THE GENETIC EVALUATION OF 130 OILSEED RAPE (*BRASSICA NAPUS* L.) CULTIVARS USING SSR (SINGLE SEQUENCE REPEAT) MARKERS

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Abstract

Oilseed rape (*Brassica napus*) has become one of the most cultivated oil crop, due to its utilization in different ways as human nutrition, as alternative biofuel source or raw material for the chemical industry. Also the residues obtained after the oil extraction are used in the animal feeding being considered important protein sources (Snowdon *et al.*, 2007).The aim of this study was to make the genetic evaluation of 130 oilseed rape cultivars provided by Center for Genetic Resources Nederland using SSR markers. For this, we used 51 SSR markers which amplified 139 specific fragments. Based on a obtained data it was calculated the genetic similarity (GS) between analyzed genotypes that concluded in one matrices which led to the generation of the SSR dendrogram. Analyzing the obtained dendrogram it was observed that a high genetic diversity between the studied cultivars.

Key words: SSR markers, Dendrogram, genetic diversity

Oilseed rape (*Brassica napus L.*) is the world' s third most important source of vegetable oils after palm and soybean (Beckman, 2005). The rapeseed production has increased during the past 25 years and presently it contributes with about 14 % of the global vegetal oils. To ensure the rapeseed production, breeders aim is to produce high yeld and high quality rapeseed oil cultivars. Information of the genetic diversity in *B. Napus* germplasm collections can give to breeders and geneticist's important information on the allelic diversity present in gene bank materials and may help to indentify genetically diverse pools for use in cross combinations to improve important agronomic traits or to better exploit heterosis (Diers and Osborn, 1994). There are many techniques used to study the genetic diversity of crop germplasm. Some examples for studing the genetic diversity are morphological traits, total seed proteins, isoenzymes and several types od DNA markers. In recent years, molecular genetic tecniques to detect DNA polymorfism had been increasingly used to characterise and identify novel germplasm for use in crop breeding (O'Neill *et al.* 2003). The genetic diversity of *B. napus* species had also being studied previous, but it had been investigated a number of limited range of genotypes. For example, Thormann *et al.* (1994) used restriction length polymorfism (RFLP) and randomly amplified polymorphic DNA markers (RAPD) to determine the genetic distances in and between cruciferous species. Diers and Osborn (1994) compared RFLP patterns in 61 winter and spring rapeseed cultivars and Hasan *et al.* (2006) used SSR markers to detect the genetic diversity among 96 *B. napus* genotypes. The aim of this study was to determine the genetic diversity of 130 *B. napus* cultivars using SSR molecular markers. The studied cultivars were provided by CGN (Center for Genetic Resources) Netherland.

MATERIAL AND METHOD

In order to determine the genetic relationships between the 130 studied oilseed rape cultivars, we used 51 SSR markers. The plants were cultivated in field, in the agricultural year 2012-2013 in order to get the necessary material for the DNA extraction. For this, fresh leaves were collected from each cultivar and then immediately frozen in liquid nitrogen. The DNA extraction was made using the CTAB procedure which was modified according to Doyle and Doyle (1990).
The polymerase chain reaction (PCR) mixture (25 µL) for SSRs contained 20 ng DNA template, 0.75 pmol of each primer, 0.2 mM dNTP mix, 1 mM MgCl2, 10XPCR reaction buffer and 5 unit of Taq DNA polymerase (Qiagen).

