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Genetic diversity of *Sinapis arvensis* L. (wild mustard) in Türkiye determined by microsatellite markers

Mikrosatellit marker ile Türkiye'de *Sinapis arvensis* L. (yabani hardal)'ın genetik çeşitliliğinin belirlenmesi

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ABSTRACT

Wild mustard (*Sinapis arvensis* L.) is a self-fertilizing weed species that exerts negative impacts on wheat production and herbicides are intensively used to manage it. Cross-fertilization may lead to genetic differentiation in this species. Therefore, this study investigated genetic diversity among wild mustard populations collected from wheat fields across various regions in Türkiye. Genetic variation was evaluated using 5 simple sequence repeat (SSR) markers in populations collected from 30 different locations. Populations were analyzed using UPGMA (unweighted pair group method with arithmetic mean) and principal component analysis (PCA). The mean genetic diversity (GD) and polymorphism information content (PIC) values were 0.752 and 0.844, respectively. High genetic variability was recorded among populations within geographic locations. The populations were categorized into two major groups by UPGMA. There was no apparent geographic isolation among tested populations, which displayed a high degree of variability. The primary source of this variability is thought to be the adaptability of wild mustard seeds dispersed through various methods across diverse locations. Despite being a predominantly self-pollinating species, wild mustard may also employ some cross-pollination mechanisms. In conclusion, SSR markers proved useful in determining genetic diversity in outcrossing species, especially where no prior genotypic information is available. The study suggests that genetic diversity is maintained in wild mustard populations even with rotational farming practices and intensive use of herbicides.

INTRODUCTION

Wheat is the staple food of many countries since it ranks first in terms of cultivation and second in production in the world among the cultivated plants used in human nutrition, has wide adaptation ability, the appropriate nutritional value of wheat grain, and the ease of storage and processing. Wheat is the second most-produced cereal after maize and the global trade of wheat is more than all other crops combined (FAO 2023). World cereal production was 2.8 billion tons in 2021-

2022, 28% of which is wheat. Globally, wheat was cultivated on 220 million hectares during 2020-2021, which produced 770 million tons of wheat grains and Türkiye accounted for 3.3% of the global wheat cultivation area and ranked 10th with ~20 million tons production (FAO 2023).

Cereals are ranked first in terms of cultivation area and production amount among field crops in Türkiye. Several factors adversely affect wheat production, which has such a large cultivation area and production potential, and cause significant losses. Weeds reduce the yield and quality of wheat by creating competition for water, light, and nutrients on the one hand, and by being intermediate hosts of diseases and pests on the other hand, are the most important of these factors. Crop loss in cereals due to competition of weeds varies between 20-40% on average in the world (Güncan 2010).

The problematic weed species in wheat cultivation areas are; *Alopecurus myosuroides* Huds., *Avena* spp., *Lolium* spp., *Bromus tectorum* L., *Hordeum murinum* L., *Phalaris* spp., *Secale cereale* L., *Setaria viridis* (L.) P.Beauv., *Sinapis arvensis* L., *Bifora radians* M. Bieb., *Galium* spp., *Chenopodium album* L., *Boreava orientalis* Jaub. & Spach, *Ranunculus arvensis* L., *Papaver rhoeas* L., *Centaurea depressa* Bieb., *Convolvulus arvensis* L., *Cirsium arvense* (L.) Scop., *Acroptilon repens* (L.) D.C., *Vicia* spp., *Capsella bursa-pastoris* (L.) Medik., *Stelleria media* (L.) Vill., *Lamium amplexicaule* L., and *Rumex crispus* L. (Anonymous 2023).

There are differences among the genotypes/populations of a species in terms of various morphological, anatomical, physiological, biochemical, and behavioral characteristics. Differences among populations are due to different alleles of a gene and different frequency distributions of these alleles among populations. Genetic diversity refers to all variations in gene composition within a species. Genetic diversity is one of the components of biological diversity and its determination is one of the most important conditions for ecosystems to be healthy and efficient and for their sustainable operation. High intra-species genetic diversity is a guarantee for adaptation to changing environmental conditions. Species and races with high genetic diversity can adapt more successfully to environmental conditions changing according to time and place (Işık 1997). Genetic diversity in weeds, which is a major problem in wheat agricultural areas, is undesirable (Kaya 2008).

