selected from thirty four* ***Camelina*** *accessions obtained from the US Gene Bank and from the Central Research Institute of Field Crops in Turkey, were used in this study. These seedlings were tested with seventy-three universal ISSR markers. Then eleven markers, which were highly polymorphic, were selected for characterization. According to characterization results, statistical analyses were performed in NTSYS-PC. Hence, genetic relationship between genotypes was shown on dendrogram which will be used in breeding purpose.*

***Keywords: Camelina sativa****, false flax, molecular characterization, ISSR.*

## *Bazı Ketencik [Camelina Sativa (L.) Crantz] Populasyonlarından Seçilen Materyallerin Moleküler Karakterizasyonu*

***ÖZ:*** *Ketencik [****Camelina sativa*** *(L.) Crantz], Akdeniz ve Orta Asya'ya özgü yağlı tohumlu bir bitkidir. Son yıllarda,* ***Camelina*** *yağı yüksek Omega-3 yağ asidi içeriği ve biyodizel yakıt olarak kullanılma olanağı nedeniyle dünya çapında popüler hale gelmiştir. Türkiye'nin bitki örtüsünde doğal olarak yetişmesi, yetiştiği alanlarda diğer bitkilerle olan rekabet gücünün fazla olması ve yüksek miktarda besin elementlerine ihtiyaç duymaması nedeniyle alternatif bir biyodizel bitkisi haline gelmiştir. Bu çalışmada, ABD Gen Bankası'ndan ve Türkiye Tarla Bitkileri Merkez Araştırma Enstitüsü'nden elde edilen otuz dört* ***Camelina*** *aksesyonundan seçilen tek bitkilerin moleküler karakterizasyonu yapılmıştır. Bu genotipler yetmiş üç universal ISSR markörü ile test edilmiştir. Daha sonra, genetik karakterizasyon çalışmaları için polimorfik olan onbir primer seçilmiştir. Elde edilen sonuçların istatistiksel analizi NTSYS-PC programında yapılmıştır. Elde edilen dendogram ile genotipler arasındaki genetik ilişki belirlenmiş olup, bu bilgi gelecekteki ıslah programlarına yön verebilecektir.*

***Anahtar Sözcükler: Camelina sativa****, ketencik, moleküler karakterizasyon, ISSR.*

## INTRODUCTION

*Camelina sativa* (L.) is named as German sesame and Siberian oil. The natural distribution area is Mediterranean and Central Asia (Putnam, *et al.,*1993; McVay, 2008). The oil is used as food, as lamp oil and for cosmetics since ancient times (Knorzer, 1978). Cultivation of the *Camelina sativa* began in the Neolithic period and was used as an oil plant throughout the Iron Age (Knorzer, 1978).

There are seven commonly known species of *Camelina* Crantz., namely *Camelina sativa* (L.) Crantz., *C. laxa* C. A. Mey, *C. rumelica, C. microcarpa* Andrz. ex DC., *C. hispida* Boiss., *C. anomala* Boiss. & Hausskn. and *C. alpkoyensis* Stars. (Mutlu, 2012). Among these species, *Camelina sativa* is the only species with economic importance (Kurt and Seyis, 2008).

*Camelina sativa* contains many natural antioxidants, such as tocopherols, which are used as oil stabilizing and edible oil. The most important feature of *Camelina sativa* is the high linolenic acid content (38%). Hence*, Camelina sativa* meets requirement of cooking oil because of rich OMEGA-3 fatty acid content (Crowley and Fröhlich, 1998). Furthermore, the plant is an important source for biodiesel and used in the machine lubricant industry. The high iodine value of the methyl ester *of Camelina sativa* oil allows the machine to be used in oil for longer time without deterioration (Frohlic and Rice, 2005). Because of that, several molecular studies about *C. sativa* genotypes were published in literature (Vollmann *et al*., 2005; Gehringer *et al.,* 2006; Ghamkhar *et al.,* 2010).

The purpose of our research is to provide information for breeding of *Camelina sativa*. Our research is the first research in Turkey which provides information on molecular basis for breeding purposes.

