

# Analysis of SSR Markers Associated with Drought Tolerance of Bread Wheat Varieties

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**Abstract** This study investigated the genetic diversity of Bread wheat cultivars by genotyping 24 SSR markers genetically linked to drought tolerance. The highest polymorphism information content values were determined for the primer pairs Wms261, Wms396, Wms148. Also, according to the results of the analysis of Ezoz and Oq Marvarid variety samples using SSR markers, the alleles formed for this trait are phylogenetically close to the Bardosh variety, which is known to have high drought tolerance, and these variety samples can also be evaluated as drought stress-tolerant genotypes.

**Keywords** Bread wheat (*Triticum aestivum* L.), Variety, Abiotic stress, SSR, Drought, DNA, Marker

## 1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the world. It also provides more than 20% of the calories and protein needed to feed more than 35% of the world's population in more than 40 countries [1]. Wheat is sensitive to heat and drought stress, especially during the flowering and late embryogenesis stages of the vegetative period, which negatively affects yield and grain quality [4]. Water deficit during the vegetative growth stage has significant negative effects on plant physiology. Drought-induced stomatal closure reduces the plant's transpiration and cooling capacity, which leads to a significant increase in leaf temperature and a decrease in photosynthetic efficiency [15]. In addition, drought stress during the reproductive stage of wheat, especially during flowering and grain filling, has a serious impact on grain yield. [7] Water deficits have been shown to affect pollen viability, impair fertilization, and reduce grain size, resulting in reduced grain yield. In addition to physiological effects, drought stress also induces a variety of molecular responses in wheat. [8] The genetic diversity of Bread wheat varieties in terms of tolerance to drought was examined by phenotypic observations and simple sequence repeats (SSR) [1]. Golabadi et al. (2011) [12] used microsatellite markers to identify QTLs with yield-trait competent such as thousand

grain weight and harvest index. [13] studied D genome-based genetic diversity research in terms of tolerance to drought using SSR markers. In addition, SNPs are the most abundant polymorphic markers in plant [14]. The bi-allelic nature, high level of polymorphisms, ubiquitous presence, uniform distribution across genomes, automated data acquisition, and analysis make SNPs the most suitable marker for genome-wide marker analysis [20].

## 2. Materials and Methods

Plant materials: 18 domestic and foreign varieties of winter Bread wheat: Asr, Bardosh, Baxmal-97, Chillaki, Do'stlik, E'zoz, KATE-1, KR12-07, KR12-5003, KR12-9023, Krasnodar 99, Mars-1, Oqmarvarid, Qayroqtosh, Shams, Surxak, Xisorak varieties were used in the research.

DNA Extraction and SSR marker assay: Varieties were grown under climate control at 25°C for 14 days for isolating DNA. The CTAB method was used to isolate genomic DNA from research samples. SSR markers specific for wheat were used to determine mutual genetic polymorphism between wheat varieties. PCR (hot-start wheat program) working mixture was prepared in a volume of 10 µl. The DNA concentrations of the samples were determined by gel electrophoresis by visual comparison with lambda (λ) phage DNA at a specific concentration (25 ng/µl) on a 0.9% agarose gel. Then, PCR amplification products were subjected to horizontal electrophoresis in 2.5% agarose gel at 100 V voltage for 90 minutes. Molecular weight marker is Invitrogen 50 bp DNA ladder (Cat. No. 10416014).

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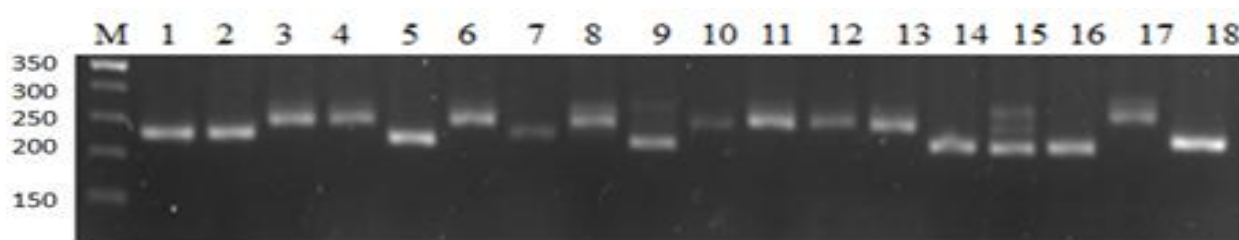
**SSR markers panel.** In order to study the level of drought tolerance of the selected variety samples for the study, a panel of DNA markers genetically linked to these traits was created. Various literature was used to compile this panel of markers, and SSR markers with a high level of polymorphism were selected.

