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Determining Close Relationships Between Wheat Cultivars (*Triticum Aestivum*) Using Dna Markers

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ABSTRACT

This study aimed to investigate the genetic diversity and phylogenetic relationships among 30 wheat cultivars. Six DNA markers (Xgwm44, Xgwm337, WMC473, Xgwm642, Xgwm111, and Xgwm635) were used to analyze the genetic variation in these cultivars. A total of 79 alleles were identified across the six markers, demonstrating a high level of genetic diversity among the wheat cultivars. The marker Xgwm337 exhibited the highest number of alleles, suggesting its potential for discriminating between different genotypes. Phylogenetic analysis revealed that the 30 wheat cultivars could be divided into two main groups. The cultivar Aliksevich was assigned to the first group. The second group was further divided into four subgroups, with Mars, Yuka, Andizhan-2, Andizhan-4, Qadr, and Krasnodar-99 belonging to different subgroups.

Key: *Wheat, Microsatellite marker, SSR markers, DNA markers, Molecular genetics*

The taxonomy of wheat has a long history, and the existence of various competing classifications, as well as the use of different botanical names for the same species, has caused confusion among researchers. The use of different classification variants for the genus Triticum L. remains a subject of discussion in molecular biological, genetic, and phylogenetic studies, identification and collection of wheat samples, as well as in breeding practice.

Carl Linnaeus is considered the first scientist to systematize wheat. However, judging by his own records, the classification of wheat has a much older history [1]. The system of wheat classification proposed by Linnaeus in 1753 was based on a number of morphological features, such as phenology (spring wheat - T. aestivum L., winter wheat - T. hybernum L.), grain morphology (spelta - T. spelta L.) and awns. Later, Dumortier supplemented the classification of wheat with an important trait related to the domestication process - the fragility of the spike. Ko¨rnicke also made a significant contribution to the classification of wheat, based on morphological differences and describing a large number of species and varieties in this genus [2]. It is known that classifications arise as a result of studying the flora. Although many floras of the 18th-19th centuries included descriptions of wheat (genus Triticum), they did not make significant innovations in its systematics.

Gancharov, revising the classification of Morris and Sears, drew attention to one shortcoming. According to the scientist, dividing species into groups without explaining how these groups differ from each other is unjustified [3]. These groups, in his opinion, are not formally incorrect, but they represent informal groups that do not have a clear nomenclatural status. "If they wanted to, they could formalize these groups by separating them into sections. However, Bouden, neither Morris nor Sears did so. Someone may disagree with their decision, but that does not mean it is wrong, it is simply different. In addition, they used the name T. dicoccon instead of T. dicoccum," - noted in the works of Gancharov. Currently, the name of this species remains a subject of discussion [4].

According to Takhtajan, the more informative the classification system, the more useful it is from both a scientific and practical point of view [5]. An uninformative classification is not only useless but also harmful. If there are no clear criteria for distinguishing individual taxa, then one cannot expect gene bank specialists to control the purity of samples[6]. Since their value is not assessed, important samples may be excluded. Indeed, the lack of formal taxonomy and nomenclature, the inability to identify individual forms, as noted by Michael Windham, can lead to the so-called "nomenclatural extinction".

According to Gancharov, even if his classification is changed in the future, the number of artificial tetraploid and hexaploid amphidiploids will remain unchanged. This is due to the fact that all possible combinations of the genomes Ab, Au, B, G, and D are already used by researchers. The advantages of Gancharov's classification for the identification and collection of wheat samples, as well as for molecular genetic and phylogenetic studies, have been widely discussed in other works [7]. However, this does not mean that the classification cannot be improved, it simply offers advantages that are not present in other proposals for infrageneric taxonomic revision of Triticum.

To accurately map valuable traits in crop plants, researchers must employ a diverse array of molecular markers (Bakhadirov et al., 2021).

Materials and Method

Thirty wheat cultivars from the genus Triticum were used as experimental material, and molecular analyses were conducted to determine their genetic relationships.

