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Full Length Research Paper

Phenotypic and molecular evaluation of genetic diversity of rapeseed (*Brassica napus* L.) genotypes

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The genetic diversity and relationships among rapeseed genotypes were evaluated using quantitative analysis and random amplified polymorphic DNA (RAPD) markers. Ten morphological and biochemical traits (plant height, height to the first branch, stem diameter, branch number, leaf number, pod number, seed yield per plant, 1000-seed weight, oil content and protein content) were analyzed in a three-year field experiment with 30 winter rapeseed genotypes. RAPD analysis was performed with 13 primers, chosen according to previous results. Significant genetic variability among the genotypes was obtained at both the morphological and molecular level. Nine out of the thirteen RAPD primers that were used for the analysis enabled the estimation of DNA polymorphism of the genotypes, while the other primers were monomorphic. Dendrograms obtained for morpho-biochemical traits and for the RAPD results were compared. In both cases partial grouping of genotypes based on their geographical origin was established.

Key words: *Brassica napus* L., genetic diversity, RAPD, cluster analysis.

INTRODUCTION

Rapeseed is the second most important oil crop in the world after soybean (Hasan et al., 2006). Besides that, rapeseed is also very important as a key crop for the raw material supply in the biodiesel (green diesel) industry (Marjanovic-Jeromela, 2005).

In the breeding process, significant improvement of quality and production was achieved, as well as utilization of rapeseed oil in human nutrition. However, the genetic basis of elite oilseed rape breeding material has been narrowed by an intensive selection of specific oil and seed quality traits (decreasing contents of eruca acid in oil and glucosinolates in seeds). As a consequence, genetic variability in this important crop is restricted with regard to many characters of value for breeding process (Cowling, 2007; Ananga et al., 2008). In order to increase the existing genetic variability, breeders collect and use the material originating worldwide. The importance of sufficient genetic diversity is especially emphasized by demand of breeding for hanging agricultural environments (climate changes, new disease distribution and market requirements).

There are various techniques available for evaluation of crop genetic variability, such as morphological, biochemical and molecular markers. Molecular (DNA) markers have many advantages over other techniques (independent of environment and plant growth stage, unlimited number, etc.) and they have been increasingly employed for analysis of genetic diversity (Prasad et al., 2000; Kondic-Spika et al., 2008; Nyende, 2008).

A variety of molecular markers have been used to study the genetic variation among the diverse group of important crops in the genus *Brassica*, such as: Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorfic DNA (RAPD), Simple Sequence Repeats (SSR), Sequence-Related Ampliphied Polymor-phism (SRAP), etc (Halldén et al., 1994; Diers et al., 1996; Negi et al., 2001; Riaz et al., 2001; Hasan et al., 2006).

Thormann et al. (1994) compared RFLP and RAPD markers and concluded that both these methods are equally useful in the determination of intraspecific relationships, while for interspecific relations the RAPD method exhibited some evasion. Förster and Knaak

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Table 1. *Brassica napus* genotypes used in the study.

Genotypes	Origin
Sremica, Banacanka, UM-1, UM-2, UM-5, UM-6, UM-8, UM-9, UM-10, UM-11, UM-12, UM-13, UM-14	Serbia
Falcon, K-571, K-1550, Alaska, Aligator, H-450, Valeska, Orkan, Pronto, Artus	Germany
Samourai, Jet Neuf, B-009, Duna	France
Oktavija, Jana	Hungary
Casino	Sweden

Table 2. Primers used for generating RAPDs in *Brassica napus* genotypes.

No.	Primer's name	Sequence (5'-3')
1.	UBC 1	CCT GGG CTT C
2.	UBC 18	GGG CCG TTT A
3.	UBC 43	AAA ACC GGG C
4.	UBC 51	CTA CCC GTG C
5.	UBC 64	GAG GGC GGG A
6.	UBC 73	GGG CAC GCG A
7.	UBC 81	GAG CAC GGG G
8.	UBC 96	GGC GGC ATG G
9.	UBC 101	GCG GCT GGA G
10.	UBC 105	CTC GGG TGG G
11.	UBC 155	CTG GCG GCT G
12.	UBC 222	AAG CCT CCC C
13.	UBC 331	GCC TAG TCA C

(1995) also compared RAPD and RFLP techniques in the determination of genetic distance among 21 winter rapeseed varieties. They calculated a highly significant correlation (r = 0.87**) between genetic distances obtained in the analyses. Summarizing the obtained results, the authors emphasized the advantages of the RAPD technique due to its simplicity and low cost. RAPD also does not require any digestion by restriction enzymes, radioactive probes or any previous knowledge about the sequence and requires a small amount of DNA (Williams et al., 1990). The significance of RAPD markers in the evaluation of genetic diversity in the genus *Brassica* has been confirmed in several other studies (Shengwu et al., 2003; Yu et al., 2005; Shiran et al., 2006; Ahmad et al., 2007). RAPD has also been used for constructing the genetic linkage map in *B. napus* (Foisset et al., 1996; Lombard and Delourme, 2001). RAPD and SCAR markers associated with low linolenic acid and other important traits in rapeseed have been identified (Rajcan et al., 1999; Zhang et al., 2003).

