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Genetic Diversity of Some Bread Wheat (*Triticum aestivum* L.) Genotypes Using SSR Markers Associated with Drought

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HIGHLIGHTS

- Seven SSR markers showed polymorphism among 32 bread wheat genotypes.
- A total of 25 polymorphic alleles were detected with a mean PIC of 0.70.
- UPGMA analysis grouped genotypes into three main clusters.
- Genetic dissimilarity ranged from 0.083 to 0.800 among genotypes.

Abstract

This study aimed to assess the genetic diversity of 32 bread wheat genotypes using SSR markers associated with drought. In the study, 10 SSR primers were applied to all genotypes; BARC 024, WMC 9, and WMC 603 showed monomorphic patterns, while seven primers were polymorphic. In total, 25 polymorphic alleles were detected, with the number of alleles per locus ranging from 2 to 6 and a mean of 3.6 alleles per SSR locus. PIC (Polymorphic Information Content) values ranged from 0.31 to 0.97, with a mean value of 0.70. The lowest and highest values were obtained from Xbarc17 and Xbarc12, respectively. The dendrogram was constructed using UPGMA analysis, and the bread wheat genotypes were divided into three groups. Cluster I is further divided into two sub-clusters, Ia and Ib. Cluster II is further divided into two other sub-clusters, IIa and IIb. The pair-wise genetic dissimilarity indices revealed a minimum difference index of 0.083 and a maximum of 0.800 between genotypes. Bayraktar 2000 and LR5 and LR6 showed genetic similarity to the drought-tolerant cultivar Gerek 79, whereas BL8 and the drought-sensitive cultivar Bezostaja 1 (C10) showed the greatest genetic distance. Sertak52 (C5) and BL7 and BL4 showed genetic similarity to the drought-tolerant cultivar Bayraktar 2000, while BL6 and Bezostaja 1 (C10) showed the greatest genetic distance. BL1, BL3, and BL5 showed genetic similarity to the drought-sensitive cultivar Bezostaja 1, whereas LR1, LR2, LR5, and LR6 showed the greatest genetic distance. Overall, the genotypes and SSR markers used in this study provide preliminary data for future breeding and genetic studies related to drought-associated traits.

Keywords: Bread wheat; Breeding; Drought; SSR markers

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1. Introduction

Wheat is one of the main cereal crops in Türkiye and a source of cash for small-scale farmers. World wheat production is 761 million tons per year from an area of 219 million ha and the average yield is 3470 kg/ha. Türkiye's wheat production is 20.5 million tons per year from an area of 6.9 million ha. As a result of various biotic (pests, diseases and weeds), abiotic (drought and heat stresses, salinity, waterlogging, low soil fertility and acidity) and socio-economic constraints, the national average yield is 2960 kg/ha, which is well below the world average (FAO 2024).

Drought is one of the most important abiotic stresses limiting wheat productivity and stability globally (Adhikari et al. 2015). In addition, global climate change increases the frequency of severe drought conditions (Dai 2013). Drought is common in dry farming areas, mainly due to irregular rainfall and the unpredictable nature of the climate. In Türkiye, including the Central Anatolia Region, wheat is grown mostly under precipitation-dependent conditions and is subject to recurrent drought and heat stresses associated with climate change. High temperature combined with low humidity reduces plant performance and leads to water scarcity, which has become an important limiting factor in increasing wheat production (Hirt and Shinozaki 2004). Globally, water scarcity has become the most critical problem today due to its detrimental effects on plant growth and performance under various environmental stresses (Janmohammadi et al. 2008). Due to drought in developing countries, wheat yield has decreased to 50–90% of its irrigated potential (Ali et al. 2013). Therefore, increasing the yield in wheat requires the evaluation of all developmental stages from grain to grain with appropriate strategies in order to target various growth stages (Triboi and Triboi-Blondel 2002).

Drought tolerance is a complex trait due to its polygenic inheritance, and its expression is influenced by various environmental factors. The genetic basis of drought tolerance traits is central to cultivar development for drought stress conditions (Farshadfar et al. 2013). The nature of gene actions involved in the inheritance of drought-related traits influences the selection of the most suitable parents, the breeding strategies to be used, and the most promising progeny from segregating breeding populations.

