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MOLECULAR CHARACTERIZATION AND GENETIC VARIABILITY IN BREAD, DURUM AND DICOCCUM WHEAT GENOTYPES BASED ON MICROSATELLITE MARKERS

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ABSTRACT

Tetraploid wheat species *Triticum durum Desf.* and *Triticum dicoccum* Schrank ex Schubler could be unique sources of favorable genes for economically important characteristics like resistance to biotic and abiotic stresses as well as quality characteristics. Molecular characterization and determination of genetic relationship among varieties and hybrid lines of bread, durum and emmer wheat is fundamental for selection of genotypes with desirable traits resilient under climatic fluctuations and development of successful crop improvement programmes. In this study, ten simple sequence repeat (SSR) markers were used to characterize 50 bread, durum, dicoccum wheat genotypes from North Macedonia. In total, 48 alleles were detected with an average of 4.8 alleles per locus. The highest number of alleles per locus was detected in genome D with 5.5, compared to 4.75 and 4.5 for genomes B and A, respectively. The mean PIC values for all 10 microsatellite markers was 0.66 with values ranging from 0.59 for marker Wmc264 to 0.85 for marker Wmc89 that are important for breeding programs. The average genetic diversity based on SSR markers was 0.51 with a range of 0.20 - 0.72. A dendrogram was constructed to determine the genetic relationship and cluster analyses separated tetraploid wheat from hexaploid wheat genotypes. The data could serve as a basis for wheat taxonomy, wheat improvement programmes and further association mapping for important agronomic traits.

Key words: wheat, hybrid lines, cluster, microsatellites, variability.

INTRODUCTION

Wheat is one of the most important crops in the world regarding the nutritional value and the important role in daily energy intake. World wheat production is almost entirely based on common or bread wheat (*Triticum aestivum* L.), which accounts for about 90% of world production and durum wheat (*Triticum durum Desf.*), which accounts for the remaining 10% of wheat production (Monneveux et al., 2012). Currently, the wheat breeding strategy is aimed at improving the tolerance of the varieties to various kinds of stress while maintaining a high level of productivity and quality. There are several different genetic resources for wheat improvement, as distant taxonomic groups that contain genes for resistance to abiotic and biotic stresses, as well as high grain quality. It is known that the genomic pool of tetraploid wheat species *Triticum durum* Desf. and *Triticum dicoccum* Schrank ex Schubler contain valuable alleles of genes controlling economically important characteristics, such as high grain protein content, drought tolerance and diseases resistance (Pascual et al.,2020; Pour et al., 2021).

Diversity in wheat genetic resources of different ploidy level can be identified by several methods such as morphological, biochemical or molecular screening. Morphological

characterization is often influenced by environmental factors. Currently, considerable attention is dedicated to the usage of molecular markers which, compared to the morphological characteristics and biochemical analysis, that allows identification of polymorphism, even among closely related forms. Microsatellites are one of the most promising molecular marker types for screening of a genetic stocks. Microsatellites have been proposed as one of the most suitable markers for the assessment of genetic variation and diversity among wheat species, varieties, lines, because they are multiallelic, chromosome-specific, evenly distributed along chromosomes and have high level of polymorphism (Röder et al., 1995). There are numerous reports suggesting the usefulness of microsatellite markers for determination of the genetic variability in a wider taxonomic range (Ehtemam et al., 2010; Hao et al., 2011; Li et al., 2012; Babay et al., 2015).

The aim of this study is to evaluate potential of ten microsatellite markers to assess levels of genetic variability among genotypes of bread, durum and dicoccum wheat genotypes and determination of their polymorphism and genetic relationships.

MATERIAL AND METHODS

Three wheat species *Triticum durum* Desf. (durum wheat), *Triticum aestivum* L. (bread wheat) and *Triticum dicoccum* Schrank ex Schübler (emmer wheat) and the lines obtained from their hybridization (Manasievska Simik, 2006) were used as a plant material for the study, Fifty genotypes were analyzed, two durum varieties (mina, iva), two bread varieties (skopjanka, MT 6/2), one landrace of *Triticum dicoccum* (*dicoccum var. pherugeneum*) and 45 lines (Table 1.)

