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MICROSATELLITES IN THE ANALYSIS OF WHEAT GENETIC DIVERSITY

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Microsatellite markers (SSR) were used to study wheat genetic diversity. A set of 87 wheat genotypes was analysed with four SSR markers. Primers used for the amplification of adequate microsatellite loci (*Xgwm*) are according to RÖDER *et al*. (2002). Results were obtained using Applied Biosystems 3130 genetic analyser. Total of 28 alleles were determined, i.e. average of 7 alleles per marker. Number of alleles for individual markers ranged from six (*Xgwm3*) to eight (*Xgwm18*). The presence of two null alleles for *Xgwm18* and *Xgwm155* was found. There were five rare alleles (frequency <2%). Polymorphism information content (PIC) values ranged from 0.52 for *Xgwm408* to 0.80 for *Xgwm18*. Mean PIC value was 0.69 for all markers, which signifies a high level of the detected polymorphism. According to the data collected through the analysis of four markers, most genotypes can be grouped in clusters. The results show usefulness of microsatellite markers in detecting polymorphism, identifying genotypes and assessing genetic diversity.

Key words: genetic variability, SSR markers,.*Triticum aestivum* L.

INTRODUCTION

Modern, genetically improved cultivars and contemporary cultivation practices have resulted in continual increase of the average wheat yields worldwide in the previous 50 years. In this period Serbia saw an average annual increase of wheat yield potential of 41 kg ha^{-1} (MLADENOV *et al*., 2011). There was also a significant improvement of technological quality parameters (HRISTOV *et al*., 2009). In order to sustain this positive trend it is necessary to further

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investigate genetic diversity of wheat at a molecular level as well as to integrate the obtained data into classic breeding programmes (CHAO *et al*., 2007).

Genetic diversity plays an important role in the intensive agricultural production scenario and it is very often the subject of study on various plant species (RAUF *et al*., 2012; ŠURLAN MOMIROVIĆ *et al*., 2013). Modern cultivation method and intensive long-term selection narrow genetic diversity of wheat, which leads to decreased possibilities of adaptation to biotic and abiotic stress factors (FU *et al*., 2006; ZUANG *et al*., 2011).

Previous decades of advanced technics, based on the application of molecular markers, have provided study of diversity, monitoring the changes during evolution process as well as changes resulting from the effects of intensive selection (LANDJEVA *et al*., 2007). Microsatellites or simple sequence repeats (SSR) belong to the group of most suitable molecular markers for the study of genetic variability. They are very informative, co-dominant, locus specific, simple to analyse, multi-allelic and distributed throughout the whole genome (RÖDER *et al*., 2002; GADALETA *et al*., 2009).

The aim of this research was to determine whether it is possible to assess genetic diversity in the collection of 87 wheat genotypes by using only a few microsatellite markers. Moreover, the aim was also to group the genotypes by similarities based on the molecular evaluation, as well as to compare different variability indices in the formed groups.

MATERIALS AND METHODS

For genetic diversity analysis 87 wheat genotypes (*Triticum aestivum* L.) were used in the study. Out of the total number, 21 genotypes originate from Serbia, while others come from various countries of the world (Table 1). The seed was produced at the experimental fields of Institute of Field and Vegetable Crops, Novi Sad, Serbia in the growing season 2008-2009 with the application of standard cultivation practices. Isolation of genome DNA was performed from wheat seedlings according to modified method by DOYLE and DOYLE (1990).

Wheat genotypes were analysed by a set of four microsatellite markers: GWM3, GWM18, GWM95, and GWM155. Primers used for the amplification of adequate microsatellite loci (*Xgwm*) are according to RÖDER *et al.* (2002). In the literature, these markers are linked to agronomically important traits (QLT loci) such as falling number and α-amylase activity, pre-harvest sprouting, disease resistance, etc. (WANG *et al*., 2009; KUMAR *et al*., 2010). Nevertheless, these markers can also be used for genetic diversity analysis.

