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Assessment of genetic diversity of wheat genotypes using microsatellite markers

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Abstract

Background and Purpose: *Genetic diversity is the material basis for crop improvement. In this study, genetic diversity of 30 wheat genotypes was evaluated at the DNA level using 24 simple sequence repeat (SSRs) markers.*

Materials and Methods: *DNA extraction was performed according to the modified CTAB-method. Microsatellite analysis was performed using fluorescent fragment detection on a LI-COR 4200 DNA.*

Results and Discussion: *The number of alleles per locus ranged from 1 to 14 with an average number of 8.44 alleles per locus. The highest number of alleles per locus was detected in the genome A with 7.2, compared to 5.9 and 5.0 for genomes B and D, respectively. The highest number of alleles was recorded at chromosome 7 (9.5), while the lowest number of alleles was detected at chromosomes 3 and 4 (5.0 and 5.3). The smallest genetic distance characterized genotypes Super Zitarka and Zitarka, Tena and Osjecanka, Tena and Bezostaja, Lela and Toras, Janica and Alka, Felix and Seka. Genotypes Pipi and Courtot showed the least genetic similiarities with rest of the genotypes.*

Conclusions: *The identification of genetic diversity should be a good tool of selecting genotypes in breeding programs.*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important crop grown in Croatia, with production approximately 3.87 t ha^{-1} on average from 1996 till 2006 *(1)*. It has one of the largest and most complex genomes of cereals. It is alohexaploid (2n=6x=42, AABBDD) with three homeologous genomes *(2)*. The haploid genome of hexaploid wheat is 16 Mb (16 billion base pairs of DNA) (in comparison with the human genome 5x more, which has approximately 3 Mb). Genetic diversity is the basis for the improvement of traits *(3, 4)*. Morphological traits can be used to characterize genetic diversity, and are often influenced by environmental factors. Today, considerable attention is dedicated to the usage of molecular markers.

In breeding programs, it is desirable to have large genetic diversity for the creation of new genotypes. The aim is to measure the genetic similarity (GS) and genetic distance (GD) among parents, which can be used to estimate the expected genetic variation in different combinations of progeny. In general, the study of genetic diversity has two major objectives: 1) analysis of the levels of polymorphism among certain individuals and 2) studies of the distribution of polymorphism

(5). Genetic diversity can be assessed form pedigree analysis, morphological traits or using molecular markers and it is the material basis for crop improvement *(6)*. DNA markers are technology that can increase breeding progress, especially for traits that are difficult to select under field conditions and that are controlled by multiple genes. Microsatellites are repeating sequences of 2–6 base pairs of DNA (SSRs; Simple sequence repeats) and are among the most stable markers of genetic variation and divergence among wheat genotypes because they are multiallelic, chromosome-specific and evenly distributed along chromosomes *(7)*. Microsatellite genotyping is used for genetic biodiversity, population genetics at the level of relatedness, genome mapping, as markers for pathogens, etc. Hypothesis is that the relationship of parents affects the genetic diversity. The aim of this research is to determine the genetic diversity of the investigated genotypes.

MATERIALS AND METHODS

Plant material

In Table 1, the origin and pedigree of tested genotypes are shown.

Molecular analysis of genetic diversity

In this study, we used microsatellite markers to investigate relationships among 30 wheat genotypes. For microsatellite analysis fresh leaves (1–2 cm long) were se-

TABLE 1

Origin and pedigree of the examined genotypes.

Genotype	Origin	Pedigree
Srpanjka	Croatia	Osk.4.50-1-77/Zg.2696
Zitarka	Croatia	Osk.6.30/2/Slavonka//Osk.6.78/1-73/Kavkaz
Golubica	Croatia	Slavonija/Gemini
Super Zitarka	Croatia	GO3135/Zitarka
Janica	Croatia	Osk.5.36/9-91/Srpanjka
Lucija	Croatia	Srpanjka/Kutjevcanka
Alka	Croatia	Osk.5.140-22-91/Sana
Divana	Croatia	Favorit/5/Cirpiz/4/Jang-Kwang/2/ Atlas-66/Comanche/3/Velvet
Lela	Croatia	Srpanjka/Super Zitarka
Pipi	Croatia	Soissons/Osk.6.83-5-91
Katarina	Croatia	Osk.5B.4-1-94/Osk.5.140-22-91
Renata	Croatia	Zitarka/Osk.7.5-4-82/Kom.Bg.160-86//Srpanjka
Aida	Croatia	Srpanjka/Rialto
Seka	Croatia	Srpanjka/Demetra
Felix	Croatia	Srpanjka/Kom.Bg.160-86
Soissons	France	Iena/HN-35
Renan	France	Mironovskaya 808/Maris Huntsman//VPM1/Moisson/3/Courtot
Sirban Prolifik	Hungary	Unknown pedigree
U1	Croatia	Carlotta Strampelli/Marquis
Libellula	Italy	Tevere/Guiliani//San Pastore
Bezostaja	Former USSR	Skorospelka 2/Lutenscens 17
Zlatna Dolina	Croatia	Leonardo/ZG 414/57
Tena	Croatia	Libellula/Bezostaja 1
Osjecanka	Croatia	Tena (EMS1.5%)
Courtot	France	Mexique-50/B-21-Versailles
Frontana	Brazil	Frontiera/Mentana
Sumai 3	China	Funo/Taiwan-wheat
Chinese Spring	China	LV/Sichuan
Toras	Germany	Taras/Stamm//Herevard/3/Tarso
Hermann	Germany	Nic90-3390A/Xanthos

