EVALUATION OF THE GENETIC DIVERSITY AMONG SOME OILSEED RAPE *BRASSICA NAPUS* CULTIVARS REVEALED BY RAPD MARKERS COMPARED WITH MORPHOLOGICAL TRAITS EVALUATION

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Abstract

In this study we have analyzed the genetic diversity and the relationships among 32 cultivars of oilseed rape (*Brassica napus*) using quantitative analysis and random amplified polymorphic DNA (RAPD) markers. For this purpose we analyzed four morphological traits (plant height, branch number, pod number and mean number of seeds per pod) at 32 oilseed rape cultivars provided from Center for Genetic Resources Netherlands (CGN). RAPD analysis was perform with 8 primers chosen after a previous screening. Significant genetic variability among those 32 cultivars was obtained both at the morphological and molecular level. We obtained a dendrogram for the morphological traits and a dendrogram for RAPD analysis and we compared them.

Key words: RAPD analysis, dendrogram, genetic similarity

Oilseed rape is consider to be one of the most important oil crop worldwide because is a high quality source of vegetable oil for the food industry and suppliers protein to the animal feed market. It is also seen as a key crop for raw material supply in the biodiesel industry (Mabel and Kamundia, 2007).

In the breeding process, the quality and production had a significant improvement as well as utilization of rapeseed oil in human nutrition.

The are many techniques available for evaluation of crop genetic variability, such as morphological, biochemical and molecular markers (Marjanovic et al, 2009). Molecular (DNA) markers technique is the most used method for the evaluation of genetic variability because it have many advantages over other techniques (independent of environment and plant growth stage, unlimited number, etc) and the method is more precise (Prasad et al, 2000; Kondic-Spika et al, 2008). In order to estimate the genetic variation among the diverse group of important crops in *Brassica* genus it have been used a variety of molecular markers such as: Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length polymorphism (AFLP), Random Amplified Length Polymorphism (RAPD) , etc (Hallden et al, 1994;, Diers et al., 1996).

The present study was to estimate the genetic diversity among some oilseed rape cultivars based on morphological and molecular characterization. For this purpose, 32 oilseed rape cultivars were analyzed and the results of genetic distances estimated by morphological and molecular evaluations were compared.

MATERIAL AND METHOD

Plant material: the plant material for this study comprised 32 genotypes of rapeseed cultivars proceeded from the Centre for Genetic Resources Netherlands. The plant were sown in the field in the year 2009 in order to obtain the morphological data and to collect the fresh tissue material for DNA extraction. Each genotype was sown in the field at 50 cm distance between rows and 50 plant on each row. Measurements and counts were made at each genotype and comprised 20 plants per genotype. The following morphological trait were analyzed: plant height, number of branches per plant, number of pods per plant, mean number of seed per pod. The average values of the morphological data are presented in *table 1*.

RAPD analysis In order to estimate the genetic similarity above the 32 oilseed rape genotypes, a number of 8 RAPD primers (*tab. 2*) were used to see the DNA polymorphism in these genotypes.

DNA extraction: Leaf samples were taken from all plants of each cultivar, bulked and immediately frozen in liquid nitrogen.

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Table 1

The average of the values for morphological traits at 32 rapeseed genotypes

Nr. Crt	Genotype	Country of origin	Plant high	Branch number	No.of pods per plant	No. of seeds per pod
1	Matador	Sweden	134	9	351	25
2	Mirander	unknown	120	8	289	23
3	Niederarn bacher	Germany	111	5	248	20
4	Norli	Germany	117	7	342	28
5	Octavia	unknown	124	10	485	30
6	Olimpiade	Italy	111	4	218	18
7	Olymp	Germany	110	8	385	22
8	Panter	Sweden	101	5	256	21
9	Perle	Germany	126	10	498	29
10	Andol	France	98	11	255	25
11	Arabella	Germany	116	11	415	28
12	Bienvenu	France	98	6	341	31
13	Brilland	Pollen	132	11	265	26
14	Bristol	France	106	12	239	23
15	Buko	unknown	104	10	377	32
16	Capricorn	Great Britain	110	10	412	33
17	Cobra	Germany	97	7	252	20
18	Collo	Germany	128	10	318	27
19	Planet	Germany	107	6	255	24
20	Prominj	Former-URSS	125	6	232	22
21	Ridana	Germany	125	14	378	35
22	Samourai	France	101	7	219	18
23	Score	Great Britain	108	7	252	23
24	Silesia	Former-Czechoslovakia	106	10	362	36
25	Silex	Germany	119	8	285	29
26	Silvia	Germany	137	10	468	38
27	Sollux	Germany	120	8	378	36
28	Susana	Germany	118	7	391	33
29	Tamara	Germany	116	6	362	31
30	Tapidor	France	106	10	421	39
31	Tor	Sweden	120	9	374	25
32	Veronika	Germany	121	8	503	37