1- Table

Thirty wheat cultivars from the genus Triticum were used as experimental material, and molecular analyses were conducted to determine their genetic relationships

№	Cultivar	№	Cultivar	№	Cultivar
1	Krasnadar-99	11	Zomin-1 (Jizzakh)	21	Antonina
2	Ok marvarid	12	Dustlik (Jizzakh)	22	Uzbekiston-25
3	Ilgor	13	Antonina (Khon tex)	23	Druzhba
4	Ezoz	14	Esaul (Andijon)	24	Yuka
5	Zomin-1 (Karshi)	15	Mars	25	Andijon-4 (Uchkurgon)
6	Jasmina	16	Nodir	26	Andijon -4 (Andijon)

7	Forboma	17	Yogdu	27	Andijon -2 (Andijon)
8	Asr (Namangan)	18	Kadr	28	Andijon-2 (Uchkurgon)
9	Семруг	19	Navbakhor	29	Vassa (VIR Tashkent)
10	Истиқлол	20	Алексевич	30	Vassa (VIR Andijon)

CTAB method (cetyltrimethylammonium bromide) was used to extract genomic DNA from the research samples (ref. 10). The quantity of genomic DNA samples was determined by gel electrophoresis, comparing it to a specific concentration (50 µL) of λ-phage DNA. Polymerase Chain Reaction (PCR) was performed using the FroFlex PCR system (Applied Biosystems, USA) based on 6 microsatellite (Simple Sequence Repeats, SSR) DNA markers: Xgwm44, WMC473, Xgwm635, Xgwm111, Xgwm337, and Xgwm642, which are closely linked in wheat. Molecular weight of PCR products was determined by gel electrophoresis (Thermo Scientific, USA) and photographed using a Molecular Imager (Bio-Rad, USA). The results of the molecular analysis were genotyped using the GelAnalyzer computer program.

2- Table

Nucleotide sequences of wheat primers

Spesies origin	**SSR-Primers**	**Nucleotide sequence**
T.aestivum	Xgwm111	F: TCTGTAGGCTCTCTCCGACTG R: ACCTGATCAGATCCCACTCG
	Xgwm635	F: TTCCTCACTGTAAGGGCGTT R: CAGCCTTAGCCTTGGCG
T.aestivum	Xgwm44	F: GTTGAGCTTTTCAGTTCGGC R: ACTGGCATCCACTGAGCTG
T.aestivum	Xgwm106	F: CTGTTCTTGCGTGGCATTAA R: AATAAGGACACAATTGGGATGG
	Xgwm337	F:CCTCTTCCTCCCTCACTTAGC R: TGCTAACTGGCCTTTGCC
T.aestivum	Xgwm44	F: GTTGAGCTTTTCAGTTCGGC R: ACTGGCATCCACTGAGCTG
T.aestivum	Xgwm111	F: TCTGTAGGCTCTCTCCGACTG R: ACCTGATCAGATCCCACTCG
T.aestivum	Xgwm635	F: TTCCTCACTGTAAGGGCGTT R: CAGCCTTAGCCTTGGCG
T.aestivum	Xgwm642	F: ACGGCGAGAAGGTGCTC R: CATGAAAGGCAAGTTCGTCA
T.aestivum	Xgwm111	F: TTCCTCACTGTAAGGGCGTT

		R: CAGCCTTAGCCTTGGCG
T.aestivum	Xgwm 473	F:TCATACGGGTATGGTTGGAC R: CACCCCCTTGTTGGTCAC

RESULTS

The genetic diversity of the wheat samples was assessed using six DNA markers: Xgwm44, Xgwm337, WMC473, Xgwm642, Xgwm111, and Xgwm635. The markers exhibited a high degree of polymorphism, with a total of 79 alleles identified among the analyzed samples.