## MATERIALS AND METHODS

Thirty four *Camelina sativa* (L.) Crantz genotypes were used in this study, which were gathered from the Seed Bank of the Agricultural Research Service of the United States Department of Agriculture (Table 1).

The seeds were planted in pots and kept in 25 $^{o}$C temperature and 45-52% humidity in the climate cabinet. After 21 days, the DNA was isolated from fresh leaves by DNeasy Plant Mini Kit from Qiagen (Hilden, Germany). DNA quality and quantity were controlled in 1% agarose gel. PCR amplification was performed according to modified Qiagen protocol; in a total volume of 25 µl master mix (2x PCR Master Mix Fermentas); containing 20–50 ng of genomic DNA, 1µl l M primer and 1 unit of Taq DNA polymerase. PCR reactions were started with an initial denaturation of 2 min at 94 $^{o}$C; followed by 30 cycles of 1 min at 94 $^{o}$C, 2 min at 52 $^{o}$C (annealing) and 40 s at 72 $^{o}$C (extension), with a final extension step at 72 $^{o}$C for 5 min. Amplified products were separated by 2 % agarose gel electrophoresis with 1x TBE buffer and stained with ethidium bromide. DNA bands were visualized by KODAK GL 200 Imaging Cabinet (Eastman Kodak Company, Rochester, USA).

Eleven primers formed polymorphic bands out of 73 oligonucleotide primers (Table 2). The GC percentages of selected primers were between of 47.1% and 66.7%. Amplified bands were coded as diallelic characters (present=1, absent= 0). Fragment sizes were determined manually by 100 bp DNA Ladder Plus (Fermentas, Carlsbad, CA, USA). The IBM PC version of NTSYS (Rohlf, 2000) was used for clustering analysis (CA). A Mantel test (Mantel, 1967) was applied to Jaccard's, Simple matching and Dice similarity coefficients. The maximum value (r=0.83819) was obtained with Jaccard's. Thus, we combined the Jaccard's similarity coefficient with the Unweighted Pair Group with Arithmetic Mean (UPGMA) clustering algorithm for CA. The complete data matrix is available on request in NTS format.

## RESULTS AND CONCLUSION

After the screening of 34 genotypes with 11 ISSR primers (UBC primers No. 841, 842, 826, 820,

811, 810, 889, 887, 878, 851 and 14), 51 polymorphic bands were obtained. The size of bands ranged from 100 bp to 1000 bp.

When the dendrogram drawn according to the Jaccard's index was examined, it was seen that the dendogram was divided into two branches (Figure 1). The cluster in the second branch was mostly from the European - Siberian floristic region. The geographical locations of our country and other countries were concordant with the clusters in the dendrogram.

The two most closely clustered accessions in the dendrogram were 14 and 16, Denmark and Germany respectively. They were similar to each other with 0.89. According to the obtained dendrogram, accessions 44 and 30, which were from Poland and Germany were the least similar to the rest of the *Camelina* accessions and formed another cluster. The similarity index score of these two accessions was 0.48. Apart from this cluster, the other cluster had another two groups. One group consisted accessions 37 and 6, and the other group consisted the rest. The similarity level of these two groups was 0.58.

To conclude, it is clear that *Camelina* has a high potential to be an alternative source for oil industry. Results of the study provide priceless value to literature of *Camelina* plant. We hope that our results will contribute the future breeding programs and to develop new varieties.

Table 1. *Camelina sativa* accessions used in our study.
Çizelge 1. Çalışmada kullanılan *Camelina sativa* aksesyonları.