lected randomly from each genotype. After drying in a lyophilizer for a period of 72 hours, they were ground in a laboratory oscillatory mill for 5–10 minutes (MM 301, Retsch, Germany).

DNA extraction

DNA extraction was performed according to the modified CTAB-method *(8)*. The quantity of DNA was measured with a UV spectrophotometer at 260 nm and adjusted to a concentration of 100 ng/µL. Amplification products were separated by electrophoresis (60V, 60 mA, 100W, 60–90 min) in 0.7% agarose gels in 1 TAE buffer stained with 2 µL/100 ml ethidium bromide. The gel was scanned with a transluminator.

Microsatellites (SSRs)

In this study, 24 SSR markers were applied. Primer sequences were obtained from Graingenes database (http://graingenes.org). Microsatellite analysis was performed using fluorescent fragment detection on a LI-COR 4200 DNA dual-dye DNA analyzer system. For this method, either of the SSR primers was directly labelled with a fluorochrome (IRD700 or IRD800) or had a M13 tail. In the latter case, a flourochrome labelled M13-30 oligo (5 'CCC AGT CAC GAC GTT G 3') was, as a third primer, added to the PCR reaction. PCR for directly labelled SSRs was done according to Roeder *et al. (7)* and for M13 tailed primers according to Steiner *et al. (9)*. The PCR products were separated in 25-cm plates of the LI-COR analyzer using 7% polyacrylamide gels (Long Ranger, FMC).

Statistical analysis

Gene diversity was calculated according to formula of Nei (1973):

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

where Pij is the frequency of the jth allele for ith locus summes across all alleles for the locus. Matrix similarity of genotypes were calculated by using NTSYSpc.2.1 *(10)* with Sanh-clustering using the UPGMA (Unweighted Paired Group Method Using Arithmetic Averages) method. We used two different coefficients: BAND *(11)* and Dice *(12,13)*. The results are presented graphically in dendrograms.

RESULTS

To assess genetic distance among 30 wheat genotypes, 24 microsatellite markers were used for 18 loci. The number of alleles per locus ranged from 1 (*Gwm888*) to 14 (*Gwm681*), with the average number of 8.44 alleles per locus (Table 2). The highest number of alleles per locus was detected in genome A with 7.17, compared to 5.86 and 5.00 for genomes B and D, respectively.

Microsatelite PIC values ranged from 0.07 to 0.90 (Table 2). Approximately 87.5% of microsatellite markers that permeate chromosomes 7 A, B and D genomes had a PIC value greater than 0.50, which indicates that the majority of markers enabled a high level of polymorphism. The most polymorphic SSR marker *Gwm681* was at chromosome location 7A with 14 alleles and the highest PIC value of 0.90. The highest number of alleles was recorded at chromosome 7 (9.50), while the lowest

TABLE 2

Microsatellite markers, their chromosomal location, the expected allele size, the amplified number of alleles and polymorphism information content.

Mm	Cl	Eas	Na	PIC	Mm	Cl	Eas	Na	PIC
Gwm164	1A	120	7	0.76	*Barc319*	5A	–	7	0.82
Gwm 642	1D	180–200	5	0.69	*Gwm 408*	5B	–	7	0.67
Gwm 558	2A	–	7	0.75	*Gwm 335*	5B	200–240	9	0.82
Wmc667	2A	–	10	0.82	*Gwm 190*	5D	200–250	2	0.24
Gwm 120	2B	150–170	9	0.82	*Barc3*	6A	–	5	0.75
Gwm 349	2D	210–260	6	0.76	*Gwm 427*	6A	180–200	6	0.79
Gwm 1071	3A	150	8	0.76	*Gwm 219*	6B	150–190	8	0.83
Barc84	3B	–	2	0.07	*Gwm 816*	6B	180–190	5	0.72
Gwm 160	4A	180	5	0.56	*Barc273*	6D	225–240	3	0.60
Gwm 610	4A	170	6	0.81	*Gwm 681*	7A	190	14	0.90
Gwm 888	4B	195	1	0.08	*Gwm 870*	7A	135	5	0.78
Gwm 624	4D	130–140	9	0.84	*Barc56*	5A	125	6	0.66

Mm – rosatellite marker; Cl – Chromosal location of a marker; Eas – Expected allele size (bp); Na – Number of alleles; PIC – Polymorphism information content

TABLE 3

The amplified number of alleles and the number of used microsatellites through genomes and chromosomes.