Genetic variation studies are not only considered in an evolutionary context. It also helps in eradication and weed control as an important part of research (Sun 1997). By knowing the genetic diversity of rapidly spreading species, the geographical origin of these plants can be determined (Meekins et al. 2001) and in light of this information, it is possible to select biocontrol agents for biological control (Nissen et al. 1995). To determine the biological control agents to be applied against species that have developed resistance, genetic variation should be determined. To choose the biological control agents to use against strains that have evolved resistance, genetic variation should be evaluated. This genetic heterogeneity in plants is shown using several molecular DNA marker techniques (Kaya 2008, Yalım 2005, Yılmaz 2021). Simple sequence repetition (SSR) markers are employed in a variety of domains, including genetic linkage mapping, plant evolution, and genetic diversity research (Özden Çiftçi and Altınkut Uncuoğlu 2019, Yorgancılar et al. 2015). In a study conducted by Ash et al. (2003), the genetic variation of *Carthamus lanatus* (L.) (safflower) was determined and it was reported that effective control was achieved by applying mycoherbicide to varieties that developed resistance to herbicides. This situation is also important because it sheds light on biological control applications against weeds and at the same time allows the use of herbicides to be reduced. Populations showing genetic diversity can develop higher rates of resistance to control agents and make control possibilities difficult (Meekins et al. 2001).

Nowadays, with the development of molecular techniques, studies on the genetic diversity of weed genotypes have allowed new openings in Weed Science (Leon et al. 2021, Ye et al. 2004). Markers that detect phenotypic and/or genotypic characteristics of an individual are defined as markers If there is more than one gene or phenotypic trait in a population, it can be said that that genotype is polymorphic, and markers can determine the polymorphism rate. An ideal marker should be polymorphic, reproducible, codominant, uniformly distributed throughout the genome, not subject to environmental influences, neutral, and economical (de Vicente and Fulton 2004). Markers are divided into three groups: phenotypic, biochemical, and molecular markers (Kaya 2008).

Microsatellites, also known as simple sequence repeats (SSR), are the smallest repeated units in DNA sequences, and repeat motifs vary between 1-6 bp. If the sequences of the flanking regions surrounding microsatellites (flanking region) are known, appropriate primers (usually 20-25 bp in length) can be designed for those regions and amplified by polymerase chain reaction (PCR). In addition, SSR primers between related species can be used in different organisms. Sequence skipping, incorrect base pairing, and unequal crossing-over events that occur during DNA replication are the main events that cause differences in microsatellite numbers and are determined by gel electrophoresis (Matsuoka et al. 2002). Microsatellite markers can be used effectively in population genetics and gene mapping studies because they require less DNA, are codominant and stable marker systems, are abundant and distributed in the genome, are reproducible and suitable for automation, contain high polymorphism, and are an informative marker system (Filiz and Koç 2011, Powell et al. 1996). Gıdık (2016) researched the determination of yield and

quality elements of *S. arvensis* and *S. nigra* species collected from the Thrace Region, employing ISSR and SSR methods to investigate the genetic diversity of these species. As a result of the study, it was identified 9 genotypes of *S. arvensis*, observing polymorphism in all 10 ISSR primers used in the research. The ISSR method revealed that the polymorphism rate within the *S. arvensis* population ranged between 58.33% and 14.58%. The study emphasized the limited number of molecular genetic characterization studies related to the intraspecific variations of *S. arvensis* and underscored the necessity of expanding such studies. Similarly, Erden (2018) investigated the phylogenetic relationships of species within the Brassicaceae family, including 1 biotype of *S. arvensis,* by amplifying the nrDNA ITS region through PCR for a total of 43 taxa belonging to 28 genera from the Brassicaceae family. The study revealed that the Brassicaceae family is a phylogenetically paraphyletic group, indicating that it is a group where a common ancestor is known, but the species belonging to this family are not fully discovered, or the relationships between species are not completely resolved. In another study, in analyses based on rDNA and cpDNA gene regions to investigate intraspecific variations of *Sinapis arvensis*, it was found that the ITS4 and ITS5 regions of the rDNA gene did not allow for the determination of subspecies. However, these regions were considered highly suitable for species diagnosis and determining species relationships. On the other hand, analyses relying on the matK region of cpDNA (trnK-710F and trnK-2R) were found to be a successful method for distinguishing subspecies of *S. arvensis*. Furthermore, the results of phylogenetic analyses based on rDNA and cpDNA regions suggested that taxonomically, *Sinapis arvensis* should be considered within the *Brassica* genus rather than the *Sinapis* genus (Ateş 2022).