Fluorescently labelled primers were used to multiply microsatellite fragments during polymerase chain reaction (PCR). Fluorescent dye was used to label the forward primer: blue-6-FAM (GWM3), red-PET (GWM18) and green-VIC (GWM95 and GWM155), while the reverse primer was left unlabelled. The obtained PCR products were size separated based on the principle of capillary electrophoretic separation on the genetic analyser Applied Biosystems 3130.

Data were analysed via Microsoft Excel-Software. Polymorphism level was determined by polymorphism information content (PIC) value calculated according to ANDERSON *et al.* (1993): $PIC= 1- \sum p_i^2$, where p_i^2 is the square of relative frequency of each allele. Amplified fragments were scored in binary format in such a way that the product presence of certain size was marked by 1 and its absence by 0. Binary data were used to calculate genetic connections among genotypes by applying similarity coefficients according to JACCARD (1908). Matrix with similarity coefficients was used for further cluster analysis and construction of dendrogram using SPSS Software Statistic version 17.0.

Table 1. List of wheat genotypes and their origin

No.	Genotype	Origin	No.	Genotype	Origin
1	Per.Gabot.	ARG	45	Flamura 80	ROM
2	Klein Forten	ARG	46	Krasnodar.39	RUS
3	Gaboto	ARG	47	Bezostaja1	RUS
4	Condor	AUS	48	Jubilajnaja 50	RUS
5	Banks	AUS	49	Stepnaja 30	RUS
6	Kite	AUS	50	Kavkaz	RUS
7	Cook	AUS	51	FTHP Redeemer	SRB
8	Jozef	AUT	52	Pesma	SRB
9	Amadeus	AUT	53	Jugoslavija	SRB
10	Priaspa	BGR	54	Balkan	SRB
11	Vraca	BGR	55	Košuta	SRB
12	Rusalka	BGR	56	Renesansa	SRB
13	Yantar	BGR	57	Sofija	SRB
14	Garazinko	BRA	58	Pobeda	SRB
15	Dobrich	BUG	59	Simfonija	SRB
16	Manitou.Ins.	CAN	60	Oda	SRB
17	Pembina	CAN	61	Brkulja 4	SRB
18	Winalta	CAN	62	Banaćanka 1	SRB
19	Chris	CAN	63	Sonata	SRB
20	Marquis	CAN	64	KG 56	SRB
21	Al-Kan-tzao	CHN	65	NS732	SRB
22	Žitarka	CRO	66	NS736	SRB
23	Lada	CZE	67	Cipovka	SRB
24	Bazalt	DEU	68	Danica	SRB
25	Roason	FRA	69	NS30/95	SRB
26	Top	FRA	70	NS900	SRB
27	Chanplein	FRA	71	Arija	SRB
28	Noe	FRA	72	Diana	SWE
29	Maris Hunt.	GBR	73	Arina	SWZ
30	Bersee	GBR	74	Sardona	SWZ
31	Sanja	HRV	75	Tom Thumb	TIB
32	Bankut 1205	HUN	76	Bolal	TUR
33	MV 21	HUN	77	Kirac 66	TUR
34	MV 19	HUN	78	Prometej	UKR
35	MV17	HUN	79	Odeska 51	UKR
36	MV 20	HUN	80	Nahodka 4	UKR
37	Irnerio	ITA	81	Tavricanka	UKR
38	Mara	ITA	82	Mirono. 808	UKR
39	Aobakomughi	JPN	83	Tarasov. 29	UKR
40	Saitama27	JPN	84	Atlas 66	USA

41	Ai-Bian	JPN	85	Florida	USA
42	Suwon 92	KOR	86	Stephens	USA
43	Siete Cerros	MEX	87	Phoenix	USA
44	Lerma rojo	MEX			

RESULTS AND DISCUSSION

Polymorphism indicators

In order to determine genetic polymorphism, 87 wheat genotypes were analysed with 4 microsatellite markers (GWM3, GWM18, GWM95, and GWM155), which are located on chromosomes 3D, 1B, 2A, and 3A (Table 2). Within four microsatellite loci total of 28 allele variants were detected. Mean number of alleles per marker was 7. Studying genetic diversity of the set of 96 genotypes from the genetic collection of Institute of Field and Vegetable Crops from Novi Sad, Serbia by using 36 microsatellite markers, KOBILJSKI *et al.* (2002) detected 7.96 alleles per locus. Similar relation of allele variants per locus confirms the statement of polymorphism of locus and stability of genetic diversity within the breeding material composed of genotypes originating from different breeding centres of the world. Besides this, it was shown that with a relatively small number of used SSR markers it was possible to determine a high level of polymorphism.