Understanding the genetics behind drought stress tolerance as a quantitative trait remains a challenge for plant biologists and geneticists (Fleury et al. 2010). Genetic control of drought tolerance traits requires intensive and integrative genetic, genomic, and molecular studies to determine the stages and mechanisms through which the underlying genes are involved. In addition, to understand the mechanisms that enable plants to tolerate stress, it is appropriate to examine the response of tolerant genotypes and compare it with other susceptible genotypes (Rampino et al. 2006).

Significant improvements have been made in the adaptation of wheat to drought-prone environments, largely achieved through field-based, experimental selection for drought tolerance. In Türkiye, wheat is grown by small-scale farmers in rain-dependent conditions. The performance of plants in various environments depends on the suitability of the varieties used for adaptation to the agro-ecology of the production areas. Therefore, in order to improve the yield and stability of wheat in these agricultural ecologies, there is a need for a specific breeding program that contributes to the development of wheat varieties with high performance in different soil moisture conditions.

Cultivation of wheat varieties that are drought tolerant and adaptable to the changing environment will provide high and stable yields. Increasing wheat yield by developing drought tolerant, high yielding and stable varieties is a continuous process that uses available genetic resources. Information obtained on germplasm diversity significantly affects the use of genetic resources in plant breeding programs for the development of new gene combinations (Ayana and Bekele 1998). Prior knowledge of genetic diversity and relationships between modern and local varieties will be beneficial for the development of new varieties. It is very important to characterize the genetic diversity among wheat germplasm collections to expand genetic diversity in future wheat breeding programs (Huang et al. 2002). Molecular markers have proven their role in plant breeding programs by providing selection precision and accelerating the process. Molecular marker-assisted breeding can improve breeding efficiency by assessing genetic diversity within narrow germplasm pools. This is likely to speed up the breeding process. Genetic variation in wheat has been investigated using

different molecular markers such as RAPDs, RFLP, AFLPs, SSR, STS, ISSRs, gene-based and MIR-based SSRs (Mehta et al, 2021; P. Sharma et al, 2021; R. Sharma et al, 2021).

SSRs have multi-allelic nature, co-dominant inheritance, reproducibility, abundance and high polymorphic information content (PIC). Few SSR markers are sufficient to distinguish closely related wheat genotypes (Plaschke et al. 1995; Russell et al. 1997; Singroha et al. 2020). Simple sequence repeats (SSRs) or microsatellite markers are sequences with a variable number of repeats of several nucleotides, usually two to five nucleotides (Valdes et al. 1993). Microsatellites are ubiquitous throughout the genome in eukaryotes and are the most informative molecular markers due to their high mutation rate. Microsatellite markers are highly polymorphic and informative, abundant throughout the genome, often codominant, and readily amenable to automation. Therefore, many genetic maps have been created in hexaploid wheat using microsatellite markers (Röder et al. 1998; Somers et al. 2004). Microsatellite markers are also used in other genetic analyses, including QTL mapping (Carter et al. 2009; Santra et al. 2008), genetic diversity (Huang et al. 2002), and genome-wide association analysis (Brbaklić et al. 2013).

In this context, this study was carried out to determine the genetic differences and similarities among some bread wheat genotypes using SSR markers associated with drought-related traits. In addition, this study aimed to identify potential parental genotypes for use in crossing programs targeting drought-related traits. Increasing the likelihood of successful selection in breeding programs highlights the importance of this study.

2. Materials and Methods

2.1. Plant Materials

In this study, seeds of 32 bread wheat genotypes were used as plant material and analyzed using SSR markers. Cultivars obtained from different Agricultural Research Institutes in Türkiye, Anatolian wheat landraces and breeding lines developed by IWWIP (International Winter Wheat Improvement Program) through hybridization were used as plant material. These bread wheat genotypes consisted of 13 cultivars, 13 breeding lines and 6 landraces. The origin and pedigrees of the bread wheat genotypes are summarized in Table 1.

2.2. DNA Marker Analysis

2.2.1. DNA isolation

DNA was extracted from the young leaves of each bread wheat genotype using the Thermo Scientific GeneJET Plant Genomic DNA Purification Mini Kit, following the manufacturer's instructions. Approximately 100 mg of plant tissue was ground in liquid nitrogen using a mortar and pestle or stainless-steel beads. The powdered tissue was transferred into a 1.5 mL microcentrifuge tube containing Lysis Buffer A, followed by the addition of Lysis Buffer B and RNase A. The samples were incubated at 65 °C for 10 min with occasional vortexing. After precipitation and centrifugation steps, the supernatant was transferred to a clean tube and mixed with Plant gDNA Binding Solution and ethanol. The mixture was loaded onto a spin column, and DNA purification was carried out using Wash Buffer I and Wash Buffer II according to the kit protocol. Genomic DNA was eluted using Elution Buffer in two consecutive steps. The quality and concentration of the extracted DNA were checked, and the DNA samples were stored at 2–8 °C until further analysis.