Table 1. Wheat genotypes included in the study

Number	Genotype	Number	Genotype
1	10/2	26	3/5
2	10/3	27	3/6
3	10/6	28	3/8
4	10/7	29	3/9
5	11/3	30	4/1
6	12/1	31	4/2
7	13/1	32	4/4
8	1/3	33	4/5
9	14/1	34	4/6
10	1/4	35	6/1
11	15/3	36	6/3
12	1/5	37	6/6
13	16/3	38	6/7
14	19/1	39	7/2
15	19/3	40	7/4
16	19/4	41	7/6
17	20/4	42	8/1
18	20/5	43	9/1
19	20/9	44	9/2
20	2/2	45	9/3
21	2/3	46	mina
22	3/10	47	iva
23	3/11	48	skopjanka
24	3/13	49	MT/6
25	3/3	50	*dicoccum var. pherugeneum*

Ten SSR markers for wheat genomes (AA, BB and DD genome) (listed in Table 2) were chosen for the evaluation, 4 SSR markers for A genome, 4 SSR markers for B genome and 2 SSR markers for D genome.

The genomic DNA was isolated from grains using NucleoSpin II Plant Kit method (Machery- Nagel,2014).The PCR reactions were carried out using Qiagen 2x HotstarTaq Mastermix containing 0.5 units of HotStarTaq DNA polymerase per reaction and 1x PCR buffer containing a final concentration of 1.5 mM $MgCl_2$ and 200 µM of each dNTP.The fragment analysis was performed using capillary electrophoresis at Size Standard GeneScan LIZ500 (Applied Biosystems) and analysis were performed on an Applied Biosystems 3730XL DNA Analyzer under the following conditions: injection time of 10 s; injection voltage of 1.6 kV; execution time of 2100 s; operating voltage 15 kV; capillary length of 50 cm; POP7 polymer; Dye Set G

Table 2. Microsatellite markers used in the study

No.	Primer Name	Chr	Primer Prefix	Primer Sequence	Primer Prefix	Primer Sequence
1	gwm294	2A	F*	5' GCAGAGTGATCAATGCCAGA 3'	R**	5' GGATTGGAGTTAAGAGAGAACCG 3'
2	wmc264	3A	F	5' CTCCATCTATTGAGCGAAGGTT 3'	R	5' CAAGATGAAGCTCATGCAAGTG 3'
3	cfd15	1A	F	5' CTCCCGTATTGAGCAGGAAG 3'	R	5' GGCAGGTGTGGTGATGATCT 3'
4	barc151	5AL	F	5' TGAGGAAAATGTCTCTATAGCATCC 3'	R	5' CGCATAAACACCTTCGCTCTTCCACTC 3'
5	barc84	3BL	F	5' CGCATAACCGTTGGGAAGACATCTG 3'	R	5' GGTGCAACTAGAACGTACTTCCAGTC 3'
6	gwm257	2B	F	5' AGAGTGCATGGTGGGACG 3'	R	5' CCAAGACGATGCTGAAGTCA 3'
7	wmc89	4B	F	5' ATGTCCACGTGCTAGGGAGGTA 3'	R	5' TTGCCTCCCAAGACGAAATAAC 3'
8	gwm408	6B	F	5' GTATAATTCGTTCACAGCACGC 3'	R	5' TCGATTTATTTGGGCCACTG 3'
9	cfd65	1D	F	5' AGACGATGAGAAGGAAGCCA 3'	R	5' CCTCCCTTGTTTTTGGGATT 3'
10	gwm642	7D	F	5' ACGGCGAGAAGGTGCTC 3'	R	5' CATGAAAGGCAAGTTCGTCA 3'

*F (Forward), **R (Reverse)

Allele number, frequency of alleles, observed heterozygosity (Ho), expected heterozygosity (He) and Polymorphic Information Content (PIC) were determined using power Marker v. 3.25 (Liu et al., 2005).

The data were standardized and Principal Component Analysis (PCA) and the scatter plot of the principal component according to variables and individuals was performed. Hierarchical cluster analysis was carried out using the unweighted pair group method using arithmetic average (UPGMA) and the dendrogram was created using the squared Euclidean distance as the similarity coefficient. The analyses were performed using the statistical software XLSTAT (Version 2015.5.01.22537).