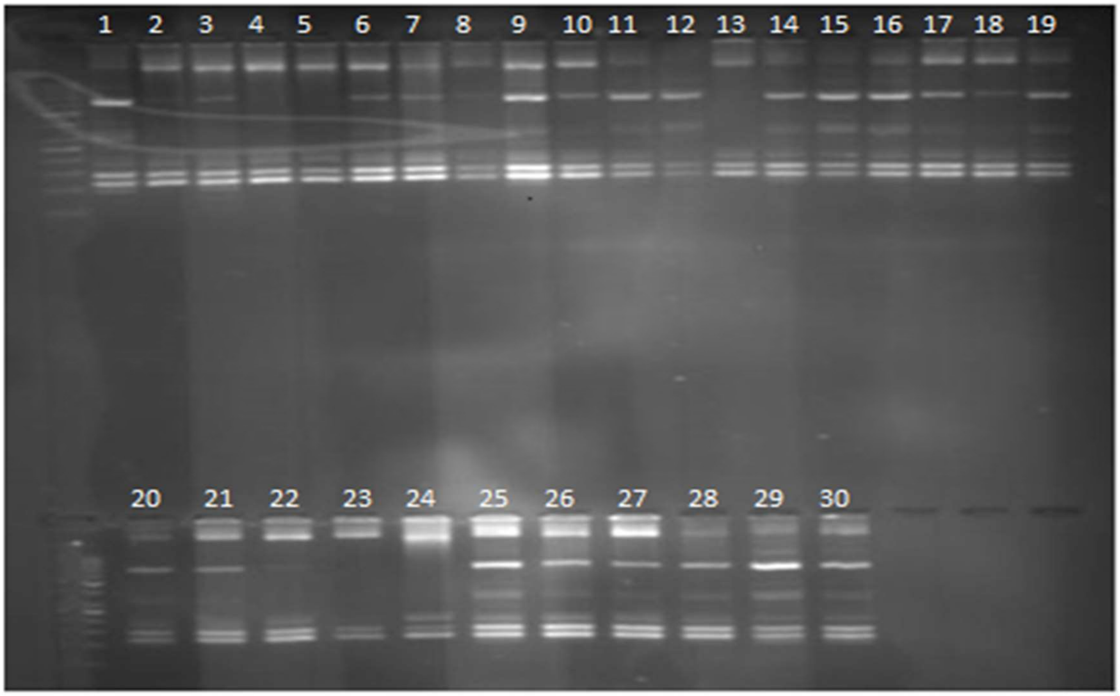

Figure-1 Electrophoregram of PCR amplicons in the analyzed samples for the DNA marker Xgwm44

The DNA marker Xgwm44 exhibited a total of ten alleles with sizes ranging from 86, 91, 133, 139, 147, 154, 212, 414 to 424 base pairs (bp) in the analyzed wheat samples.

The DNA marker Xgwm337 displayed a total of nineteen alleles ranging from 28, 30, 32,36,83,84,128,147,152,157, 160, 180, 183, 219, 220, 229, 234, 239, 244 to 689 base pairs (bp) in the examined wheat samples.

The DNA marker WMC473 presented two alleles with sizes of 335 and 407 base pairs (bp), respectively, in the analyzed wheat samples.

The DNA marker Xgwm642 showed a total of twelve alleles with sizes ranging from 216, 221, 269, 270, 276, 282, 368, 427, 428, 429, 436 to 442 base pairs (bp) in the tested wheat samples.

The DNA marker Xgwm111 exhibited eight alleles with sizes ranging from 149, 151, 153, 193, 197, 213, 218 to 272 base pairs (bp) in the analyzed wheat samples.

The DNA marker Xgwm635 displayed eight alleles ranging from 112, 116, 119, 149, 156, 160, 228 to 246 base pairs (bp) in the tested wheat samples.

The marker Xgwm337 displayed the highest number of alleles (19), indicating its potential for discriminating between different genotypes. The remaining markers also exhibited a significant level of polymorphism, suggesting that they are suitable for characterizing genetic variation within the wheat population.

