



e-ISSN: 1308-8769, ANAJAS Haziran 2023, 38 (2): 267-280

# Genetic Diversity Analysis of Avena sterilis L. Germplasm By Microsatellite Markers

# Avena sterilis L.'in Mikrosatellit İşaretleyicileri ile Genetik Çeşitlilik Analizi

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Makale Bilgisi/Article Information

Makale Türü/Article Types: Araştırma Makalesi/Research Article Geliş Tarihi/Received: 16 Kasım/November 2022 Kabul Tarihi/Accepted: 27 Şubat/February 2023 Yıl/Year: 2023 | Cilt-Volume: 38 | Sayı-Issue: 2 | Sayfa/Pages: 267-280

Atıf/Cite as: Altop Kaya, E. "Genetic Diversity Analysis of Avena sterilis L. Germplasm By Microsatellite Markers" Anadolu Journal of Agricultural Sciences, 38(2), Haziran 2023: 267-280.

https://doi.org/10.7161/omuanajas.1115743

## GENETIC DIVERSITY ANALYSIS OF AVENA STERILIS L. GERMPLASM BY MICROSATELLITE MARKERS

## **ABSTRACT**

*Avena sterilis* is an important grassy weed that causes problems in wheat cultivation areas, and herbicides are used intensively to control this weed. The knowledge of genetic diversity is important to have information on the effect of weed species on environmental factors and herbicides. The high level of genetic diversity within the species is a guarantee for adaptation to changing environmental conditions. This study was conducted based on complaints of the loss of effectiveness during the chemical control. Analyses were carried out on 24 selected populations collected from wheat cultivation areas in Turkey's central Black Sea region. Seventy-seven alleles from microsatellite loci were detected, and the average number of alleles per locus was determined as five using 17 microsatellite primers. The average genetic diversity (GD) and polymorphism information content (PIC) values were 0.894 and 0.732, respectively. According to the UPGMA dendrogram generated using Average Linkage, the populations were clustered into two main groups. A high degree of diversity was found among the studied sterile oat genotypes, and it has been observed that there is no geographical isolation. It is understood that this situation is primarily caused by the adaptation of sterile oat seeds transferred between regions in several ways. Although they are highly self-pollinating species, they may also have some cross pollination mechanism as well. It was concluded that there might be gene escape between resistant and susceptible sterile oat populations, as resistant weeds can show high genetic diversity. According to these findings, issues such as crop rotation, cultural control methods, encouraging the use of certified seeds, and the use of herbicides with different mechanisms of action can be listed among the measures that can be taken to control the high genetic diversity, which is an indicator of herbicide resistance.

*Keywords:* Sterile Oat*,* Wheat, Genetic Diversity, SSR.

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## ÖZ

*Avena sterilis,* buğday ekim alanlarında sorun olan önemli bir yabancı ot olup, mücadelesinde yoğun herbisit kullanımı söz konusudur. Herbisitlerin ve çevresel faktörlerin yabancı ot türleri üzerindeki etkisi hakkında bilgi sahibi olmak açısından genetik çeşitlilik çalışması önem taşır. Türler içindeki yüksek düzeyde genetik çeşitlilik, değişen çevre koşullarına adaptasyonun garantisidir. Bu çalışma ile *A. sterilis*'in kimyasal mücadelesindeki etkinlik kaybına ilişkin üretici şikayetleri doğrultusunda, genetik çeiştliliğin belirlenerek herbisit dayanıklılık potansiyelinin ortaya konulması amaçlanmıştır. Analizler Türkiye'nin Orta Karadeniz bölgesindeki buğday ekim alanlarından toplanan 24 seçilmiş popülasyon üzerinden gerçekleştirilmiştir. 17 mikrosatellit primeri kullanılarak yapılan çalışmada, lokuslarda toplam 77 allel tespit edilmiş, lokus başına ortalama allel sayısı beş olarak belirlenmiştir. Ortalama gen çeşitliliği (GD) ve polimorfizm bilgi içeriği (PIC) değerleri sırasıyla 0.894 ve 0.732'dir. Average Linkage kullanılarak oluşturulan UPGMA dendrogramına göre popülasyonlar iki ana gruba ayrılmıştır. İncelenen kısır yabani yulaf genotipleri arasında yüksek derecede çeşitlilik tespit edilmiş olup, coğrafi izolasyonun olmadığı görülmüştür. Bu durumun öncelikle yabancı ot tohumların çeşitli yollarla taşınarak o coğrafi bölgelere adaptasyonuyla ilişkili olduğu anlaşılmaktadır. Dayanıklı yabancı otlar yüksek genetik çeşitlilik gösterebileceğinden, dirençli ve duyarlı steril yulaf popülasyonları arasında gen kaçışı olabileceği sonucuna varılmıştır. Bu bulgulara göre; ekim nöbeti, kültürel kontrol yöntemleri, sertifikalı tohum kullanımının teşvik edilmesi, farklı etki mekanizmalarına sahip herbisitlerin kullanımı gibi hususlar, herbisit dayanıklılığında göstergeci olan yüksek genetik çeşitliliğin kontrol altında tutulması için alınabilecek önlemler arasında sıralanabilir.