Table 2. Description of the used microsatellite markers including allele number and PIC value

Marker	Chromosome	Product size (bp)	Dominant allele (bp) and its frequency	Total no. of alleles (rare alleles)	PIC
GWM 3	3D	71-81	75 (0.66)	6 (1)	0.52
GWM 18	1B	Null, 178-196	186 (0.35)	8 (2)	0.80
GWM 95	2A	104-122	116 (0.49)	7 (2)	0.65
GWM 155	3A	Null, 124-148	140 (0.33)	7 (0)	0.77
Total				28 (5)	
Mean				7 (1.25)	0.69

Determined level of DNA polymorphism in this study was higher than polymorphism found in European elite wheat cultivars research by other authors (PLASCHKE *et al.* 1995; STACHEL *et al.* 2000). These authors found between 5.2 and 6.2 alleles per locus. Similar values for mean number of alleles per locus were reported by LANDJEVA *et al.* (2006) in Bulgarian winter wheat cultivars (6.8) and CHAO *et al.* (2007) in U.S. elite wheat cultivars (7.2). However, study of 559 French wheat genotypes found as many as 14.5 alleles per locus (ROUSSEL *et al*., 2004), while in Chinese cultivars there were 11.7 alleles per locus (GUO *et al*., 2011).

The smallest number of alleles (6) was found at locus *Xgwm*3, size ranging from 71 to 81 bp, while at locus *Xgwm*18 there was the largest number of allele forms (8) size ranging from 178 to 196 bp (Table 2, Figure 1). Locus *Xgwm95* had total of 7 alleles sized from 102 bp to 144 bp, while the locus *Xgwm155* had 7 alleles sized from 124 bp to 148 bp. Two markers were found to have null alleles (Gwm18 and Gwm155). There were total of 5 rare alleles whose frequency was below 2%, with the exception of *Xgwm155* which had no rare alleles. PIC values ranged from 0.52

for *Xgwm3* to 0.80 for *Xgwm18*. Mean PIC value was 0.69 which points to a high level of the detected polymorphism.

