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Genetic diversity of hexaploid wheat cultivars estimated by RAPD markers, morphological traits and coefficients of parentage

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Abstract

Estimates of genetic diversity can be based on different types of data. The aim of this research were to study genetic diversity among Croatian wheat cultivars by random amplified polymorphic DNA (RAPD) markers, morphological traits and pedigree records; to analyse differences between wheat cultivars from two breeding centres; and to evaluate usability of RAPD markers for estimation of genetic diversity among wheat cultivars in comparison with morphological traits and pedigree record data. Studies were conducted on 14 wheat cultivars and breeding lines from two breeding centres in Croatia. For the RAPD analysis 36 primers were screened and the 14 most polymorphic ones yielded 341 polymorphic bands. Twelve morphological traits were used for morphological analysis. Pedigrees were composed of seven generations of ancestors. RAPD markers showed a high level of polymorphism among the cultivars examined and the breeding lines. No significant correlations were observed among the methods tested.

Key words: *Triticum aestivum* — genetic diversity — morphological traits — pedigree records — random amplified polymorphic DNA markers

Genetic diversity in cultivated crops is essential for successful breeding and creation of new cultivars. According to Franco et al. (2001) the study of phenotypic and genetic diversity to identify groups with similar genotypes is important for conserving, evaluating and utilizing genetic resources; for studying the diversity of pre-breeding and breeding germplasm; and for determining the uniqueness and distinctness of the phenotypic and genetic constitution of genotypes with the purpose of protecting a breeder's intellectual property rights. Knowing the genetic diversity of wheat germplasm is necessary for identifying diverse parental combinations and creating segregating progeny with high genetic variability for selection. Criteria for the estimation of genetic diversity can be different: pedigree records (Cox et al. 1985), morphological traits (Marić et al. 1998) or molecular markers.

Molecular markers detect variation of the DNA sequences among cultivars and therefore directly bypass problems connected with environmental effects. In hexaploid wheat, large genome size, a high proportion of repetitive DNAs, continuous inbreeding caused by self-pollination and a narrow genetic base represent the difficulties for use of molecular markers (Joshi and Nguyen 1993). Different molecular marker techniques such as restriction fragment length polymorphism (RFLP; Paull et al. 1998), simple sequence repeat (SSR; Plaschke et al. 1995), sequence tagged site (STS; Talbert et al. 1994) and amplified fragment length polymorphism (AFLP; Barret and Kidwell 1998) have been used for the estimation of genetic diversity in wheat. The random amplified polymorphic DNA (RAPD) technique, regardless of its sensitivity to reaction conditions and problems with repeatability and amplifying of non-homologous sequences (Devos and Gale 1992), has been successfully used for the assessment of genetic diversity in diploid, tetraploid and hexaploid wheat (He et al. 1992, Myburg et al. 1997, Liu et al. 1999, Sivolap et al. 1999).

Two main wheat breeding centres in Croatia are the 'Bc Institute' in Zagreb and the 'Agricultural Institute' in Osijek. They run independent breeding programmes and have used different parents for obtaining breeding populations by hybridization. Quantitative and qualitative morphological traits and pedigree records have been the basis for the estimation of genetic diversity among various cultivars. However, differences among these cultivars at the DNA level are not known. There is generally a lack of information on the genetic diversity of winter wheat germ plasm. Consequently, research work on estimating the genetic diversity in Croatian wheat germ plasm was carried out.

The aims of this research were: to study genetic diversity in the Croatian wheat germplasm using RAPD markers, morphological traits and pedigree records; to analyse differences among wheat cultivars from the two breeding centres; and to compare results based on RAPD markers, morphological traits and pedigree results.

Materials and Methods

Plant materials: For this study a total of 14 cultivars and breeding lines of wheat, *Triticum aestivum* L., were taken from the two Croatian breeding centres (Table 1).

