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COMPARISON OF ISOZYME, RAPD AND AFLP MARKERS IN GENETIC SIMILARITY ASSESSMENT OF CMS *OGURA* F_1 HYBRIDS OF WINTER OILSEED RAPE (*BRASSICA NAPUS* L.) PARENTAL LINES

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Isozyme, RAPD and AFLP markers were evaluated and compared for their ability to determine genetic similarity in a set of 18 parental lines of winter oilseed rape F_1 hybrids developed using CMS *ogura*. Five isozyme systems, 64 RAPD starters and 23 *Eco*RI+3/*Mse*I+3 AFLP primer combinations generated 597 polymorphic markers. These polymorphic fragments were chosen for estimation of genetic similarity. Of the total number of polymorphic products, polymorphic zymograms constituted only 3.0% of the markers, 57 RAPD primers 37.7%, and 23 AFLP primer combinations 59.3%. The size of RAPD polymorphic products ranged from 564 to 2100 bp. On average there were four amplified bands per primer, with 61.0% polymorphism. The AFLP polymorphic fragments ranged from 72 to 1352 bp in size. AFLP assays generated 15 bands per primer pair on average and detected roughly four times more bands than with RAPD analysis. The genetic similarity coefficients based on all marker data range from 0.52 to 0.84. The correlation of genetic similarities based on RAPD and AFLP markers was 0.58. Estimated genetic similarity based on isozyme data was not correlated with genetic similarity derived from the two DNA-based markers. The dendrogram constructed with the three types of markers taken together grouped all the winter oilseed rape parental lines into several similar clusters. The genomic distribution and frequency of the RAPD and AFLP markers can serve well as estimators of genetic similarity between parental lines of F_1 CMS *ogura* hybrids.

Key words: Winter oilseed rape, *Brassica napus*, F_1 hybrids CMS *ogura*, genetic similarity, molecular markers.

INTRODUCTION

Molecular genetics techniques have completely changed how we study plant genomes and plant breeding. Molecular markers enable researchers to analyze genotypes without regard to environmental conditions and plant development stage. These markers include isozyme and polymorphic markers of DNA. Isozymes, the use of which was proposed by Hunter and Market (1957), have many advantages: they are direct products of the action of genes, they segregate according to Mendel's laws, and they usually are codominant. Besides studies on the genus *Brassica* (Yu et al., 2005), isozyme markers have been applied in a wide range of research on a variety of plant species (Weeden, 1989; Krzymińska et al., 2008; Ouji et al., 2011).

Markers based on DNA analysis have advantages similar to those of isozyme markers but they have a wider polymorphism spectrum and theoretically their number is unlimited. Markers employing PCR technique, such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), single sequence repeat (SSR) and single nucleotide polymorphism (SNP), support the selection of quantitative traits whose expression is considerably affected by environmental conditions (Javidfar et al., 2006; Hasan et al., 2008). They are used to construct genetic maps employed in many ways: as sources of genetic information describing species (Snowdon and Friedt, 2004; Chen et al., 2011), in research on the genetic diversity/similarity of breeding material (Kozak et al., 2011; Ouji et al., 2011; Zhao et al., 2012), and in constructing genet-

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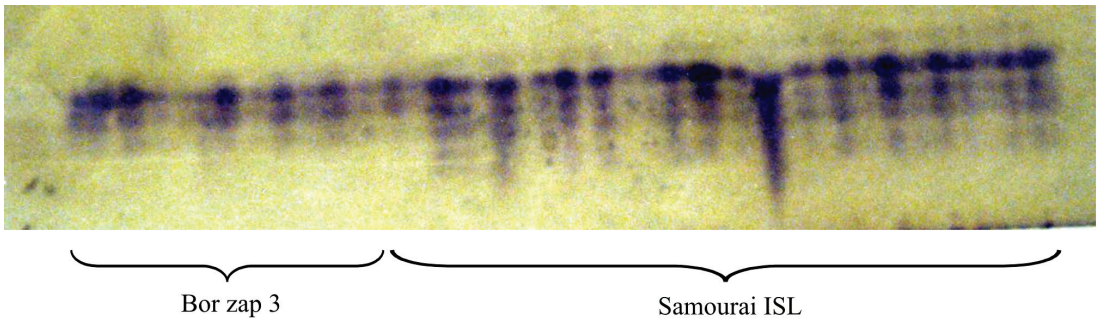

Fig. 1. Zymogram of malate dehydrogenase (MDH) in paternal line Bor zap 3 without restorer gene and CMS *ogura* maternal line Samourai ISL of CMS *ogura* hybrids of winter oilseed rape.

ic pools for various breeding programs (Seyis et al., 2003; Hasan et al., 2005; Ofori et al., 2008; Wei et al., 2009; Krajewski et al., 2012).

Identifying a parental combination with strong yield heterosis is the most important step in the development of hybrid cultivars (Basunanda et al., 2010). The level of genetic diversity/similarity between parents has been proposed as a predictor of F_1 performance and heterosis (Moll et al., 1965; Falconer and Mackay 1996). Prediction of heterosis from molecular-based distance has been reported in oilseed brassicas (Diers et al., 1996; Yu et al., 2005). Becker and Engqvist (1995) noted the usefulness of RAPD-based genetic distance estimates to predict heterosis for leaf dry matter production in *B. napus*. Shen et al. (2003) found a positive association between hybrid seed yield and genetic distance estimated by AFLP analysis.

In this study we (i) estimated the genetic similarity of F_1 CMS *ogura* hybrid parental lines based on isozymes, RAPD and AFLP markers, (ii) compared the genetic similarity and dendrograms obtained using those different markers, and (iii) assessed the usefulness of genetic similarity (GS) estimated by isozymes and molecular markers as a criterion for selection of parental components for F_1 hybrid breeding.