*Anahtar Kelimeler:* Kısır Yabani Yulaf**,** Buğday, Genetik Çeşitlilik, SSR.

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### 1. INTRODUCTION

Wheat is a widely grown grain product and major staple foods in several countries worldwide. Common wheat (*Triticum aestivum* L.), durum wheat (*Triticum durum* Desf.) and club wheat (*Triticum compactum* Host) are the most important wheat genotypes among thousands of known wheat varieties. Wheat is grown as a cash crop as it gives a good yield per unit area, grows well in a temperate climate with a moderately short growing season and is a versatile crop. Wheat, which constitutes 20% of the calories taken from food worldwide and is the staple food of 35% of the population, is also vital to Turkey (Yağdı et al., 2020).

Wheat is the second most produced cereal grain after maize, and the global trade of wheat is greater than all other crops combinedly (FAO, 2022). In the 2020- 2021 production season, while the worldwide grain production is 2.7 billion tons, 28% of this is wheat. In the 2019-2020 production season, while the world has a planting area of 217 million hectares and a wheat production of 764 million tons, Turkey constitutes 3.2% of the world's wheat cultivation area and ranks tenth with a production of approximately 20 million tons (FAO, 2022).

 Wheat production is limited by many biotic and abiotic factors. It is stated that the yield loss caused by biotic factors such as disease agents, pests (fungi, bacteria, nematodes, viruses, insects, etc.) and weeds can exceed 30% worldwide (Özer et al., 2001). Since the competitiveness of weeds is generally high, cereal products remain weak and yield decreases. Yield loss in cereals due to weed competition varies between 20-40% on average in the world (Güncan, 2010).

Weeds pose a major problem in wheat cultivation as in other cultivated plants. Among these weeds, *Lolium* spp., *Bromus tectorum* L., *Alopecurus myosuroides*, *Phalaris* spp., *Avena sterilis* L., *Raphanus raphanistrum*, *Vicia sativa* L., *Sinapis arvensis* L., *Silybum marianum* (L.) Gaertn., *Convolvulus arvensis* L., *Fumaria officinalis* L., *Galium aparine* L., *Matricaria chamo*milla L., *Papaver rhoaes* L., *Capsella bursa-pastoris* (L.) Medical, *Cirsium arvense* (L.) Scop, *Avena fatua* L. and many narrow and broad-leaved weeds cause significant losses in wheat cultivation areas (Aksoy et al., 2005).

The species belonging to the genus *Avena*, which is a problematic weed in wheat cultivation areas, especially *A. sterilis* (Sterile oat), are among the first weeds that pose problem in wheat-growing areas in Turkey as well as all over the world (Anonymous, 2017). Chemical control is the most effective control method to reduce yield loss caused by weeds in wheat cultivation areas. The emergence of resistance in weeds due to the continuous use of chemical herbicides carries a significant risk (Anonymous, 2017).

 Herbicide resistance is a situation that emerges due to the use of the same effective substances against the target weed for many years in a row and the application of high doses (Demirkan, 2009). Excessive use of herbicides leads to an increase in the cost of production, changes in the natural ecosystem, environmental pollution problems and the formation of resistant populations in weeds, loss of effectiveness of current control methods and severe yield losses.

In Turkey, wheat ranks first among the plants in which herbicides are used intensively, and as a result, resistance develops among these weeds (Türkseven, 2015; Kaya Altop et al., 2017 a,b; Kaya Altop et al., 2022). Resistance to herbicides started with sterile oats (*Avena sterilis* L.) in wheat cultivation areas for the first time in Turkey (Uludağ et al., 2001). Afterwards, resistant populations of sterile oat continued to emerge in several new populations as well.