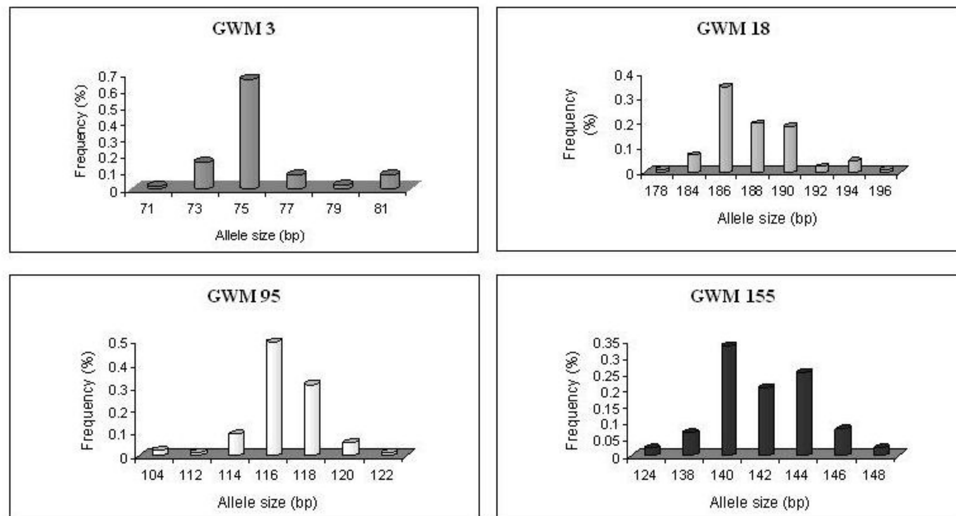

Figure 1. Allele frequencies at four microsatellite loci

The results on total number of alleles, mean allele number per marker, number of rare alleles and PIC values were very similar to those of RÖDER *et al*. (2002) who analysed 500 European wheat cultivars with 20 SSR markers. RÖDER *et al*. (2002.) determined the following values: number of alleles for *Xgwm3* was 5, PIC 0.55*, Xgwm18* had 10 alleles, and PIC value was 0.75, 9 alleles were at locus *Xgwm95,* PIC value 0.63, while *Xgwm*155 also had 9 alleles, and PIC value was 0.68. Even though RÖDER *et al.* (2002) carried out a much more extensive research, they too found the highest polymorphism at locus *Xgwm18*. Similar number of allele forms and a relatively high level of polymorphism per loci were also reported by KOBILJSKI *et al*. (2002) and MACCAFERRI *et al*. (2008).

Microsatellite markers were additionally used to characterise and assess the genetic diversity of 40 wheat cultivars developed in Serbia (KONDIĆ-ŠPIKA *et al*., 2008). Total of 115 alleles were detected with the mean allele number 6.05 per locus. Presence of null alleles was not detected at any of 19 loci. PIC value of individual markers ranged from 0.096 for *Xgwm408* to 0.850 for *Xgwm18*, with average of 0.641 for all markers. These results show a high level of polymorphism. PIC values were not connected with the number of alleles at a certain locus, which is in accordance with the results of PRASAD *et al*. (2000).

Nonetheless, HUANG *et al*. (2002) found significant correlation between PIC values and number of alleles. They showed that it takes a large number of samples to determine a reliable

correlation. Sample size in their analysis was 998 genotypes, while the study of PRASAD *et al.* (2000) included 55 samples.

Cluster analysis

Similarity coefficient among genotypes was calculated according to JACCARD (1908). Based on genetic similarity, genotypes were grouped in four clusters designated I, II, III, and IV (Figure 2). Cluster I comprises genotypes from Sonata to Kite (36 genotypes), cluster II comprises genotypes from Klein Forten to line NS900 (27 genotypes), cluster III comprises genotypes from Nahodka 4 to Dobrich (4 genotypes), and cluster IV from Kondor to Rusalka (17 genotypes). Each cluster consists of larger number of sub-clusters, e.g. cluster II has three sub-clusters (IIa, IIb, and IIc). Some of the genotypes stand out separately not belonging to any of the four clusters (Per. Gabot, Atlas 66, Stepnaja 30). Stepnaja 30 is connected to other genotypes only at the highest hierarchical level, meaning that it is genetically most distant.

Genotype grouping was not completely connected to geographical origin, but certain regularity arises. Cluster I can be considered most divergent regarding origin of genotypes; it contains the largest number of Serbian cultivars (11) and surrounding countries, but there are also genotypes originating from other parts of Europe, Asia, North and South America. Cluster II contains 10 Serbian cultivars, while the rest are mostly genotypes from the East, with the exception of several genotypes from Argentina (2), Canada (1) and Brazil (1). Clusters III and IV contain no Serbian genotypes. Cluster III actually comprises only four genotypes, two of which are from Bulgaria, one from Croatia and one from Ukraine. Cluster IV mostly comprises genotypes from Western Europe and North America. There are five genotypes, however, originating from the East (Bulgaria, Japan, Korea and Tibet). Complete or partial grouping of genotypes into clusters according to their geographical origin or breeding centre was also reported by RÖDER *et al.* (2002), LANDJEVA *et al.* (2006), HUDCOVICOVA *et al.* (2013), VANZETTI *et al.* (2013).

Molecular evaluation showed that some genotypes seemed genetically identical due to determined similarity coefficient value 1 (Sonata and Danica, NS732 and Cipovka, Mara and Al-Kan-Tzao, Condor and Stephens, Bankut and Sonata, etc). This certainly is not the case of identical genotypes, but results from the fact that the used markers were inadequate for distinguishing between them. Their divergence is expressed at the level of other loci that were not encompassed in this study. Similar observations were given by PRASAD *et al.* (2000), HUANG *et al.* (2002), and AKKAYA and BUYUKUNAL-BAL (2004).