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Amplifications were performed using a standard amplification cycle in a GeneAmp PCR System 9700 thermal cycler, and SSR polymorphisms were separated and visualized using a LI-COR GeneReadir 4200 (MWG Biotech, Ebersberg). For the SSR analysis it was used M13-tailing technique. In this method the fluorescently labeled universal M13 primer 5'-AGGGTTTTCCCAGTCACGACGTT- 3' is added to the PCR reaction, and the forward primer of each SSR is appended with the sequence 5'-TTTCCCAGTCACGACGTT-3'. The amplification was performed after a touch-down PCR cycle as follows: an initial denaturation was performed at 95°C for 2 min, followed by five cycles of denaturation for 45 s at 95°C, annealing for 5 min beginning at 68°C and decreasing by 2°C in each subsequent cycle, and extension for 1 min at 72°C. Then five cycles were performed with 45 s denaturation at 95°C, 1 min annealing beginning at 58°C and decreasing 2°C in each subsequent cycle, and 1 min of extension at 72°C. The PCR was then completed with an additional 27 cycles of 45 s denaturation at 94°C, 2 min of annealing at 47°C, and 30 s of extension at 72°C, with a final extension at 72°C for 10 min.

The visualization of the amplified fragments was made using Saga generation software version1. Each primer was scored manual, for the presence of the band using '1"and 0 were the band was absent. This data were concretized in a matrix which was used for the 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).

RESULTS AND DISCUSSIONS

In order to estimate the genetic similarity of the 130 oilseed rape genotypes, it were used 51 SSR markers which lead to the amplification of 139 scorable fragments ranging from 80-340 bp. The smallest number of fragment which were amplified per one marker was 1 (Ol10-E12, CB 10437, Na 14-H12) and the biggest was 7 (Na12-B07). The level of polymorphism was of 100% (*tab.1*).

Table 1

SSR results after the fragment scoring

Nr. Crt	Primer	Nr de benzi amplificate	Minim	Maxim
1.	CB-10065	2	210	230
2.	Na10-G08	3	310	340
3.	Ol10-B02	2	80	170
4.	Na12-C01	3	40	110
5.	BRMS-30	2	210	220
6.	Na10-D11	2	218	220
7.	CB 10536	2	145	150
8.	Ol10-E12	1	280	280
9.	MD 60	2	180	190
10.	CB 10028	5	170	255
11.	CB10206	2	240	245
12.	CB 10437	1	190	190
13.	Cb 10097	2	210	220
14.	CB 104347	2	220	230
15.	Na12-H06	3	210	265
16.	CB 10611	2	170	190
17.	BRMS 20	1	200	200
18.	Ol10-D08	2	180	185
19.	BRMS 309	4	200	230
20.	Na10-B11	4	200	240
21.	Na12-D08	4	90	145
22.	OL10-C10	6	190	280
23.	Ra2-F04	2	110	150
24.	Na14-H12	1	257	257
25.	Ol10-D01	2	270	275
26.	CB10600	1	310	310
27.	Na12-A01	3	155	165
28.	Ol10-D03	3	155	235
29.	Ol10-F02	1	155	155
30	Na14-G06	2	240	245
31.	Ol11-B05	3	140	160
32.	Ni2-C12	1	80	80
33.	Na12-B11	1	130	130
34.	Ol13-E08	2	170	190
35.	Ol10-G06	3	130	165
36.	OL10-E05	6	130	170
37.	OL13-F08	2	140	145
38.	Na12-B07	7	130	147
39.	Ra12-E12	5	150	240
40.	Na12-A02	5	150	226
41.	Na12-B05	2	220	230
42.	HMR416	4	240	265
43.	Ra2-F11	5	210	245
44.	Ol11-H02	2	200	210
45.	Na10-C01	1	100	100
46.	HMR354	6	260	315
47.	Na14-G10	2	170	180
48.	HMR562	2	210	215
49.	HMR585	5	170	195
50.	Na12-G05	3	120	230
51.	CB10536	2	145	150