The present study was therefore undertaken to estimate the genetic diversity of rapeseed genotypes based on morphological and biochemical traits and molecular (RAPD) characterization. For this purpose, 30 rapeseed (*B. napus* L.) genotypes were analyzed and the results of genetic distances estimated by morphological and molecular evaluations were compared.

MATERIALS AND METHODS

Plant material

Thirty rapeseed genotypes with different geographic origin and properties were tested in this study (Table 1). Their significance in breeding programs was the basic reason for detailed genetic analysis.

A three-year experiment was conducted at the Rimski Sancevi Experimental Field of the Institute of Field and Vegetable Crops using a randomized block design with three replications. The sowing arrangement was 25 x 5 cm. Each genotype was sown in four rows (4 m long) per replicate. In the course of the growing season, the common cultivation practices were applied. Measurements and counts were done on samples comprised of 33 plants per genotype. The following morphological and agronomic traits were analyzed: plant height, height to the first branch, stem diameter, branch number, leaf number, pod number, seed yield per plant and 1000-seed weight. Oil content was determined using NMR, while protein content was obtained using the classical method by Kjeldahl.

RAPD analysis

DNA polymorphism was estimated on the basis of 13 RAPD primers (Table 2), which had already been proven to be polymorphic in rapeseed (Pankovic et al., 2000). Total DNA was extracted from fresh leaf tissue according to Dellaportha et al. (1983).

Amplification was carried out in a volume of 25 µl containing: 1x buffer, 0.2 mmol/l each of dNTPs (dATP, dGTP, dCTP and dTTP), 0.5 µmol/l of primer, 30 ng of genomic DNA, 3.0 mmol/l of $MgCl_2$ and 1 U of Taq polymerase. PCR reaction was performed in 45 cycles: denaturation - 1 min at 95°C, annealig - 1 min at 36°C, extension - 2 min at 72°C. The amplified products were separated by electrophoresis in 2% agarose gel to which EtBr (0.5 µl/ml) was added. The separated PCR products were then visualized through UV-Transluminator. The molecular weights of RAPD-PCR bands were determined using the DNA size marker (100 bp-DNA Ladder; Pharmacia Biotech). The obtained results were photographed and stored by the BIO-PRINT system.

Statistical analysis

The results of phenotypic evaluation of 30 rapeseed genotypes were analyzed using factorial ANOVA and significance of differentces was determined by the LSD test. Genotype groups were divided by cluster analysis according to UPGMA (Unweighted Pair Group Method using Arithmetic averages). This is an additive method, which forms a matrix of Euclidean distances among the mean values of the groups (genotypes) for the construction of a dendrogram (Fox and Rosielle, 1982). The statistical calculations were done by the System for Statistics SYSTAT Ver. 9.0 (Wilkinson, 1999), software modules STATS and CLUSTER. For clarity, we used the five-group K means analysis with the Euclidean

Table 3. Three-year average values for morpho-biochemical traits in 30 rapeseed genotypes.