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Sonata           72   -+
  Danica           83   -+
  Bankut 1205      17   -+-------------------+
  Lada             30   -+                   |
  Banaćanka 1      61   -+                   |
  Mara             64   -+-------------------+-----+
  Al-Kan-tzao      78   -+                   |     +-+
  Chris            18   ---------------------+     | |
  FTHP Redeemer    13   ---------------------------+ |
  Oda              52   -+                           |
  Tarasov. 29      69   -+                           |
  Sardona          21   -+                           |
  Sofija           43   -+-----------------+         +---+
  Pobeda           46   -+                 |         |   |
  Balkan           31   -+                 +-+       |   |
  Prometej         11   -+                 | |       |   |
  Brkulja 4        56   -+-----------+     | +-----+ |   |
  Manitou.Ins.      8   -+           +-----+ |     | |   |
  MV 21            22   -------------+       |     +-+   |
  Jozef            12   ---------------------+     |     +-+
  Renesansa        42   ---------------------------+     | |
  Winalta          16   ---------------------+-----------+ |
  Phoenix          76   ---------------------+           | |
  MV 19            28   -------------+-------------------+ |
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  Lerma rojo        51   -------------+                    +---+
  Diana             14   -------------+---------------------+   |
  Sanja             63   -------------+                     |   |
  Tavricanka        57   ---------------------+-------------+   |
  NS30/95           84   ---------------------+                 |
  Bolal              2   ---------------------------------------+-+
  Banks             37   ---------------------+---------+       | |
  Kavkaz            60   ---------------------+         +---+   | |
  Siete Cerros      36   ---------------------+         |   |   | |
  Irnerio           53   ---------------------+---------+   +---+ |
  Arina             15   ---------------------+             |     |
  Kite              40   -----------------------------------+     |
  Klein Forten       7   -+----------+                            |
  Košuta            41   -+          +------------+
+---+
  Arija             87   -----------+           +--------+        |
|
  Odeska 51         25   -----------------------+       +---+     |
|
  Krasnodar.39       3   ---------------------+------+   |   |     |
|
  Saitama27         77   ---------------------+     +---+   |     |
|
