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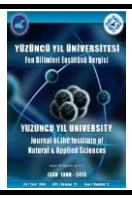

Research Article

The Applicability of Wheat SSR Markers to Analyzing the Molecular Diversity and Distribution of Orchardgrass (*Dactylis glomerata* L.) Genotypes from Eastern Anatolian Habitats

Ahmad NABHAN[1], Ösmetullah ARVAS[2], M. Alp FURAN*[3]

[1]Agriculture-Plant Biotechnology Shafak Organization, Gaziantep, Türkiye
[2] Department of Field Crops, Faculty of Agriculture, Van Yuzuncu Yil University, Van, Türkiye
[3]Department of Agricultural Biotechnology, Faculty of Agriculture, Van Yuzuncu Yil University, Van, Türkiye
Ahmad NABHAN, ORCID No: 0000-0002-8125-8913, Ösmetullah ARVAS, ORCID No: 0000-0001-8713-2388, M. Alp FURAN, ORCID No: 0000-0002-0171-0405
*Corresponding author e-mail: alpfuran@gmail.com

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Abstract: Türkiye is one of the world's nations with the greatest flora diversity in different plants. Moreover, it possesses a high level of plant genetic diversity. Orchardgrass (*Dactylis glomerata* L.) is one of the most important fodder species used during cool seasons since it is a hardy and perennial plant. The identification of Orchardgrass cultivars is essential for maximizing cultivar utilization, and safeguarding breeders' intellectual property. *Dactylis glomerata* L. is an allogamous, variable, monospecific genus with multiple subspecies distinguished by morphology, chromosomal count, and distribution. This genus has a single species, *Dactylis glomerata* L, which is comprised of multiple subspecies whose traits have not been exhaustively characterized. Using DNA assays that evaluated the transferability of nine SSR primers designed for wheat loci, the genetic diversity of 44 orchardgrass genotypes from eight naturally distributed locales in the Eastern Anatolia Area of Türkiye was calculated. On average, 6.78 alleles were discovered for each of the nine SSR primers, for a total of 61 alleles. A total of 54 polymorphic alleles were identified, with an average of 6.78 per primer. Polymorphism information content (PIC) values ranged from 0.320 (WMC96) to 0.626% (XBARC187). The average polymorphism rate of 88.89% suggests a high amount of genetic diversity among all studied genotypes. The average expected heterozygosity (He) ranged between 0.178 (Ağrı) to 0.882 (Erzurum). The genetic separation ranged from 0.01 to 0.66. In conclusion, our findings indicate that the *Dactylis glomerata* L genotypes gathered in Eastern Anatolia are a rich source of genetic variability, supplying a vast array of genetic material for orchardgrass breeding efforts.

Doğu Anadolu Habitatlarından Domuz ayrığı (*Dactylis glomerata* L.) Türlerinin Moleküler Çeşitliliğinin ve Dağılımının Analizinde Buğday SSR Markerlerinin Uygulanabilirliği

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Öz: Türkiye, dünyanın flora çeşitliliği en fazla olan ülkelerinden biridir. Ayrıca, yüksek düzeyde bitki genetik çeşitliliğine sahiptir. Domuz ayrığı (*Dactylis glomerata* L.), dayanıklı ve çok yıllık bir bitki olması nedeniyle serin mevsimlerde kullanılan en önemli yem türlerinden biridir. Orchardgrass çeşitlerinin tanımlanması, çeşit kullanımını en üst düzeye çıkarmak ve yetiştiricilerin fikri mülkiyetini korumak için gereklidir. *Dactylis glomerata* L., morfoloji, kromozom sayısı ve dağılımı ile ayırt edilen çok sayıda alt türü olan allogam, değişken, monospesifik bir cinstir. Bu cinsin tek bir türü vardır,

Anahtar Kelimeler
Dactylis glomerata,
Genetik çeşitlilik,
Meyve bahçesi çimi,
SSR

Dactylis glomerata L, özellikleri kapsamlı bir şekilde karakterize edilmemiş birden fazla alt türden oluşur. Buğday lokusları için tasarlanan dokuz SSR primerinin aktarılabilirliğini değerlendiren DNA tahlilleri kullanılarak, Türkiye'nin Doğu Anadolu Bölgesi'nde doğal olarak dağılmış sekiz yerel bölgeden 44 Domuz ayrığı genotipinin genetik çeşitliliği hesaplandı. Ortalama olarak, dokuz SSR primerinin her biri için toplam 61 alel için 6.78 alel keşfedildi. Primer başına ortalama altı olmak üzere toplam 54 polimorfik alel tanımlandı. Polimorfizm bilgi içeriği (PIC) değerleri %0,320 (WMC96) ile %0,626 (XBARC187) arasında değişmektedir. %88.89'luk ortalama polimorfizm oranı, çalışılan tüm genotipler arasında yüksek miktarda genetik çeşitlilik olduğunu göstermektedir. Beklenen ortalama heterozigotluk (He) 0,178 (Ağrı) ile 0,882 (Erzurum) arasında değişmekteydi. Genetik ayırma 0.01 ile 0.66 arasında değişmektedir. Sonuç olarak, bulgularımız, Doğu Anadolu'da toplanan *Dactylis glomerata* L. genotiplerinin, Domuz ayrığı bitkisi ıslah çabaları için çok çeşitli genetik materyal sağlayan zengin bir genetik çeşitlilik kaynağı olduğunu göstermektedir.

