Relationship of yielding ability and heterosis effect of winter rapeseed F_1 hybrids with genetic distance of parental lines

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Abstract

Breeding of oilseed rape hybrid varieties in Poland is based on CMS *ogura* hybridization system. The factor that has a decisive role in the advancement of this type of breeding is effective selection of parental forms of hybrids. Theoretically, greater heterosis effect is obtained in case of the crossing of lines with substantial genetic distance. Taking this into account, investigations of the relation between genetic distance of parental lines of hybrids, heterosis effect and seed yield of F_1 hybrids were initiated. The plants material were 14 F_1 hybrids of oilseed rape and their parental lines. These hybrids and their parental forms were evaluated for seed yield, agronomically important traits and their mid-parent heterosis (MPH) in field trials conducted in two localities for 2 years. Genetic distance between CMS *ogura* and restorer lines was evaluated on the basis of DNA polymorphism delineated with RAPD and AFLP markers and on the basis of enzymatic protein polymorphism. Phenotype similarity of parental forms was estimated on the basis of 9 phenotypic traits by Mahalanobis distance. The obtained results revealed statistically significant relationship between the genetic distance of the parental lines estimated by: AFLP markers; AFLP, RAPD markers and AFLP, RAPD, isozymes markers and seed yield of hybrids created with these lines. There was no association between genetic distance and mid-parent heterosis for seed yield. However, the information in this study points out to the possibility of selection of F_1 hybrid combination, which assures high yield on the basis of genetic distance. The correlation between Mahalanobis distance and genetic distance has been recorded.

Key words: winter oilseed rape, F_1 hybrids, yield of seeds, heterosis effect, genetic distance, molecular markers, correlations

Introduction

Former results of hybrid breeding of winter oilseed rape showed the possibility to increase yielding ability of this plant by utilization of heterosis effect (Lefort-Buson et Datté, 1982, 1983; Grant et Beversdorf; 1985, Bartkowiak-Broda, 1991; Krzymanski et al., 1993, 1994). However, the genetic base of the oilseed rape breeding material has been considerably reduced by the intensive breeding aiming at specific quality traits and developing double low winter oilseed rape varieties i.e.: low in erucic acid and low in glucosinolate content. The factor that has a decisive role in the advancement of hybrid varieties breeding is an effective selection of parental forms of hybrids. The knowledge of general and specific combining ability helps in selection of parental forms, and theoretically greater heterosis effect is achieved by the crossing of lines with substantial genetic distance (GD). Therefore the idea of constructing separate genetic pools for the oilseed rape hybrid breeding on the basis of DNA polymorphism of breeding materials was generated. Numerous molecular techniques have been applied to study the differences among genotypes and define genetic diversity within various plant species. The most frequent are RFLP, RAPD, AFLP, SSR and also enzymatic protein polymorphism. Relationship of genetic distance of parental lines of F_1 hybrids with yielding ability and heterosis effect has been examined by many authors for different crops such as: maize, rice, wheat, oilseed rape and others (Bernardo, 1992; Diers et al., 1996; Liu et al., 1999; Reif et al.,. 2003; Nowakowska et al., 2005; Yu et al., 2005).

The aim of this study was to establish a relationship between phenotype and genetic distance of parental lines estimated using AFLP, RAPD and isozymes polymorphism and yielding ability and heterosis effect of CMS *ogura* F_1 hybrids.

Materials and Methods

The plants material were 14 F_1 hybrids of oilseed rape (4 non-restored hybrids – Kaszub F_1, Mazur F_1, Pomorzanin F_1, Lubusz F_1, 10 restored hybrids and their parental lines: 10 paternal lines – 6 restorers, 4 without restorer gene and 8 CMS *ogura* lines). These hybrids and their parental forms were evaluated in field trials in randomized block design in four replications during two crop seasons of 2002–2003 and 2003–2004 in two localities. During the growing season the following factors were measured: data of flowering, flowering period and after the harvest seed yield of each plot (surface of plot 10 m^2), 1000 seeds weight, oil content, alkenyl glucosinolate and sum of glucosinolates content. 25 pods of each plot were used to measure the number of seeds per pod and length of pods. In this material, oil content was determined with NMR and the analyses of glucosinolates were performed with the method of gas chromatography of silyl derivatives of desulfo-glucosinolates (Michalski et al., 1995). Statistical analyses of field trials results were carried out using SERGEN and Statistica programs. The mid-parent heterosis (MPH) was computed using the formula MPH=100 × (F_1–MP)/MP, where F_1 is the hybrid mean and MP is the mid-parent mean. Phenotype similarity of parental forms was estimated on the basis of 9 phenotypic traits by Mahalanobis distance.