The results of this study demonstrate that the six DNA markers employed were highly polymorphic, with a wide range of allele sizes and frequencies observed. This suggests that these markers are effective for capturing genetic variation within the wheat population. The marker Xgwm337 exhibited the highest number of alleles, indicating its potential for discriminating between different genotypes.

The observed genetic diversity has important implications for wheat breeding and conservation. The presence of a large number of alleles for each marker suggests that there is a rich reservoir of genetic variation available for breeders to select for desirable traits. Additionally, the genetic diversity observed in this study highlights the importance of conserving genetic resources to ensure the long-term sustainability of wheat production.

3- Table

A set of microsatellite markers and their characteristics: polymorphism information content (PIC) and heterozygosity (He).

№	DNA markers	Molecular weight (b.p.)	Number of alleles	PIC	HE
1	Xgwm44	86-424	10	0.8455	0.86
2	Xgwm337	28-689	21	0.9195	0.9239
3	WMC473	335-407	2	0.3726	0.4953
4	Xgwm642	216-442	12	0.9041	0.9112
5	Xgwm111	149-272	8	0.8553	0.8697
6	Xgwm635	112-246	8	0.8488	0.8642

The phylogenetic relationships of 30 wheat samples were investigated based on 6 DNA markers and their allelic variants using the Hierarchical Clustering dendrograms function in the NCSS 2022 statistical analysis software.

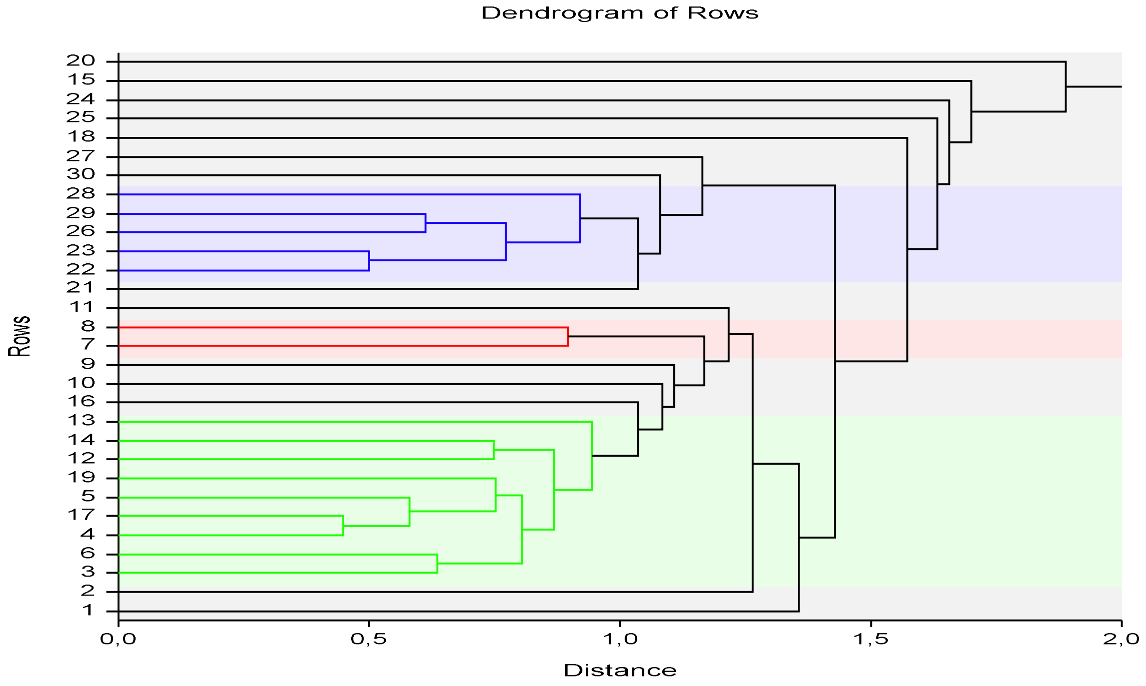

Figure 2. Phylogenetic tree of 30 wheat varieties based on polymorphism of closely related DNA markers.