Genome	Na	Number of used microsatellites
A	7.17	12
B	5.86	7
D	5.00	5
Chromosome		
1	6.50	2
2	8.00	4
3	5.00	2
4	5.25	4
5	6.20	5
6	5.40	5
7	9.50	2

Na – Number of alleles

number of alleles was detected at chromosomes 3 and 4 (5.00 and 5.25) (Table 3).

The lowest genetic similarity was found between genotypes Pipi and Chinese Spring (0.00), Pipi and Sumai 3 (0.05), Janica and Courtot (0.05), Janica and Pipi (0.05), while the values were the same for Dice and Band coefficient. The highest genetic similarity was obtained between the genotypes Super Zitarka and Zitarka (0.65), Tena and Osjecanka (0.65), with the same values for Dice and Band coefficient. Similar values were found for Tena and Bezostaja (Dice = 0.60, Band = 0.62), Lela and Toras (0.65, 0.60), Janica and Alka (0.60, 0.61), Felix and Seka (0.57, 0.62), with slight differences in coefficients (Figure 1, 2).

DISCUSSION

This study determined the genetic diversity among 30 winter wheat genotypes. 24 markers amplified a total of 152 alleles, with the average number of 6.33 alleles per locus. These results are comparable with the results of other authors *(14)*. The largest number of alleles (7.17) per locus was determined in A genome, somewhat less alleles were found in B genome (5.86), and D genome (5.00). These values are higher than those found by Dreisigacker *et al. (15)*. They found that the average number of alleles was 5.90 (A genome), 6.80 (B genome) and 5.80 (D genome). A slightly higher average number of alleles was found by Dvojkovic *(16)* (D = 9.65, A = 8.86, B = 8.93). PIC values of microsatellite markers ranged from 0.07 to 0.90, which is similar to findings of other authors *(17)*. Observing the values calculated using Dice and Band coefficient was expected to be high among Super Zitarka and Zitarka because one parent Super Zitarka is Zitarka. A mutation was made on genotype Tena (with 1.5% EMS), and genotype Osjecanka was created, which is the reason for their genetic similarity.

High genetic similarity coefficients were obtained between genotypes Tena and Bezostaja because Bezostaja is one of the parents of Tena. Genotypes Seka and Felix were also similar in genetic structure because they have a common parent Srpanjka. Genotypes Janica and Alka