MATERIALS AND METHODS

The genetic polymorphism of 18 parental lines of F_1 CMS *ogura* hybrids of winter oilseed rape was estimated with the use of molecular markers. The investigated lines were 8 CMS *ogura* lines (Samourai ISL, PN 9294, PN 9288, PN 2824, PN 2850, BO 83a, BO 120a, BO 238a) and 10 paternal lines: 6 restorer lines containing the *Rfo* restorer gene (PN 4533, PN 4537, PN 4539, PN 4555, BO 110-RJ-4-103, PN 1265) and 4 lines without the restorer gene (Bor zap 1, Bor zap 2, Bor zap 3, Bor zap 4).

The plant material pedigrees and methods of analyzing isozymes and molecular markers were described in detail by Liersch (2005) and Bocianowski et al. (2011). The polymorphism of enzymatic protein was analyzed following methods developed by Shields et al. (1983) and Vallejos (1983). Enzyme separation was done by horizontal electrophoresis on 10.5% starch gel. Five isozyme systems were examined: isocitrate dehydrogenase – IDH (E.C.1.1.1.42), malate dehydrogenase – MDH (E.C.1.1.1.37), 6-phosphogluconate dehydrogenase – 6PGD (E.C.1.1.1.44), leucine aminopeptidase – LAP (E.C.3.4.11.1), and phosphoglucoisomerase – PGI (E.C.5.3.1.9).

RAPD AND AFLP ANALYSES

Genomic DNA was extracted from 8-day-old leaves of 10 plants for each investigated line by a modified CTAB procedure according to Doyle and Doyle (1990). RAPD analysis followed the method of Williams et al. (1990) method. The DNA samples were analyzed using 64 primers (Operon Technologies, Alameda, CA, USA) (Tab. 1). The amplified products were separated by electrophoresis for 3 h in 1.8% agarose gel. Each sample was analyzed in two replicates. The majority of these primers were selected from the linkage map for rapeseed described by Lombard and Delourme (2001).

AFLP analysis followed standard methods in accordance with the manufacturer's instructions (Gibco BRL, AFLP Analysis Reagent Kit, AFLP Analysis System I) and as previously described by Vos et al. (1995). Twenty-three primer combinations including 6 *Eco*RI and 7 *Mse*I primers were used (Tab. 2). PCR products were resolved on 13.35% denaturing polyacrylamide gels, silver-stained.

DATA ANALYSIS

Genetic similarity (GS) of investigated lines was calculated according to Nei and Li's (1979) coefficient, defined as $GS = 2N_{AB}/(N_A+N_B)$, where N_{AB} is the number of fragments shared by accessions A and B, N_A is the number of amplified fragments in sample

TABLE 1. RAPD primers used for analysis of parental lines of winter oilseed rape F_1 hybrids

Primer	(a)	(b)	(c)	Primer	(a)	(b)	(c)
OPA-01	9	5	55.56	OPL-12	10	6	60.00
OPA-07	9	8	88.88	OPN-01	–	–	–
OPA-08	6	5	83.33	OPN-02	9	9	100.00
OPA-09	3	2	66.67	OPN-07	3	1	33.33
OPA-11	9	5	55.56	OPN-13	4	3	75.00
OPA-14	–	–	–	OPN-18	6	5	83.33
OPA-15	8	7	87.50	OPN-20	11	9	81.82
OPA-16	3	2	66.67	OPP-03	6	6	100.00
OPA-18	11	6	54.55	OPP-05	4	1	25.00
OPC-02	8	2	25.00	OPP-07	6	3	50.00
OPC-04	4	2	50.00	OPP-08	6	4	66.67
OPC-09	6	2	33.33	OPP-09	3	2	66.67
OPC-15	–	–	–	OPP-11	7	4	57.14
OPC-18	5	4	80.00	OPP-14	7	5	71.43
OPD-08	6	5	83.33	OPW-02	10	5	50.00
OPF-01	8	7	87.50	OPW-03	–	–	–
OPF-04	5	3	60.00	OPW-05	6	5	83.33
OPF-06	3	2	66.67	OPW-08	6	6	100.00
OPF-09	7	2	28.57	OPW-09	11	7	63.64
OPF-14	–	–	–	OPW-11	5	2	40.00
OPF-15	–	–	–	OPW-13	6	4	66.67
OPF-20	4	1	25.00	OPW-15	5	4	80.00
OPG-03	6	4	66.67	OPW-19	5	2	40.00
OPG-04	6	6	100.00	OPW-20	10	1	10.00
OPG-05	6	4	66.67	OPV-07	6	1	16.67
OPG-11	10	6	60.00	OPY-01	8	6	75.00
OPG-13	3	2	66.67	OPY-02	8	3	37.50
OPG-12	–	–	–	OPY-04	10	3	30.00
OPG-14	6	4	66.67	OPY-05	6	2	33.33
OPG-15	6	5	83.33	OPY-10	4	3	75.00
OPJ-07	4	2	50.00	OPY-13	9	4	44.44
OPK-08	5	4	80.00	OPY-15	9	4	44.44
Mean							61.00

(a) – total number of amplified DNA fragments; (b) – number of polymorphic DNA fragments; (c) – (b/a × 100) – % of polymorphism

A, and N_B is the number of amplified fragments in sample B. Genetic similarity (GS) was computed for each of three marker types separately as well as for the three marker types together. The genotypes were grouped using the unweighted pair group method with arithmetic mean (UPGMA). Similarities among parental lines were visualized with dendrograms. Minima, maxima, means and coefficients of variation were estimated for all GS coefficients. In addition, correlation coefficients were estimated to determine the relationship between GS calculated for all three marker systems. All calculations were done with GenStat v. 7.1 (Payne et al., 2003).