Genotype	Plant height (cm)	Height to the first branch (cm)	Stem diameter (cm)	Branch no.	Pood no.	Leaf no.	Protein content (%)	Oil content (%)	TSW (g)	Seed yield per plant (g)
Sremica	126.6	52.6	1.1	6.8	147.9	9.4	21.8	44.0	3.8	11.4
Banacanka	124.8	52.4	1.2	6.7	120.0	11.1	18.4	46.4	3.8	8.8
Samourai	109.6	40.5	1.0	6.1	118.0	9.0	18.9	46.2	3.3	8.9
Falcon	125.4	56.4	1.1	6.2	130.9	10.0	18.4	45.1	3.5	10.4
Jet Neuf	116.6	47.3	0.9	6.9	144.3	9.1	19.5	46.2	4.1	11.0
Oktavija	122.3	46.9	1.0	7.1	140.8	8.8	20.1	47.7	4.1	10.0
Jana	122.8	51.4	1.0	6.6	114.4	10.2	19.4	47.0	3.4	8.3
B-009	128.0	46.5	1.1	7.1	165.7	9.4	17.6	46.2	3.5	11.2
UM-1	127.0	62.1	0.9	6.8	104.9	11.0	19.4	45.4	3.9	9.3
UM-2	119.5	57.5	0.9	6.1	108.5	10.4	19.5	45.5	4.2	7.4
UM-5	116.4	52.3	1.1	6.4	112.0	10.2	21.3	43.0	3.4	7.9
UM-6	124.7	59.9	1.0	6.3	117.2	10.6	20.0	43.0	3.6	8.8
UM-8	126.9	58.1	1.1	6.9	142.6	10.8	20.0	43.0	3.6	10.0
UM-9	119.7	42.9	0.9	6.2	122.0	9.5	18.0	45.0	3.5	7.8
UM-10	124.6	54.7	1.0	6.4	136.6	10.0	20.1	43.0	4.0	9.1
UM-11	131.5	68.8	1.0	8.1	125.6	12.5	19.0	46.	3.5	7.55
UM-12	118.8	61.6	1.1	5.8	170.8	11.9	22.0	43.4	3.6	6.9
UM-13	119.6	57.2	0.9	7.1	108.0	11.4	21.0	41.0	3.9	6.5
UM-14	119.8	53.2	0.9	7.3	107.9	11.0	20.5	42.4	3.9	6.2
K-571	132.1	59.7	1.1	7.2	138.0	10.6	19.2	46.1	3.5	9.8
K-1550	117.4	50.9	1.1	6.2	126.6	9.8	18.2	44.9	3.5	10.1
Alaska	123.6	61.2	1.1	5.6	135.4	10.1	17.8	45.0	3.5	9.6
Aligator	132.1	61.7	1.0	5.2	121.2	10.2	19.4	45.2	4.0	7.6
H-450	123.5	53.6	1.0	6.4	126.3	10.1	19.1	43.5	3.7	9.6
Casino	129.0	59.5	0.9	6.1	124.9	10.4	19.2	43.9	4.0	8.7
Valeska	130.1	61.9	1.1	5.4	112.3	11.4	18.6	46.0	3.8	9.5
Duna	124.7	47.9	1.1	6.0	157.8	9.9	18.9	43.6	3.7	9.5
Orkan	124.1	53.3	1.0	5.4	122.6	10.3	18.5	45.6	4.0	8.35
Pronto	116.4	59.5	1.1	6.1	120.1	10.1	18.8	43.5	3.5	9.8
Artus	127.3	46.8	1.111	7.0	117.3	9.3	18.6	44.0	3.8	9.5
Average	123.5	54.6	1.0	6.4	128.0	10.3	19.5	44.8	3.8	9.0
CV (%)	4.6	8.6	6.1	11.8	11.0	10.0	4.7	2.6	4.0	13.0
LSD 0.05	5.293	4.35	0.059	0.706	13.08	0.953	0.85	1.11	0.14	1.09
0.01	6.984	5.74	0.776	0.932	17.25	1.258	1.12	1.46	0.18	1.41

distance treated as a metric distance.

Highly reproducible polymorphic RAPD bands were scored as "+" (present) or "–" (absent) for each genotype. These results were used for calculation of Simple Matching coefficients (Staub et al., 2000), using the formula:

SM = (a+d)/n

Where a = number of bands present in both genotypes ("+ +" comparisons); d = number of null alleles in both genotypes ("- - " comparisons); and n = total number of bands ("+ +", "+ -", "- +" and "- - " comparisons). Genetic distance (GD) was calculated via SM co-efficients according to the formula:

GD = 1 – SM (Spooner et al., 1996).

A pair-wise difference matrix was constructed using calculated values for GD between every pair of genotypes. The matrix was used to produce a UPGMA dendogram representing relationships among the rapeseed genotypes.

RESULTS

Analysis of quantitative traits

Three-year average values of 10 quantitative traits recorded in the 30 rapeseed genotypes are presented in Table 3. The genotypes were found to differ significantly with regard to the traits concerned. Grain yield per plant as the most important agronomic trait ranged from 6.2 g in the cultivar UM-14 to 11.4 g in the variety Sremica and