1. Introduction

Türkiye is one of the world's wealthiest countries in terms of plant diversity. In addition, it contains a high amount of plant genetic diversity. Several variables are responsible for this diversity: temperature variances, topographic diversity, geological diversity, diversity of water sources, and altitude differences (Arı et al., 2014; Kaya, 2015). Using natural resources in plant breeding projects has given them a great deal of significance, particularly in terms of enhancing plant productivity and pastures, which are still deteriorating due to overgrazing (Ünal & Mutlu, 2016). According to Aygün et al. (2009), the region of Eastern Anatolia in Türkiye is home to numerous grasslands and forage crop species. In addition, it comprises 35 percent of the total pasture and meadow areas in Türkiye, which are considered a gene source for numerous plants utilized in pasture and meadows. To offer appropriate and affordable animal products by ensuring high-quality fodder for our animals, must develop pastures and cultivate forage crops within the agricultural sector (Uysal et al., 2015). Therefore, it is vital to establish, through breeding programs, varieties of high-quality fodder plants that may be grown in agricultural fields or other environmental settings (Jiang et al., 2013).

Dactylis glomerata L. is an allogamous, changeable, monospecific genus with numerous subspecies characterized by morphology, number of chromosomes, and range. The most common name for *D. glomerata* L. is orchardgrass (Garcia & Lindner, 1998; Bushman et al., 2011; Jiang et al., 2013). This genus has one species, *Dactylis glomerata* L, which consists of numerous subspecies whose characteristics have not been fully described (Yan et al., 2016), it is widely utilized for grazing and hay production around the globe (Xie et al., 2012; Jiang et al., 2013).

Orchardgrass (*Dactylis glomerata* L.) is one of the most important cool-season perennial and persistent feed grasses in Türkiye and many other temperate and cold-climate nations (Last et al., 2014; Yan et al., 2016). It is a major feed crop with high agronomic value in the eastern portion of Türkiye and other locations with climates similar to the east (Yan et al., 2016). Its economic worth derives from its excellent yield and disease resistance in a variety of environmental situations. Orchardgrass is a widespread grass that is well-adapted to severe water deficit situations because it maintains a greater photosynthetic rate than the majority of temperate pasture during the harshest drought conditions. In addition to its high fodder quality, sugar and protein levels, shade tolerance, and persistence (Last et al., 2014; Madesis et al., 2014). Identification of orchardgrass cultivars is necessary to guarantee purity for farmers, effective cultivar utilization, and intellectual property security for breeders.

Information on the genetic distance of plant germplasm will be an essential resource for identifying populations by determining the genetic relationship at a particular germplasm source. Information on the genetic link between genotypes can be utilized as a supplemental tool to phenotypic information at the beginning of the breeding program to improve breeding populations (Parmaksız, 2004). Genetic variability between genotypes can help breeders decide what materials to utilize when developing new genetic combinations (Parmaksız, 2004). Genotype collections from natural populations of agriculturally significant plant species have been utilized extensively in the

creation of better cultivars. Morphological markers and molecular markers have been utilized extensively for the development of genetic resources and the investigation of rebreeding and breeding genotypes (Madesis et al., 2014). Because they are reproducible, reliable, and independent of environmental variables, molecular markers are utilized for the direct investigation of genetic variation (Madesis et al., 2014). Molecular markers can advance our understanding of plant genetics and plant breeding techniques. It is believed that the knowledge gathered through these new technologies will be used to improve global food safety. Particularly, DNA markers have the potential to increase the efficacy of plant breeding programs in a variety of areas, such as removing the fingerprints of genetic stocks, determining genetic diversity, enhancing selection efficiency, and the application of quantitative trait loci (QTL) (Gedil & Menkir, 2019). Bushman et al. (2011) developed an EST library and SSR markers from salt-, drought-, and cold-stressed tissues for the enhancement of orchardgrass genetic resources. Of the 1,162 SSR markers developed, approximately 80% exhibited amplification products in orchardgrass germplasm and 40% in Festuca and Lolium species. Based on the putative orthology, the Unigens reported that the total number of genotyped bands was higher for tetraploid accessions than for diploid accessions, and that Mediterranean subspecies and Central Asian subspecies were in the same groups based on clustering analyses using information annotated from the NCBI database for rice, Triticeae grasses, other Poaceae, and Arabidopsis. Numerous researchers have conducted previous research on SSR efficacy between species within the same family. In order to examine and quantify genetic diversity and interrelationships in orchardgrass germplasm, SSR markers have been successfully utilized to determine the genetic diversity and interrelationships of orchardgrass germplasm from diverse geographic locations (Xie et al., 2010a and 2010b; Xie et al., 2012; Jiang et al., 2013; Madesis et al., 2014; Yan et al., 2016). Differently in this study, we tested wheat-specific SSR primers on orchardgrass, a member of the same family as wheat. Using the tranferability of wheat SSR markers, the purpose of this study was to determine the genetic diversity of *Dactylis glomerata* L. genotypes that occur naturally in the Eastern Anatolia Region of Türkiye.