RAPD analysis: The DNA was extracted from lyophilized leaves using a modified sodium disulphite method (Bolarić and Posselt 1999). Each cultivar was represented by four bulked leaf samples consisting of leaves from 10 plants that were used to prepare genomic DNA extracts for each cultivar. This made a total set of 56 samples. After screening 36 primers, RAPD analysis was conducted using the 14 most polymorphic primers, 12 primers with 10 bp, one primer with 17 bp and another primer with 18 bp (Table 2). The reaction mixtures for polymerase chain reaction (PCR) contained 10× PCR reaction buffer (10 mM TRIS–HCl pH 8, 50 mM KCl, 1.5 mM $MgCl_2$), 100 μM dNTP, 2 μl bovine serum albumin (BSA), 0.2 μl of primer, 1 U *Taq* polymerase and 10 ng of genomic DNA. Temperature cycling was performed using a thermal cycler (MJ Research PTC-100, MJ Research Inc., Massachusetts, USA) programmed specially for 10-bp

primers and specially for primers with more than 10-bp according to Sivolap et al. (1999). Amplification products were analysed by electrophoresis on 1.4% agarose gels in 0.5× TBE buffer, stained by ethidium bromide, visualized and photographed under UV light.

Table 1: Group, pedigree and origin of cultivars examined

Cultivars	Group	Pedigree	Origin
Slavonija	I	Osk 4.216/2-76/Osk 20	Institute in Osijek
Ana	I	Osk 4.216/2-76/Zg 2877/74	Institute in Osijek
Srpanjka	I	Zg 2696/Osk 4.50/1	Institute in Osijek
Golubica	I	Slavonija/Gemini	Institute in Osijek
Kata	I	Osk 6.30/20/Slavonka/3/Ephrat M68/Osk 154/19/Kavkaz	Institute in Osijek
Osk	I	Snaša//KB 160-86/Žitarka	Institute in Osijek
Žitarka	I	Osk 6.30/20/Slavonka/3/Ephrat M68/Osk 154/19/Kavkaz	Institute in Osijek
Sana	II	Mura/C.I. 14123//Zg 2413/72	Institute in Zagreb
Marija	II	Zg 4572/68/Kavkaz/Zg 1971/70	Institute in Zagreb
Patrija	II	Odeskaya 51/Zg IPK 82-10// GK3282	Institute in Zagreb
Adriana	II	Zg 1758/70/TpR-349	Institute in Zagreb
Anita	II	Zg 513/80/Gala	Institute in Zagreb
Bc	II	L-7580/Bc 570/80	Institute in Zagreb
Tina	II	Sana/Gala	Institute in Zagreb

Table 2: Nucleotide sequences of 14 polymorphic primers, total number of bands per primer and number of polymorphic bands per primer

Primers	Total number of random amplified polymorphic DNA (RAPD) bands	Number of polymorphic bands
AGG GGT CTT G	16	13
CAA TCG CCG T	24	21
GAG GAT CCC T	23	23
GGT GAT CAG G	19	18
CCG AAT TCC C	37	36
GGC TGC AGA A	25	23
TGC CCG TCG T	32	31
GAC GCC ACA C	14	12
ACC AGG TTG G	39	38
GAG CAA GTT CAG CCT GG	23	20
CCG AAT TCG C	26	23
CTG ACC AGC C	22	22
GGA CCC CGC C	34	34
GGC AGC AAC ATG GCA TTC	32	27
Total	366	341

Morphological traits and pedigree records: Plant breeders from both Institutes provided data for 12 morphological traits (plant growth habit, time of ear emergence, flag leaf and ear glaucosity, stem and ear length, pith in cross-section of straw, ear shape, ear density, awns presence, ear colour, grain colour) recorded as in official DUS (distinctness, uniformity and stability) testing. For 13 cultivars, seven generations of ancestors were found. For the cultivar 'Patrija' seven generations of ancestors were not found.