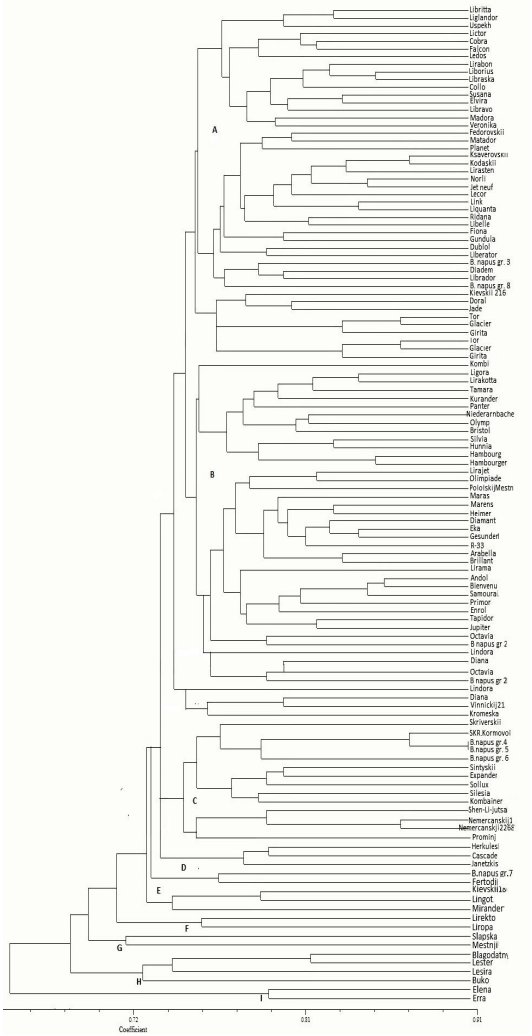

Figure 1 **The obtained dendrogram of 130 oilseed rape cultivars**

Based on the UPGMA analysis of the obtained data after the SSR analysis it was obtained a dendrogram which comprised 9 clusters: cluster "A", "B", "C", 'D", "E", "F", "G", "H" and "I" (*fig. 1*). Analyzing the obtained dendrogram it can be observed that the studied cultivars grouped after the country of origin.

The first cluster "A" grouped 37 cultivars and it is divided in three subcusters, most of them originating from Germany. The closest cultivars proved to be „Ksaverovskii" and „Kodaskii" having a similarity coefficient of 0.80, both originating from Ucraine.

Cluster "B", grouped 36 cultivars, this cluster being divided in more subculsters. It can be also observed that in this case, the cultivars were also grouped after the country of origin. In this case, the most closest cultivars were "Andol" and "Bienvenu" with a similarity coefficient of 0.85 both originating from France.

The most different cultivar in this cluster was "Lindora" which originating from Germany and grouped separate from the other cultivars.

Cluster "C" grouped 12 cultivars form different countries. In this cluster, the closest cultivars were "B. napus gr. 4" and "B. napus gr.5" with a similarity coefficient of 0.91.

Cluster "D" grouped just two cultivars being different than the other cultivars, having CS of 0.75. As it can be observed, the last five cultivars are the smallest, most of them grouping only two or three cultivars. In this clusters, the cultivars which were grouped are different, having CS between 0.68-0.80.

For determining the genetic diversity, 51 markers SSR had been used which amplified 139 polymorphic fragments. The number of polymorphic fragments at one marker oscilated between 1 and 6 and the level of polymorphism was of 100%. The high level of polymorphism obtained among 130 oilseed cultivars can be explained by the genetic fond and the different origin of the cultivars.

This technique had been used in previous studies for determining the genetic diversity at different species from *Brassica* genus like broccoli and cauliflower (Hu *el al.*, 1991; Li *et al.*, 1997; Hasan *et al.*, 2003).

CONCLUSIONS

Knowing the genetic diversity among the different plant species proved to be a useful tool in the breeding programs for identifying new genotypes with interest treats. This new genotypes can be used in the future studies as germplasm sources for improving some agronomical traits (Diers and Osborn, 1996).

Analyzing the obtained dendrogram, it can be observed a high genetic diversity between the studied cultivars which demonstrates the useful of this technique for determining the genetic relationships between the individual of the same genus.

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