2. Material and Methods

2.1. Plant material

Throughout the months of July and August, *Dactylis glomerata* L. genotypes were collected from 43 naturally scattered locations encompassing 8 cities across the Eastern Anatolian Region of Türkiye (Figure 1). In our investigation, the *Dactylis glomerata* L. (Amba) cultivar served as the control. The study's genotype numbers and information about the geographical region's characteristics are listed in Table 1.

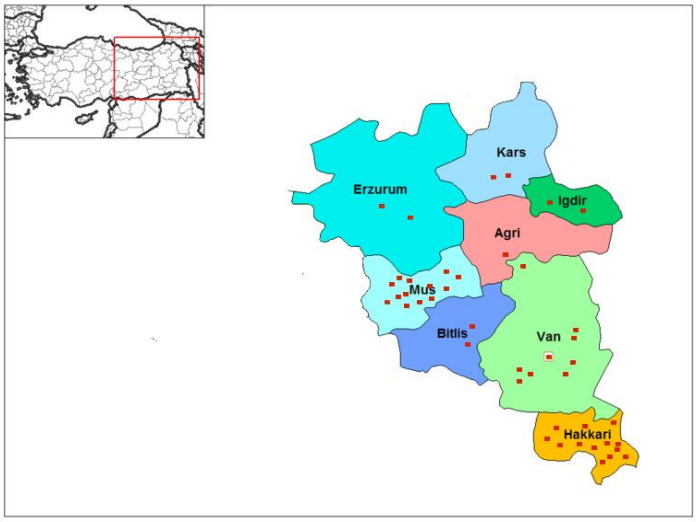

Figure 1. The study's plants and their locations on the map.

Table 1. The study's genotypes and the geographic regions from where they were obtained

No	Genotype	Location-District	Latitude	Longitude	Height (m)
1	H2	Hakkari - Merzan	37° 33.639'	043°41.629'	2166
2	H3	Hakkari - Ademan	37° 33.502'	043° 40.417'	2543
3	H5	Hakkari - Kamışlı köyü	37° 34.259'	043° 32.195'	1717
4	H6	Hakkari - Cevzdibi köyü	37° 32.511'	043° 29.609'	1575
5	H9	Hakkari - Mergers -Adaman	37° 33.888'	043° 39.352'	2283
6	H21	Hakkari- Durkankaya 1	37° 37.980'	043° 37.165'	2999
7	H23	Hakkari - Durkankaya 2	37° 37.015'	043° 37.718'	2932
8	H26	Hakkari - Durkankaya 3	37° 34.835'	043° 38.185'	2499
9	H27	Hakkari - Durkankaya 4	37° 37.648'	043° 37.355'	3011
10	H41	Hakkari - Merkez 1	37° 43.755'	043° 58.169'	2347
11	H43	Hakkari - Merkez 2	37° 40.449'	043° 58.635'	1870
12	H45	Hakkari - Merkez 3	37° 43.478'	043° 59.139'	2238
13	H47	Hakkari - Merkez 4	37° 43.872'	043° 59.270'	2202
14	M61	Muş - Varto 1	39° 08.007'	041° 42.258'	2163
15	M67	Muş - Varto 2	39° 08.652'	041° 42.228'	2160
16	M71	Muş - Varto 3	39° 12.570'	041° 41.411'	1916
17	M72	Muş - Varto 4	39° 12.569'	041° 41.481'	1649
18	M74	Muş - Varto 5	39° 09.297'	041° 41.311'	2073
19	M75	Muş - Varto 6	39° 09.489'	041° 41.010'	2088
20	M79	Muş - Varto 7	39° 06.385'	041° 43.835'	2268
21	M80	Muş - Varto 8	39° 06.442'	041° 45.848'	2259
22	M81	Muş - Merkez 1	38° 35.980'	041° 33.786'	1438
23	M85	Muş - Merkez 2	38° 42.874'	041° 29.540'	1763
24	M110	Muş -Bulanık 1	38° 52.128'	041° 56.754'	1766
25	M113	Muş - tiğem	38° 47.539'	041° 23.257'	1260
26	M115	Muş - Bulanık 2	38° 49.314'	041° 72.540'	1532
27	A121	Ağrı - Patnos	39° 14.116'	042° 54.890'	1637
28	V141	Van- Erçiş	39° 05.549'	043° 37.911'	1750
29	R163	Iğdır - Merkez 1	38° 49.922'	043° 40.526'	1725
30	R175	Iğdır - Merkez 2	38° 49.111'	043° 40.311'	1680
31	V181	Van - Kampüs 1	38° 34.032'	043° 16.869'	1658
32	V189	Van - Kampüs 2	38° 57.119'	043° 28.818'	1665
33	V202	Van - Bostanci 1	38° 52.552'	043° 44.658'	1688
34	V207	Van - Bostanci 2	38° 52.890'	043° 44.995'	1692
35	K225	Kars - Dağpınar 1	40° 46.940'	043° 31.527'	2100
36	K240	Kars - Dağpınar 2	40° 47.510'	043° 31.681'	2119
37	V241	Van - Gavaş 1	38° 29.934'	043° 10.640'	1750
38	V247	Van - Gavaş 2	38° 30.179'	043° 10.777'	1752
39	V253	Van - Gavaş 3	38° 30.925'	043° 11.174'	1760
40	B261	Bitlis - Merkez 1	38° 42.022'	042° 12.354'	1558
41	B269	Bitlis - Merkez 2	38° 42.623'	042° 12.291'	1595
42	E283	Erzrum - Merkez-1	39° 90.226'	041° 97.740'	1870
43	E288	Erzrum- Merkez -2	39° 91.692'	041° 25.672'	1860
44	Con	Control			