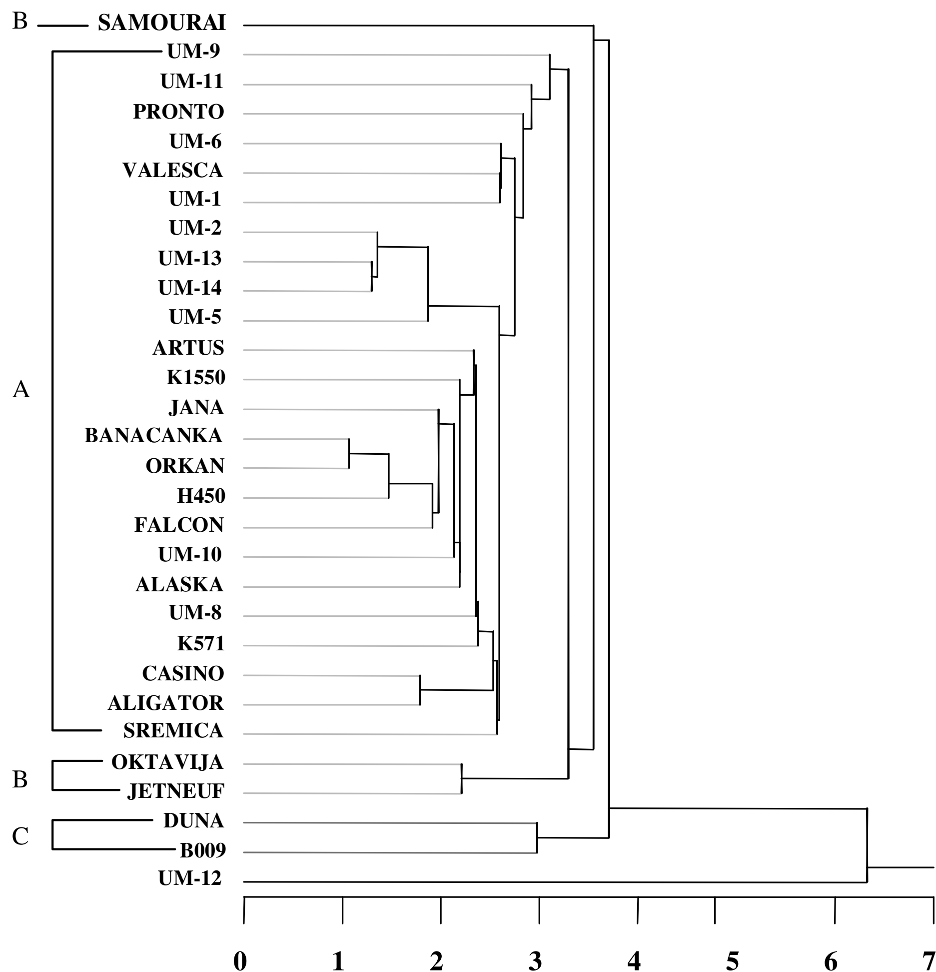

Figure 1. Dendogram constructed for 30 rapeseed genotypes based on analysis of 10 morpho-biochemical traits.

had the greatest range of variation (CV 13.0%) of all the traits under study. The smallest average variation over the three study years was found for protein content (CV 2.6) and oil content (CV 4.0). Protein content ranged from 17.6% (B-009) to 22.0% (UM-12) and oil content from 41.0% (UM-13) to 47.7% (Oktavija).

Using the average values of the 10 quantitative traits, an aggregate dendrogram was created that divided the genotypes into three uneven clusters plus two standalone genotypes. The biggest group, Cluster A, included 24 genotypes, while Clusters B and C consisted of two each. The large number of hierarchical levels indicates that the material used in the study is heterogenous. The diverse linkage among the genotypes suggests that there is no single cultivar in which all the positive or negative traits are concentrated (Figure 1). The first group is characterized by clustering based on geographic origin and includes all the genotypes developed by the Institute of Field and Vegetable Crops except the line UM-12 as well as the genotypes originating from Germany. Cluster B consists of two genotypes, Oktavija, originating from Hungary and Jet Neuf from France. Cluster C, comprised of the genotypes Duna and B-009 originating from France and connects with the other clusters and the standalone French cultivar Samourai at a high hierarchical level.

The line UM-12 from the Novi Sad breeding program set itself apart from the other genotypes by linking with them only at the uppermost hierarchical level.

DNA polymorphism

Out of the 13 RAPD primers assessed in the study, nine were used for DNA polymorphism assessment in the rapeseed genotypes, while the rest were monomorphic. In the DNA polymorphism analysis, only clear and reproducible bands were used. Of the 54 amplified RAPD fragments, 23 were polymorphic. The average number of polymorphic fragments per primer varied from 1 to 6, averaging 2.5, while the size of the fragments ranged between 0.4 and 2.0 kb. The average percentage of polymorphism was calculated for each primer individually and

Table 4. Numbers of amplified and polymorphic fragments, their size range and percentage of polymorphism detected by 9 RAPD primers in 30 rapeseed genotypes.

Primer	Amplified fragments	Polymorphic fragments	Fragment size range (bp)	Percentage of polymorphism (%)
UBC 1	6	3	600 – 1500	50
UBC 18	4	2	400 – 900	50
UBC 43	4	2	600 – 1500	50
UBC 51	5	1	700 – 1100	20
UBC 64	6	6	500 – 1000	100
UBC 105	10	2	300 – 1400	20
UBC 155	6	1	300 – 1500	17
UBC 222	8	4	500 – 1400	50
UBC 331	5	2	600 – 2000	40
Total	54	23		
Average	6	2.5		44