Sinapis arvensis is a species that is mostly self-pollinated but it can also exhibit some cross-pollination (Stewart 2002). A review of *Brassica* species, cross-pollination, and implications for pure seed production in New Zealand. Cross-pollination may cause genetic differentiation of this species. To determine all these differences, this study aims to determine the genetic diversity of *S. arvensis* by using the Microsatellite (SSR-Simple Sequence Repeats) method, one of the PCR-based marker techniques, and to contribute to the determination of strategies for control.

MATERIALS AND METHODS

Sampling and breeding of populations

The seeds of *Sinapis arvensis* were collected from 48 locations in Amasya, Balıkesir, Bilecik, Bursa, Çorum, and Samsun provinces and from 5 different parts of each field to represent the field at the end of the summer period. A 5-10 km distance between the locations was ensured while taking samples from locations in the same province (Barret 1982). For pre-germination, *S. arvensis* seeds of each population were placed in Petri dishes 9 cm in diameter with a double layer of moistened blotting paper and placed in an incubator at +22 ^{0}C with a 12/14 light period. The germinated populations were transferred to pots and grown in sterile soil in a greenhouse under controlled conditions. For further research, 30 populations were selected (11 from Amasya (AMS-1, AMS-2, AMS-3, AMS-4, AMS-5, AMS-6, AMS-7, AMS-8, AMS-9, AMS-10, AMS-11) 4 from Samsun (SAM-1,- SAM-2, SAM-10, SAM-12) 1 from Çorum (ÇOR-1) 5 from Bursa (BUR-1, BUR-2, BUR-5, BUR-9, BUR-10) 7 from Balıkesir (BAL-1, BAL-2, BAL-3, BAL-4, BAL-5, BAL-6, BAL-7) and 2 from Bilecik (BİL-1, BİL-3)) (Figure 1).

Figure 1. Seed collection sites of *Sinapis arvensis* in Türkiye

DNA extraction and PCR application

For extraction, genomic DNA was extracted from leaf samples when the plants grown under greenhouse conditions reached the 4-6 leaf stage using a DNeasy DNA extraction kit (Qiagen, Germany) according to the kit protocol. Genomic DNA extraction was performed from 100 mg of fresh leaf tissue. Following the DNA protocol, the DNAs obtained were stored at -80 ^{o}C until SSR-PCR application.

PCR for SSR molecular marker testing was performed in a total volume of 25 µl. The SSR-PCR reaction mixture consisted of; 2 µl (1.0 ng/µl-1) Genomic DNA, 3 µl (25 ng) of each forward and reverse primers (Table 1), 0.5 µl 10 mM dNTP, 0.2 µl Taq DNA Pol, 2.5 µl 10XPCR buffer, 13.7 µl sdH_2O. The temperature values and times to be applied for PCR were established as follows: (1) 94 ^{o}C→ 3 min (Initial denaturation), 35 cycles (2) 94 ^{o}C→ 1 min (Separation of DNA strands), (3) 38 ^{o}C→ 1 min (Primer adhesion to the strands), (4) 72 ^{o}C→ 1 min (Completion of the strand), and (5) 72 ^{o}C→ 10 min (Final incubation). For the analysis of DNA fragments formed after PCR, 3.5% agarose gel was run on a horizontal maxi electrophoresis device (BioRad) using 1 x TBE buffer (100 mM Tris, 100 mM boric acid, 2 mM EDTA, pH 8.3), 2 g agarose (Serva Agarose) (Serva, Germany), 1.5 g microporous

agarose (Nusseive GTG Agarose) (Combrex, USA). The bands obtained with reference to a 1 Kb DNA marker (New England Biolabs®UK) were photographed with the help of a gel imaging device (Vilber Lourmat).

Evaluation of the bands

The determination of SSR bands was based on the appearance or absence of the band in the gel analyzed after electrophoresis. In this study, optimal PCR conditions were established by repeating the amplifications several times, and conditions that gave a stable band profile for each primer were selected. To obtain the phenogram, monomorphic and polymorphic bands in the gels were detected.