2.2. Genomic DNA extraction

Young, fresh leaf specimens from the chosen plant were collected and wrapped in aluminum foil paper. These samples were kept at –80 °C prior to DNA analysis. To isolate the DNA, a commercial DNA purification kit (Thermo Scientific GeneJET) was utilized, and DNA concentration was determined using Thermo NanoDrop 2000 devices. The quality of the extracted DNA was determined using the A260/A280 ratio. It was determined that the quality of DNA was between 1.8 and 2.0 (Sambrook & Russell, 2001).

2.2. SSR Markers

The GrainGenes Probe Report at https://wheat.pw.usda.gov was utilized to select randomly nine SSR primers generated from wheat two SSRs per linkage cluster (Furan et al., 2017a) (Table 2). PCR reactions were conducted in a total volume of 25 µl containing 16.2 µl water, 2.5 µl 10X DNA polymerase buffer, 1 ml dNTPs (2.5 mM each), 0.5 µl each primer at 10 M, 0.3 µl Taq polymerase at 10 U/ µl (Fermentas), and 2.5 µl genomic DNA. The thermocycler was programmed as follows: an initial cycle at 94 ^{0}C for 2 minutes, 40 cycles at 94 ^{0}C for 30 seconds, 60 ^{0}C (depending on the primer used) for 60 seconds, and 72 ^{0}C for one minute, followed by a 10-minute extension at 72 ^{0}C. The amplicons were size-fractionated on a 2% (w/v) high-resolution agarose gel in TBE buffer for 3–4 hours and visualized with ethidium bromide. Thermo ScientificTM products. As a molecular weight marker, GeneRuler 100 bp Plus DNA Ladder was utilized, and the gel was screened using a Quantum ST4 UV transilluminator (Furan et al., 2017b).

Table 2. Names and sequences of randomly selected SSR primers from the Grain Genes database

No	Primer Name	Forward Primer (5´→3´)	Reverse Primer (5´→3´)
1	WMC96	GTACAACGGAGGCATTCTCAAGTT	TGTACATGGTGGTCTTGTTGAGGT
2	WMC147	ATATGCATTGCCTGGAACTGGAAGGA	AATTCAAACACGCCTCCCGAGTGT
3	WMC153	CTACCTATCCAAGCGATGGGGA	CGTGCAAATAATTCCCCGTGGGA
4	WMC276	TTGTCTTTCTTCCTCCACAAGCAGCGAA	ATTTCCAGTTGCCACCGACGAAGAACTT
5	WMC322	CACAAAGAGCAGCCCACTTT	AAGTTGCTGACATCGATCCA
6	XBARC187	GCTCCAGGTCGGAGATGTGA	CACAACACATCCAGTGACCAGAGT
7	XBARC263	TTGCTCGGTATGAAGAAAATAGTCTTTCC	ATCTTGCAACTAGACTGAGGCAACCA
8	XGWM630	CTGTCGTAAGAGCGCCAACAG	GTCTGAACGATGAACAGTACACGC
9	XGWM319	TAGCGACAGGATGGCCTCTTCT	GGGGAGCACGCCTTCGTTCT

2.3. Data analysis

All amplicons were scored on the basis of their presence or absence to generate a binary matrix (1-presence/0-absence). Only distinct and robust bands were captured and utilized for further research. For each location, the number and size ranges of SSR-amplified alleles were computed. To evaluate genetic diversity, the polymorphism rate (P), number of total alleles, and polymorphic information content (PIC) were utilized. PIC was computed using the formula PIC = 1 - ∑P2ij, where Pij is the frequency of the jth allele over all alleles of the locus (Nei, 1973). The software GeneAlEx 6 (Peakall & Smouse, 2006) was utilized to determine the Pairwise Region Matrix of Nei Genetic Distance and gene diversity (expected heterozygosity, HE).