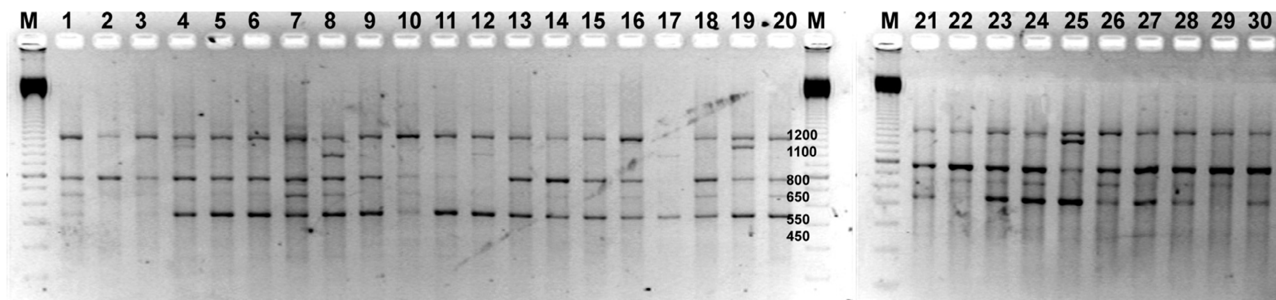

Figure 2. RAPD amplification products generated by primer UBC 64. The order of samples: Lane M, 100 bp ladder; lane 1, Sremica; lane 2, Banacanka; lane 3, Samourai; lane 4, Falkon; lane 5, Jet Neuf; lane 6, Oktavija; lane 7, Jana; lane 8, B009; lane 9, UM-1; lane 10, UM2; lane 11, UM-5; lane 12, UM-6; lane 13, UM-8; lane 14, UM-9; lane 15, UM-10; lane 16, UM-11; lane 17, UM-12; lane 18, UM-13; lane 19, UM-14; lane 20, K-571; lane 21, K-1550; lane 22, Alaska; lane 23, Aligator; lane 24, H-450; lane 25, Casino; lane 26, Valesca; lane 27, Duna; lane 28, Orkan; lane 29, Pronto; lane 30, Artus.

ranged from 17 to 100% (Table 4).

Results of amplification with the UBC 64 primer are an example of reaction with the primers that produced polymorphic fragments. Six polymorphic RAPD fragments 450-1200 bp in size are clearly visible. The first fragment, 1200 bp in size, was found only in UM-12. The second one (1100 bp) was observed in UM-14 and Casino. A 800 bp fragment was observed only in B-009. The 650 bp fragments were found in all the genotypes except UM-2, UM-5, UM-6 and UM-12. Fragments around 550 bp in size were recorded in 13 of the 30 genotypes studied (Sremica, Jana, B-009, UM-13, K-571, K1550, Alaska, Aligator, H-450, Valesca, Orkan, Pronto, Artus), while the smallest fragment, about 450 bp in size, was found in all the genotypes except Banaćanka, Samourai, UM-2, Alaska and Orkan (Figure 2).

Genetic distances (GD) among the genotype pairs were expressed as percentage in the form of a matrix, as shown in Table 5 ("pairwise difference matrix"). The values of GD among the genotypes ranged from 38% (between UM-5 and UM-6) to 80% (between UM-11 and Casino), averaging 64%.

Cluster analysis according to UPGMA was used to construct a dendrogram (Figure 3) showing GD inter-relationships among the genotypes. Looking at Figure 3, we can see that there are two major clusters, A and B, positioned at a genetic distance of about 67%. Cluster A consists of ten newer genotypes from a number of breeding programs across Europe. Within the group, the genotypes H-450 and Aligator, both from Germany, are the closest (GD = 45%), while Artus (Germany) and Duna (France) are the most distant (GD = 55%).

The remaining 20 genotypes are grouped into Cluster B, which is further divided into two sub-clusters and two standalone genotypes, Jet Neuf and Sremica. The two separate genotypes are at a genetic distance of 65% and are linked to the other Cluster B genotypes at a GD of about 66.5%. The smallest GD in terms of DNA polymorphism (38%), that is, the greatest similarity, was found between the genotypes UM-5 and UM-6 from the first sub-cluster, which were developed at the Institute of Field and Vegetable Crops in Novi Sad. It should be

Table 5. Pairwise difference matrix (%) for 30 *Brassica napus* genotypes.