RESULTS AND DISCUSSION

The genetic diversity of 50 wheat genotypes within three different wheat species (*Triticum aestivum*, *Triticum dicoccum* and *Triticum durum*) were analyzed using 10 polymorphic microsatellite markers. The markers were selected to cover all 3 wheat genomes with a minimum of 2 markers per genome. Fragment analyses of PCR products were used for detection of 48 allelic variants in all investigated loci (Table 3). The number of alleles per locus ranged from 3 (Gwm294, Cfd15, Gwm408, Gwm257) to 9 (Wmc89), with average 4.8 alleles per locus. The highest number of alleles per locus was detected in genome D with 5.5, compared to 4.75 and 4.5 for genomes B and A, respectively. Similar results for the number of alleles per locus were obtained by other authors, recording 3-12 alleles for all polymorphic microsatellite loci (Christov et al., 2022; Ganeva and Korzun, 2012).

Table 3. Genetic variability parameters of microsatellite loci: number of alleles (NA), major allele frequency (MAF), observed (Ho) heterozygosity, expected (He) heterozygosity, polymorphism information content (PIC)

Nr	Locus	Chr	Allele sizes (bp)	NA	MAF	Ho	He	PIC
1	gwm294	2A	67-99	3	0.45	0.03	0.60	0.59
2	wmc264	3A	133-141	6	0.29	0.03	0.59	0.60
3	cfd15	1A	161-177	3	0.89	0.02	0.20	0.75
4	barc151	5AL	214-246	6	0.31	0.01	0.79	0.74
5	gwm408	5B	155-189	3	0.52	0.02	0.59	0.61
6	gwm257	2B	195-199	3	0.60	0.05	0.50	0.53
7	wmc89	4B	125-179	9	0.34	1	0.67	0.85
8	barc84	3BL	105-129	4	0.49	0.98	0.63	0.62
9	cfd65	1D	156-202	5	0.33	0.82	0.72	0.70
10	gwm642	1D	168-199	6	0.50	1	0.47	0.69
Total				48				
Mean				4.8	0.47	0.39	0.51	0.66
A genome				4,5	0.49	0.02	0.54	0.64
B genome				4,7	0.72	0.99	0.59	0.65
D genome				5,5	0.54	0.91	0.60	0.69

The data of microsatellite loci and the corresponding alleles were used to calculate the polymorphic information content (PIC) and heterozygosity (H) to evaluate a marker system for its ability to detect high levels of DNA polymorphism in an analysis of genetic diversity. The mean PIC for all 10 microsatellite markers was 0.66 with values ranging from 0.59 for marker Wmc264 on chromosome 3A to 0.85 for marker Wmc89 on chromosome 4B. These results indicate that the majority of markers enabled a high level of polymorphism. The genetic diversity index (He or GD) ranged from 0.20 (cfd15-1A) to 0.72 (cfd65-1D) with a mean of 0.51. The average observed heterozygosity (Ho) across all 10 loci was 0.20 with the highest values of 1 in locus wmc89-4B and in locus gwm642-1D. The lowest observed heterozygosity was recorded in locus barc151-5AL (0.01). The microsatellite markers used showed different levels of gene diversity between the wheat genomes. The highest PIC and GD values were observed in the D genome (0.69 and 0.60) compared to B (0.65 and 0.59) and A (0.64 and 0.54) genomes, respectively (Table 3).

Previous studies show that when the PIC > 0.5, the marker has the maximum diversity, indicating high allelic diversity among germplasms. Contrarily when PIC < 0.25, the marker has minimum diversity (Raza et al., 2019; Sonmezoğlu et al., 2012). Thus, these results with an average PIC value of 0.69 indicate a sufficient amount of gene variability and diversity among analyzed genotypes.

The study of the genomic structure of the wheat lines derived from different crossing combinations of hexaploid and tetraploid wheat of distant taxonomic groups, it is necessary to take into account that the formation of fertile and stable hybrids of wheat is accompanied by complex genome reorganization, in which the level of inclusion of foreign material depends on the degree of relatedness of genomes (Orlovskaya et al.,2011) The most objective assessment of the genomic composition of hybrid forms is provided by molecular techniques (Leonova et al.,2013). A dendrogram based on the relationship values among the varieties and lines derived from ten crossing combinations of bread, durum and emmer wheat was constructed using microsatellite marker information. according to A, B and D genome phylogenetics (Figure 1). All genotypes were grouped clearly into two main clusters based on ploidy levels and the members of each group had relatively close relationships among each other.