3. Results

3.1. Mobility of SSR markers between species

The nine SSR primers employed in eight Orchardgrass areas yielded 61 alleles, averaging 6.78 per SSR primer and ranging from 4 to 8. The range of polymorphism allele counts was between 3 and 7 with an average of 6.0 (Table 3). Indicating a high level of genetic variation, the proportion of polymorphism per primer ranged from 75% to 100%, with an average of 88.69%. The average value of polymorphism information content (PIC) ranged from 0.320 for (WMC96) to 0.626 for (XBARC187), with a mean value of 0.48 (Table 3).

Table 3. Total numbers of alleles, numbers of polymorphism alleles, percentage of polymorphism alleles, and polymorphism information content (PIC) values of 9 SSR primers used on Orchardgrass genotypes in 43 naturally spread locations spanning 8 districts

Primer number	Total numbers of alleles	Numbers of polymorphism alleles	Percentage of polymorphism alleles	Polymorphism information content (PIC)
WMC96	7	6	85.71%	0.320
WMC147	6	6	100.00%	0.488
WMC153	8	6	75.00%	0.543
WMC276	6	6	100.00%	0.483
WMC322	8	6	75.00%	0.563
XBARC187	7	7	100.00%	0.626
XBARC263	8	7	87.50%	0.426
XGWM630	7	7	100.00%	0.522
XGWM319	4	3	75.00%	0.363
Total	61	54		
Mean	6.78	6.00	88.69%	0.48

3.2. Genetic diversity

One of the ways used to determine genetic diversity is the criterion known as "expected heterozygosity" and characterized as "genetic diversity" (Nei, 1973). The values calculated for each SSR primer are displayed in (Table 4). The range of the Nei gene diversity index, or predicted heterozygosity per location, was between 0.178 and 0.882. (Table 4). To generate the germplasm similarity matrix, Nei's technique for genetic consistency and genetic distance was used to the amplification data.

Table 0. Expected heterozygosity (He) values according to (Nei, 1973) for each SSR primers for each region

regions /primers	WMC96	WMC147	WMC153	WMC276	WMC322	XBARC187	XBARC263	XGWM630	XGWM319	Mean
Hakkari	0.537	0.620	0 .635	0.586	0.642	0.642	0.635	0.635	0.635	0.619
Muş	0.555	0.515	0.635	0.555	0.549	0.515	0.623	0.320	0.635	0.545
Ağrı	0.580	0.178	0.735	0.580	0.198	0.180	0.735	0.735	0.615	0.504
Van	0.320	0.383	0.650	0.320	0.531	0.490	0.650	0.650	0.642	0.515
Iğdır	0.405	0.700	0.540	0.438	0.700	0.716	0.568	0.494	0.540	0.567
Kars	0.593	0.670	0.667	0.560	0.670	0.670	0.650	0.650	0.642	0.641
Bitlis	0.265	0.593	0.611	0.265	0.550	0.550	0.615	0.611	0.615	0.519
Erzurum	0.745	0.875	0.675	0.745	0.882	0.875	0.645	0.675	0.675	0.755
Mean	0.500	0.567	0.643	0.506	0.590	0.580	0.640	0.596	0.625	

The average genetic distance (GD) for the eight Orchardgrass regions was 0.28, ranging from 0.01 to 0.66. The genetic gap between Van and Bitlis was the smallest (0.01), while the genetic distance between Bitlis and Hakkari was the most (0.66) (Table 5).

Table 5. Matrix of pairwise distances between 44 orchardgrass genotypes scattered throughout 8 districts

	Hakkari	Muş	Erzurum	Kars	Iğdır	Ağri	Van	Bitlis
Hakkari	0.00							
Muş	0.05	0.00						
Erzurum	0.19	0.19	0.00					
Kars	0.35	0.45	0.10	0.00				
Iğdır	0.22	0.23	0.10	0.08	0.00			
Ağri	0.24	0.25	0.22	0.20	0.07	0.00		
Van	0.48	0.40	0.36	0.26	0.38	0.50	0.00	
Bitlis	0.66	0.62	0.38	0.34	0.41	0.45	0.01	0.00

3.3. Phylogenetic construction

Phylogenetic analysis is typically employed to classify gathered germplasm. Either randomly or in a way that maximizes genetic distance can be used to generate groups (Uysal et al., 2015). To illustrate the links between Orchardgrass genotypes, a UPGMA dendrogram was constructed and graded as six groups labeled A-F (Figure 2).