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```
  Flamura 80       32   -+--------------------+     |       |     |
|
  Priaspa          50   -+                    +-----+       |     |
|
  Gaboto           70   ----------------------+             |     |
|
  NS736            80   ----------------------+             |     |
|
  MV17             29   -------------+---+                  +-----+
|
  KG 56            73   -------------+   +-------------+    |
|
  Marquis          65   -----------------+             +-+  |
|
  MV 20            54   -------------+---------------+ | |  |
|
  Ai-Bian          82   -------------+               +-+ |  |
+-+
  Bezostaja1        5   -+---------------------+     |   |  |
| |
  Jugoslavija      27   -+                     +-----+   |  |
| |
  Jubilajnaja 50    9   -+--------------+      |         |  |
| |
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  Garazinko        19   -+             +-------+         +-+
| |
  Cook             58   -----------+---+                 |
| |
  Mirono. 808      62   -----------+                     |
| |
  NS732            79   -+-----------+                   |
| |
  Cipovka          81   -+         +-----+               |
| |
  Simfonija        49   -------------+     +-------+     |
| |
  Pesma            24   -------------------+       +---+ |
| |
  Kirac 66         20   ---------------------------+   +-+
| |
  NS900            85   -------------------------------+
| |
  Per.Gabot.        1   -----------------------------------+------
---+ |
  Atlas 66          6   -----------------------------------+
|
  Nahodka 4        38   ---------------------+--------------------
---+ |
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  Yantar            75   ----------------------+
+-+
  Žitarka            4   -----------------------------------+------
---+ |
  Dobrich           26   -----------------------------------+
+-+
  Condor            35   -+---------------------------+
| |
  Stephens          74   -+                           +-+
| |
  Top               45   -+-----------------+         | |
| |
  Chanplein         55   -+                 +---------+ |
| |
  Suwon 92          44   -+-----------+     |           +---+
| |
  Aobakomughi       48   -+           +-----+           |   |
| |
  Amadeus           71   -------------+                 |   +------
---+ | |
  Pembina           10   -------------------------------+   |
| | |
  Florida           59   ---------------------+-----------+ |
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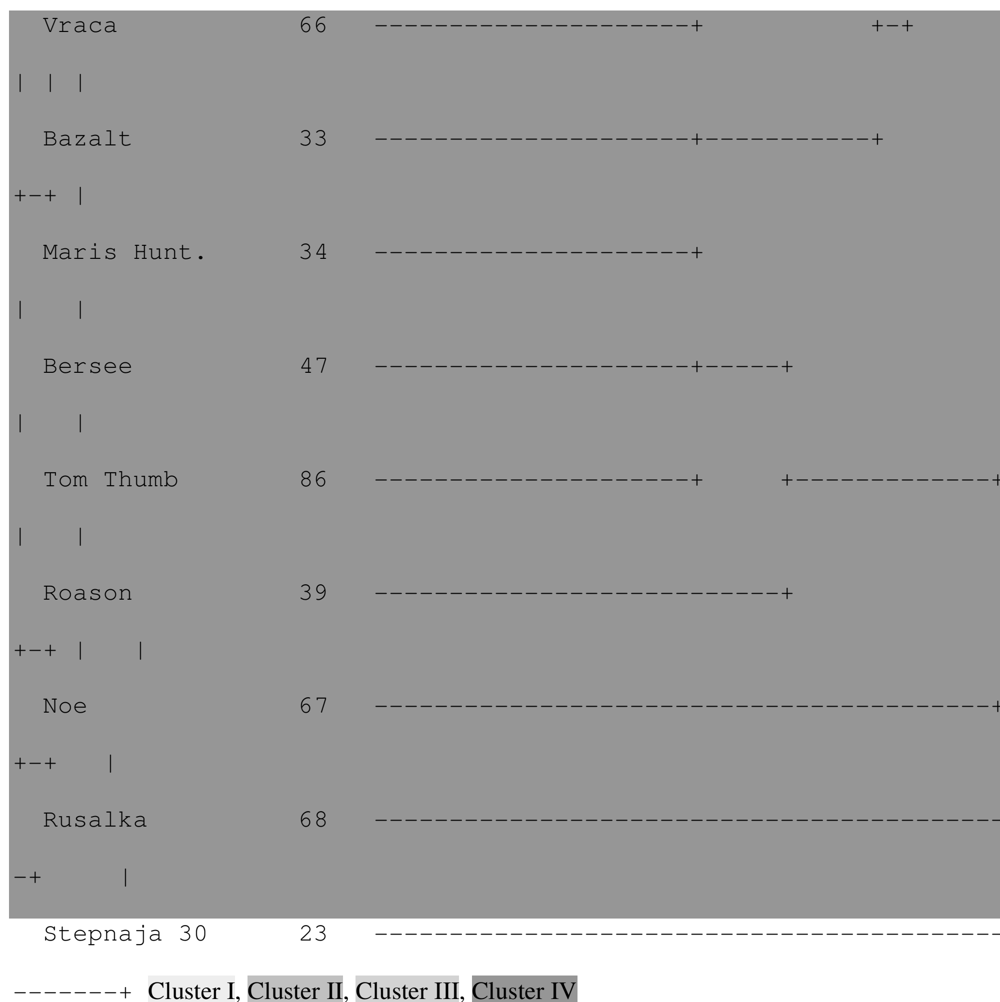