2.2.2. PCR amplification

Ten previously described SSR primers (Gupta et al. 2002; Song et al. 2005) known to be linked to putative QTL affecting drought tolerant (Ciucă and Petcu, 2009; El-Maghraby et al. 2005) were selected for evaluation. The chromosomal location, annealing temperatures, and primer sequences of the SSR markers are presented in Table 2.

Table 1. Bread wheat cultivars, breeding lines, and landraces used in this study.

No	Cultivar/Pedigree	Origin
C1	Ankara 093-44	FCCRI, Türkiye
C2	Sürak 1593-51	FCCRI, Türkiye
C3	Yektay 406	TZARI, Türkiye
C4	Kıraç 66	TZARI, Türkiye
C5	Sertak 52	TZARI, Türkiye
C6	Köse 220/39	FCCRI, Türkiye
C7	Melez 13	TZARI, Türkiye
C8	Bayraktar 2000	FCCRI, Türkiye
C9	Gerek 79	TZARI, Türkiye
C10	Bezostaja 1	MRI, Türkiye
C11	Dağdaş 94	BDIARI, Türkiye
C12	Karahan 99	BDIARI, Türkiye
C13	Konya 2002	BDIARI, Türkiye
BL1	093-44/AU	BDIARI, Türkiye
BL2	TMP64/YY305	BDIARI, Türkiye
BL3	Arapahoe/3/Brule//Hiplains/Newton	IWWIP
BL4	Cham6/TK13//LND	IWWIP
BL5	LOV29/3/JSV6/LOV13//JSW3/7/SOWT/GB//L/3/K58/N//FR/4/CNO "S"/PJ62/5/LUT/6/LİB/PCİ/8LOV29/3/JSW6/LOW13	IWWIP
BL6	Momtchill/Newton	IWWIP
BL7	Rose/22375	IWWIP
BL8	TRAKIA/4LOV29/3/JSW6/LOV13/JSW3	IWWIP
BL9	VRATZA5/4-11/KVZI4/KRC/BEZ/3/1150-18/P101//1150-18/VGDWF	IWWIP
BL10	TAST/SPRW//CA8055/3/CSM	IWWIP
BL11	VORONA/OPATA85	IWWIP
BL12	BEIJING-837/GEREK79	IWWIP
BL13	BUL5327-1/3/BEZ/NAD//KZM(ES85-24)	IWWIP
LR1	Tir	Eastern Anatolia Region, Türkiye
LR2	YG192	Central Anatolia Region, Türkiye
LR3	YG 264	Central Anatolia Region, Türkiye
LR4	YG 141	Central Anatolia Region, Türkiye
LR5	YG 62	Central Anatolia Region, Türkiye
LR6	Akbuğday	Central Anatolia Region, Türkiye

C: Cultivar, BL: Breeding Line, LR: Landrace, FCCRI: Field Crops Central Research Institute, TZARI: Transitional Zone Agricultural Research Institute, MRI: Maize Research Institute, BDIARI: Bahri Dagdas International Agricultural Research Institute, IWWIP: International Winter Wheat Improvement Program.

PCR reactions were carried out in a total volume of 20 μL containing 1.0 μL template DNA, 1.0 μL of each primer, 0.4 μL dNTP mix, 1.75 μL $MgCl_2$, 2.0 μL 10× PCR buffer, 0.2 μL Taq DNA polymerase, and sterile distilled water.

PCR was performed as follows: 94°C for 10 min; then 94°C for 1 min, 49-61°C (different annealing temperatures of primers) for 1 min, five cycles of 72°C for 1 min; then 94°C for 30 s, 49-61°C (different annealing temperatures of primers) for 30 s, thirty cycles of 72°C for 50 s; then 72°C for 10 min and storage at 4°C. PCR products were separated using 1.5 % agarose gels with ethidium bromide. Electrophoresis was performed at a constant voltage of 100 V for 2–3 h, and a 10X TBE buffer was used during electrophoresis.