	Sremica	Banacanka	Samourai	Falcon	Jet Neuf	Oktavija	Jana	B-009	UM-1	UM-2	UM-5	UM-6	UM-8	UM-9	UM-10	UM-11	UM-12	UM-13	UM-14	K-571	K-1550	Alaska	Aligator	H-450	Casino	Valeska	Duna	Orkan	Pronto	Artus
Sremica	0																													
Banacanka	61	0																												
Samourai	70	63	0																											
Falcon	65	64	51	0																										
Jet Neuf	65	67	66	66	0																									
Oktavija	66	62	54	53	58	0																								
Jana	64	68	56	58	61	49	0																							
B-009	63	71	66	61	63	54	49	0																						
UM-1	65	64	46	48	67	50	55	59	0																					
UM-2	76	58	62	63	67	56	62	67	64	0																				
UM-5	69	68	61	63	59	53	54	60	54	61	0																			
UM-6	76	75	68	70	67	59	62	68	62	68	38	0																		
UM-8	66	65	49	50	67	52	62	63	48	64	60	67	0																	
UM-9	66	66	57	52	69	56	57	57	51	63	61	66	52	0																
UM-10	68	57	67	57	66	60	66	70	58	54	66	73	63	60	0															
UM-11	72	61	75	65	63	62	69	73	70	59	69	68	72	73	51	0														
UM-12	76	74	62	59	73	54	56	62	57	61	52	47	61	58	73	67	0													
UM-13	62	67	61	56	69	50	53	58	55	68	63	58	59	59	64	62	54	0												
UM-14	71	71	59	50	68	49	56	62	53	64	60	59	60	59	63	59	48	51	0											
K-571	62	63	61	51	64	50	47	53	55	61	61	65	59	59	60	62	58	47	47	0										
K-1550	67	71	61	61	73	63	60	61	62	70	64	70	64	65	73	76	64	64	64	61	0									
Alaska	72	65	65	70	73	71	69	67	70	69	68	68	71	72	72	70	68	69	74	71	49	0								
Aligator	66	69	63	58	64	60	59	62	64	73	66	67	66	63	76	72	57	58	62	60	54	58	0							
H-450	66	70	63	59	71	57	54	57	65	69	63	64	67	63	72	70	58	53	63	56	54	59	45	0						
Casino	67	71	60	56	73	63	65	63	62	73	69	74	64	60	73	80	64	64	60	65	56	66	58	58	0					
Valeska	74	69	69	69	75	70	67	70	69	72	62	61	71	71	71	69	67	67	67	69	53	51	63	63	69	0				
Duna	64	68	67	67	65	68	66	69	71	76	69	70	73	69	70	72	70	65	70	71	68	73	69	66	62	71	0			
Orkan	61	70	63	59	71	65	63	62	65	78	71	72	67	63	76	74	63	57	62	64	54	64	50	62	53	63	59	0		
Pronto	68	62	71	71	69	68	70	69	75	62	74	74	78	77	65	62	74	65	70	67	62	54	70	66	71	62	63	65	0	
Artus	64	72	71	71	69	72	70	69	71	79	69	68	73	73	78	72	70	70	69	71	59	61	58	65	66	59	55	53	61	0

noted that all the genotypes developed at the Novi Sad Institute are found in Cluster B, as are most of the European genotypes that were used in their development as parental components.

DISCUSSION

Using cluster analysis, a dendrogram was constructed based on the results concerning ten morpho-biochemical traits of the 30 genotypes studied (Figure 1). The first cluster was the most numerous and was comprised for the most part of genotypes from the Novi Sad Institute as well as some originating from Germany. The second cluster consisted of cultivars developed in France and Hungary, while the third incorporated cultivars developed in French breeding programs. The most divergent populations are found at the far ends of the dendrogram. Thus, the French cultivar Samourai clearly set itself apart from the rest of the material due to its extremely low values of four of the traits studied: plant height, height to the first branch, leaf and 1,000-seed weight. It also had low values of the other traits, with the exception of seed oil content. At the other end of the dendrogram is UM-12, which had the highest values of pod number, leaf number and seed protein content, a very high value of first branch height, the lowest branch number and very low values of plant height, seed oil content, 1000-seed weight and seed yield per plant. This is a good illustration of how interrelationships among genotypes within a dendrogram