### 3. Results

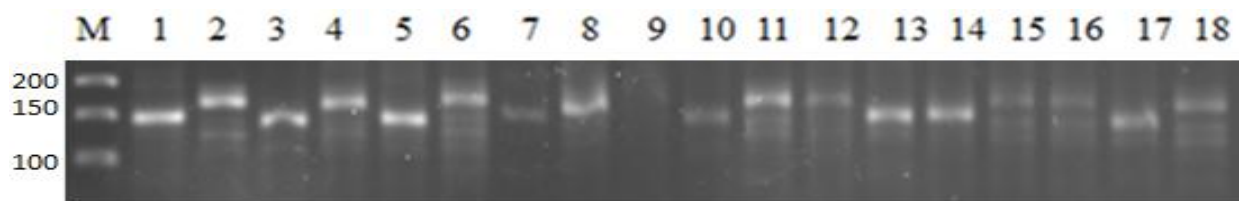
It was observed that 24 out of 45 pairs of SSR microsatellite markers showed polymorphism, while the remaining 21 SSR markers were monomorphic. Bread wheat varieties showing polymorphism were involved as donors in selection for creating new varieties with high productivity and grain quality.

Research samples were screened with SSR markers using the polymerase chain reaction (PCR) method.

PCR screening was performed on the identified DNA samples using primers genetically associated with drought tolerance. The following data were obtained as a result of PCR analysis of the WMS261 SSR marker, which is associated to valuable economic traits, in particular, the drought tolerance. In this case, the presence of three different alleles for a specific marker trait in a study samples indicates high polymorphism. Based on the results, it was noted that samples 3-, 4-, 6-, 8-, 11-, 12-, 13-, 17-with a 215-pair nucleotide resistance allele, samples 1-, 2-, 5-, 9-, 14-, 15-, 16-, 18-with a 205-pair nucleotide allele, and samples 7-, 10-with a 210-pair nucleotide allele were similar to each other (Figure 1).



**Figure 1.** Electrophoregram of the polymorphic WMS261 microsatellite marker. 1-Baxmal- 97, 2-Oqmarvarid, 3-E'zoz, 4-Asr, 5-Qayroqtosh, 6-Bardosh, 7-Surxak, 8-Yaksart, 9-Do'stlik, 10-KATE-1, 11-Mars-1, 12-Chillaki, 13-Krasnodarskaya-99, 14-Xisorak, 15-Shams, 16-KR12-07, 17-KR12-5003, 18-KR12-9023



**Figure 2.** Electrophoregram of the polymorphic WMS148 microsatellite marker. 1-Baxmal- 97, 2-Oqmarvarid, 3-E'zoz, 4-Asr, 5-Qayroqtosh, 6-Bardosh, 7-Surxak, 8-Yaksart, 9-Do'stlik, 10-KATE-1, 11-Mars-1, 12-Chillaki, 13-Krasnodar 99, 14-Xisorak, 15-Shams, 16-KR12-07, 17-KR12-5003, 18-KR12-9023

The following data were obtained as a result of PCR analysis of the WMS148 DNA marker, which is associated to valuable economic traits, in particular, the drought tolerance. In this case, two different alleles for a specific marker trait in a study samples indicates high polymorphism. According to the results, it was observed that samples 2-, 4-, 6-, 8-, 10-, 11-, 12-, 15-, 16-, 17- had a resistance allele of 175 pairs of nucleotides, and samples 1-, 3-, 5-, 7-, 9-, 13-, 14-, 17- had a similar allele of 150 pairs of nucleotides (Figure 2).

The genetic differences between the samples, based on SSR primers, were genotyped according to allele sizes using the GelAnalyzer 19.1 bioinformatics program. Molecular markers are typically used to detect genetic differences between two or more individuals, reflecting polymorphism in genetically related samples. If a marker fails to reveal genetic differences between samples, its use is ineffective. From a qualitative perspective, a marker is considered polymorphic if it has at least two alleles, with the most

common allele having a frequency of no more than 99% [10]. The degree of polymorphism is estimated using the heterozygosity index (H) and the Polymorphic Information Content (PIC). During the study, the PIC value of polymorphic SSR markers was calculated using the following formula, as described by Caetano *et al.* (2020):

$$1 - \sum_{i=1}^n p_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^n 2p_i^2 p_j^2$$

The heterozygosity value is calculated according to the following formula according to Nei, *et al.* [8]:

$$H = 1 - \sum_{i=1}^k p_i^2$$

According to Botstein *et al.* (1980), markers with a PIC value higher than 0.5 have a high degree of polymorphism and are considered reliable for use in molecular genetic studies.