DNA was extracted using the CTAB procedure modified according to Doyle and Doyle (1987). DNA content was measured using a Nano Drop 3300 florospectrophotometer. Based on these data DNA was diluted to a concentration of 5ng/µl for RAPDs and 25ng/ µl for RAPD. Polymerase chain reaction (PCR) mixture (20 µl) for RAPDs contained 5 ng genomic DNA, 10 µM of each dNTP, 25 mM $MgCl_2$, 5pmol/ µl decamer primer (ROTH) and 0,1 Units Taq DNA-polymerase (Go Taq Polymerase – Promega) and 10X respective reaction buffer. Amplification was performed in a Palm Cycler Corbett. PCR conditions were: 94°C for 4 min followed by 45 cycles consisting of 1 min at 94°C, 1 min at 36°C, and 2 min at 72°C. The amplified products were separated by electrophoresis in 2% agarose gel and strained with Ethidium bromide (0,5 µl/ml).

Statistical analysis With the values obtained we formed a matrix of Euclidian distances among the mean values of the genotypes for the construction of a dendrogram (Fox and Rosielle, 1982). The statistical calculations were done by the System of Statistic SYSTAT 13, software modules STATS and CLUSTER.

RAPD amplification products were scored using the software RFLPscan 2.1 assuming that each band of different size reflect a single locus. Only unambiguously scored fragments were used for the estimation of genetic similarity according to Nei and Li (1979). Based on these data UPGMA-clustering (unweighted pair group method using arithmetic averages) was carried out using the software package NTSYS-pc 2.1 (Rohlf 1992).

Table 2

Primers used for generating RAPDs in *Brassica napus* genotypes

No.	Primer's name	Sequence (5'-3')
1.	ROTH A08	GTG ACG TAG G
2.	ROTH A10	GTG ATC GCA G
3.	ROTH A13	CAG CAC CCA C
4.	ROTH B04	GGA CTG GAG T
5.	ROTH B07	GGT GAC GCA G
6.	ROTH B10	CTG CTG GGA C
7.	ROTH B11	GTA GAC CCG T
8.	ROTH B18	CCA CAG CAG T

RESULTS AND DISSCUSIONS

Based on the morphological traits measured on each genotype, we constructed a dendrogram (*fig.1*) that divided the genotype into three clusters (cluster A, cluster B, cluster C). Looking at the morphological dendrogram it can be observed that the cultivars were grouped in three clusters: cluster A, B and C. The biggest group is Cluster B that includes 15 genotypes originating from different counties, while cluster B comprised 13 genotypes and Cluster C only four.

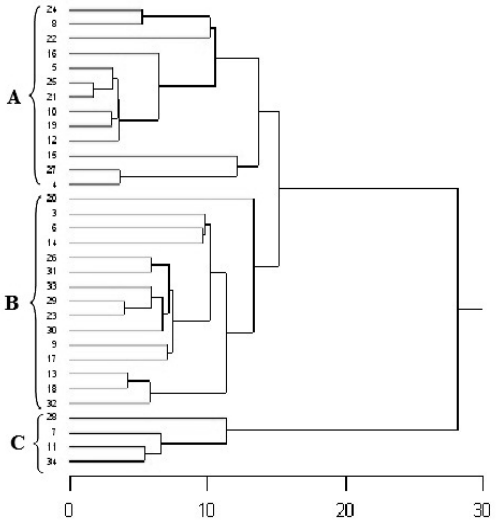

Figure 1 **Dendrogram constructed for 32 oilseed rape cultivars based on morphological traits**

RAPD analysis lead to the amplification of 166 scorable fragments ranging from 101 to 1310 bp resulting in 139 polymorphic bands. The number of amplicons ranged between 13 (ROTH A13) to 27 (ROTH A10) and presented molecular weight between 100-1116 bp. The distance matrices were made based on the two similarity coefficients according to Nei and Li (1979). Similarly matrices were used to cluster analysis using UPGMA method and a dendrogram was constructed. Results of UPGMA cluster analysis is presented in *figure 2*.