Table 2. SSR markers used in the study including their chromosomal locations, primer sequences, and annealing temperatures

SSR Primer	Chromosomal location		Primer sequences (5′–3′)	Annealing temperature (°C)
Xbarc4	5B	F	GCG TGT TTG TGT CTG CGT TCT A	51
		R	CAC CAC ACA TGC CAC CTT CTT T	
Xbarc12	3A	F	CGA CAG AGT GAT CAC CCA AAT ATA A	51
		R	CAT CGG TCT AAT TGT CAA TGT A	
Xbarc17	1A	F	GCG CAA CAT ATT CAG CTC AAC A	49
		R	TCC ACA TCT CGT CCC TCA TAG TTT G	
Xbarc18	2B	F	CGC TTC CCA TAA CGC CGA TAG TAA	51
		R	CGC CCG CAT CAT GAG CAA TTC TAT CC	
Xbarc24	6B	F	CGC CTC TTA TGG ACC AGC CTA T	52
		R	GCG GTG AGC CAT CGG GTT ACA AAG	
Xbarc108	1A	F	GCGGGTCGTTTCCTGGAAATTCATCTAA	50
		R	GCGAAATGATTGGCGTTACACCTGTTG	
Wmc9	7A	F	AACTAGTCAAATAGTCGTGTCCG	59
		R	GTCAAGTCATCTGACTTAACCCG	
Wmc596	7A	F	TCAGCAACAAACATGCTCGG	61
		R	CCCGTGTAGGCGGTAGCTCTT	
Wmc603	7A	F	ACAAACGGTGACAATGCAAGGA	61
		R	CGCCTCTCTCGTAAGCCTCAAC	
TaLEA3	1A	F	CGGCGAGAAGACAGAGATG	59
		R	ACGACCAAACAGGACTAAAGGA	

2.2.3. Statistical Analysis

SSR bands were scored as presence (1) or absence (0) for each genotype (Nei and Li 1979). Polymorphic Information Content (*PIC*) was calculated by Smith et al. (1997) based on the number of alleles and their frequency distribution according to the following formula:

$$PIC = 1-\Sigma P_i^2$$

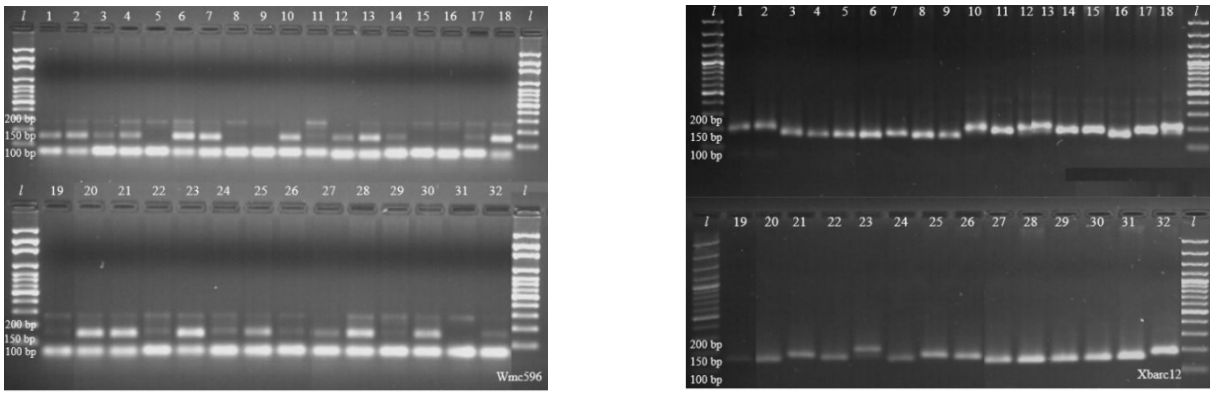
In the formula, P_i represents the frequency of the i-th allele.

Comparison of genotypes was generated from genotyping data of selected polymorphic SSR markers using DARwin software (version 6.0.021) based on genetic distances. Genetic similarity between genotypes was estimated from the dissimilarity matrix. The resulting dissimilarity matrix was further analyzed using the UPGMA clustering algorithm to construct a dendrogram. Similarly, the neighbor-joining tree was constructed based on the dissimilarity matrix using the unweighted neighbor-joining algorithm from DARwin software (Perrier and Jacquemoud-Collet 2006). The genetic similarity index among wheat genotypes was calculated according to Jaccard (1908).