RESULTS

In estimating genetic similarity between the parental lines of F_1 winter oilseed rape we obtained 597 markers, of which 18 (3.0%) characterized isozyme loci: three polymorphic zymograms each in the IDH, 6PGD, LAP and MDH (Fig. 1) isozyme systems, and

TABLE 2. AFLP primer combinations used for analysis of parental lines of winter oilseed rape F_1 hybrids

Primer combination*	Code	(a)	(b)	(c)
E-AAC : M-CAA	E1M1	45	4	8.89
E-AAC : M-CAG	E1M3	54	19	31.19
E-AAC : M-CAT	E1M4	47	12	25.53
E-AAC : M-CTA	E1M5	43	13	30.23
E-AAC : M-CTT	E1M7	52	7	13.46
E-AAG : M-CAA	E2M1	31	6	19.35
E-AAG : M-CAC	E2M2	46	15	32.61
E-AAG : M-CAT	E2M4	48	3	6.25
E-AAG : M-CTC	E2M6	41	14	34.15
E-AAG : M-CTT	E2M7	37	11	29.73
E-ACC : M-CAA	E3M1	51	13	25.50
E-ACC : M-CAC	E3M2	51	18	35.29
E-ACC : M-CAG	E3M3	46	26	56.52
E-ACC : M-CTC	E3M6	42	22	52.38
E-ACT : M-CAT	E4M4	54	26	48.15
E-ACT : M-CTC	E4M6	37	17	45.95
E-ACT : M-CTT	E4M7	49	18	36.73
E-AGG : M-CAC	E5M2	38	13	34.21
E-AGG : M-CAG	E5M3	33	14	43.75
E-AGG : M-CAT	E5M4	43	16	37.21
E-AGG : M-CTA	E5M5	40	20	50.00
E-AGG : M-CTC	E5M6	51	31	60.78
E-ACA : M-CTT	E6M7	47	16	34.04
Mean				34.50

*Core sequences for selective amplification were E-5'-GACTGCGTACCAATTC-3' (*Eco*RI primer) and M-5'-GATGAGTCCTGAGTAA-3' (*Mse*I primer). Every *Eco*RI and *Mse*I was designed by adding to the 3' end three randomly selected nucleotides;
(a) – number of amplified DNA fragments; (b) – number of polymorphic DNA fragments; (c) – (b/a × 100) – % of polymorphism.

TABLE 3. Comparison of polymorphism revealed by isozymes, RAPD and AFLP markers

Parameter	Markers			Total
	Isozyme	RAPD	AFLP	
Number of primers	5 systems	64	23	
Total number of bands	18	369	1026	
Polymorphic bands	18	225	354	
Monomorphic bands	–	144	672	
Mean number of polymorphic bands per primer	3.6	3.9	15.4	
Max. similarity	0.91	0.84	0.87	0.84
Min. similarity	0.00	0.05	0.50	0.52
Mean similarity	0.43	0.63	0.64	0.63

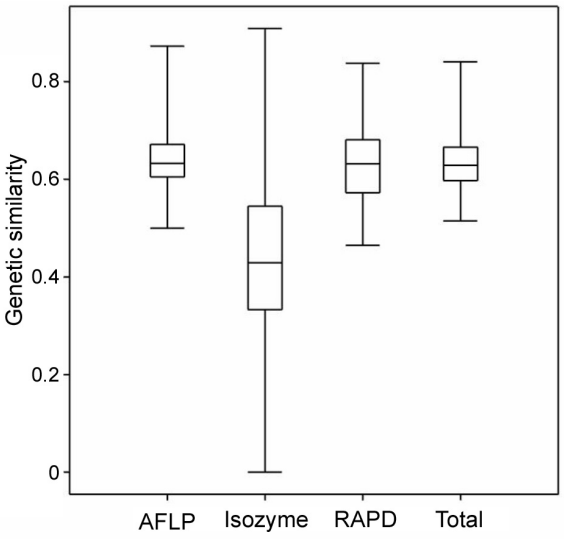

Fig. 2. Genetic similarity of parental lines of F_1 CMS *ogura* hybrids of winter oilseed rape (*Brassica napus*), classified by marker type.

six polymorphic zymograms in PGI. The DNA markers we found comprised 225 RAPD markers (37.7% of all markers) and 354 AFLP markers (59.3%). Fifty-seven of the 64 RAPD primers differentiated the DNA of the investigated lines. The polymorphic products ranged from 564 bp to 2100 bp. On average there were four amplified bands per primer, with 61.0% polymorphism (Tab. 1). The polymorphic AFLP fragments ranged from 72 bp to 1352 bp. The total number of polymorphic bands scored per primer combination varied from 3 to 31, with mean polymorphism of 34.5% (Tab. 2). AFLP assays generated 15 bands per primer pair on average and produced four times more bands per reaction than we obtained by RAPD analysis. The characteristics of polymorphism revealed by three investigated marker systems are presented in Figure 2 and Tables 3, 4 and 5.

The coefficient of correlation between GS estimated by RAPD and by AFLP markers was 0.58 ($p < 0.001$; Tab. 6). The correlation coefficient for the whole set of markers gave a GS estimate strongly correlated with estimates based on RAPD and AFLP markers but not the GS from isozyme markers (Tab. 6).

Genetic similarity based on each of the three types of markers and the whole set are presented in Figures 3 (isozymes), 4 (RAPD), 5 (AFLP) and 6 (all markers). The dendrogram based on AFLP was basically the same as the one based on RAPD. Clustering based on GS for all markers was close to the one based on AFLP markers. The paternal line