 Herbicide resistance has become an increasingly common problem. With the increased resistance to herbicides worldwide, weeds have become one of the important problems in agricultural production. Currently, there are 266 herbicide-resistant weed species (153 broad-leaved and 113 narrow-leaved) worldwide (Heap, 2022).

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With the increase in resistance to herbicides, the concern that chemical warfare is insufficient in the fight against weeds is increasing, and there is a need for more advanced methods to be used in the diagnosis and identification of these weeds (Kaya, 2008).

Like many weed species, *A. sterilis* shows high genetic differences due to its reproduction and morphological characteristics depending on the area where it grows. There are differences between the genotypes of a species in terms of morphological, anatomical, physiological, biochemical and behavioural characteristics.

Variations between populations derive from alleles of a gene and the different frequency distributions of these alleles among populations. All of the genetic differences within a species are called genetic diversity. Determining genetic diversity, one of the components of biological diversity, is one of the most important conditions for healthy and efficient ecosystems and their sustainable operation. The high level of genetic diversity within the species is a guarantee for adaptation to changing environmental conditions. Species and races with high genetic diversity can adapt more successfully to changing ecological conditions according to time and place (Işık, 1997). Genetic diversity is undesirable for weeds. When evaluated in this context, the high genetic diversity plays a triggering role in the phenomenon of resistance to herbicides. Also, it supports the increase in the adaptation abilities of resistant populations (Kaya, 2008).

Genetic variation studies not only evaluated in an evolutionary context but also assist as an essential part of research in eradication and weed control (Sun, 1997). With knowing the genetic diversity of species that spread very rapidly, the geographical origins can be determined (Meekins et al., 2001), and it is possible to select biocontrol agents with this information (Nissen et al., 1995). Genetic variation should be determined to choose the biological control agents to be applied against strains that have developed resistance. Different molecular DNA marker techniques are used to reveal this genetic variation among plants (Yalım, 2005; Kaya, 2008; Yılmaz, 2021). The simple sequence repetition (SSR) markers are used in many fields such as the evolution of plants, genetic diversity studies, genetic linkage mapping, etc. (Özden Çiftçi and Altınkut Uncuoğlu, 2019; Yorgancılar et al., 2015).

Genetic diversity study is essential to know how herbicides and environmental factors affect weed species (Sterling et al., 2004). Studies on detecting genetic variation in many weed species, as well as in species belonging to the genus *Avena*, using the SSR method are being carried out rapidly around the world. Although *A. sterilis* is a self-pollinating plant, cross pollination, which is seen at a low rate, may cause genetic differentiation of this species.

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This study was conducted with the prediction that genetic differentiation may cause an increase in herbicide resistance of sterile oats. In order to develop more effective control methods against *A. sterilis*, genetic diversity was determined and its effect on the resistance phenomenon was investigated.

## 2. MATERIAL AND METHOD

## 2.1. Sampling and Breeding of Populations

Seeds of *A. sterilis* were collected from 150 different locations of wheat cultivation areas of Samsun (60), Sinop (10), Çorum (25), and Amasya (55) provinces. While collecting samples that survived after the herbicide application during the wheat growing period from the locations belonging to the same province, care was taken to ensure that the distance between the locations was at least 5-10 km (Barret, 1982).



**Figure 1.** Survey locations

Collected samples were kept at  $+4^{\circ}$ C for three months for cooling requirements. For the pre-germination process, the seeds of each population were placed in Petri dishes with a diameter of 9 cm with a double layer of moistened blotter paper and germinated in the incubator at +22  $\rm{^oC}$  at a 12/14 light period. Germination was achieved in 24 out of 150 populations and these populations were grown in sterile soil under controlled conditions in the greenhouse. Twenty-four populations (4 Samsun (SAM-19, SAM-35, SAM-8, SAM-58), 1 Sinop (SIN-4), 13 Çorum (COR-1, COR-3, COR-7, COR-10, COR-12, COR-14, COR-15, COR-17, COR-19, COR-

20, COR-21, COR-22, COR-24), 6 from Amasya (AMS-3, AMS-5, AMS-8, AMS-12, AMS-50, AMS-55) were included for further study (Figure 1).

#### 2.2. DNA Extraction and PCR Application

When the plants grown under greenhouse conditions reached the 4-6 leaf stage leaves samples were taken and genomic DNAs were extracted using the DNeasy DNA extraction kit (Qiagen, Germany) according to the kit protocol. Genomic DNA extraction was performed from 100 mg of fresh leaves tissues. The DNAs obtained following the applied DNA protocol were stored at -80 °C until the SS-R-PCR application.