Statistical analysis

The genetic diversity was assessed by using 11 microsatellite (SSR) primers. As a result of the optimization studies, a total of 5 SSR primers were found to work. The band images obtained from the gel were evaluated with reference to the marker sizes, and band matrices were created. The band sizes of the polymorphic bands were entered as present (1) or absent (0). Thus, the band matrices to be used in the following stages were created. The number of alleles, mean number of alleles per locus (NA), expected heterozygosity (HE), observed heterozygosity (HO), gene diversity (GD), and polymorphism information content (PIC) was calculated using the genetic analysis program NTSYSpc2.1. 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)

RESULTS AND DISCUSSION

SSR markers were used to determine the genetic diversity within and between populations in *S. arvensis* and it was determined that the populations of this species have high genetic diversity.

In this study, 11 SSR markers were tested to determine the genetic diversity of *S. arvensis*. The binding temperature of each SSR primer was determined firstly by using the formula and then by using a thermal cycling device. Other PCR parameters were also optimized separately and it was determined which primers work in *S. arvensis*. As a result of the optimization studies, a total of 5 SSR primers were found to work in *S. arvensis*. The SA1 showed banding in the range of 155-185 bp (base pair). The expected heterozygosity was 0.626 and the observed heterozygosity was 0.9. SA2 showed banding in the range of 150-200 bp (base pair). The expected heterozygosity was 0.356 and the observed heterozygosity was 0.62. SA3 showed banding in the range of 200-315 bp (base pair). The expected heterozygosity was 0.874 and the observed heterozygosity was 0.543. SA4 showed banding in the range of 120-160 bp (base pair). The expected heterozygosity was 0.456 and the observed heterozygosity was 0.123. SA5 showed banding in the range of 150-185 bp (base pair). The expected heterozygosity was 0.325 and the observed heterozygosity was 0.675. As a result of the analyses, the mean genetic diversity value was determined 0.752 and the mean PIC value was 0.844. While the SA1 locus showed the highest genetic diversity, the SA4 locus was at the lowest value, 0.954 and 0.486 respectively (Table 1).

Table 1. The primers used in SSR application and amplification results.

Locus	Repeatmotif	Primer sequence (5′–3′)	Amplification					
			Size range (bp)	*NA*	*HO*	*HE*	*GD*	*PIC*
SA1	(A)7–10	F: TCAATTGCACATTCTAGAATTCTAAG R: CAATTCAATATGGTTATATATTAGAG	157–185	5	0.900	0.626	0.954	0.966
SA2	(T)8–13	F: GGTTCCGTCGTTCCCATCGC R: CATAATAATTAGATAAATCTGTTCC	150-200	7	0.620	0.356	0.568	0.679
SA3	(T)7–10	F: AATGGTATGACTAGCTTATAAGG R: CTTAACAATGAGATGAGGCAATC	273–311	8	0.543	0.874	0.879	1.090
SA4	(C)3–8 (T)6–12 (T)7–9	F: CGGATCTATTATGACATATCC R: GAAATATGAATACACTAGATTAGG	127–155	8	0.123	0.456	0.486	0.598
SA5	(A)7–8(T)5–6	F: GAAGGAATAGTCGTTTTCAAG R: CATAAATAGAGTTCCATTTCGG	155–164	5	0.675	0.325	0.874	0.886
Mean				6.6	0.572	0.527	0.752	0.844

Here, NA = number of alleles, HO = observed heterozygosity, HE = expected heterozygosity, GD = gene diversity, and PIC = polymorphism information content

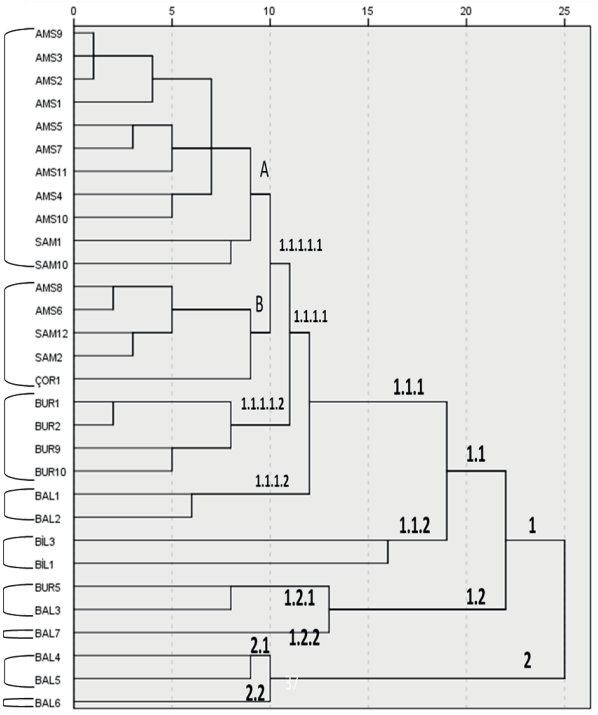

Figure 2. Dendrogram indicating genetic relationship among *Sinapis arvensis* populations generated by hierarchical clustering