| No | Code | Origin | No | Code | Origin |
|---|---|---|---|---|---|
| 2 | Ames 31220 | Georgia | 30 | PI 650152 | Germany |
| 5 | Ames 31232 | Georgia | 31 | PI 650153 | The Former Soviet Union |
| 6 | PI 258366 | The Former Soviet Union | 32 | PI 650154 | The Former Soviet Union |
| 7 | PI 258367 | The Former Soviet Union | 33 | PI 650155 | Poland |
| 10 | PI 304270 | Swiss | 36 | PI 650158 | Poland |
| 11 | PI 304271 | Swiss | 37 | PI 650159 | Poland |
| 13 | PI 311736 | Poland | 38 | PI 650160 | The Former Soviet Union |
| 14 | PI 597833 | Denmark | 39 | PI 650161 | The Former Soviet Union |
| 15 | PI 633192 | Germany | 40 | PI 650162 | Poland |
| 16 | PI 633193 | Germany | 41 | PI 650164 | Austria |
| 17 | PI 633194 | Germany | 42 | PI 650165 | The Former Soviet Union |
| 20 | PI 650142 | Denmark | 44 | PI 650167 | Poland |
| 23 | PI 650145 | Germany | 46 | PI 652885 | Slovenia |
| 25 | PI 650147 | Swiss | 47 | PI 652886 | Slovenia |
| 27 | PI 650149 | Germany | 48 | Einfact (Leindotter) | Germany |
| 28 | PI 650150 | Denmark | 49 | K-49 | Germany |
| 29/2 | PI 650151 | Swiss | 52 | K-52 | Ukraine |

Table 2. Sequences of the primers used in our study.
Çizelge 2. Çalışmada kullanılan primer dizileri.

| Primers | Sequence 5′ ⟶ 3′ |
|---|---|
| UBC841 | GAGAGAGAGAGAGAGAYC |
| UBC842 | GAGAGAGAGAGAGAGAYG |
| UBC826 | ACACACACACACACACC |
| UBC820 | GTGTGTGTGTGTGTGTC |
| UBC811 | GAGAGAGAGAGAGAGAC |
| UBC810 | GAGAGAGAGAGAGAGAT |
| UBC889 | DBDACACACACACACAC |
| UBC887 | DVDTCTCTCTCTCTCTC |
| UBC878 | GGATGGATGGATGGAT |
| UBC851 | GTGTGTGTGTGTGTGTYG |
| UBC814 | CTCTCTCTCTCTCTCTA |

B = (C, G, T); D = (A, G, T); V = (A, C, G); Y = (C, T).

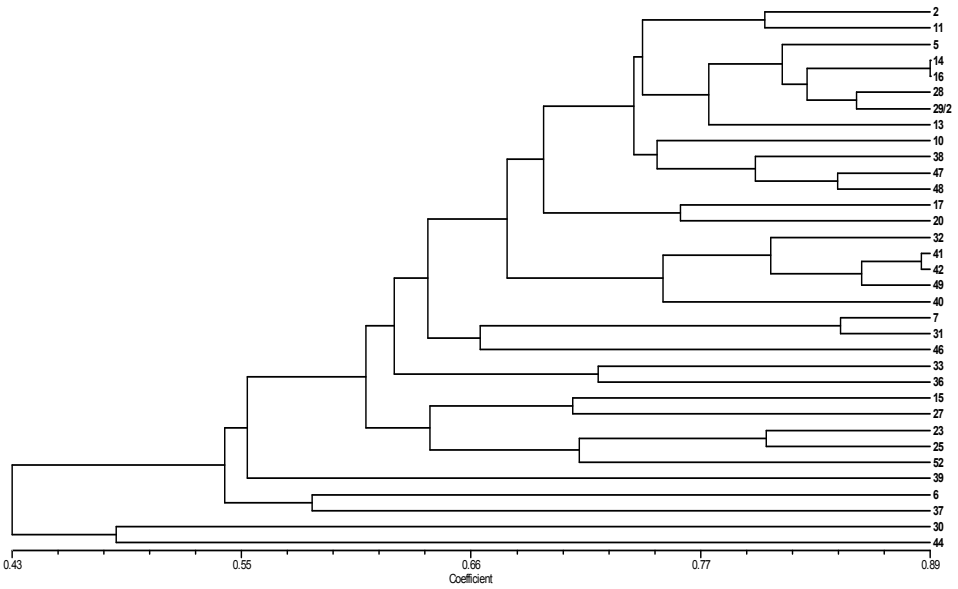


Figure 1. CA dendrogram of *Camelina sativa* accessions created with UPGMA.
Şekil 1. *Camelina sativa* aksesyonları için UPGMA ile oluşturulan kümeleme analiz dendogramı.

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