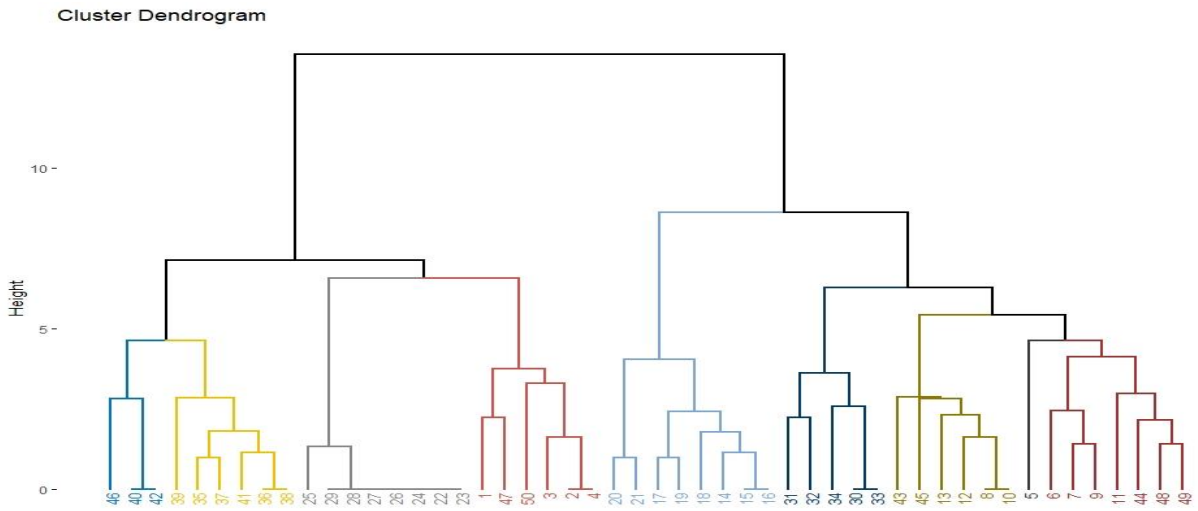

Figure 1. Cluster analysis of wheat genotypes based on microsatellite pattern

Triticum durum and *Triticum. dicoccum* genotypes were grouped in the same cluster (Cluster 1) by reflecting their genetic similarity with each other, as they are tetraploid wheat species. A total of 23 genotypes belonging to *Triticum dicoccum* and *Triticum durum* were present in this cluster. The cluster was further divided into four subgroups. The first subcluster includes the Triticum durum (variety Mina) and lines 7/4, 8/1. The lines 7/4 and 8/1 did not differ from each other in the figures of allele composition of microsatellite locuses. The second subcluster contains six genotypes (6/1, 6/3, 6/6, 6/7, 7/2,7/6). The third subcluster contains genetically very similar lines (3/3, 3/9, 3/8, 3/6, 3/5, 3/13, 3/11, 3/10). *Triticum durum* wheat variety (Iva), *Triticum dicoccum* and lines (10/2, 10/3, 10/6, 10/7) were classified into fourth subcluster.

A total of 27 *Triticum aestivum* genotypes were present in Cluster II, which was further subdivided into four subgroups with different number of genotypes The first subcluster includes the wheat lines 19/1,19/3,19/4 and lines 20/4, 20/5, 20/9, 2/2, 2/3. The lines 19/3 and 19/4 showed small genetic distance based on allele composition of microsatellite loci. The second subcluster contains five (4/1, 4/2, 4/4, 4/5, 4/6) lines. Lines 1/3, 1/4, 1/5, 9/1, 9/3, and line 16/3 are grouped in third subcluser. The fourth subcluster combined varieties (Skopjanka and MT 6/2) and hybrid lines derived from different combintions of crossing. The most genetically different genotype was line 11/3. Lines 15/3, 9/2 showed high levels of heterogeneity. It is noteworthy that, according to the microsatellite analysis, the lines differed fairly strongly from the wheat varieties used in the crossings. This is probably due to the reorganization of hybrid genomes that accompany the formation of stable forms during hybridization. Despite this, cluster analysis showed that the resulting hybrid lines fall into clusters according to their origin.