3. Results

3.1. SSR polymorphism

In the study, a total of 32 bread wheat genotypes were tested using 10 SSR primers, and Figure 1 shows representative SSR marker profiles obtained using two polymorphic primers (Xbarc12 and Wmc596) selected from the ten primers used in this study. Among these primers, seven showed polymorphic features. A total of 33 alleles were detected from the seven polymorphic primer pairs, of which 25 were polymorphic, with an average of 3.6 alleles per primer pair. The number of alleles produced by each primer pair ranged from 2 to 6.



(**a**) (**b**)

Figure 1. Representative SSR marker profiles of bread wheat genotypes using (a) Wmc596 and (b) Xbarc12 primers.

The polymorphic information content (*PIC*) values of the analyzed SSR markers ranged from 0.31 (Xbarc17) to 0.97 (Xbarc12), with an average of 0.70. The *PIC* values of the Xbarc4, Xbarc12, Xbarc18, Xbarc108, Wmc596, and TaLEA3 primers were greater than 0.5 and were therefore considered informative. Allelic diversity and PIC values for all genotypes are presented in Table 3.

Table 3. SSR primers, number of amplified bands, number of polymorphic bands, and *PIC* values.

SSR primer	Number of amplified bands	Number of polymorphic bands	Polymorphism %	*PIC*
Xbarc4	6	4	67	0.95
Xbarc12	3	2	67	0.97
Xbarc17	2	2	100	0.31
Xbarc18	7	6	86	0.80
Xbarc108	7	5	71	0.60
Wmc596	4	3	75	0.54
TaLEA 3	4	3	75	0.70
Total	33	25		-
Average	4.7	3.6	77	0.70

3.2. Genetic similarity analysis using UPGMA

A dendrogram based on genetic similarity among bread wheat genotypes was constructed using SSR marker information. (Fig. 2.). According to the SSR-based dendrogram, three main groups were obtained based on genetic similarity (Fig. 2.). The pair-wise genetic dissimilarity indices revealed a minimum difference index of 0.083 and a maximum of 0.800 between genotypes.

The UPGMA-based dendrogram grouped 32 bread wheat genotypes into three main clusters (I, II, and III), consisting of 19, 9, and 4 genotypes, respectively (Table 4.). Cluster I is further divided into two sub-clusters, Ia and Ib, with 16 and 3 genotypes. Among the 16 genotypes in the Ia sub-cluster were BL2, BL6, BL8, BL10; BL9, BL11, BL12, BL13, LR4; and C12, BL7, LR1, LR2, LR3, LR5, and LR6, clustered in three different positions. The genotypes clustered in the Ia sub-cluster consist of breeding lines obtained through hybridization by IWWIP, as well as landraces and C12 (Karahan 99). Ib sub-cluster consists of three cultivars (C1; Ankara 093-44, C3; Yektay 406, and C4; Kıraç 66). Ankara 093-44 (C1) and Yektay 406 (C3) have co-maternal parents (http://wheatpedigree.net/). Cluster II with 9 genotypes is further divided into two other sub-clusters, IIa and IIb, consisting of 7 and 2 genotypes, respectively. Breeding lines (BL1, BL3, BL4, BL5) and C7 (Melez13), C10 (Bezostaja 1), C13 (Konya 2002) cultivars were included among 7 genotypes in IIa sub-cluster. The IIb sub-cluster consists of C2 (Sürak 1593-51) and C6 (Köse 220/39). Köse 220/39 (C6) is the paternal parent of Sürak

1593-51 (C2). Cluster III with 4 genotypes consists of C5 (Sertak 52), C8 (Bayraktar 2000), C9 (Gerek 79) and C11 (Dağdaş 94).