**Table 1.** Panel of SSR markers associated with drought tolerance in wheat

№	Marker name	F/R	PIC	He	Molecular weight (b.p.)	Genetically linked allele, b.p.	Reference
1	Cfd38	Tggccattcgatattcaaaa Gtgagttgagcgcatgata	0,321	0,386	215, 230	215	[10]
2	Gwm148	Gtgaggcagcaagagagaaaa Caaagcttgactcagacaaaa	0,753	0,801	210, 230, 350	350	[10]
3	Gwm292	Tcaccgtgtgcaccgac Ccaccgagccgataatgtac	0,494	0,541	205, 215	205	[10]
4	Gwm294	Ggattggagttaagagagaaccg Gcagagtgatcaatgccaga	0,613	0,671	80, 100, 145	80	[10]
5	Wms144	Tttgctgtgtgtacgaacatac Actcacaatgtctataaaac	0,454	0,512	180, 250	205	[10]
6	Wms148	Gtgaggcagcaagagagaaaa Caaagcttgactcagacaaaa	0,881	0,515	150, 175	175	[10]
7	Wms165	Tgcagtggtcagatgtttcc Cttttcttcagattgcgcc	0,671	0,732	270, 280, 295, 315	315	[10]
8	Wms218	Cggcaaacggatcgcac Aacagtaactctcgccatagcc	0,782	0,841	130, 140, 150, 165, 180, 210	150	[10]
9	Wms261	Ctccctgtacgcctaaggc Ctcgcgctactagccattg	0,813	0,650	205, 210, 215	215	[10]
10	Wms340	Gcaatctttttctgaccacg Acgaggcaagaacacacatg	0,761	0,802	90, 95, 100, 110, 130, 140	110	[10]
11	Wms245	Gctcagatcatccaccaattc Agatgctctgggagagtcctta	0,663	0,700	110, 115, 120	110	[10]
12	Wms396	Tgcactgttttaccttcacgga Caaagcaagaaccagaccact	0,814	0,875	150, 165, 170, 180, 190, 200	165	[9]
13	Wms479	Gacctaaagccagtgatcag Agactcttggttgatacgg	0,590	0,870	235, 250, 260, 290	260	[9]
14	Wms702	Gaatcacatcgaatggatctca Gaggcctttttcgatatctgc	0,730	0,791	195, 230, 240, 280	230	[9]
15	Wms790	Aattaagatagacgtccatatcca Cgacaacgtacgcgcc	0,750	0,801	135, 140, 155, 180, 200	155	[9]
16	Xgwm108	Cgacaatggggtcttagcat Tgcacactaaattacatccgc	0,540	0,600	210, 265, 290,	210	[2]
17	Xgwm11	Ggatagtcagacaattctgtg Gtgaattgtgtgtatgcctcc	0,471	0,503	120, 140	140	[3,11]
18	Xgwm186	Gcagagcctggttcaaaaag Cgcctctagcgagagctatg	0,404	0,470	115, 135	115	[10]
19	Xgwm389	Atcatgtcgtatccttgacg Tgccatgcacattagcagat	0,642	0,702	110, 135, 160	160	[3,11]
20	Xgwm603	Acaaacggtgacaatgcaagga Cgcctctctgtaagcctcaac	0,273	0,303	105, 115	115	[3]
21	Xgwm626	Gatctaaaatgtttttctctc Tgactatcagctaaacgtgt	0,540	0,600	105, 135	110	[3,11]
22	Xpsp3200	Gttctgaagacattacggatg Gagaatagctggtttgtgg	0,491	0,910	160, 170, 175, 190, 200, 205, 210, 230	185	[11]
23	Xwmc78	Agtaaatctcccttcggcttc Agcttcttgctagtcggtgc	0,613	0,666	160, 180, 300	160	[17]
24	Xwmc89	Atgtccacgtgctaggaggtta ttgcctccaagacgaaataac	0,594	0,652	95, 105, 120	95	[17]

In this study, 17 markers had PIC values greater than 0.5, with the primer pairs Wms261 (0.88), Wms396, and Wms148 (0.81) exhibiting the highest levels of polymorphism.