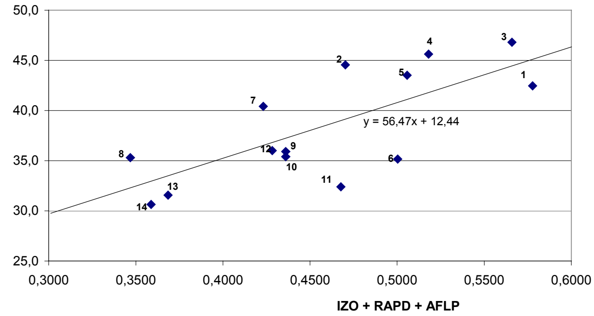
Analyses of isozymes in starch gel electrophoresis were performed as described by Schields et al. (1983) and Vallejos (1983). Five isozymes systems were examined: isocitrate dehydrogenase (IDH, EC 1.1.1.42), malate dehydrogenase (MDH, EC 1.1.1.37), 6 phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), leucine aminopeptidase (LAP, EC 3.4.11.1) and phosphoglucoisomerase (PGI, EC 5.3.1.9). The isolation of DNA for RAPD and AFLP markers was performed according to the modified method by Doyle and Doyle (1990). To detect polymorphism of DNA samples 64 arbitrary 10-bp-long oligonucleotides as RAPD primers (Operon Technologies) were investigated using Williams et al. (1990) method. AFLP amplification products were generated using the AFLP Kit of GIBCO BRL (AFLP Analysis System I; Gibco BRL Life Technologies Inc.) according to the manufacturer's instructions and Vos et al. (1995) method. Two restriction enzymes, *Eco*RI (primer : E) and *Mse*I (primer : M) were used in 23 primer combinations. The isozyme, RAPD and AFLP bands, which were polymorphic in 18 parental lines, were scored as 0 (absent) or 1 (present) for each parent. A binary matrix was constructed to estimate GD between a pair of parental lines using the formula of Nei and Li (1979).

Results and Discussion

The seed yield and heterosis effect of F_1 hybrids were differentiated. Non-restored hybrids yielded higher than restored hybrids in spite of the lowest heterosis effect. Restored hybrid revealed the lower yield despite very high mid-parent heterosis for seed yield (Figure 1A, 1B).

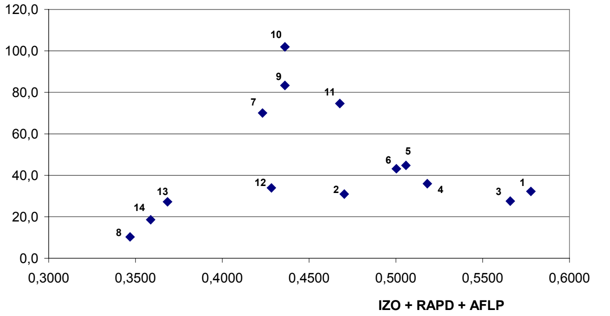
A

Yield of seeds [dt·ha^{-1}]



B

Heterosis effect [%]



GD GD

1.	Mazur F_1	4.	Lubusz F_1	7.	PN 4540/01	10.	MR 153	13.	MR 320
2.	Kaszub F_1	5.	PN 4534/01	8.	PN 4556/01	11.	MR 226	14.	MR 390
3.	Pomorzanin F_1	6.	PN 4538/01	9.	MR 124	12.	MR 289		

Fig. 1. Relationship between genetic distance of parental lines of hybrids estimated by isozymes (IZO), RAPD, AFLP markers and yield of seeds (A) and heterosis in F_1 seed yield (B)

Phenotype distance estimated on the basis of 9 phenotypic traits ranged from 2,0643 to 29,7421 with an average 17,8363 (Table 1). There was no association between phenotype distance (Mahalanobis) and seed yield of F_1 hybrids. However, correlation between Mahalanobis distance and genetic distance estimated by the molecular markers and isozymes has been recorded (Table 2). It points to the importance of assessment of phenotypic traits for proper identification of parental lines of F_1 hybrids.

Table 1. Phenotype distance (Mahalanobis) and genetic distance values of parental lines F_1 CMS *ogura* hybrids estimated by isozymes, (IZO), RAPD and AFLP markers