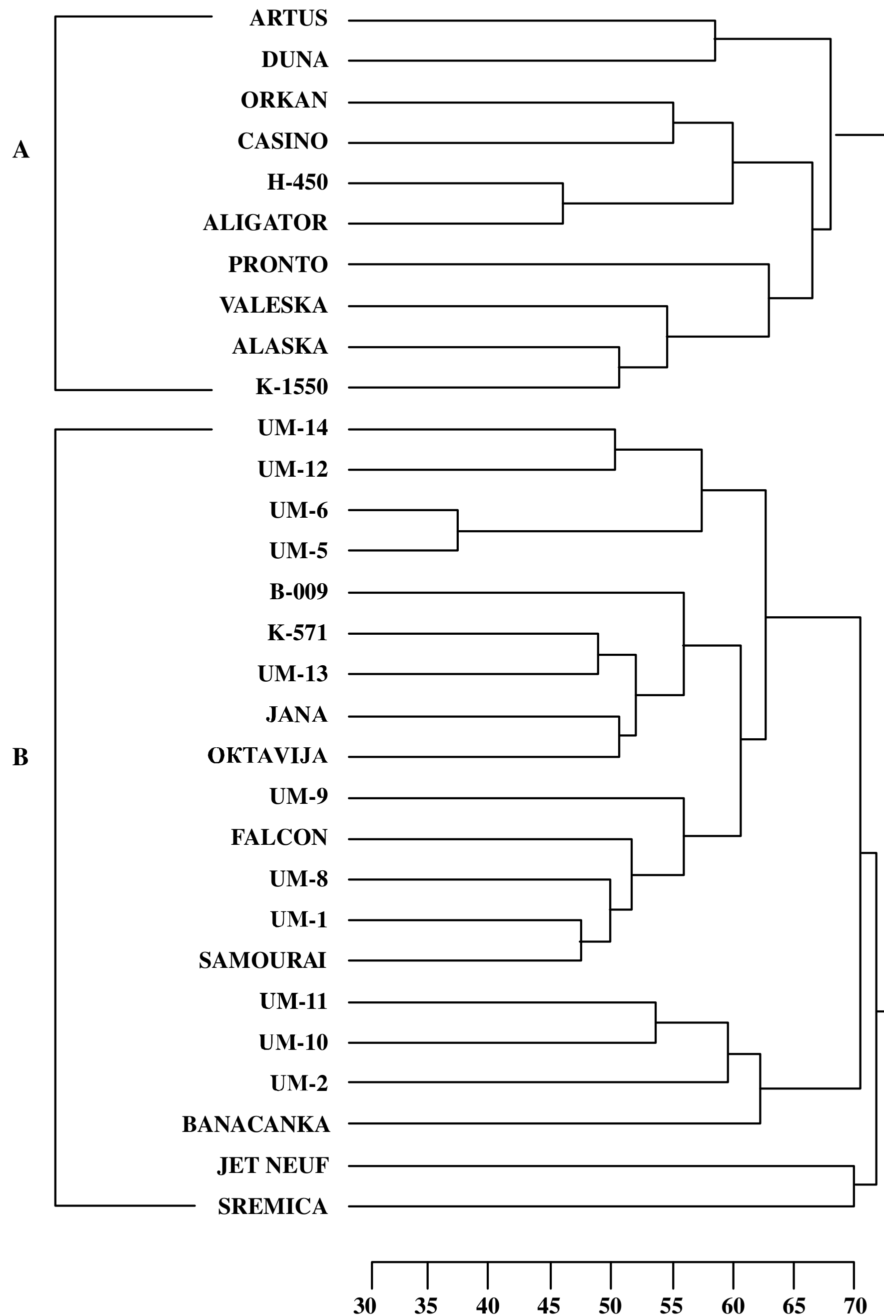

Figure 3. Dendogram illustrating genetic relationship among 30 rapeseed genotypes generated by UPGMA cluster analysis of polymorphic RAPD fragments.

are influenced not only by extreme values but also by the other characteristics, showing that the use of cluster analysis of qualitative and quantitative traits graphically depicts similarities and differences among genotypes, which agrees with the findings of other authors as well (Crossa and Cornelius, 1997; Hristov, 1999; Marjanovic-Jeromela et al., 2003; Mahasi and Kamundia, 2007).

In the present paper, DNA polymorphism was assessed using 13 RAPD primers, nine of which proved reproducible and there were 54 fragments amplified in total. The high percentage of polymorphic primers indicates that they have been well selected in the study. The results were processed and the SM coefficient of similarity was obtained and further calculations showed that the percentage of genetic distance between individual genotypes was 64%. These results are in agreement with the genetic divergence percentage of 65% as reported by McGrath and Quiros (1992) and are within

the value ranges cited by Geraci et al. (2001) and Ahmad et al. (2007). The GD value was the lowest between the genotypes UM-5 and UM-6, both developed at the Institute of Field and Vegetable Crops in Novi Sad and the highest between Casino, originating from Sweden and UM-11, bred at the Novi Sad Institute.

In the dendrogram constructed based on the results obtained by the use of RAPD markers, there are two major clusters visible. The first, Cluster A, incorporates genotypes from foreign breeding programs. The second, Cluster B, which is less numerous and has a more complex hierarchical structure, consists of genotypes developed at the Novi Sad Institute as well as those developed in various European breeding centers that have been used in the Institute's program on rapeseed in the past.

A number of authors have successfully used the RAPD technique for determining genetic similarity/distance. Results obtained by the use of this and other molecular marker techniques are used for further calculations and the construction of dendrograms, which makes it possible to visualize and more easily interpret the findings and compare them with those obtained using other techniques (Geraci et al., 2001; Xu and Gai, 2003, Pankovic et al., 2004; Prasad et al., 2009). Thus, Yuan et al. (2004) used RAPD markers to group rapeseed genotypes according to not only their geographic origin but by using the breeding method as a criterion to determine genetic distance as well. Recurrent selection has produced a broader genetic basis than pedigree selection. Analyzing a large number of genotypes using molecular markers (AFLP and SSR), Seyis et al. (2004) and Hasan et al. (2004) constructed dendrograms showing the distribution of genotypes within the clusters based primarily on their form (winter or spring), followed by geographic origin and partially, pedigree as well.