A dendrogram of the phylogenetic relationships among wheat varieties was constructed using polymorphic DNA markers. Molecular-genotypic analysis was performed using polymorphic microsatellite DNA markers linked to drought

tolerance. Hierarchical cluster analysis was conducted using the bioinformatics program NCSS Statistics 2021, revealing the phylogenetic relationships between the studied samples (Figure 3).

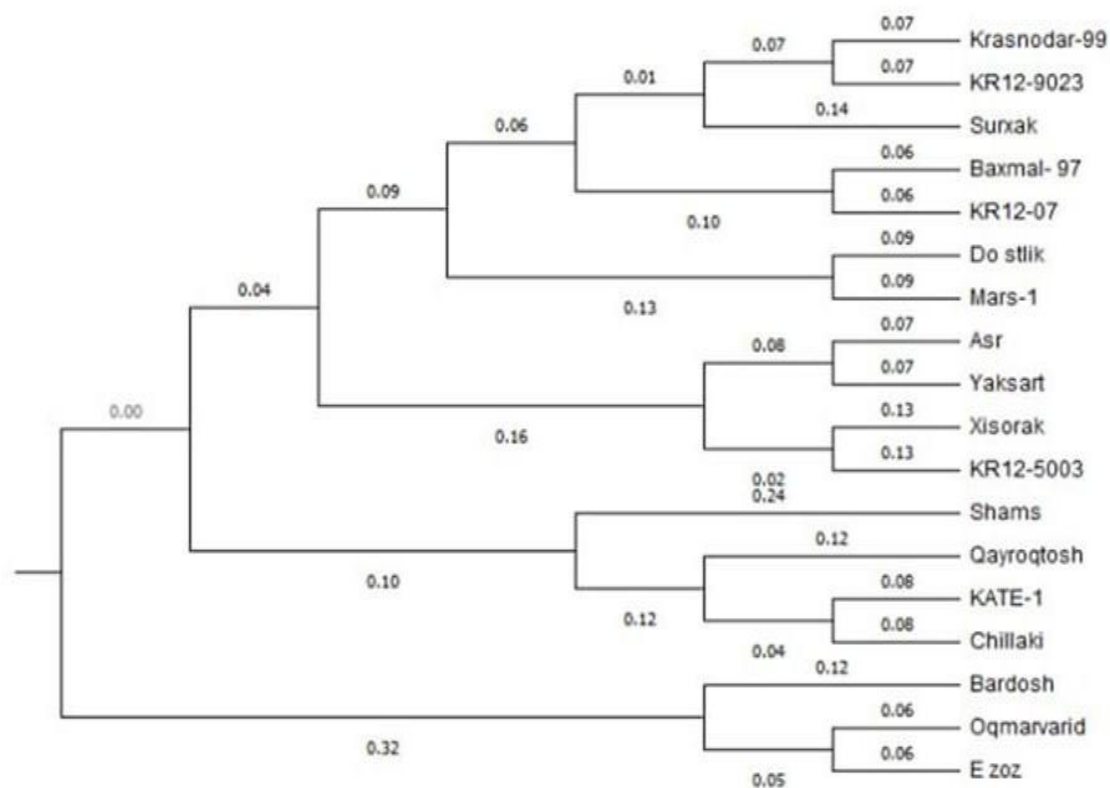


Figure 3. Phylogenetic tree of wheat samples

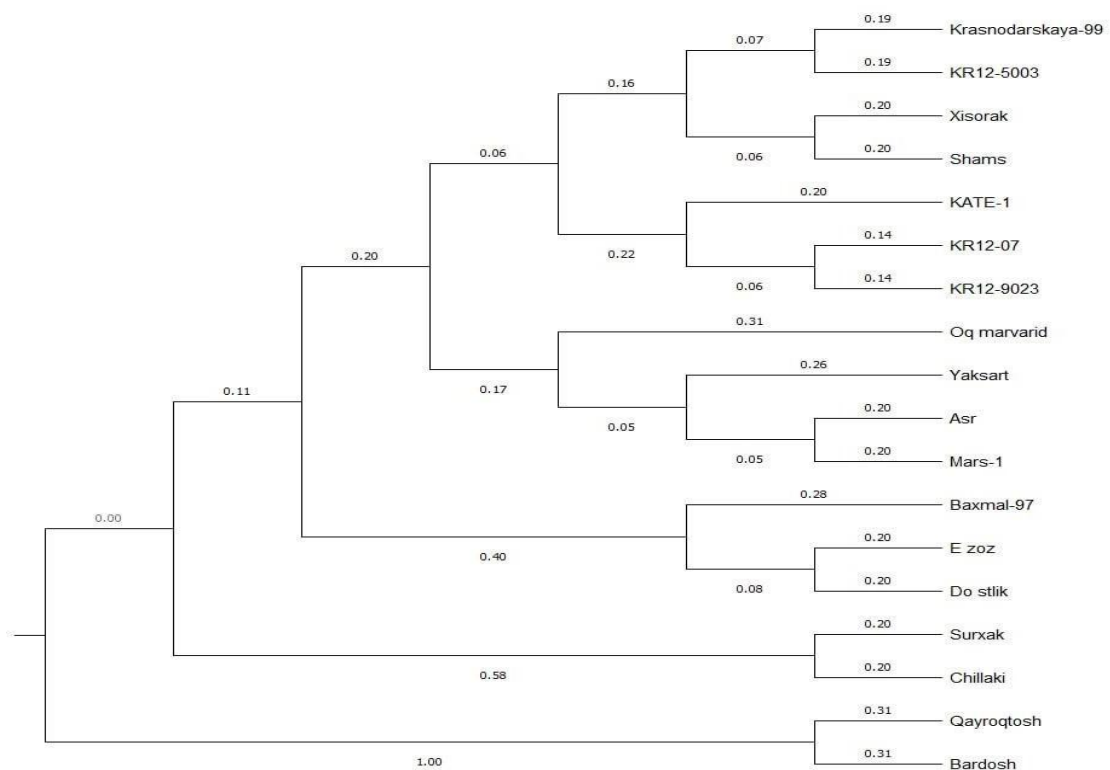


Figure 4. Cluster analysis of drought tolerance alleles of the study samples

Cluster analysis was used to classify the study samples phylogenetically and determine the genetic distances between them. This analysis revealed the degree of genetic relatedness among the 18 wheat samples in terms of drought tolerance. Analysis results showed that the genotypes were phylogenetically divided into two clusters. The Bardosh variety exhibited significant genotypic differences from other varieties in terms of drought tolerance. Furthermore, molecular marker analysis revealed phylogenetic proximity between the alleles of Ezoz and Oq Marvarid varieties and the drought-tolerant Bardosh variety, suggesting that these varieties may also possess drought-tolerant characteristics. The second cluster is also divided into several subgroups and the high variation in water deficit tolerance among varieties indicates the existence of extensive genetic diversity among genotypes for this trait. This cluster analysis shows the genetic distances between plant varieties or populations and represents their division into clusters based on their tolerance alleles. The distance between