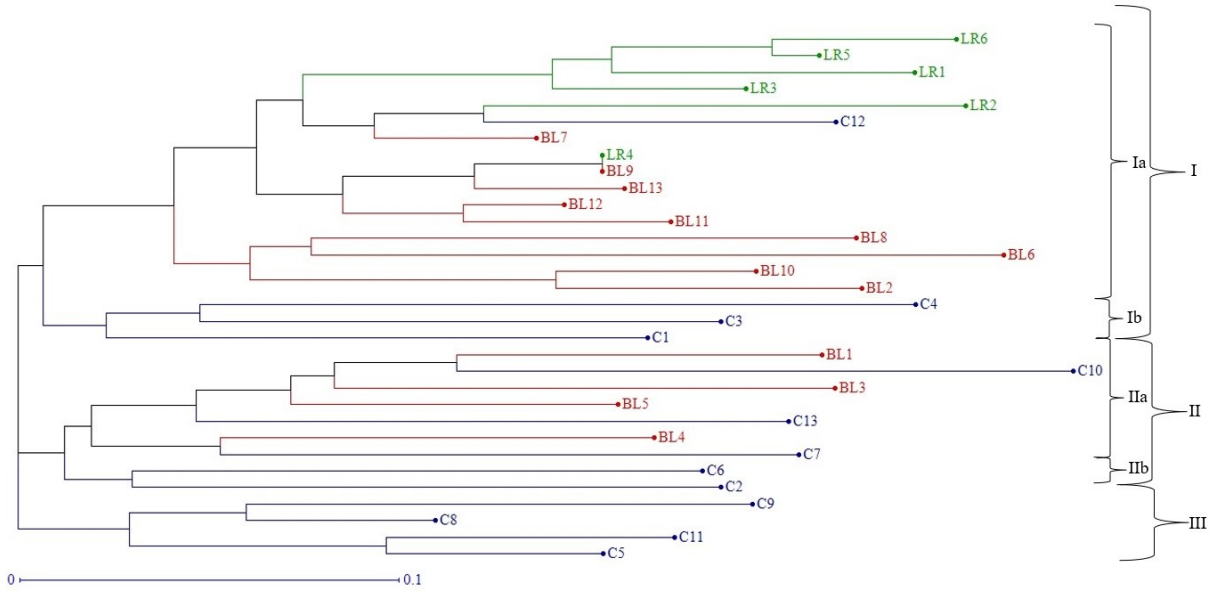


Figure 2. UPGMA dendrogram representing the clustering pattern of 32 bread wheat genotypes.

Table 4. Cluster analysis of 32 genotypes of bread wheat based on the UPGMA.

Cluster	Subcluster	Genotypes
Cluster I	Subcluster Ia	BL2, BL6, BL7, BL8, BL9, BL10, BL11, BL12, BL13, LR1, LR2, LR3, LR4, LR5, LR6, C12 (Karahan 99)
	Subcluster Ib	C1 (Ankara 093-44), C3 (Yektay 406), C4 (Kıraç 66)
Cluster II	Subcluster IIa	BL1, BL3, BL4, BL5, C7 (Melez 13), C10 (Bezostaja 1), C13 (Konya 2002)
	Subcluster IIb	C2 (Sürak 1593-51), C6 (Köse 220/39)
Cluster III		C5 (Sertak 52), C8 (Bayraktar 2000), C9 (Gerek 79), C11 (Dağdaş 94)

A neighbor-joining tree showing genetic relationships between bread wheat genotypes was also constructed based on alleles detected from 7 SSR markers (Fig. 3.). Genetic distance-based results seen in the neighbor-joining tree revealed three major clusters similar to UPGMA-based dendrogram clusters.

In relation to drought-associated traits, Bayraktar 2000 (C8) and Gerek 79 (C9) cultivars are widely preferred in Central Anatolia. Bayraktar 2000 and LR5, LR6 showed genetic similarity to the drought-tolerant cultivar Gerek 79 and showed the greatest genetic distance from this cultivar were BL8 and the drought-sensitive cultivar Bezostaja 1 (C10). Sertak52 (C5) and BL7, BL4 showed genetic similarity to the drought-tolerant cultivar Bayraktar 2000 and showed the greatest genetic distance from this cultivar were BL6 and Bezostaja 1 (C10). BL1, BL3, and BL5 showed genetic similarity to the drought-sensitive cultivar Bezostaja 1, and showed the greatest genetic distance from this cultivar were LR1, LR2, LR5, and LR6.

When the data obtained from the primers used were evaluated, it was determined that the genetic similarity coefficient between the drought sensitive Bezostaja 1 cultivar and the drought tolerant Gerek 79 cultivar was 0.263, and the genetic similarity coefficient between the drought-sensitive Bezostaja 1 cultivar and the drought-tolerant Bayraktar 2000 cultivar was 0.353.