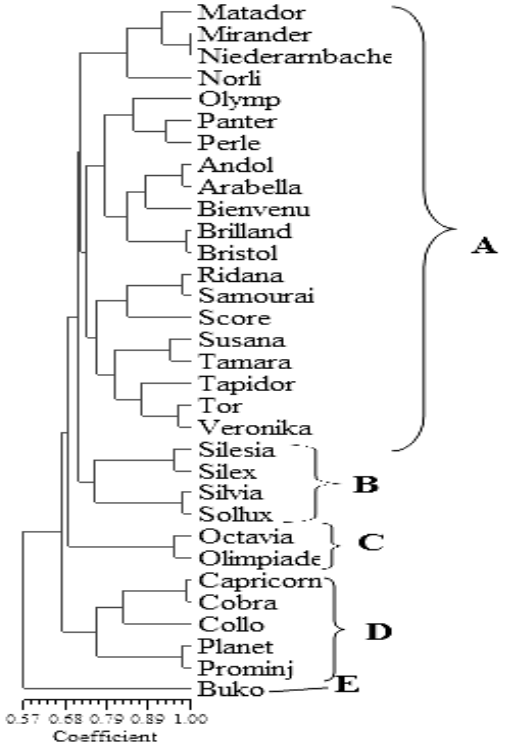

Figure 2 **Dendrogram illustrating genetic relationships between 32 rapeseed cultivars generated by UPGMA cluster analysis of polymorphic RAPD fragments**

Looking at the UPGMA dendrogram we can observe that there are five major clusters (cluster A, cluster B, cluster C, cluster D and cluster E). The biggest group (cluster A) contain 20 *Brassica napus* cultivars and it is divided in five sub clusters. In all of these sub clusters we can observe that most of the grouped cultivars are originating from the same country and made from the same breeder and this demonstrate a uniformity of the germplasm. In this group we can observe that the cultivars „Mirander" and „Niederarnbacher"are the most related cultivars and we can suppose that between these two cultivars is a very close similarity. These two are linked to genotype "Matador" meaning that the three cultivars may have common ancestry.

The other clusters are smaller Cluster B having four genotypes three of them originating from Germany, cluster D five genotypes and Cluster E one genotype. Most of the cultivars ere grouped after the country of origin. The most different cultivar seems to be "Buko" that is alone in cluster E which means that had different ancestry than the other cultivars.

Looking at the two dendrograms based on the morphological traits and on the molecular markers we can observe that both dendrograms shows a genetic variation among the cultivars. Both RAPD and morphological characters were sufficient to assess variability among 32 oilseed rape cultivars. No correlation was found between the matrices obtained by molecular and morphological traits. Despite the low correlation between those two dendrograms it can be observed that some cultivars were grouped in the same cluster in both dendrograms. For example the cultivars "Tapidor", "Tor'' and" Veronica" appear to be grouped in the same cluster in both dendrograms. The same results were obtained by other authors in different studies (Maric et al.,2004). The low correlation between RAPD dendrogram and morphological dendrogram had been also reported in other studies in European barley varieties (Schut et al, 1997), synthetic hexaploid wheat and their parents (Lage et al., 2003) and Squash (Ferriol et al., 2004). Normally until now, germplasm has been classified on the basis of morphological and agronomical traits, but recently the use of molecular markers to study diversity and characterization of the plants has become more common. This differences between the two dendrograms can be due to the fact that the morphological traits can be influenced by many factors such as: environmental conditions, the sample size, the time of making the measures, etc.

CONCLUSIONS

This study has shown that significant genetic variability was found among the genotypes on both morphological and molecular analysis, but the molecular analysis is more precise, due to the fact that the genotype is not influenced by the environmental conditions. Also, this study shows that PCR-based techniques such RAPD can be successfully used for detecting genetic variability in rapeseed. This study demonstrated that for determining the genetic variability among some cultivars, the molecular markers technique is more precise than the morphological traits.

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