varieties with close similarity in a cluster is the smallest. For example, the genetic distance between the varieties KR12-5003 and Hisorak is 0.07. This indicates a high level of genetic similarity between them. The distance between KR12-07 and KR12-9023 is 0.14, which indicates a slightly greater proximity between them. At the top of the cluster, the varieties Krasnodar99, KR12-5003, Hisorak and Shams are grouped together and the distances between them are very small (0.16 and 0.07). This means that these varieties are very similar in terms of resistance alleles. The varieties KATE-1 and KR12-07 are also genetically close to each other, linked by a distance of 0.14. The distance of the Oq marvarid variety to other clusters is 0.31. This makes it different from the first cluster above. The distance of the Yaksart variety to other varieties is 0.26, which indicates that it is located in an average proximity among the clusters of other varieties. The Asr and Mars-1 varieties are located close to each other with a distance of 0.20. The distance between Baxmal-97 and Ezoz is 0.28. Although they are related, they are located at a greater genetic distance than the above clusters. The distance between the Chillaki and Qayroqtosh varieties is 0.20 which indicates a certain relationship between them. The distance of the Bardosh variety to all other plants is very large that is around 1.00. This indicates that it differs sharply from all other varieties in terms of resistance alleles (Figure 4).

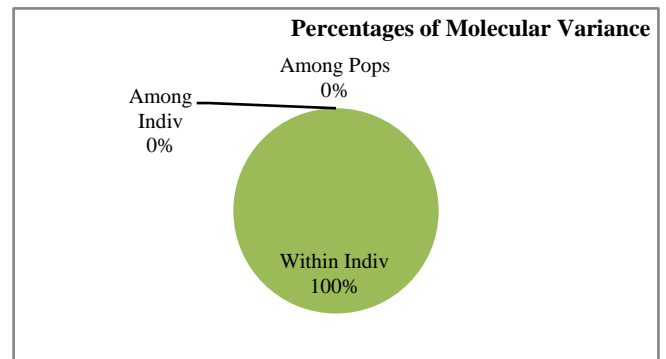
So, according to the results of the dispersion, the genetic diversity among populations was 0%, indicating the absence of genetic differences between them (Table 2).