The PCR applications in which the SSR molecular marker tests were carried out in a total volume of 25  $\mu$ L. PCR reaction mix; 2  $\mu$ l (1.0 ng/ $\mu$ L-1) genomic DNA, 1  $\mu$ l (25 ng) of each forward and reverse primers ((Table 1), Li et al., 2007) PCR master mix 2x: 12.5  $\mu$ l, 8.5  $\mu$ l sdH<sub>2</sub>O. The temperature values and times to be applied for PCR were established as follows: (1) 94 °C  $\rightarrow$  3 min., First step; 5 cycles (2) 94 °C  $\rightarrow$ 1 min., (3) 55 °C  $\rightarrow$  1 min. (decreased 1 °C per cycle up to 40 degrees), (4) 72 °C  $\rightarrow$ 1 min., Second step; 30 cycles (5) 94 ºC → 1 min., (6) 55 ºC→ 1 min., (7) 72 ºC→10 min. and (8) 72 °C $\rightarrow$  10 min. (last incubation). 1 x TBE buffer (100 mM Tris, 100 mM boric acid, 2 mM EDTA, pH 8.3) for 3.5% agarose gel for analysis of DNA fragments formed after PCR, 2 g agarose (Serva Agarose) (Serva, Germany) was carried out on a horizontal type maxi electrophoresis device (BioRad) using 1.5 g micropore agarose (Nusseive GTG Agarose) (Combrex, USA). Photographs of the bands obtained under the reference of 1Kb DNA marker (New England Biolabs®UK) were taken with the help of a gel imaging device (Vilberlurm).







### 2.3. Statistical Analysis

The genetic diversity of sterile oat was assessed by using 17 microsatellite (SSR) primers. The band images obtained from the gel were evaluated with reference to the marker sizes, and band matrices were created. The number of alleles, gene diversity (GD) and polymorphism information content (PIC) was calculated using the genetic analysis program NTSYSpc2.1 software. Further processing of data was done by carrying out sequential agglomerative hierarchical non-overlapping clustering (SAHN) on a squared Euclidean distance matrix. Dissimilarity matrices were used to construct the UPGMA (Unweighted Pair Group Method with Arithmetic average) dendrogram. In addition, the genetic relationships among genotypes were represented using a PCA (Backhaus et al., 1989) analysis with SPSS 21.0 software (IBM Corp. Released, 2012) ( Claerhout et al., 2015; Karn and Jasieniuk, 2017).

## 3. RESULTS AND DISCUSSIONS

The genetic diversity of twenty-four sterile oat populations collected from wheat cultivation areas in the central Black Sea region of Turkey was determined using 17 microsatellite primers. All microsatellites showed inter and/or intraspecific polymorphism. The total number of alleles per locus detected in sterile oat is given in Table 2.

A total of 77 alleles from microsatellite loci were detected, and these alleles ranged from 2 to 10 alleles per locus. The average number of alleles was determined as five. In a genetic diversity study with SSR analysis of sterile oats, Li et al. (2007) detected 36 alleles from nine microsatellite loci in *A. fatua*, with the number of alleles per locus ranging from 2 to 12. The average number of alleles per microsatellite locus is 4.0.The same as Li et al. (2000) identified 3.8 alleles/loci in 12 *Avena* species and 3.4 alleles/loci in 20 *Avena sativa* populations. Data (5 alleles/locus) from this study were higher than Li et al. (2000; 2007). These results showed that microsatellite markers of cultivated oat cultivars have high efficiency in examining and determining their genetic diversity.

In the studies, aiming at detecting genetic diversity in different species., it was seen that SSR analysis revealed strong gene expression (Randazzo et al., 2019; Xiong et al., 2019; Singh et al., 2020; Guo et al., 2022). When the genetic makeup of *Centaurea corymbosa*, an endemic plant, was compared, it was stated that SSR analysis using six microsatellite loci revealed a broad differentiation between populations. These microsatellites were founded more potent than allozyme loci in detecting gene flow coverage (López-Vinyallonga et al., 2011).