Hybrid	Seed yield [dt·ha^{-1}]	Phenotype distance Mahalanobis	Genetic distance values estimated by:				
			IZO	RAPD	AFLP	RAPD + AFLP	IZO+RAPD+AFLP
Mazur F_1	42,45	23,6909	0,4055	0,5878	0,5810	0,5837	0,5778
Kaszub F_1	44,55	29,7421	0,4925	0,4055	0,5127	0,4697	0,4703
Pomorzanin F_1	46,80	26,5000	0,2513	0,6286	0,5463	0,5775	0,5659
Lubusz F_1	45,62	21,4565	0,2513	0,4533	0,4055	0,4238	0,5181
PN 4534/01	43,51	14,5884	0,4055	0,6370	0,4400	0,5120	0,5058
PN 4538/01	35,16	19,2837	0,5878	0,5183	0,4848	0,4977	0,5003
PN 4540/01	40,42	12,2837	0,8102	0,5258	0,3478	0,4133	0,4232
PN 4556/01	35,30	2,0643	0,5878	0,3409	0,3399	0,3403	0,3469
MR 124	35,92	26,1259	0,4055	0,5108	0,3928	0,4370	0,4361
MR 153	35,40	25,5115	0,2513	0,5719	0,3680	0,4424	0,4361
MR 226	32,40	21,7654	0,0572	0,5958	0,4183	0,4836	0,4677
MR 289	36,01	9,5472	0,9445	0,4463	0,3970	0,4159	0,4283
MR 320	31,58	12,0172	0,4925	0,4055	0,3399	0,3649	0,3685
MR 390	30,65	5,1315	0,5878	0,3102	0,3804	0,3525	0,3588
Mean	38,27	17,8363	0,4665	0,4956	0,4253	0,4510	0,4574

Genetic distance between CMS *ogura* and paternal lines was evaluated on the basis of DNA polymorphism delineated with RAPD, AFLP markers and on the basis of enzymic protein polymorphism (isozymes). There were obtained 18 isozymes, 225 RAPD and 354 AFLP polymorphic markers. Genetic distance was computed for all three marker types, RAPD and AFLP markers and three marker types together (Table 1). Genetic distance based on all markers (IZO, RAPD, AFLP) varied from 0,3469 to 0,5778 with an average 0, 4574 (Table 1). The obtained results revealed statistically significant relationship between the genetic distance of the parental lines estimated by AFLP markers, AFLP, RAPD markers and AFLP, RAPD, isozymes markers and seed yield of F_1 hybrids created with these lines. It was a linear relationship (Figure 1A). There was no association between genetic distance and mid-parent heterosis for seed yield (Figure 1B). However, Riaz et al. (2001) found that the GD in American *B. napus* inbred lines was significantly correlated with hybrid yields and heterosis. In maize Smith et al. (1990) and Betran et al. (2003) reported that yield heterosis was significantly correlated with parental molecular diversity but Shieh and Thseng (2002) obtained the opposite results.

Table 2. Correlation coefficients between seed yield and phenotype distance (Mahalanobis) and genetic distance estimated by isozymes (IZO), RAPD and AFLP markers

	Yield	Distance Mahalanobis	Marker				
			IZO	RAPD	AFLP	RAPD +AFLP	IZO+RAPD+AFLP
Yield	1						
Distance Mahalanobis	0,494	1					
IZO	–0,171	–0,595*	1				
RAPD	0,419	0,572*	–0,467	1			
AFLP	0,601*	0,611*	–0,270	0,469	1		
RAPD+AFLP	0,604*	0,688**	–0,415	0,822**	0,888**	1	
IZO+RAPD+AFLP	0,741**	0,692**	–0,425	0,741**	0,852**	0,936**	1

* significant at the level α=0,05 **significant at the level α=0,01

Conclusion

Positive correlations between genetic distance of parental lines of F_1 hybrids obtained with the use of three types of genetic markers (AFLP, RAPD, isozymes) and the yield of hybrids indicate the possibility of selection of high yielding hybrid combinations on the basis of genetic distance. However, for effective application of evaluation of genetic distance of breeding materials to the breeding of oilseed rape hybrid varieties on the basis of molecular markers further methodological research is necessary.

References

Bartkowiak-Broda I. (1991). Studia nad systemami meskiej nieplodnosci u rzepaku *Brassica napus* L. var. *oleifera*. (Studies on male sterility systems in oilseed rape *Brassica napus* L. var. *oleifera*.). Hodowla Roslin Aklimatyzacja i Nasiennictwo, **35**, ¾, 3–6.

Bernardo R. (1992). Relatioship between single-cross performance and molecular marker heterozygosity. Theor. Appl. Genet., **83**, 628–634.

Betrán F.J., Ribaut J.M., Beck D., Gonzalez de Léon D. (2003). Genetic diversity, specific combining ability, and heterosis in tropical maize under stress and nonstress environments. Crop Sci., **43**, 797–806.

Diers B.W., Mc Vetty P.B.E., Osborn T.C. (1996). Relatioship between heterosis and genetic distance based on restriction fragment length polymorphism markers in oilseed rape (*Brassica napus* L.). Crop Sci., **36**, 79–83.

Doyle J. J., Doyle J.L. (1990). Isolation of plant DNA from fresh tissue. Focus, **12**, 13–15.