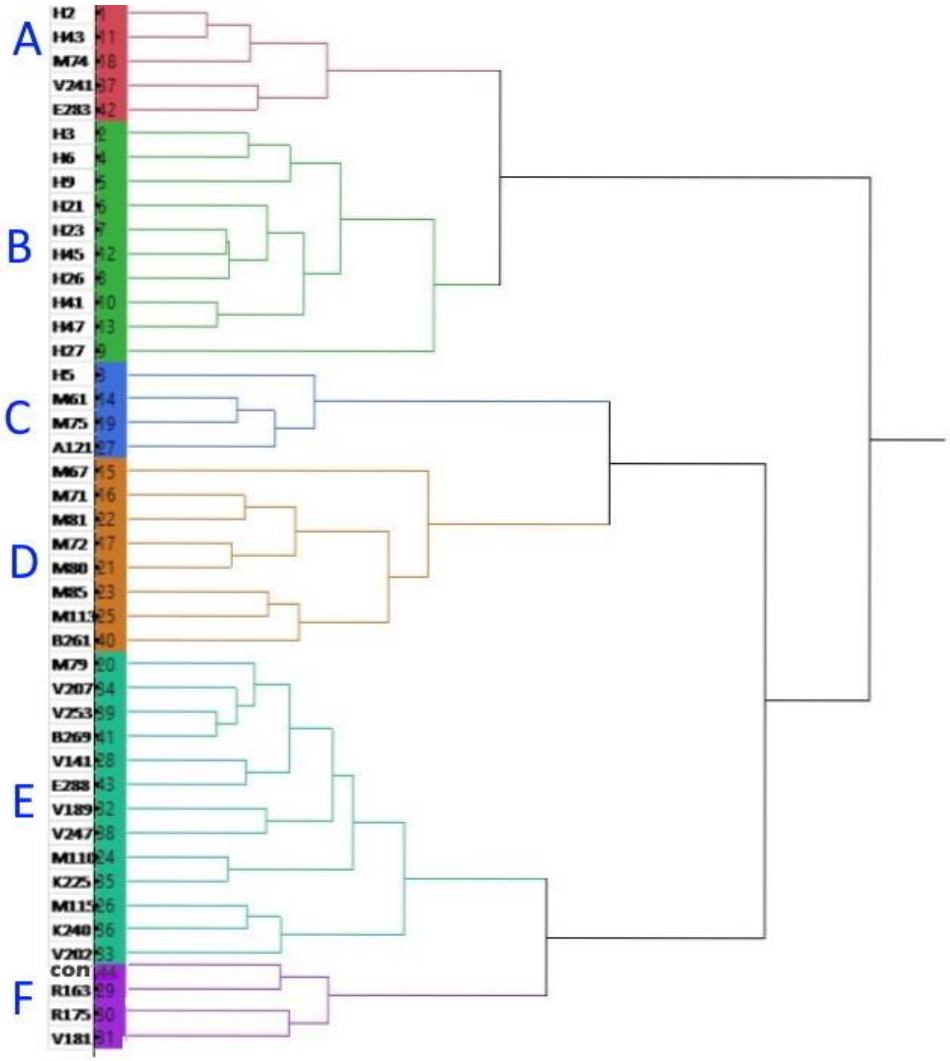

Figure 2. UPGMA study of shared allele distance for 44 orchardgrass genotypes Group A is represented by red branches, Group B by green branches, Group C by blue branches, Group D by brown branches, Group E by turquoise branches, and Group F by purple branches.

Group A consisted of the genotypes H2, H43, M74, V241, and E283. The genotypes H3, H6, H9, H21, H23, H45, H26, H41, H47, and H27 comprised Group B. The following group members, C, were H5, M61, M75, and A121. Group D was made up of the phenotypes M67, M71, M81, M72, M80, M85, M113, and B261. Group E is comprised of the following genotypes: M79, V207, V253, B269, V141, E288, V189, V247, M110, K225, M115, K240, and V202. In addition to the control, the last group, F, included the three genotypes R163, R175, and V181.

The range of the number of genotypes per group was from 4 (Group F) and 13 (Group E). The largest group was E, with 13 genotypes gathered from 13 places and five regions (provinces) (Muş, Van, Bitlis, Erzurum, Kars), while the smallest was F, with three genotypes acquired from three locations and two regions (Iğdır, Van).

4. Discussion and Conclusion

The UPGMA dendrogram classified the genotypes into six groups, illustrating the relationships between orchardgrass genotypes from various regions of East Anatolia. Group A was comprised of the genotypes H2, H43, M74, V241, and E283. Whereas H3, H6, H9, H21, H23, H45, H26, H41, H47, and H27 genotypes comprised group B, H5, M61, M75, and A121 genotypes comprised Group C, Group D is made up of the genotypes M67, M71, M81, M72, M80, M85, M113, and B261, respectively. Ultimately, it was included in group E with the genotype combinations of M79, V207, V253, B269, V141, E288, V189, V247, M110, K225, M115, K240, and V202, respectively. Based on "Characterization of Orchardgrass (*Dactylis glomerata* L.) Using Morphological Traits and Molecular Markers" it has been determined that the genotypes comprising these subgroups share similar morphological characteristics. While the genotypes in Group A had the smallest glume length, the shortest lemma length, and the shortest flag leaf width, the genotypes in Group B had the shortest plant height, the shortest length of the upper internode, the shortest lemma length, the fewest nodes per plant, and the least amount of seed weight per 1000 seeds. The members of Group C had the greatest plant height, the longest flag leaf length, the longest flag leaf width, the longest length of the upper internode, the longest panicle length, and the longest glume length, Group D comprised the genotypes M67, M71, M81, M72, M80, M85, M113, and B261, with the least number of tillers per plant, the longest panicle length, the most node number as well as the greatest number of spikelets per panicle. Group E contains the most significant number of genotypes: M79, V207, V253, B269, V141, E288, V189, V247, M110, K225, M115, K240 and V202. These genotypes had the longest duration to heading, the longest length of the upper internode, the shortest flag leaf length, the shortest flag leaf width, the most node number, the shortest panicle length, and the highest of 1000 seed weight. The last group, F, included the three genotypes R163, R175, and V181 in addition to the control, which had the greatest number of tillers per plant, the least number of spikelets per panicle, and the longest lemma length. According to differences in physical traits between cluster groups (Aygün et al., 2009), these differences may not be caused by the environment. One more likely explanation is that only genetic characteristics differentiate these groups. Cluster analysis (CA) showed eight groupings and subgroups with 80% phenotypic similarity between hybrids and parents, according to the findings of Zhouri et al. (2017), who categorized genotypes and parents graphically based on measured attributes.