TABLE 4. Genetic similarity among the studied genotypes based on isozymes (below diagonal) and RAPD markers (above diagonal)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	Bor zap 1	1	0.67	0.53	0.59	0.48	0.68	0.62	0.51	0.56	0.64	0.57	0.63	0.58	0.61	0.65	0.64	0.59	0.65
2	Bor zap 2	0.77	1	0.59	0.72	0.61	0.71	0.73	0.55	0.65	0.58	0.65	0.69	0.66	0.81	0.70	0.77	0.63	0.70
3	Bor zap 3	0.67	0.73	1	0.52	0.53	0.60	0.56	0.54	0.53	0.53	0.52	0.50	0.55	0.57	0.52	0.56	0.55	0.54
4	Samourai	0.50	0.36	0.60	1	0.62	0.63	0.71	0.51	0.63	0.57	0.69	0.68	0.67	0.71	0.67	0.72	0.56	0.70
5	PN 4537	0.43	0.31	0.17	0.33	1	0.62	0.68	0.52	0.80	0.47	0.57	0.58	0.59	0.52	0.57	0.62	0.60	0.63
6	PN 9294	0.50	0.18	0.40	0.60	0.33	1	0.72	0.66	0.67	0.73	0.75	0.76	0.65	0.63	0.68	0.68	0.71	0.74
7	PN 4539	0.46	0.50	0.36	0.18	0.62	0.18	1	0.57	0.78	0.60	0.76	0.65	0.68	0.66	0.66	0.72	0.64	0.74
8	PN 2824	0.46	0.33	0.36	0.73	0.31	0.36	0.17	1	0.54	0.52	0.60	0.61	0.56	0.47	0.53	0.53	0.58	0.58
9	PN 4533	0.53	0.43	0.46	0.31	0.40	0.15	0.57	0.57	1	0.52	0.64	0.62	0.64	0.55	0.59	0.66	0.62	0.67
10	PN 9288	0.62	0.33	0.36	0.18	0.31	0.55	0.33	0.50	0.57	1	0.68	0.68	0.57	0.57	0.57	0.59	0.61	0.67
11	PN 4555	0.47	0.38	0.40	0.53	0.35	0.27	0.50	0.63	0.67	0.38	1	0.76	0.66	0.65	0.63	0.68	0.64	0.84
12	PN 2850	0.62	0.33	0.36	0.18	0.46	0.36	0.50	0.33	0.43	0.67	0.50	1	0.68	0.64	0.69	0.70	0.62	0.81
13	Bor zap 4	0.50	0.55	0.80	0.60	0.33	0.40	0.18	0.36	0.46	0.18	0.27	0.18	1	0.63	0.66	0.71	0.60	0.66
14	BO 83a	0.46	0.50	0.73	0.55	0.31	0.55	0.33	0.33	0.29	0.50	0.38	0.33	0.55	1	0.63	0.65	0.60	0.64
15	BO 120a	0.46	0.33	0.36	0.36	0.31	0.36	0.33	0.67	0.57	0.83	0.50	0.50	0.18	0.67	1	0.83	0.55	0.68
16	BO 238a	0.31	0.33	0.18	0.18	0.46	0.18	0.50	0.50	0.57	0.67	0.50	0.33	0.00	0.50	0.83	1	0.60	0.74
17	BO 110-RJ-4-103	0.33	0.36	0.20	0.00	0.33	0.20	0.55	0.36	0.62	0.73	0.40	0.36	0.00	0.36	0.73	0.91	1	0.66
18	PN 1265	0.67	0.57	0.46	0.46	0.27	0.15	0.43	0.57	0.63	0.43	0.78	0.57	0.31	0.43	0.57	0.43	0.31	1

TABLE 5. Genetic similarity among the studied genotypes based on AFLP markers (below diagonal) and all markers together (above diagonal)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	Bor zap 1	1	0.72	0.57	0.61	0.63	0.63	0.63	0.61	0.63	0.61	0.59	0.71	0.66	0.61	0.72	0.66	0.58	0.61
2	Bor zap 2	0.70	1	0.61	0.63	0.63	0.69	0.68	0.67	0.63	0.62	0.64	0.65	0.67	0.74	0.63	0.67	0.65	0.67
3	Bor zap 3	0.56	0.6	1	0.59	0.62	0.56	0.60	0.59	0.60	0.56	0.52	0.58	0.61	0.56	0.59	0.55	0.50	0.55
4	Samourai	0.60	0.66	0.56	1	0.63	0.59	0.62	0.59	0.63	0.57	0.58	0.65	0.71	0.63	0.62	0.57	0.60	0.59
5	PN 4537	0.57	0.61	0.58	0.62	1	0.61	0.68	0.65	0.87	0.6	0.58	0.65	0.65	0.57	0.64	0.61	0.60	0.58
6	PN 9294	0.65	0.69	0.58	0.60	0.61	1	0.69	0.80	0.60	0.72	0.65	0.71	0.65	0.64	0.65	0.68	0.62	0.69
7	PN 4539	0.62	0.70	0.58	0.65	0.68	0.69	1	0.72	0.67	0.66	0.68	0.63	0.67	0.64	0.63	0.68	0.65	0.69
8	PN 2824	0.57	0.62	0.57	0.57	0.60	0.74	0.66	1	0.66	0.71	0.61	0.70	0.66	0.64	0.62	0.67	0.61	0.63
9	PN 4533	0.60	0.64	0.57	0.62	0.83	0.62	0.71	0.61	1	0.62	0.61	0.66	0.63	0.54	0.62	0.59	0.57	0.60
10	PN 9288	0.62	0.60	0.54	0.56	0.54	0.72	0.63	0.64	0.58	1	0.65	0.70	0.67	0.59	0.58	0.61	0.61	0.65
11	PN 4555	0.58	0.64	0.52	0.62	0.57	0.68	0.70	0.60	0.62	0.65	1	0.73	0.63	0.67	0.64	0.70	0.70	0.81
12	PN 2850	0.67	0.66	0.54	0.65	0.62	0.73	0.64	0.66	0.64	0.69	0.73	1	0.66	0.70	0.73	0.71	0.71	0.77
13	Bor zap 4	0.63	0.67	0.59	0.69	0.62	0.65	0.67	0.62	0.63	0.62	0.63	0.66	1	0.62	0.61	0.62	0.59	0.59
14	BO 83a	0.61	0.77	0.57	0.66	0.54	0.64	0.64	0.57	0.54	0.58	0.65	0.67	0.62	1	0.64	0.68	0.67	0.69
15	BO 120a	0.68	0.65	0.56	0.64	0.61	0.66	0.64	0.59	0.61	0.58	0.64	0.71	0.62	0.64	1	0.85	0.64	0.69
16	BO 238a	0.65	0.70	0.55	0.62	0.61	0.67	0.69	0.61	0.62	0.60	0.69	0.70	0.65	0.67	0.84	1	0.71	0.73
17	BO 110-RJ-4-103	0.58	0.64	0.52	0.58	0.60	0.65	0.64	0.59	0.59	0.61	0.67	0.67	0.59	0.63	0.61	0.67	1	0.74
18	PN 1265	0.63	0.68	0.54	0.63	0.59	0.70	0.71	0.61	0.63	0.66	0.82	0.78	0.62	0.66	0.68	0.73	0.7	1