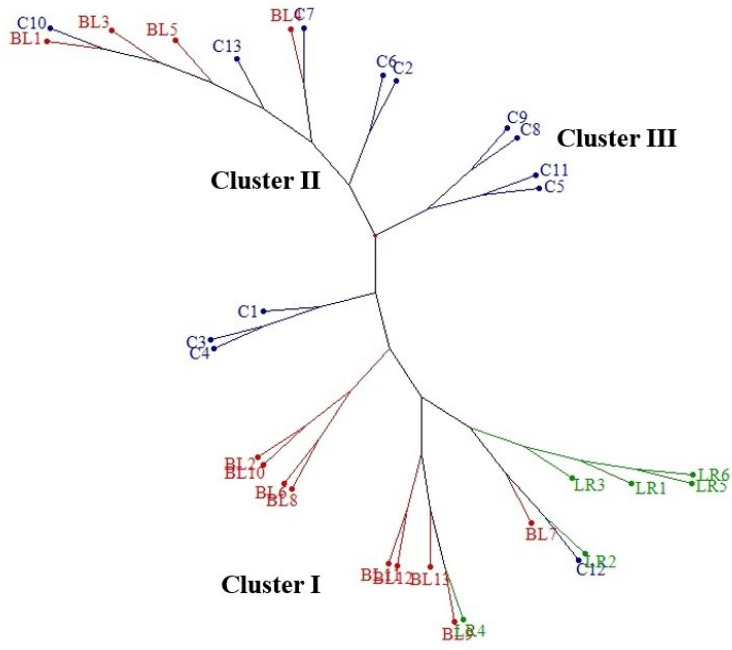


Figure 3. UPGMA Neighbor-joining tree representing grouping pattern of 32 bread wheat genotypes.

4. Discussion

The PIC provides an estimate of the discriminative power of a locus, taking into account not only the number of alleles expressed but also the relative frequencies of those alleles. PIC values range from 0 (monomorphic) to 1 (very highly discriminating with many alleles at equal frequencies). PIC values can be used to assess the level of genetic variation in a plant. A locus with a PIC value of >0.50 is considered high-diversity, and a PIC value of <0.25 is considered a low-diversity locus (Botstein et al. 1980).

These SSR markers may be useful for studies targeting drought-related traits. In addition, the PIC values recorded in this study were similar to the high PIC values reported in the studies of Röder et al. (1995), Dodig et al. (2010), Faheem et al. (2015) and Tomar et al. (2016), and statistically significantly higher values were also observed. In addition, simple sequence repeats (SSRs) represent the most suitable marker system in wheat and have been successfully used to characterize genetic diversity in advanced wheat breeding materials (Dreisigacker et al. 2005; Dreisigacker et al. 2004).

It is noteworthy that the breeding lines developed by hybridization and the landraces clustered within themselves in the Ia sub-cluster. The genotypes collected under this cluster consist of cultivars, many of which have been grown in Central Anatolia for many years to adapt to drought. Gerek 79 (C10) is Bayraktar 2000 (C8)'s paternal parent (http://wheatpedigree.net/).

Genotypes grouped based on SSR marker data are expected to provide useful information for studies related to drought-associated traits. Therefore, marker-assisted selection using SSR markers may assist in identifying genotypes with potential relevance to drought-related traits.

5. Conclusions

Drought is common in dry areas of the Central Anatolian Region, mainly due to irregular rainfall and the unpredictable nature of the climate. Wheat is grown in this region mostly under precipitation-dependent conditions and is subject to recurrent drought and heat stresses associated with climate change. This study, which assessed genetic variation among bread wheat genotypes using SSR markers associated with drought-related traits, provides useful genetic information. This study demonstrated the usefulness of 10 SSR markers

associated with drought-related traits to assess genetic diversity of 32 bread wheat genotypes comprising cultivars, breeding lines and landraces. The most polymorphic SSR marker was Xbarc12 (0.97) with the highest PIC value. The UPGMA-based dendrogram grouped 32 bread wheat genotypes into three main clusters. Genetic similarity coefficients ranging from 0.083 to 0.800 were obtained using SSR primers.

This study showed that SSR markers can be useful for genetic characterization in studies related to drought-associated traits. It was determined that SSR markers can be used successfully in the characterization of genetic resources related to drought-associated traits. As a result, the findings obtained from the genotypes evaluated in this study and the SSR markers used are expected to provide preliminary data for future breeding and genetic studies targeting drought-associated traits.

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Conflicts of Interest: The authors declare no conflict of interest.

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