Orchardgrass is a widespread fodder plant in mild regions. Robins et al. (2012) have demonstrated the significance of introducing genetic and phenotypic variety into breeding plans in previous research. According to Erfani et al. (2012) and Zhang et al. (2012), SSR markers are one of the most prevalent methods for examining genetic diversity. Using 29 SSR markers, Last et al. (2014) determined the genetic diversity of 20 populations of orchardgrass gathered from the Swiss Alps. These results are consistent with our research. In addition, numerous studies on orchardgrass genotypes have demonstrated that SSR markers are an effective method for determining the genetic diversity of a population (Ma et al., 2001; Mao et al., 2016; Yan et al., 2016; Demirkol, 2017). All the aforementioned study indicated that SSR markers are present in the majority of eukaryotic genomes and are regarded as extremely efficient, informative, credible, reproducible, and polymorphic. These benefits make it a useful method for determining and assessing the diversity of closely related kinds. This study employed nine SSR primer pairs to evaluate the genetic diversity of Orchardgrass genotypes gathered from several locales in Eastern Anatolia. Our SSR primers discovered an average

of 6.78 alleles per primer, for a total of 61 alleles. The identification of 54 polymorphic alleles, with an average of six alleles per primer, enabled us to obtain accurate results for distinguishing Orchardgrass genotypes and locating genetic diversity in various regions of Eastern Anatolia. Madesis et al. (2014) employed seven SSR primers to analyze the genetic diversity of Orchardgrass samples obtained from three distinct areas (northern, central, and southern Greece). SSR primers produced an average of six polymorphic bands per primer, ranging from two to ten. The results of this study are also consistent with the notion that a substantial level of polymorphism can be observed when a sufficient number of primers are used. Moreover, our results were comparable to those of Xie et al. (2010b), who employed 21 primer pairs to evaluate the genetic diversity of 16 accessions of *Dactylis glomerate* L. and obtained an average of 6.8 polymorphic alleles per locus from 143 polymorphic alleles. Earlier investigations of the genetic diversity of Orchardgrass plants from several geographic regions, which showed 7.9 alleles per locus (Jiang et al., 2013) and 7.8 alleles per locus (Mao et al., 2016), discovered that the average polymorphic allele of each marker was greater. 9.3 alleles per locus (Yan et al., 2016), 6.3 bands per primer (Xie et al., 2010a). The average polymorphism rate of the studied Orchardgrass genotypes was 88.69% (Table 3), indicating a substantial degree of genetic variation across the genotypes utilized in this study. In addition, when the polymorphism values obtained in this study are compared with those from previous research, EST-SSR polymorphism (P = 74.14%) (Xie et al., 2010a), SRAP polymorphism (P = 84.38%) (Zeng et al., 2008), and AFLP polymorphism (P = 84.0%) (Peng et al., 2008) confirm that the SSR markers we used in our study provide a highly accurate and reliable technique for assessing genetic diversity between and within *Dactylis glomerata* L genotypes. The transferability of the primers chosen for this investigation to orchardgrass species has also been demonstrated by these findings. In this investigation, the polymorphism information content varied between 0.320 and 0.626, with a mean of 0.48. Typically, the PIC value is a useful metric for comparing various molecular markers. It can also be used as an evaluation metric to determine the efficiency of primers in any DNA marker (Hongtrakul et al., 1997; Manifesto et al., 2001; Garcia et al., 2004). Based on the information content, Vaiman et al. (1994) established three scales of polymorphism information content: high (PIC > 0.5), intermediate (0.5 > PIC > 0.25), and low (PIC 0.25). Xie et al. (2011) reported a mean polymorphism information content (PIC) of 0.33, which was in good accord with the results of this investigation (0.48). In contrast, the genetic variation amongst Orchardgrass plants gathered from different geographic regions was determined to be lower than in earlier studies: 0.848 (Jiang et al., 2013), 0.62 (Last et al., 2013), and 0.909 (Yan et al., 2016). Our findings indicated that the SSR technique was an effective means of determining the genetic differences across orchardgrass varieties. In addition, the results revealed that orchardgrass has a large number of genetic loci and a broad range of genetic variances among its genotypes. In addition, the SSR molecular marker identified genetic differences and interactions between eight orchardgrass groups effectively and reliably. The rising number of plants and genotype performance could be viewed as additional proof of how to adapt to different environmental circumstances and environments (Booy et al., 2000; Crawford & Whitney, 2010; Drummond & Vellend, 2012). Using genetic markers, a previous study on outbreeding in pasture genotypes such as Lolium spp. and Festuca pratensis revealed a high average predicted heterozygosity ranging from He = 0.3 to 0.7 (Balfourier et al., 1998; Peter Schmid et al., 2008; Litrico et al., 2009). In our study the majority of locations, the mean anticipated heterozygosity (He) ranged from 0.178 (Ağrı) to 0.882 (Erzurum). These findings are consistent with Last et al. (2013) and Madesis et al. (2014) who reported He values ranging from 0.44 to 0.59, 0.51 to 0.56, and 0.494 to 1.360, respectively. According to previous research on pastures and meadows, the patterns of genetic diversity are influenced by numerous factors, including space and time, habitat age, connectivity, and past use in a landscape and historical context (Münzbergová et al., 2013). Bitlis and Hakkari regions had the greatest genetic distance between them, as measured by GD = 0.66. Bitlis and Van genotypes had the smallest genetic distance between regions, with GD = 0.01. The average genetic distance (GD) reported for all locations was 0.28. These results align with those of Mao et al. (2016), who found that the average genetic distance amongst 19 Orchardgrass cultivars was 0.2629. While it was greater than the result of Last et al. (2013), who reported an average genetic distance of GD = 0.01 to 0.02, Last et al. (2014) reported that the genetic distance between genotypes was extremely small (D = 0.01 to 0.02) and that there was no significant correlation between genetic distance among genotypes. The high genetic variation observed in our study may be attributed to the fact that the genotypes studied originated from a large eco-geographic range with wide variation in