**Table 2.** Summary statistics of AMOVA analysis

Source	df	SS	MS	Est.Var.	(%)
Among Pops	2	6,087	3,044	0,000	0%
Among Indiv	15	59,607	3,974	0,000	0%
Within Indiv	18	132,500	7,361	7,361	100%
Total	35	198,194	-	7,361	100%

The genetic diversity within populations is 100%, indicating

a high level of genetic variation within individuals. (figure 5).



**Figure 5.** Distribution of variance according to AMOVA results

The level of differentiation between the studied populations was found to be low ( $F_{st} = -0.025$ ).  $P = 0.726$  indicates that this difference is statistically significant. (Table 3).

**Table 3.** Results of F-statistics based on AMOVA

Indicator Value	Indicator Value	P(rand $\geq$ data)
Fst (between populations)	-0,025	0,726
Fis (between individuals)	-0,299	1,000
Fit (total variance)	-0,332	1,000
Fst max	0,517	-
F'st	0,168	-

The AMOVA results show that the populations analyzed are genetically close to each other, with significant gene flow between them. Although the differentiation between populations is significant, it is very low, indicating that the genetic structure is not sufficiently separated.

## 4. Discussion

This research provides comprehensive molecular characterization of drought tolerance in 18 Bread wheat varieties using SSR markers, offering valuable insights for wheat improvement programs. The results show significant genetic diversity in drought-responsive alleles among the studied varieties, with important implications for MAS (marker assisted selection). The polymorphism analysis revealed that 24 out of 45 SSR markers showed polymorphism, consistent with previous reports of SSR polymorphism rates in wheat [19] Notably, SSR markers WMS261, WMS396 and WMS148 showed particularly high polymorphism ( $PIC > 0.8$ ), [18] The observed allele patterns at these loci suggest distinct drought tolerance mechanisms among the cultivars, with the 215-bp allele of WMS261 and 175-bp allele of WMS148 potentially representing valuable markers for breeding. Cluster analysis revealed two major groups with distinct drought tolerance profiles. The close clustering of KR12-5003, Hisorak and Krasnodar99 (genetic distance  $< 0.16$ ) suggests shared genetic mechanisms for drought tolerance, possibly through common ancestral origins or convergent selection. These findings align with [12] report of co-localized QTLs

for drought tolerance in similar wheat genotypes. The exceptional position of Bardosh (genetic distance = 1.00) confirms its unique drought tolerance characteristics, supporting previous phenotypic observations [5]. The intermediate positions of Ezoz and Oq Marvarid near Bardosh suggest they may share some tolerance mechanisms, making them potential candidates for pyramiding drought tolerance genes. The genetic distance patterns observed (0.07-1.00) reflect the wide diversity in drought response strategies among wheat varieties, consistent with the findings of [13]. The marker-trait associations identified in this study, particularly the high-PIC markers linked to drought tolerance, provide practical tools for breeding programs. The panel of polymorphic SSR markers developed here offers a cost-effective solution for marker-assisted selection in resource-limited settings, complementing newer SNP-based approaches [14]. The clear separation of varieties based on tolerance alleles supports the potential for developing molecular breeding strategies targeting specific drought response mechanisms.

These findings advance our understanding of drought tolerance genetics in Bread wheat and provide a foundation for developing drought resistant varieties. Future work should validate these markers in diverse genetic backgrounds and environments, and explore their relationships with physiological drought tolerance traits. Integration of these SSR markers with high-throughput SNP genotyping could further enhance breeding efficiency for drought tolerance.

## 5. Conclusions

The cluster analysis demonstrates the genetic similarity between wheat varieties and their grouping patterns based on resistance alleles. The dendrogram reveals that varieties positioned at the upper cluster (including KR12-5003, Hisorak, and Krasnodar99) show close genetic proximity, indicating shared resistance alleles. Bardosh, located at the lower cluster, exhibits significant genetic divergence from other varieties in terms of resistance alleles, while demonstrating notable drought resistance characteristics. These results provide valuable genetic markers for developing drought-resistant wheat varieties through selective breeding programs.

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