The dendrogram based on the presence or absence of band indices divided the populations into two main groups (Figure 2). Based on the genotypes studied, the family tree is divided into 2 main groups. According to UPGMA analysis, high similarity (96%) was determined between AMS-2/ Amasya-Merkez and AMS-9/Amasya-Suluova populations.

The most distant genetic similarity (2%) was determined between BAL-1/Balıkesir-Bandırma and BAL-6/Balıkesir-Gönen, BAL-2-BAL-6/Balıkesir-Gönen-1,2 BAL-2/ Balıkesir-Gönen and BAL-5/Balıkesir-Manyas, AMS-9/ Amasya-Suluova and BAL-6/Balıkesir-Gönen and AMS-11/ Amasya-Merkez and BAL-6/Balıkesir-Gönen populations. Moderate similarity was found between the other populations (Table 2).

SSR markers were used to determine the genetic diversity within and between populations in *S. arvensis* and it was determined that the populations of this species have high genetic diversity.

As a result of the optimization studies, a total of 5 SSR primers were found to work in *S. arvensis*. In the dendrogram created by using hierarchical clustering analysis of molecular parameters, the sampled genotypes were taxonomically classified into a total of 9 groups, including 2 main groups.

Table 2. Correlation matrix of *Sinapis arvensis* populations based on SSR-PCR band results

	BAL6	BAL4	BAL5	BAL7	BİL3	BUR5	BAL3	BAL1	BAL2	AMS8	AMS6	SAM1	AMS1	ÇOR1	AMS9	AMS2	AMS4	AMS5	AMS7	AMS3	AMS10	1	SAM10	BUR1	BUR2	BUR9	BİL1	0	SAM12	SAM2
BAL6	100%																													
BAL4	70%	100%																												
BAL5	66%	70%	100%																											
BAL7	33%	36%	49%	100%																										
BİL3	49%	46%	43%	62%	100%																									
BUR5	21%	15%	36%	70%	62%	100%																								
BAL3	20%	15%	27%	43%	41%	73%	100%																							
BAL1	2%	6%	10%	27%	39%	39%	57%	100%																						
BAL2	2%	11%	2%	33%	49%	38%	46%	79%	100%																					
AMS8	23%	19%	16%	25%	39%	32%	39%	72%	66%	100%																				
AMS6	23%	28%	16%	17%	39%	23%	30%	64%	66%	91%	100%																			
SAM1	19%	15%	11%	29%	44%	17%	25%	59%	70%	79%	70%	100%																		
AMS1	6%	19%	7%	24%	41%	29%	37%	62%	74%	83%	83%	79%	100%																	
ÇOR1	23%	36%	24%	7%	23%	12%	29%	54%	57%	75%	83%	62%	83%	100%																
AMS9	2%	23%	19%	19%	37%	33%	49%	66%	70%	71%	71%	66%	87%	79%	100%															
AMS2	6%	28%	24%	24%	41%	29%	46%	62%	66%	66%	66%	70%	83%	74%	96%	100%														
AMS4	15%	28%	32%	15%	32%	21%	29%	54%	57%	58%	66%	62%	66%	74%	79%	83%	100%													
AMS5	11%	15%	28%	19%	29%	33%	32%	58%	62%	71%	71%	66%	70%	70%	74%	70%	87%	100%												
AMS7	7%	19%	23%	23%	34%	36%	44%	61%	66%	76%	76%	71%	83%	75%	87%	83%	75%	87%	100%											
AMS3	6%	19%	15%	15%	41%	29%	46%	71%	74%	75%	75%	70%	83%	74%	96%	91%	83%	79%	83%	100%										
AMS10	6%	19%	24%	33%	41%	38%	46%	62%	66%	58%	58%	62%	66%	57%	79%	83%	83%	70%	66%	83%	100%									
AMS11	2%	7%	20%	29%	36%	35%	34%	59%	62%	70%	61%	74%	70%	53%	75%	79%	79%	83%	79%	79%	79%	100%								
SAM10	19%	24%	20%	39%	34%	29%	36%	69%	71%	70%	61%	74%	71%	62%	67%	71%	71%	76%	72%	71%	71%	74%	100%							
BUR1	23%	28%	24%	41%	49%	38%	37%	54%	57%	66%	58%	70%	66%	49%	62%	66%	66%	70%	66%	66%	74%	79%	71%	100%						
BUR2	24%	28%	23%	40%	43%	36%	36%	44%	49%	59%	59%	62%	58%	49%	53%	58%	66%	70%	66%	58%	66%	71%	63%	92%	100%					
BUR9	28%	41%	20%	31%	52%	20%	27%	51%	62%	61%	61%	66%	71%	53%	67%	71%	62%	58%	63%	71%	62%	66%	74%	79%	72%	100%				
BİL1	23%	20%	25%	27%	47%	26%	24%	30%	32%	31%	31%	44%	41%	23%	37%	41%	41%	37%	34%	41%	49%	53%	43%	67%	60%	69%	100%			
BUR10	28%	41%	20%	39%	61%	29%	36%	60%	71%	70%	70%	74%	71%	53%	67%	71%	62%	58%	63%	71%	62%	66%	65%	79%	72%	83%	52%	100%		
SAM12	28%	41%	20%	20%	44%	26%	34%	59%	62%	79%	87%	57%	70%	70%	66%	62%	62%	66%	71%	70%	53%	57%	57%	70%	71%	74%	44%	83%	100%	
SAM2	32%	28%	16%	25%	48%	41%	48%	64%	58%	83%	74%	61%	66%	58%	62%	58%	49%	62%	67%	66%	49%	61%	61%	75%	67%	70%	48%	78%	87%	100%