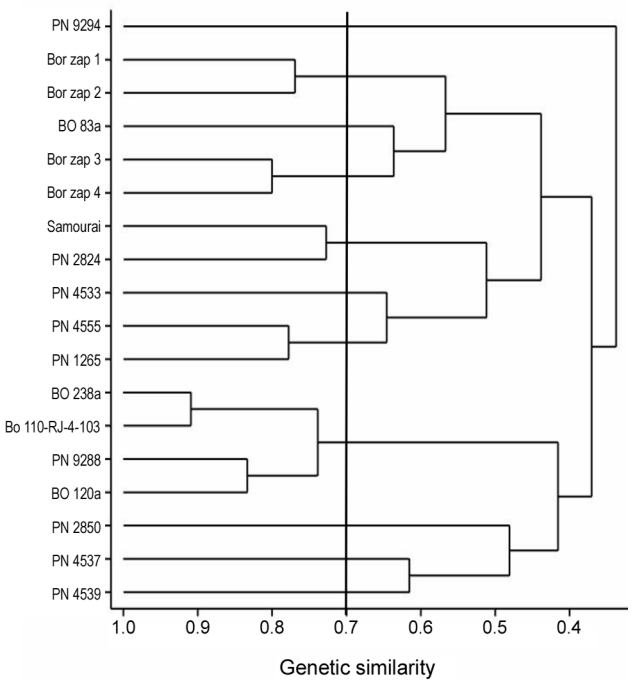

Fig. 3. Dendrogram for 18 parental lines of winter oilseed rape (*B. napus*) constructed for isozyme markers.

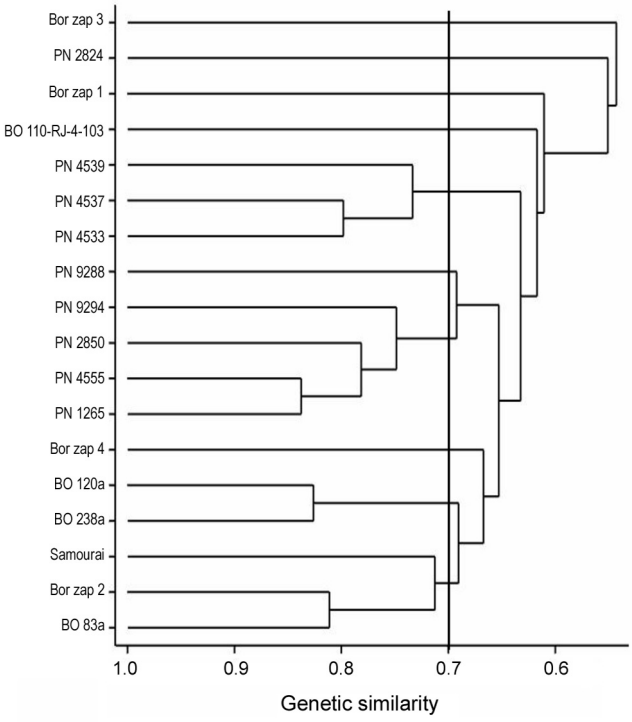

Fig. 4. Dendrogram for 18 parental lines of winter oilseed rape (*B. napus*) constructed for RAPD markers.

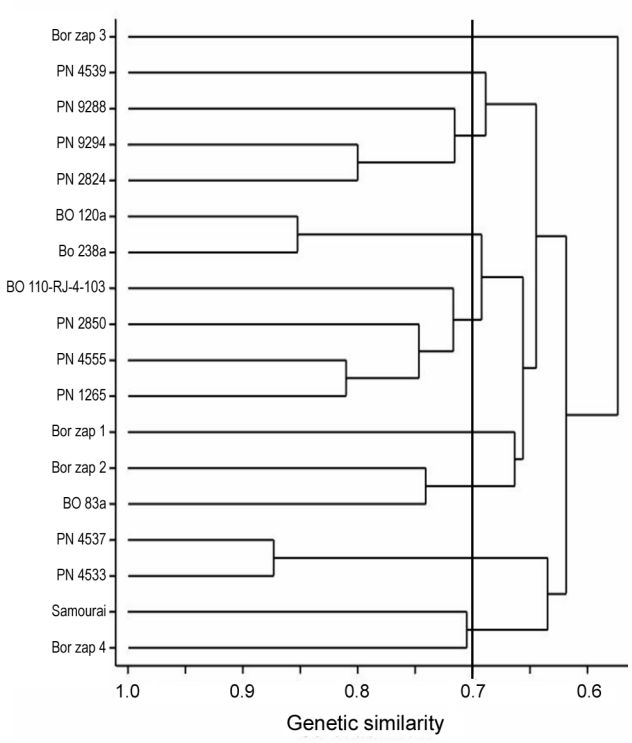

Fig. 5. Dendrogram for 18 parental lines of winter oilseed rape (*B. napus*) constructed for AFLP markers.