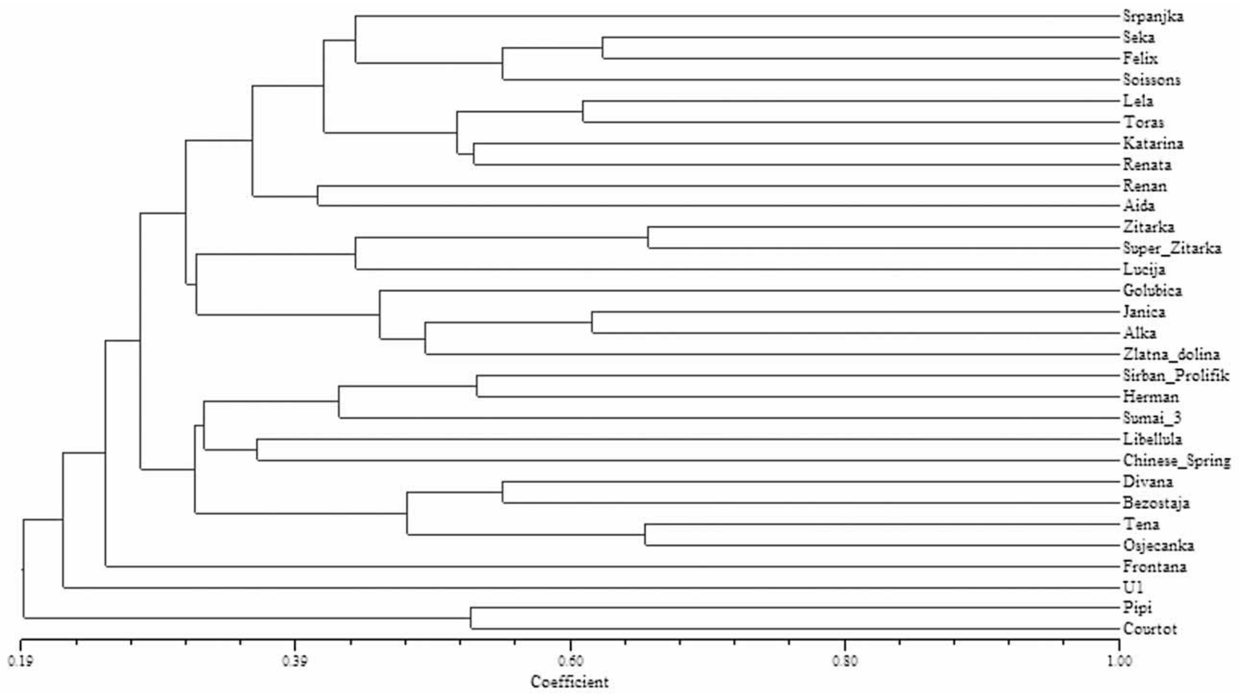

Figure 1. *Dendrogram of 30 wheat genotypes based on 24 SSR markers. X-axis values correspond to Band's coefficient of similarity.*

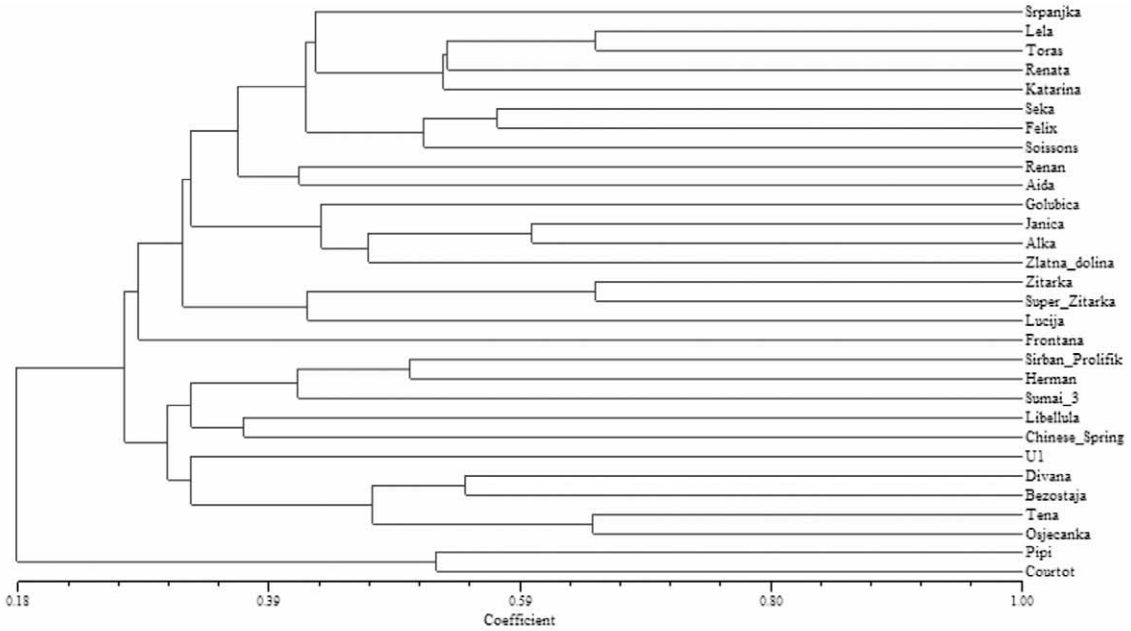

Figure 2. *Dendrogram of 30 wheat genotypes based on 24 SSR markers. X-axis values correspond to Dice's coefficient of similarity.*

also a share a high similarity, also as a result of a common parent (Alka has a parent-Osk.5.140 22–91, which is originally related to genotype Srpanjka, and Srpanjka is one of the parents to Janica). It is difficult to explain high genetic similarity between Lela and Toras; it probably originates from some distant common alleles. Dendogram is based on the similarity of genotypes divided into two large groups. One group, including Pipi and Courtat, stood apart from other groups, as they had U1 genotype separated from other genotypes. For more precise genetic distance between genotypes, it is necessary to use a larger number of microsatellite markers.

Although 570 microsatellite sequences have been developed, This number is insufficient due to the large genome of wheat *(18)*. This paper confirmed the hypothesis that the relationship of parents placed genotypes into the same groups on the basis of common alleles. This type of investigation about information on genetic diversity is helpful for developing appropriate science-based strategies for wheat breeding *(19)* and it can be a good tool of selecting genotypes in breeding programs.

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