This is a similarity matrix

When the genotypes were analyzed based on geographical locations, genotypes from the same locations were grouped into the same subgroups.

Freville et al. (2001) investigated the genetic structure of *Centaurea corymbosa*, a narrow endemic plant, using six microsatellite loci and compared their results with allozyme analysis. Microsatellite analysis revealed that there is a wide differentiation among populations. It was also reported that allozyme loci are less powerful than microsatellites in determining the extent of gene flow (Çağlar 2010). Naghavi et al. (2009) used 21 SSR primers to determine the genetic relationship between 52 *Triticum aestivum* and 13 *Aegilops* species collected from various regions of Iran using SSR markers.

It was observed that SSR analysis demonstrated significant gene expression in the investigations aimed at identifying genetic diversity in different species (Guo et al. 2022, Randazzo et al. 2019, Singh et al. 2020, Xiong et al., 2019). It was reported that when the genetic composition of the indigenous plant *Centaurea corymbosa* was analyzed, SSR analysis employing six microsatellite loci indicated a significant divergence across populations. According to Lopez-Vinyallonga et al. (2011), these microsatellites were found to be more effective than allozyme loci at detecting gene flow coverage.

In this study, 11 SSR primers were used to determine the genetic relationship between 30 *S. arvensis* populations. In the study of Çağlar (2010), 3 SSR primers were used and it was observed that the genetic similarity rate in *Centaurea nivea* biotype varied between 26% and 76%. Kaya-Altop (2012) determined the genetic relationship between *Cyperus difformis* populations, which is a problem in paddy cultivation areas, using RAPD primers and determined that genetic similarity varied between 0.01% and 96%. In addition, Gıdık (2016) identified 9 genotypes of *S. arvensis*, observing polymorphism in all 10 ISSR primers used in the research. The ISSR method revealed that the polymorphism rate within the *S. arvensis* population ranged between 58.33% and 14.58%. In another study, in analyses based on rDNA and cpDNA gene regions to investigate intraspecific variations of *Sinapis arvensis*, it was found that the ITS4 and ITS5 regions of the rDNA gene did not allow for the determination of subspecies. However, these regions were considered highly suitable for species diagnosis and determining species relationships. On the other hand, analyses relying on the matK region of cpDNA (trnK-710F and trnK-2R) were found to be a successful method for distinguishing subspecies of *S. arvensis*. Furthermore, the results of phylogenetic analyses based on rDNA and cpDNA regions suggested that taxonomically, *Sinapis arvensis* should be considered within the *Brassica* genus rather than the *Sinapis* genus (Ateş 2022). In another study with 5 SSR primers, the genetic diversity rate among 62 *E. oryzoides* populations was found to support the present study (Altop et al. 2018). In another study, genetic diversity studies were carried out on 40 different *Alopecurus myosuroides* populations with 5 different SSR primers, and it was determined that genetic diversity was detected at a high rate and had similar results and backing up to the current study (Boylu and Kaya Altop 2021). For *S. arvensis*, while the genetic similarity rate varied between 2% and 96%, it was determined that the closest populations to each other genetically were AMS-2/ Amasya-Merkez and AMS-9/ Amasya-Suluova populations with 96% similarity rate. The most distant genetic similarity was determined between BAL-1/Balıkesir-Bandırma and BAL-6/Balıkesir-Gönen, BAL-2-BAL-6/Balıkesir-Gönen-1,2 BAL-2/Balıkesir-Gönen and BAL-5/Balıkesir-Manyas, AMS-9/Amasya-Suluova and BAL-6/Balıkesir-Gönen and AMS-11/Amasya-Merkez and BAL-6/ Balıkesir-Gönen populations with a value of 2%.

The variation among populations was highly diverse, and the clarity of geographical isolation was remarkable. This can be interpreted primarily in terms of adaptation to geographical areas and the concept that even if there are self-fertilized species, some cross-fertilization may also be present in them. Other important factors may include the transport of seeds between regions by humans and tools and the resistance developed by the weed against herbicides used in weed control methods. The high rate of genetic diversity in the findings obtained points to the potential of the species to come up with the problem of resistance. The possibility of gene escape between the resistant forms and wild or susceptible populations is considered a strong possibility. The present study may shed light on the next researchers and issues.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Yabani hardal (*Sinapis arvensis* L.), buğday üretimi üzerinde olumsuz etkiler yaratan, kendi kendine döllenen bir yabancı ot türüdür ve bu türle mücadelede yoğun olarak herbisitler kullanılmaktadır. Yabancı döllenme bu türde genetik farklılaşmaya yol açabilir. Bu nedenle bu çalışmada Türkiye'nin çeşitli bölgelerindeki buğday tarlalarından toplanan yabani hardal popülasyonları arasındaki genetik çeşitlilik araştırılmıştır. Yabani hardal popülasyonlarının genetik varyasyon derecesi, Türkiye'nin 30 farklı lokasyonundan

alınan örneklerde 5 basit dizi tekrarı (SSR) işaretleyici kullanılarak değerlendirilmiştir. Popülasyonlar hiyerarşik kümeleme analizi (UPGMA) ve temel bileşen analizi (PCA) kullanılarak analiz edilmiştir. Ortalama genetik çeşitlilik (GD) ve polimorfizm bilgi içeriği (PIC) değerleri sırasıyla 0.752 ve 0.844 olarak bulunmuştur. Sonuçlar, coğrafi konumlar içinde bireysel genotipler arasında yüksek genetik değişkenlik göstermiştir. Popülasyonlar UPGMA dendrogramı tarafından gösterildiği gibi, iki ana grupta kategorize edilmiştir. İncelenen yabani hardalın genotipleri arasında belirgin coğrafi izolasyon belirlenememiş ve yüksek derecede değişkenlik göstermiştir. Bu değişkenliğin ana kaynağının, farklı lokasyonlara çeşitli yöntemlerle dağıtılan yabani hardal tohumlarının adaptasyonu olduğu düşünülmektedir. Çoğunlukla kendi kendine döllenme yapan bir tür olmalarına rağmen, bazı yabancı döllenme mekanizmalarını da kullanabilirler. Sonuç olarak, SSR belirteçlerinin, özellikle önceden genotipik bilginin mevcut olmadığı durumlarda, geçiş yapan türlerdeki genetik çeşitliliğin belirlenmesinde yararlı olduğu belirlenmiştir. Çalışma, rotasyonel tarım uygulamaları ve yoğun herbisit kullanımına rağmen yabani hardal popülasyonlarında genetik çeşitliliğin korunduğunu göstermektedir.

Anahtar kelimeler: SSR, yabani hardal, moleküler marker, genetik çeşitlilik

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