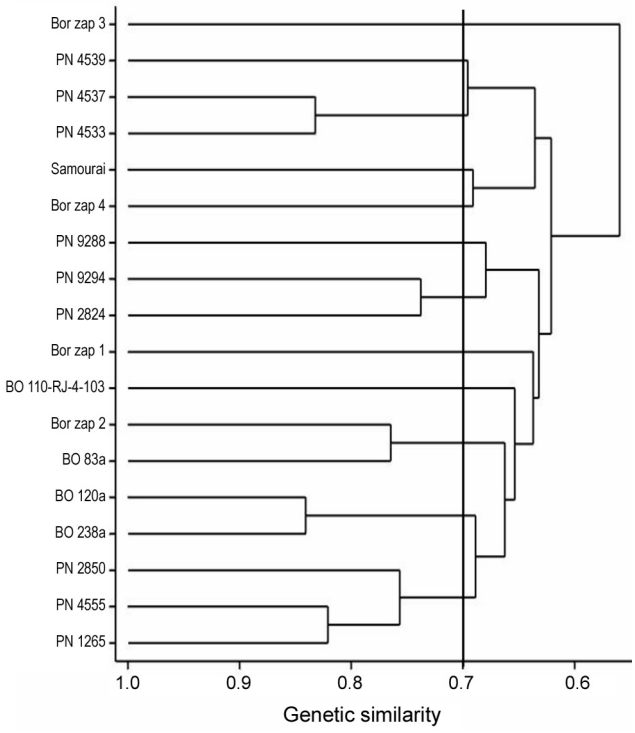

Fig. 6. Dendrogram for 18 parental lines of winter oilseed rape (*B. napus*) constructed for all markers.

TABLE 6. Correlations between GS estimated from analyses using isozymes, RAPD and AFLP markers; correlation coefficients, with p values in parentheses

	RAPD	AFLP	All markers
Isozymes	0.01 (0.945)	0.14 (0.091)	0.14 (0.088)
RAPD	1	0.58 (<0.001)	0.87 (<0.001)
AFLP		1	0.91 (<0.001)

without restorer gene Bor zap 3 (paternal lines of the high-yielding composite hybrid Pomorzanin) had a separate branch on the dendrograms for RAPD, AFLP, and all markers analyzed together, indicating its difference from the other lines. Interestingly, isoenzyme markers did not separate it, probably due to too few isozyme banding patterns. Figures 5 and 6 show several similar small clusters containing 15 of the 18 lines.

DISCUSSION

Mohammadi and Prasanna (2003) stressed three important elements of evaluating genetic diversity in plant material: (i) selection of an appropriate statistical measure to estimate the relationships among the studied genotypes, (ii) selection of molecular techniques, and (iii) the number of molecular markers used. We tested three types of markers differing in the number of polymorphic markers generated, the repeatability of results, their cost, and their ability to test a large number of samples quickly. Isozymes, RAPD and AFLP markers have been used to estimate genetic diversity/similarity in various crop species (Foisset et al., 1996; Corbellini et al., 2002) and species of the genus *Brassica* (Snowdon and Friedt, 2004; Hu et al., 2007). The RAPD method is one of the simplest and cheapest molecular techniques; RAPD markers have been used successfully to analyze genetic diversity, identify, quantify and characterize genetic variation in all available germplasm resources (Ćwiklińska et al., 2010; Bocianowski and Seidler-Łożykowska, 2012). The AFLP method has been applied in research involving, for example, identification of sequence variability (Vos et al., 1995) and prediction of hybrid performance in maize using AFLP and SSR molecular markers (Schrag et al., 2010). Lefort-Buson et al. (1987) determined the genetic distances of parental lines of F_1 hybrids of spring and winter rapeseed based on eleven isoenzymatic systems, seven of which were studied in detail. They showed the potential of isozyme analysis for selecting components of hybrid rapeseed cultivars. Yu et al. (2005) reported similar results but stated that the use of isozyme markers for selection of parental lines of hybrids of the Schaan 2A system was limited by their low frequency of occurrence. That is the most important factor limiting the use of this type of marker. For example, the maximum number of loci is 30 in wheat, 36 in maize, and 26 in tomato (Lefort-Buson et al., 1988). This explains the small share of isozyme markers – only 3% – in our pool of markers.

The RAPD and AFLP molecular markers we used differ in number, polymorphism level and repeatability. Genetic similarity estimates can differ due to differences in the number of marker observations and in the character of polymorphic markers. Generally, the percentage of polymorphic DNA bands is smaller for AFLP and yet this technique is more efficient than RAPD and isozymes for analyzing genetic divergence, thanks to the large number of polymorphic DNA fragments per assay unit (Lombard et al., 2000; Simoniuc et al., 2002). Our study confirmed this: the RAPD method generated 61.0% polymorphic DNA fragments, and the AFLP method only 34.5% (Tabs. 1, 2). Studying *Brassica campestris*, Das et al. (1999) reported 79.2% polymorphism for RAPD markers and 66.8% for AFLP markers.

For the studied parental lines of CMS *ogura* F_1 hybrids we found correlations between the genetic similarity estimates based on RAPD and AFLP markers (r = 0.58), RAPD and all markers (r = 0.87), and AFLP and all markers (r = 0.91). There was no such correlation between the analyses based on isozyme markers and either RAPD or AFLP markers. A number of authors have studied genetic relationships within and between *Brassica* species using techniques such as RFLP and RAPD (dos Santos et al., 1994; Thormann et al., 1994). Estimating the relationships in 18 lines of *Brassica* species, Thormann et al. (1994) reported a strong correlation (r = 0.97) between the results from RFLP and from RAPD markers. In a study of *Brassica oleracea* genotypes, dos Santos et al. (1994), found quite a strong correlation between the results from RFLP and from RAPD markers (r = 0.74).

The dendrograms for the various markers (Figs. 3–5) show differences in genotype groupings, especially for the isozyme markers. The differences in genotype clustering between marker types correspond to the differences between genetic similarity coefficients.

The dendrograms based on RAPD and AFLP markers as well as on all markers taken together group the genotypes into clusters generally in agreement with the known pedigrees. The results show that genetic variation among the selected CMS *ogura* breeding lines was small. The classification of these lines is based on pedigree.

In their study of *Brassica oleracea* genotypes, dos Santos et al. (1994) noted some inconsistency between RAPD and RFLP analyses in clustering based on genetic similarity but reported correspon-

dence between genotype grouping and genotype origin. Studying carrot (*Daucus carota* L.), Nakajima et al. (1998) discussed some differences between dendrograms constructed on the basis of AFLP and RAPD markers, which may have been due to differences in PCR efficiency or to small band sample size. According to Simoniuc et al. (2002), some differences between RAPD and AFLP clustering may be due to the fact that AFLP analyses are based on restriction sites and therefore scan different parts of the genome. In their study, pedigree relationships were best reflected in the combined cluster of AFLP and RAPD analyses (Simoniuc et al., 2002). The same was true in our present study.