The gene diversity of *A. sterilis* ranged from 0.764–1.199. Polymorphism information content values changed from 0.381-1.018. The average gene diversity and PIC values were 0.894 and 0.732, respectively (Table 2). Different studies confirm the high genetic diversity detected in different populations. These studies investigating genetic variation, population structure and diversity of *A. sterilis* alleles have generally concluded that resistance develops by differentiation in the process, and resistance is distributed through gene flow (Menchari et al., 2006; 2007). Weed species with high levels of genetic diversity exhibit considerable potential for weed adaptation; therefore, the effectiveness of frequently used weed-control techniques may be reduced (Dekker, 1997; Holtand Hochberg, 1997).

Naghavi et al., (2009) studied 21 SSR primers to determine the genetic relationship between 52 *Triticum aestivum* and 13 *Aegilops* species using SSR markers. Boylu and Kaya Altop, (2021) looked at 11 SSR primers to assess the genetic relationship of 40 *Alopecurus myosuroides* populations. Çağlar (2010) used 3 SSR primers in his study and it was seen that the genetic similarity rate in the *Centaurea*  *nivea* biotype varied between 26%- 76%. In another study conducted with 5 SSR primers, the genetic diversity ratio among 62 *Echinochloa oryzoides* populations supported the results of the current study (Altop et al., 2018).

Locus	<b>Number of Allels</b>	GD	PIC Value
LOC1	$\overline{4}$	0.995	0.764
LOC2	$\,$ 8 $\,$	0.902	0.511
LOC3	$\,$ 8 $\,$	0.764	0.441
LOC4	3	1.199	1.018
LOC5	3	0.770	0.674
LOC6	3	0.920	0.987
LOC7	$\mathfrak{Z}$	0.854	0.971
LOC8	3	1.025	0.893
LOC9	$\sqrt{2}$	0.804	0.658
LOC10	5	0.782	0.639
LOC11	$\sqrt{2}$	0.809	0.893
LOC12	3	0.826	0.830
LOC13	6	0.851	0.619
LOC14	5	1.002	0.686
LOC15	$\mathfrak{Z}$	0.927	0.846
LOC16	10	0.782	0.381
LOC17	6	0.993	0.635
Mean	5	0.894	0.732

**Table 2.** Genetic characterization of *A. sterilis* populations

According to the UPGMA dendrogram, constructed using the Average Linkage, populations divided into two major clusters. Genetic distance ranged from 0 to 0.25. The first cluster formed different levels of branching and included 22 of the 24 populations (Figure 2). This main cluster was characterized by a wide geographical distribution. It is not possible to talk about clear geographical isolation. The second main cluster consisted of COR7 and COR24 populations (belonging to Çorum province).



**Figure 2.** Dendrogram created by hierarchical cluster analysis method

When the correlation matrix created according to the SSR-PCR band and results of *A. sterilis* populations were examined, the correlation between populations representing the second main group was determined as 83% (Table 3). Amasya, AMS55-AMS50 and Çorum COR22-COR15 populations were defined as genetically closest populations. Genetic similarity was considered to be expected since the distance between them was relatively close. But, despite belonging to the same province COR1 and COR20 populations, only 2% similarity was observed. These results showed that geographical distance could not lead to a clear inference in interpreting genetic differences. In addition, COR7 with SIN4 from Sinop province (distance of about 478 km) and SAM48 (Samsun's population) with COR17 (distance of about 250 km) were genetically the most distant populations. Cluster analysis results of the US and Chinese populations of *Avena* also supported our findings (Li et al., 2007).



**Table 3.** Correlation matrix of *A. sterilis* populations created according to SSR-PCR band results

## 4. CONCLUSION

A high degree of diversity was detected among the investigated *A. sterilis* genotypes, and there was no geographical isolation among genotypes. The main reason of this situation is the adaptation ability of sterile oat seeds transferred from different regions. Although they are highly self-pollinating species, they may also have some cross pollination. In addition, it has been concluded that resistant weeds may cause high genetic diversity and a high probability that gene escape may occur between resistant and susceptible populations. This study can shed light on the next researchers and issues. Suggestions for the solutions to these problems, which emerged as a result of the findings, can be listed as follows; a) Crop rotation should be done in wheat fields. b) Cultural control methods (soil cultivation, use of clean seeds, selection of early or late varieties according to the growing period of the weed, fallow application, good soil preparation, and attention to the cleanliness of the used equipment) must be applied. c) Seeds should be well controlled within the country. d) The use of herbicides with different mechanisms of action should be recommended.

### Conflict of Interest

The author declare that there is no conflict of interest.

#### **Ethics**

This study does not require ethics committee approval.

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https://doi.org/10.7161/omuanajas.1205286 doi