Data analysis: The RAPD markers were scored on the presence (1) or absence (0) of amplification products for each of 56 samples. To reduce estimation bias, amplified bands were analysed according to Lynch and Milligan (1994). The RAPD binary matrix was used to calculate squared Euclidean distances (Excoffier et al. 1992). Squared Euclidean distances were calculated using the NTSYS-pc program (Rohlf 1990). The squared Euclidean distance matrix was used as input data for AMOVA (Excoffier et al. 1992) which is incorporated in ARLEQUIN ver. 1.1 (Schneider et al. 1997). The average genetic distance (GD) between any two cultivars is represented by its F_{ST} value, and refers to as intercultivar distance. F_{ST} values were used as input data for two-dimensional principal coordinate analysis (2D PcoA) (Huff 1997).

Data for morphological traits were standardized as described by Roldan-Ruiz et al. (2001) and then used to calculate Euclidean distances. The 2D PcoA was made on the basis of a distance matrix. Coancestry coefficients were calculated according to Falconer and Mackay (1997). The 2D PcoA was made on the basis of coancestry coefficients. The Mantel (1967) test was used for the estimation of the correlation between three distance matrixes. Mantel's test was performed in the NTSYS-pc program.

Results

RAPD polymorphisms and genetic distance

The size range of amplified bands was between 400 and 1900 bp. The average number of polymorphic bands per primer was 24.35. The highest number of polymorphic bands was achieved with primer number 9, 10.6% of the total number of amplified bands. There were no bands specific for group or cultivar separation.

A total of 366 RAPD bands were amplified, among which 341 (93.2%) were polymorphic. Within the groups, higher polymorphism was achieved in group I (23.9% polymorphic bands) compared with group II (22% polymorphic bands).

The average GD (based on F_{ST} values) among the 14 cultivars was 0.421 (Table 3). GD values ranged from 0.16 to 0.67. The average distance within the groups was 0.36 for group I and 0.42 for group II. The average GD between the two groups was 0.45.

Table 3: Average genetic distances (based on F_{ST} values) among the 14 cultivars (upper triangle) and coefficients of parentage $f \times 100$

	Slavonija	Ana	Srpanjka	Golubica	Kata	OSK	Žitarka	Sana	Marija	Patrija	Adriana	Anita	Bc	Tina
Slavonija		0.16	0.19	0.20	0.26	0.45	0.46	0.39	0.32	0.40	0.45	0.43	0.45	0.58
Ana	30.77		0.16	0.23	0.29	0.48	0.47	0.39	0.36	0.41	0.45	0.40	0.45	0.56
Srpanjka	7.13	0.21		0.24	0.31	0.49	0.48	0.39	0.34	0.42	0.43	0.43	0.46	0.55
Golubica	50.00	30.77	7.13		0.32	0.47	0.47	0.39	0.35	0.40	0.44	0.42	0.46	0.58
Kata	1.08	0.42	17.10	1.08		0.32	0.43	0.34	0.19	0.38	0.44	0.45	0.44	0.58
Osk	1.41	1.44	17.30	1.41	65.62		0.59	0.39	0.35	0.41	0.53	0.51	0.54	0.67
Žitarka	1.08	0.42	17.10	1.08	100.00	65.62		0.52	0.51	0.54	0.59	0.51	0.52	0.55
Sana	6.11	24.57	0.07	6.11	0.40	0.91	0.40		0.21	0.33	0.38	0.39	0.46	0.55
Marija	1.34	0.21	6.76	1.34	14.59	14.59	14.59	1.13		0.37	0.42	0.42	0.44	0.59
Patrija	–	–	–	–	–	–	–	–	–		0.28	0.32	0.41	0.59
Adriana	2.00	0.93	2.17	2.00	0.78	0.98	0.78	0.14	0.38	–		0.24	0.37	0.61
Anita	6.11	12.07	0.07	6.11	0.14	0.65	0.14	5.96	0.07	–	0.14		0.27	0.51
Bc	6.11	12.07	0.07	6.11	0.14	0.65	0.14	5.96	0.07	–	0.14	5.96		0.57
Tina	6.11	24.57	0.07	6.11	0.40	0.91	0.40	50.00	1.13	–	0.14	29.67	5.96	