Figure 2. Dendrogram representing 86 wheat genotypes

Genotype grouping was not completely connected to geographical origin, but certain regularity arises. Cluster I can be considered most divergent regarding origin of genotypes; it contains the largest number of Serbian cultivars (11) and surrounding countries, but there are also genotypes originating from other parts of Europe, Asia, North and South America. Cluster II contains 10 Serbian cultivars, while the rest are mostly genotypes from the East, with the exception of several genotypes from Argentina (2), Canada (1) and Brazil (1). Clusters III and IV contain no Serbian genotypes. Cluster III actually comprises only four genotypes, two of which are from Bulgaria, one from Croatia and one from Ukraine. Cluster IV mostly comprises genotypes from Western Europe and North America. There are five genotypes, however, originating from the

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If more markers were used in the study, these genotypes would certainly differentiate, even though they are probably related with similar pedigree. For example, cultivars Sonata and Danica have Bezostaja 1 in their pedigree, but not as a direct parental component. Low similarity coefficient showed genetically most distant genotypes (Pembina and Roason 0.15, Žitarka and Nahodka 0.143, Per. Gabot and Banks 0.129, Per. Gabot and Žitarka 0.121, Per. Gabot and Pembina 0.100, Per. Gabot and Stepnaja 0.046). Such genotypes can be recommended for crossing if new gene recombination and widening of genetic variability are desired.

The largest number of alleles was detected in cluster II (19) as compared to cluster I (18), cluster III (10) and cluster IV (16) (Table 3). Average number of alleles per cluster was 15.7, while average per locus for all clusters was 3.94. The largest number of rare alleles was recorded in clusters II (8) and IV (7). Clusters also differed regarding the presence of null alleles per loci. Marker GWM3 had one null allele in cluster II and one in cluster III. The highest number of null alleles was detected by GWM18 marker (two in cluster I, four in cluster II, one in cluster III and three in cluster IV). One null allele was found in cluster I by using marker GWM155, while no null alleles were detected using marker GWM95. The highest mean PIC value was found in cluster III and cluster IV (0.52). Mean PIC value for all clusters (0.49) was significantly lower than the PIC value determined in total material prior to grouping into clusters (0.69). This difference is a logical consequence of grouping genotypes in clusters according to their similarity, which significantly decreased polymorphism within clusters.

Table 3. Polymorphism indicators in different clusters

	Cluster I	Cluster II	Cluster III	Cluster IV	Mean
No. of genotypes	36	27	4	17	21
Total no. of alleles	18	19	10	16	15.7
Mean no. of alleles at locus	4.5	4.75	2.5	4	3.9
No. of rare alleles (frequency <2%)	5	8	4	7	6
Mean no. of rare alleles by marker	1.25	2	1	1.75	1.5
No. of null alleles (locus)	2 (*Xgwm*18) 1 (*Xgwm*155)	1 (Xgwm3) 4 (Xgwm18)	1 (Xgwm3) 1 (Xgwm18)	3 (Xgwm18)	
Heterozygosity	1	2	1	1	1.25
Mean PIC value	0.44	0.46	0.52	0.52	0.48

Analysis of allele distribution in clusters for each locus shows differences between them (Table 4). Dominant allele at locus *Xgwm3* (75 bp) had the highest frequency in clusters I and II. *Xgwm18* locus had dominant allele sized 188 bp with the highest frequency only in cluster IV. Dominant allele (116 bp) at locus *Xgwm95* had the highest frequency in cluster I, while dominant allele at locus *Xgwm155* (144 bp) had frequency 0.65 in cluster IV.

Table 4. Distribution of dominant alleles by clusters and their frequencies

Marker	Cluster I	Cluster II	Cluster III	Cluster IV
GWM 3	75 (0.80)	75 (0.96)	73 (0.80)	73 (0.50)
GWM 18	186 (0.59)	186 (0.44)	190 (0.50)	188 (0.86)
GWM 95	116 (0.89)	118 (0.82)	116 (0.50)	116 (0.47)
GWM 155		142 (0.20)	146 (0.50)	144 (0.65)

For marker GWM3, which is connected to the falling number and α-amylase production in wheat, two alleles were dominant among clusters (Table 4). The most dominant allele 75 bp was detected in clusters I and II. Frequency of this allele is 0.85 for cluster I, and 0.96 for cluster II. Allele sized 73 bp was more present in cluster III (0.8) and cluster IV (0.5).