Wu and Sheng (1999) used cluster analysis to show the results of a study of 6259 genotypes originating from a Chinese genetic resources collection of the genus *Brassica* (*B. campestris* L., *B. juncea* Czern. and Coss., *B. napus* L.). Major morphological traits, qualitative characters and resistance to dominant diseases were assessed. The recorded variability was used to create clusters within groups differing in their geographic origin. Distribution into clusters among and within the groups was double-checked and verified by the RAPD and AFLP methods.

Using AFLP molecular markers for constructing UPG MA clusters, Negi et al. (2004) established genetic distances for the species *Brassica nigra* L. within a gene bank in India. Vasic et al. (2003) created dendrograms based on different primers, compared them with the latest taxonomic classification and concluded that the RAPD technique can be used to determine genetic similarities between populations within and among the species of the same genus (*Helianthus*). To set off a European project on *Brassica* genetic resources (RESGEN, Lühs et al., 2003), 96 rapeseed genotypes were characterized using SSR (Simple Sequence Repeat) markers. The research within the project has continued and has been expanded to include a much greater number of genotypes and primers (Hasan et al., 2004).

This underscores the importance of molecular markers as a technique for determining intraspecific genetic variability (Prasad et al., 2000; Snowdon and Friedt, 2004). Given that in rapeseed, as well as in other species, a positive correlation has been found between genetic distances determined by molecular markers and heterosis (Diers et al., 1996; Shen et al., 2004), it is expected that the use of molecular markers in the selection of new genotypes for crossing purposes will speed up the breeding process.

By comparing the dendrogram obtained using RAPD markers and the aggregate dendrogram obtained by analyzing ten quantitative traits, certain regularities can be noted. In both cases, genotypes are clustered according to geographic origin, although the clustering is more pronounced in the dendrogram resulting from quantitative trait analysis. Clustering according to geographic origin has also been reported by various other authors for different species of the genus *Brassica* (Srivastava et al., 2001). Qi et al. (2008) attribute deviations in the geographic clustering of genotypes detected by molecular markers to the fact that the pressures of artificial selection may have limited mutation effects on the DNA sequence; in other words, numerous changes have occurred in the process of breeding as a result of artificial selection relative to natural mutations, resulting in a loss of distinctive traits that should characterize genotypes from the same geographic area. A lack of correlation between geographic origin and genetic distance has been established in other species as well (Johnson et al., 2007; Khan et al., 2009).

Similarities in GD values and ordering of genotypes were not found by comparing two different dendograms. Other authors have obtained similar results when comparing the findings of morphological, biochemical and molecular analyses in different species (Vollman et al., 2005; Johnson et al., 2007; Khan et al., 2009). The accuracy of determining genetic similarity/distance based on molecular markers depends on many factors, such as the number of markers used, their distribution along the genome, and the degree of precision with which the results are analyzed (Schut et al., 1997). In addition to that, molecular markers cannot be used for the purpose of drawing conclusions about interallelic and intraallelic interactions that lead to the expression of particular traits within the genome. Given that a relatively small number of RAPD primers were used in the present study, it is reasonable to expect that a significant portion of the rapeseed genome was not covered by the analysis, hence no major similarity was found between the dendrograms.

There are other possible reasons for the lack of correlation between RAPD analysis and morphological

data. One of them is that RAPD markers detect polymorphism in coding as well as non-coding gene regions, so it is possible that the bulk of the detected polymorphism was from the non-coding regions. Also, plants may be morphologically similar, but this does not necessarily imply genetic similarity, since different genetic bases can result in similar phenotypic expression (Khan et al., 2009). This is especially the case with traits that are significantly influenced by environmental factors. The link between molecular markers and phenotypic traits can be significant if the markers are selected based on their linkage to particular known loci (Persson and Gustavsson, 2001).

In summary, significant genetic variability was found among the genotypes on morphological, biochemical and molecular levels, meaning they can be used for further rapeseed advancement through the breeding program. Also, the study has shown that PCR-based techniques such as RAPD can be successfully used for detecting genetic variability in rapeseed. The technique's simplicity makes it particularly suitable for breeding programs in which a large number of lines need to be analyzed. Many other authors have reached similar conclusions on the use of RAPD markers in the breeding of rapeseed and other crops as well (Shengwu et al., 2003; Shiran et al., 2006; Ahmad et al., 2007, Debnath, 2007, Guo et al., 2007; Ananga et al., 2008). Because this is the first study of this kind performed on the actual rapeseed breeding material of Southeastern Europe, relatively small number of genotypes and RAPD primers were utilized. The findings of this preliminary study indicate that such research should be continued. Future studies should include a greater number of genotypes and markers in order to cover a large portion of the rapeseed genome and to get valuable information on genetic variability of this important crop.

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