habitat characteristics, such as slope or exposure, and relatively long distances between regions, as well as a large altitudinal and latitudinal range. Geographic distance tends to enhance genetic distance in a proportional manner. Our study determined that the average genetic distance between eight regions of Orchardgrass genotypes was 0.28 and that the average polymorphism rate of the studied Orchardgrass genotypes was 88.69%. These results demonstrated that plant samples' genetic diversity increased proportionally to their geographical origin. The introduction of new genetic resources and the release of new varieties is an essential necessity to adapt to agricultural settings and climate change-related environmental conditions.

Characterization of orchardgrass is vital for the protection and maintenance of this plant, which has a significant impact on the enhancement of animal husbandry and agricultural applications. This study examined the morphological and genetic variation of orchardgrass (*Dactylis glomerata* L.) gathered from numerous Eastern Anatolian locations. In addition, the study examined the ability of the gathered data to differentiate genotypes and regions of genotypes, as well as the relationships between traits. Researchers and plant breeders will be able to better understand and alter the vegetative and reproductive features of wild Orchardgrass genotypes thanks to the findings of this study. Regarding the characteristics evaluated in this study, Orchardgrass plants obtained from diverse regions of Eastern Anatolia display a wide range of variance. The wild populations of *Dactylis glomerata* L (Eastern Anatolia) displayed a high level of genetic diversity. This genetic variation can now be utilized in new variety of breeding initiatives. These *Dactylis glomerata* L. genotypes from our study may serve as valuable sources for plants that are adapted to distinct environmental circumstances. This study revealed that orchardgrass inhabits harsh situations and diverse habitats and that these populations retain a high degree of phenotypic and genetic variation. In addition, the study demonstrated that orchardgrass grown in stressful situations can supply beneficial genes for adaptation features, while populations from more favorable habitats can provide useful genes for breeding programs' quantitative and qualitative traits. Due to the significance of orchardgrass, extra care must be taken to conserve the natural settings in which its wild genotypes spread. Hence, all Orchardgrass genotypes gathered from natural habitats must be stored in a gene bank for greater protection and effective in situ preservation of the current prime germplasm through the protection of wild Orchardgrass. According to our findings, there is a vast range of variance among Orchardgrass plants collected from diverse regions of Eastern Anatolia in terms of the analyzed traits, and this has significant implications for genetic diversity. The SSR results are extremely valuable for generating and enhancing new orchardgrass types. Our study's findings will provide further information for breeding programs and the advancement of orchardgrass genotypes; they may also assist in enhancing and modernizing the variety of current germplasm resources. In particular, transferable primers allow researchers to perform genetic analyses in different species or species groups using previously characterized primers. This can increase efficiency in genetic research, saving cost and time. Even though our study gave a good basis for describing orchardgrass genotypes, future research should focus on collecting and storing orchardgrass genotypes from all over the world to create a database of genotype fingerprints for germplasm protection and control, as well as to improve animal husbandry and agriculture.

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