Grant I., Beversdorf W.D. (1985). Heterosis and combining ability estomates in spring-planted oilseed rape (*Brassica napus* L.). Can. J. Genet. Cytol., **27**, 472–478.

Krzymanski J., Pietka T., Krotka K. (1993). Zdolnosc kombinacyjna i heterozja mieszancow diallelicznych rzepaku ozimego podwojnie ulepszonego I. Pokolenia F_1. (Combining ability and heterosis in diallel crosses of double low winter oilseed rape F_1 generation). Postepy Nauk Rolniczych, **5**, 41–52.

Krzymanski J., Pietka T., Krotka K. (1994). Zdolnosc kombinacyjna i heterozja mieszancow diallelicznych rzepaku ozimego podwojnie ulepszonego II. Pokolenia F_1 i F_2. (Combining ability and heterosis in diallel crosses of double low winter oilseed rape II. F_1 and F_2 generation). Rosliny Oleiste – Oilseed Crops, **XV** (1), 21–32.

Leffort-Buson M., Dattée Y. (1982). Genetic study of some agronomic characters in winter oilseed rape (*Brassica napus* L.). J. Heterosis. Agronomie, **2** (4): 315–322.

Lefort-Buson M., Dattée Y. (1983). L'hétérosis chez le colza oleagineux (*Brassica napus* L.). Proc. 6th International Rapeseed Conference, 17-19 May 1983, Paris, France, Vol. **1**, 558–564.

Liu Z.Q., Pei Y., Pu Z.J. (1999). Relationship between hybrid performance and genetic diversity based on RAPD markers in wheat, *Triticum aestivum* L. Plant Breeding, **118**, 119–123.

Michalski K., Kolodziej K., Krzymanski J. (1995). Quantitative analysis of glucosinolates in seeds of oilseed rape – effect of sample preparation on analytical results. Proc. 9th International Rapeseed Congress, 4-7 July 1995, Cambridge, UK, **3**, 911–913.

Nowakowska J., Bartkowiak-Broda I., Ogrodowczyk M. (2005). Wstepne badania zwiazku miedzy efektem heterozji mieszancow F_1 rzepaku ozimego (*Brassica napus* L.) a dystansem genetycznym linii rodzicielskich. (Preliminary investigations of relatioship between heterosis effect of F_1 winter rapeseed hybrids (*Brassica napus* L.) and genetic distance of parental lines). Rosliny Oleiste – Oilseed Crops, **XXVI** (1), 19–33.

Nei M., Li W. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci., USA **76**, 5256-5273.

Reif J.C., Meichinger A.E., Xia X.C., Warburton M.L., Hoisington D.A., Vasal S.K., Srinivasan G., Bohn M., Frish M. (2003). Genetic distance based on Simple Sequence Repeats and heterosis in Tropical Maize populations. Crop Science, **43**, 1275–1282.

Riaz A., Li G., Queresh Z., Swati M.S., Qiuros C.F. (2001). Genetic diversity of oilseed *Brassica napus* inbred lines based on sequence-related amplified polymorphism and its relation to hybrid performance. Plant Breeding, **120**, 411–415.

Shieh G.J. Thseng F.S. (2002). Genetic diversity of Tainan-white maize inbred lines and prediction of single cross hybrid performances using RAPD markers. Euphytica, **124**, 307–313.

Shields C.R., Orton C.J., Stuber C.W. (1983). Isozymes in plants genetics and breeding. In: Tanksley S.D. and Orton T.J. (Eds). Part A, Elsevier Sciences Publishers, B.V., Amsterdam, 443–458.

Smith O.S., Smith J.S.C., Bowen S.L., Tenborg R.A., Wall S.J. (1990). Similarities among a group of elite maize inbreds as measured by pedigree, F_1 grain yield, heterosis and RFLPs. Theor. Appl. Genet., **80**, 833–840.

Williams J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A., Tingey S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res., **18**, 6531–6535.

Vallejos C.E. (1983). Enzyme activity staining. In: Tanksley S.D. and Orton T.J. (Eds) Isozymes in plants genetics and breeding, Part A, Elsevier Sciences Publishers, B.V., Amsterdam, 469–516.

Vos P., Hogers R., Sleeker M., Reijans M., Lee T., Homes M., Freiters A., Pot J., Peleman J., Kuiper M., Zabeau M. (1995). AFLP: a new concept for DNA fingerprinting. Nucl. Acids Res., **23**, 4404–4414.

Yu C.Y., Hu S.W., Zhao H.X., Guo A.G., Sun G.L. (2005). Genetic distance revealed by morphological characters, isozymes, proteins and RAPD markers and their relatioships with hybrid performance in oilseed rape (*Brassica napus* L.). Theor. Appl. Genet., **110**, 511–518.