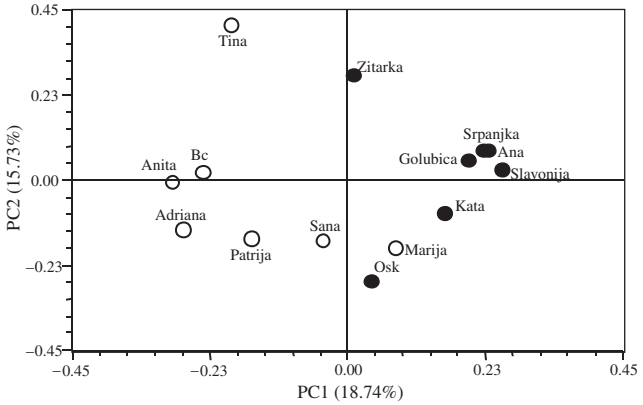

Fig. 1: Two-dimensional principal coordinate analysis of cultivars examined (group I, ● and group II, ○) based on random amplified polymorphic DNA (RAPD) data

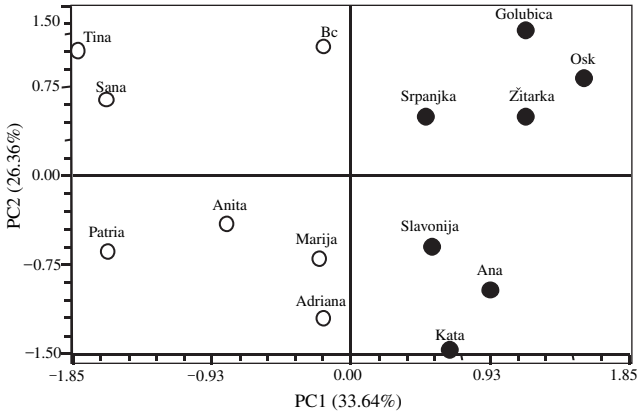

Fig. 2: Two-dimensional principal coordinate analysis of cultivars examined (group I, ● and group II, ○) based on morphological traits

2D principal coordinate analysis

In order to study genetic relationships among the 14 cultivars, a matrix of F_{ST} values was used for 2D PcoA (Fig. 1). The cultivars formed two main groups: one group included six cultivars from group II; and the other group included all the cultivars from group I and one ('Marija') from group II.

Morphological results

The average distance among 14 cultivars based upon the morphological data matrix was 8.59. The distance among the cultivars in group I was 6.42 and among the cultivars in group II, 6.14. The distance between the two groups of cultivars was 11.22. The 2D PcoA based on morphological traits is shown in Fig. 2. The cultivars formed two clearly divided groups: one group included all the cultivars from group II and the other, all the cultivars from group I.

Coefficient of parentage

Values of f varied between 0.07 and 100 for the cultivars derived from the same cross (Table 3). The average f value between the two groups of cultivars was 4.54. The average f value for cultivars from group I was 20.41, and for cultivars from group II, 7.12. In the 2D PcoA cultivars did not form clearly divided groups (Fig. 3.).

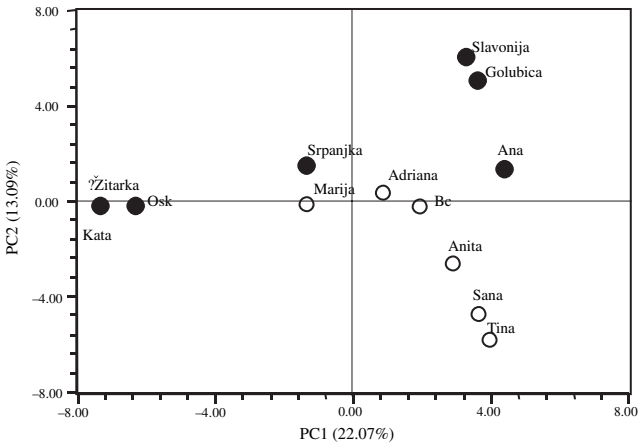

Fig. 3: Two-dimensional principal coordinate analysis of cultivars examined (group I, ● and group II, ○) based on pedigree records

Table 4: Results of Mantel's test

	Random amplified polymorphic DNA (RAPD)	Morphological traits
RAPD	–	–
Morphological traits	0.121	–
Coefficient of parentage	0.198	0.262

Mantel test results

Results of Mantel's test are presented in Table 4. Mantel's test showed weak correlation among the three distance matrixes examined.