Phylogenetic analysis revealed that the 30 wheat cultivars studied were divided into two main groups. The cultivar Aliksevich was assigned to the first group. The second group, in turn, was divided into four subgroups. The Mars cultivar was included in the first subgroup, Yuka in the second, and Andizhan-2 and Andizhan-4 in the third. The fourth subgroup was also divided into two parts. The Qadr cultivar was included in the first part, while the second part was further divided into three subgroups. The first of these three subgroups included eight cultivars, the second - fifteen cultivars, and the third - one cultivar, Krasnodar-99. In conclusion, it follows that the 30 wheat cultivars, being close relatives, were divided into two groups: Russian cultivars formed a separate group, while our local cultivars ended up in one group.

Discussion

The results of this study demonstrate that the six DNA markers employed were highly polymorphic, with a wide range of allele sizes and frequencies observed. This suggests that these markers are effective for capturing genetic variation within the wheat population. The marker Xgwm337 exhibited the highest number of alleles, indicating its potential for discriminating between different genotypes.

The phylogenetic analysis of the 30 wheat cultivars revealed a clear division into two main groups. This clustering suggests distinct evolutionary pathways and potentially different genetic characteristics associated with each group.

The first group, which includes the cultivar Aliksevich, may represent a distinct lineage within the wheat species. Further investigation into the genetic makeup of this group could provide insights into its unique evolutionary history and potential adaptations.

The second group, divided into four subgroups, showcases a more complex evolutionary pattern. The Mars, Yuka, Andizhan-2, and Andizhan-4 cultivars form distinct subgroups, suggesting shared genetic ancestry and potentially similar agronomic traits. The Qadr cultivar, placed in a separate part of the fourth subgroup, might represent a distinct lineage within this group.

The further division of the fourth subgroup into three subgroups with varying numbers of cultivars highlights the genetic diversity within this group. The eight cultivars in the first subgroup, the fifteen cultivars in the second subgroup, and the single cultivar Krasnodar-99 each represent unique genetic lineages.

The overall results of the phylogenetic analysis suggest that while the 30 wheat cultivars are closely related, they have diverged over time through evolutionary processes. The observed grouping into two main groups and further subdivisions indicates distinct genetic lineages and potentially different adaptations to specific environmental conditions. Genomic Analysis: Sequencing the genomes of these cultivars would provide a more detailed picture of their genetic variation and identify specific genes associated with the observed clustering.

Phenotypic Characterization: Examining the phenotypic traits (e.g., yield, disease resistance, quality) of these cultivars could help identify associations between genetic variation and agronomic performance.

Population Genetics: Analyzing the genetic structure of these cultivars could provide insights into their evolutionary history, population dynamics, and gene flow patterns.

Comparative Genomics: Comparing the genomes of these wheat cultivars with those of other wheat species or closely related grasses could help identify conserved genes and regions of the genome that have undergone rapid evolution.

By combining phylogenetic analysis with genomic, phenotypic, and population genetic data, researchers can develop a more comprehensive understanding of the genetic diversity and evolutionary history of these wheat cultivars, which can be valuable for breeding programs and agricultural practices.

Conclusion

The phylogenetic analysis of the 30 wheat cultivars revealed a clear evolutionary structure, with the cultivars clustered into two distinct groups. This suggests that despite being closely related, these cultivars have diverged over time through evolutionary processes.

The first group, which includes the cultivar Aliksevich, may represent a distinct lineage within the wheat species. The second group, divided into four subgroups, showcases a more complex evolutionary pattern. The Qadr cultivar and the three subgroups within the fourth subgroup further highlight the genetic diversity within this group.

Overall, the results of the phylogenetic analysis suggest that the 30 wheat cultivars, while closely related, have evolved along different pathways, potentially adapting to specific environmental conditions. These findings provide valuable insights into the evolutionary history and genetic diversity of these cultivars, which can inform future breeding programs and agricultural practices.

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