Dominant alleles by clusters for marker GWM18 were alleles sized 188 (frequency 0.86) for cluster IV, 186 bp, (frequency 0.59 for cluster I and 0.44 for cluster II) and allele 190 bp (frequency 0.5) for cluster III (Table 4). Rare alleles were sized 178, 184 bp for all four clusters, as well as alleles sized 192 and 194 bp (Figure 3). The presence of two dominant allele peaks in clusters I and II is explained by a large number of sub-clusters in these groups (Figure 2).

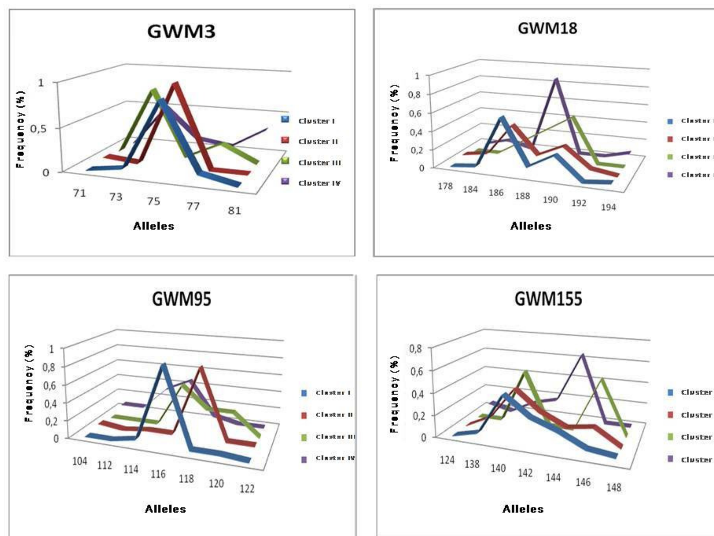

Figure 3. Distribution of alleles by clusters in four microsatellite loci

For marker GWM95 dominant alleles in clusters were sized 116 bp and 118 bp. The rare allele was sized 122 bp (Figure 3). Dominant allele for marker GWM155 in clusters I and II was sized 142 bp, allele sized 146 bp in cluster III, while in cluster IV the dominant allele was sized 144 bp.

This study showed that by using a smaller number of highly-polymorphic microsatellites, genetic variability can be studied and genotypes can be grouped according to similarity. Although the genotypes were not completely differentiated by the used SSR markers, a high level of polymorphism was detected in the breeding material, and at the same time genotypes were preliminarily grouped according to similarity. Polymorphic genotypes probably carry unique and potentially useful genes, and can thus be recommended for further breeding efforts.

The results showed reliability, usefulness, and efficiency of microsatellites. Amplification of microsatellite markers in the studied genotypes and high level of polymorphism enable further study of potential connection between these markers and agronomically important traits in our agro-ecological conditions.

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MIKROSATELITI U ANALIZI GENETIČKOG DIVERZITETA PŠENICE

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Izvod

Mikrosatelitski markeri (SSR) korišćeni su za proučavanje genetičkog diverziteta. Set od 87 genotipova pšenice analiziran je sa 4 SSR markera. Prajmeri za amplifikaciju odgovarajućih mikrosatelitnih lokusa (Xgwm) su po Röder et al. (1998). Rezultati su dobijeni na Applied Biosystems 3130 genetskom analizatoru. Ukupno 28 alela bilo je zabeleženo, što je u proseku 7 alela po markeru. Broj alela za pojedinačne markere kretao se u rasponu od šest (Xgwm3) do osam (Xgwm18). Utvrđeno je prisustvo i dva nulta alela za Xgwm18 i Xgwm155. Retkih alela (frekvencije <2%) bilo je 5. Vrednosti polimorfizma (PIC) bile su u rasponu od 0,52 za Xgwm408 do 0,80 za Xgwm18. Prosečna PIC vrednost bila je 0,69 za sve markere, što ukazuje na visok nivo detektovanog polimorfizma. Na osnovu informacija dobijenih analizom 4 markera, većina genotipova može biti podeljena u klastere. Rezultati pokazuju korisnost mikrosatelitnih markera za detekciju polimorfizma, za identifikaciju genotipa i za procenu genetičkog diverziteta.

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