The principal component analysis (PCA) apart from cluster analysis was performed for grouping of genotypes (Figure 2). Applying both methods was recommended to extract the maximum amount of information from the matrix data.

Clustering was useful in detecting relationships among genotypes, while PCA allowed a view on the relationships between groups. PCA was carried out using the genetic distances data set based on microsatellite data showed similar clustering of the fifty genotypes as was evident from cluster analysis. *Triticum aestivum* genotypes formed a separate group. Tetraploid wheat revealed lower genetic distance compared to bread wheat and they were present close to each other in both clustering algorithms.

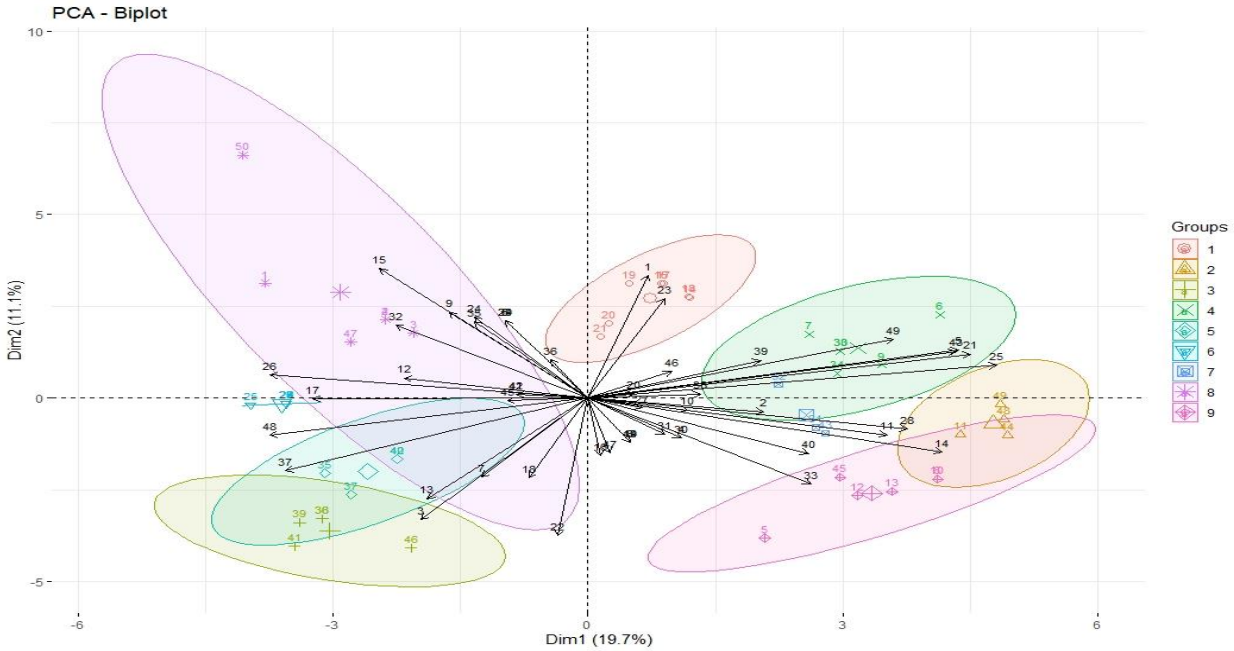

Figure 2. PCA analysis of wheat genotypes based on microsatellite pattern

CONCLUSIONS

The results obtained in our study provided new information on the relationships between wheat genotypes grown in North Macedonia. The set of the used microsatellite markers showed a high level of polymorphism and sufficient information to discriminate studied genotypes The results are in conformity with expectations and provide the first basis for further research. SSR markers successfully discriminated wheat cultivars and wheat lines. The high level of genetic diversity reported in this study should be taken into account in the development of wheat breeding programs. These results demonstrate the reliability, usefulness, and effectiveness of microsatellites SSRs in the analysis of genomic diversity. They can be successfully employed in assaying the level of polymorphism and diversity and serves to facilitate the development of better genotypes and conservation strategies of wheat germplasm.

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