CONCLUSIONS

1. Our analyses of genetic similarity between parental lines of F_1 CMS *ogura* hybrids confirmed the general observation that isozyme markers are useful in this type of research only as markers complementary to molecular markers, owing to the small number of loci they differentiate.
2. AFLP markers are the most useful ones for studying the genetic similarity of winter oilseed rape breeding lines, because of their high precision and the large number of markers they yield for a single genotype.
3. Approaches combining markers such as RAPD and AFLP may provide more accurate information on genetic diversity and relationships in *B. napus* genotypes.

REFERENCES

Basunanda P, Radoev M, Ecke W, Friedt W, Becker HC, and Snowdon RJ. 2010. Comparative mapping of quantitative trait loci involved in heterosis for seedling and yield traits in oilseed rape (*Brassica napus* L.). *Theoretical and Applied Genetics* 120: 271–281.

Becker H, and Engqvist GM. 1995. The potential of resynthesized rapeseed for hybrid breeding. *Proceedings of 9th International Rapeseed Congress* 4–7 July 1995, vol. 1, 118–121. Cambridge, UK.

Bocianowski J, Kozak M, Liersch A, and Bartkowiak-Broda I. 2011. A heuristic method of searching for interesting markers in terms of quantitative traits. *Euphytica* 181: 89–100.

Bocianowski J, and Seidler-Łożykowska K. 2012. The relationship between RAPD markers and quantitative traits of caraway (*Carum carvi* L.). *Industrial and Crops Production* 36: 135–139.

Corbellini M, Perenzin M, Accerbi M, Vaccino P, and Borghi B. 2002. Genetic diversity in bread wheat, as revealed by coefficient of parentage and molecular markers, and its relationship to hybrid performance. *Euphytica* 123: 273–285.

Chen W, Zhang Y, Yao J, Ma C, Tu J, and Tingdong F. 2011. Quantitative trait of loci mapping for two seed yield component traits in an oilseed rape (*Brassica napus*) cross. *Plant Breeding* 130: 640–646.

Ćwiklińska A, Broda Z, Bocianowski J, and Dobrzycka A. 2010. The usefulness of RAPD and AFLP markers for determining genetic similarity in rye (*Secale* L.) species and subspecies. *Acta Biologica Cracoviensia Series Botanica* 52(1): 19–25.

Das S, Rajagopal J, Bhatia S, Srivastava PS, and Lakshmikumaran M. 1999. Assessment of genetic variation within *Brassica campestris* cultivars using amplified fragment length polymorphism and random amplification of polymorphic DNA markers. *Journal of Biosciences* 24/2: 433–440.

Diers BW, Mc Vetty PBE, and Osborn TC. 1996. Relationship between heterosis and genetic distance based on restriction fragment length polymorphism markers in oilseed rape (*Brassica napus* L.). *Crop Science* 36: 79–83.

dos Santos JB, Nienhuis P, Skroch J, Tivang J, and Slocum MK. 1994. Comparison of RAPD and RFLP genetics markers in determining genetic similarity among *Brassica oleracea* L. genotypes. *Theoretical and Applied Genetics* 87: 909–915.

Doyle JJ, and Doyle JL. 1990. Isolation of plant DNA from fresh tissue. *Focus* 12: 13–15.

Falconer DS, and Mackay TFC. 1996. *Introduction to Quantitative Genetics*. 4th ed. Longman, England.

Foisset N, Delourme R, Barret P, Hubert N, Landry BS, and Renard M. 1996. Molecular-mapping analysis in *Brassica napus* using isozyme, RAPD and RFLP markers on a doubled-haploid progeny. *Theoretical and Applied Genetics* 93: 1017–1025.

Hasan M, Seyis F, Badani AG, Pons-Kühnemann J, Lühs W, Friedt W, and Snowdon RJ. 2005. Surveying genetic diversity in the *Brassica napus* gene pool using SSR markers. *Genetic Resources and Crop Evolution* 53: 793–802.

Hasan M, Friedt W, Freitag NM, Link K, Pons-Kühnemann J, and Snowdon RJ. 2008. Association of gene-linked SSR to seed glucosinolates content in oilseed rape (*Brassica napus* ssp. napus). *Theoretical and Applied Genetics* 116: 1035–1049.

Hu S, Yu Ch, Zhao H, Sun G, Zhao S, Vyvadilova M, and Kucera V. 2007. Genetic diversity of *Brassica napus* L. germplasm from China and Europe assessed by some agronomically important characters. *Euphytica* 154: 9–16.

Hunter RL, and Markert CL. 1957. Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels. *Science* 125: 1294–1295.

Javidfar F, Ripley VL, Roslinsky V, Zeinali H, and Abdmishani C. 2006. Identification of molecular markers associated with oleic and linolenic acid in spring oilseed rape (*Brassica napus*). *Plant Breeding* 125: 65–71.

Kozak M, Bocianowski J, Liersch A, Tartanus M, Bartkowiak-Broda I, Piotto F, and Azevedo RA. 2011. Genetic divergence is not the same as phenotypic divergence. *Molecular Breeding* 28(2): 277–280.

Krajewski P, Bocianowski J, Gawłowska M, Kaczmarek Z, Pniewski T, Święcicki W, and Wolko B. 2012. QTL for yield components and protein content: a multienviron-

ment study of two pea (*Pisum sativum* L.) populations. *Euphytica* 183: 323–336.

KRZYMIŃSKA A, GAWŁOWSKA M, WOLKO B, and BOCIANOWSKI J. 2008. Genetic diversity of ornamental *Allium* species and cultivars assessed with isozymes. *Journal of Applied Genetics* 49(3): 213–220.