Discussion

In this investigation, RAPD showed a high level of polymorphism and a high number of clearly amplified bands. A high level of polymorphism is in accordance with the research of Joshi and Nguyen (1993) and He et al. (1992). The two main centres of winter wheat breeding in Croatia have used different parents for the creation of new cultivars. Therefore, it would be expected that parental cultivars from these two centres would form two separate and distant groups of new cultivars. The present results, based on RAPD analysis, clearly showed that cultivars analysed from the two centres belong to two separate groups. The only exception was the cultivar, 'Marija', from group II that fell in group I. In its pedigree 'Marija' has the cultivar 'Kavkaz', which was a parent used in crosses for several cultivars from group I, which might explain the grouping obtained. RAPD analysis also showed a capability for distinguishing highly related cultivars. The cultivars 'Žitarka' and 'Kata' were developed from the same cross, but they are clearly distinguished with RAPD markers. Average genetic diversity was higher between than within the two groups, which suggest different breeding programmes and points to a narrow genetic base in the modern winter wheat cultivars. Genetic diversity based on morphological data and on pedigree records was higher between than within the two groups of cultivars. Pairs of cultivars 'Kata' and 'Žitarka', 'Osk 253' and 'Žitarka', and 'Slavonija' and 'Golubica' were closely related in the pedigree-based analysis but much less related in RAPD analysis. Similar results were reported by Soleimani et al. (2002) in AFLP and pedigree-based genetic diversity estimates for the cultivars of durum wheat.

Weak correlations between RAPD data and pedigree records in research for wheat and other cultivars were reported by several authors (Tinker et al. 1993, Barrett et al. 1998). There are several possible explanations for such results. Some of them are connected with the RAPD technique and some with the coefficient of parentage. Sensitivity to the working conditions and equipment used (Devos and Gale 1992) can influence the results of RAPD analysis. Another problem is the possibility of overestimation of genetic similarity because fragments with the same size could have different origins. This could be the case particularly in hexaploid wheat, because of the complexity of its genome. On the contrary, some assumptions made in calculating coefficients of parentage can be wrong. One of them is that ancestors are not correlated because there are no records about them. According to Soleimani et al. (2002) the assumption of no genetic relationship between ancestors without a known pedigree may be a major factor contributing to the disparity between pedigree and molecular marker-based diversity estimates, especially when comprehensive pedigree information is not available. Another assumption is that each parent contributes 50% of its genetic base to the offspring. But, as a result of selection pressure that ratio can be considerably different (Barrett et al. 1998). Some disadvantages of the coefficient of parentage are clearly visible in the current research. The coefficient of parentage between the cultivars 'Kata' and 'Žitarka' was one because they are developed from the same cross and they have the same parents. But they are recognized as different cultivars because of gene recombination and the development of different traits. These differences are not visible with the coefficient of parentage but RAPD analysis clearly separates these two cultivars.

There was no correlation between the GD matrices obtained by molecular and morphological data. There are several possible reasons for this lack of correlation. Some are connected with the RAPD technique and some with the analysis of morphological traits. When morphological traits are used it is assumed that alleles with the same phenotype have the same origin (Cox et al. 1985). That is not always true and could result in wrong conclusions. Quantitative morphological traits are used for the genetic diversity analysis. These traits are influenced by environmental conditions and they can show considerable variation. Also, some traits can be incorrectly measured and so cause problems in the estimation of genetic diversity. Finally, the number and choice of morphological traits and sample size can also affect the correlation. In this study, 12 morphological traits were examined. It is possible that if more morphological traits had been used a better correlation with RAPDs would have been obtained.

The results of this study indicate that RAPD analysis could be successfully used for the estimation of genetic diversity among wheat cultivars. Thus, it could serve as an efficient tool for the selection of genetically diverse parents.

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