LEFORT-BUSON M, GUILLOT-LEMOINE B, and BARTKOWIAK-BRODA I. 1987. A *Brassica* study of rapeseed (*Brassica napus*) F_1 hybrids from lines of same and different geographic origins. *Proceeding of the 7th International Rapeseed Congress* 11–14 May 1987: 63–68. Poznan, Poland.

LEFORT-BUSON M, HEBERT Y, and DAMERVAL C. 1988. Les outils d' évaluation de la divérsité génétique et phénotypique. *Agronomie* 8(3): 173–178.

LIERSCH A. 2005. Influence of genetic variability on heterosis effect in winter oilseed rape (*Brassica napus* L.). Ph.D. dissertation, Plant Breeding and Acclimatization Institute, Poznan, Poland [in Polish].

LOMBARD V, BARIL CP, DUBREUIL P, BLOUET F, and ZHANG D. 2000. Genetic relationships and fingerprinting of rapeseed cultivars by AFLP. *Crop Science* 40: 1417–1425.

LOMBARD V, and DELOURME R. 2001. A consensus linkage map for rapeseed (*Brassica napus* L.): construction and integration of three individual maps from DH populations. *Theoretical and Applied Genetics* 103: 491–507.

MOHAMMADI SA, and PRASANNA BM. 2003. Analysis of genetic diversity in crop plants-salient statistical tools and considerations. *Crop Science* 43: 1235–1248.

MOLL RH, LOONQUIST JH, VELEZ FORTUNO J, and JOHNSON EC. 1965. The relationship of heterosis and genetics divergence in maize. *Genetics* 52: 139–144.

NAKAJIMA Y, OEDA K, and YAMAMOTO T. 1998. Characterization of genetic diversity of nuclear and mitochondrial genomes in *Daucus* varieties by RAPD and AFLP. *Plant Cell Reports* 17: 848–853.

NEI M, and LI W. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the USA* 76: 5269–5273.

OFORI A, BECKER HC, and KOPISH-OBUCH FJ. 2008. Effect of crop improvement on genetic diversity in oilseed *Brassica rapa* (turnip-rape) cultivars, detected by SSR markers. *Journal of Applied Genetics* 49: 207–212.

OUJI A, SUSO MJ, ROUAISSI M, ABDELLAOUI R, and GAZZAH ME. 2011. Genetic diversity of nine faba bean (*Vicia faba* L.) populations revealed by isozyme markers. *Genes & Genomics* 33: 31–38.

PAYNE R, MURREY D, HARDING S, BAIRD D, SOUTOU D, and LANE P. 2003. GenStat for Windows (7th edition) – Introduction. VSN International, Oxford, England.

SCHRAG TA, MÖHRING J, MAURER HP, DHILLON BS, MELCHINGER AE, PIEPHO HP, SØRENSEN AP, and FRISCH M. 2009. Molecular marker-based prediction of hybrid performance in maize using unbalanced data from multiple experiments with factorial crosses. *Theoretical and Applied Genetics* 118: 741–751.

SEYIS F, SNOWDON RJ, LÜHS W, and FRIEDT W. 2003. Molecular characterization of novel resynthesized rapeseed (*Brassica napus*) lines and analysis of their genetic diversity in comparison with spring rapeseed cultivars. *Plant Breeding* 122: 473–478.

SHEN J, LU G, FU T, and YANG G. 2003. Relationship between heterosis and genetic distance based on AFLPs in *Brassica napus*. *Proceedings of the 11th International Rapeseed Congress* 6–10 July 2003, vol. 1, 342–346. Copenhagen, Denmark.

SHIELDS CR, ORTON CJ, and STUBER CW. 1983. An outline of general resource needs and procedures for the electrophoretic separation of active enzymes from plant tissue. Isozymes. In: Tanksley SD, Orton TJ [eds.], *Isozymes in Plant Genetics and Breeding*, Part A, 443–458. Elsevier Science Publishers, B.V, Amsterdam.

SIMONIUC D, UPTMOOR R, FRIEDT W, and ORDON F. 2002. Genetic diversity and relationships among pea cultivars revealed by RAPDs and AFLPs. *Plant Breeding* 121: 429–435.

SNOWDON RJ, and FRIEDT W. 2004. Molecular markers in *Brassica* oilseed breeding: current status and future possibilities. *Plant Breeding* 123: 1–8.

THORMANN CE, FERREIRA ME, CAMARGO LEA, TIVANG JG, and OSBORN TC. 1994. Comparison of RAPD and RFLP markers for estimating genetic relationships within and among cruciferous species. *Theoretical and Applied Genetics* 88: 973–980.

VALLEJOS CE. 1983. Enzyme activity staining. In: Tanksley SD, Orton TJ [eds.], *Isozymes in Plant Genetics and Breeding*, Part A, 469–516. Elsevier Science Publishers, B.V, Amsterdam.

VOS P, HOGERS R, BLEEKER M, REIJANS M, VAN DE LEE T, HORNES M, FRIJTERS A, POT J, TELEMAN J, KUIPER M, and ZABEAU M. 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research* 23: 4407–4414.

WILLIAMS JGK, KUBELIK AR, LIVAK KJ, RAFALSKI JA, and TINGEY SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18: 6531–6535.

WEEDEN NF. 1989. Applications of isozymes in plant breeding. *Plant Breeding Reviews* 6: 11–54.

WEI LB, ZHANG HY, ZHENG YZ, MIAO HM, ZHANG TZ, and GUO WZ. 2009. A genetic linkage map construction for sesame (*Sesamum indicum* L.). *Genes & Genomics* 31: 199–208.

YU CY, HU SW, ZHAO HX, GUO AG, and SUN GL. 2005. Genetic distance revealed by morphological characters, isozymes, proteins and RAPD markers and their relationships with hybrid performance in oilseed rape (*Brassica napus* L.). *Theoretical and Applied Genetics* 110: 511–518.

ZHAO W, LEE GA, KWON SW, MA KH, LEE MC, and PARK YJ. 2012. Development and use of novel SSR markers for molecular genetic diversity in Italian Millet (*Setaria italica* L.). *Genes & Genomics* 34: 51–57.