Relationship of yielding ability and heterosis effect of winter rapeseed
F1 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 F1 hybrids were initiated. The
plants material were 14 F1 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 F1
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, F1 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 F1 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 F1 hybrids.

Materials and Methods

The plants material were 14 F1 hybrids of oilseed rape (4 non-restored hybrids – Kaszub F1, Mazur F1, Pomorzanin F1, Lubusz F1, 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 m2),
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 × (F1–MP)/MP, where F1 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, EcoRI (primer: E) and MseI (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 F₁ 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).

Table 1. Phenotype distance (Mahalanobis) and genetic distance values of parental lines F₁ CMS ogura hybrids estimated by isozymes (IZO), RAPD, AFLP markers and yield of seeds (A) and heterosis in F₁ seed yield (B)

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Seed yield [dt·ha⁻¹]</th>
<th>Phenotype distance Mahalanobis</th>
<th>Genetic distance values estimated by:</th>
<th>GD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mazeur F₁</td>
<td>42.45</td>
<td>23,6909</td>
<td>IZO: 0.4055, RAPD: 0.5878, AFLP: 0.5810</td>
<td>0.5837, 0.5778</td>
</tr>
<tr>
<td>Kaszub F₁</td>
<td>44.55</td>
<td>29,7421</td>
<td>IZO: 0.4925, RAPD: 0.4055, AFLP: 0.5127</td>
<td>0.4697, 0.4703</td>
</tr>
<tr>
<td>Pomorzanin F₁</td>
<td>46.80</td>
<td>26,5000</td>
<td>IZO: 0.2513, RAPD: 0.6286, AFLP: 0.5463</td>
<td>0.5775, 0.5659</td>
</tr>
<tr>
<td>Lubusz F₁</td>
<td>45.62</td>
<td>21,4565</td>
<td>IZO: 0.2513, RAPD: 0.4533, AFLP: 0.4055</td>
<td>0.4238, 0.5181</td>
</tr>
<tr>
<td>PN 4534/01</td>
<td>43.51</td>
<td>14,5884</td>
<td>IZO: 0.4055, RAPD: 0.6370, AFLP: 0.4400</td>
<td>0.5120, 0.5058</td>
</tr>
<tr>
<td>PN 4538/01</td>
<td>35.16</td>
<td>19,2837</td>
<td>IZO: 0.5878, RAPD: 0.5183, AFLP: 0.4848</td>
<td>0.4977, 0.5003</td>
</tr>
<tr>
<td>PN 4540/01</td>
<td>40.42</td>
<td>21,2837</td>
<td>IZO: 0.9102, RAPD: 0.2528, AFLP: 0.3478</td>
<td>0.4133, 0.4232</td>
</tr>
<tr>
<td>PN 4556/01</td>
<td>35.30</td>
<td>20,643</td>
<td>IZO: 0.5878, RAPD: 0.3409, AFLP: 0.3399</td>
<td>0.3403, 0.3469</td>
</tr>
<tr>
<td>MR 124</td>
<td>35.92</td>
<td>26,1259</td>
<td>IZO: 0.4055, RAPD: 0.5108, AFLP: 0.3928</td>
<td>0.4370, 0.4361</td>
</tr>
<tr>
<td>MR 153</td>
<td>35.40</td>
<td>25,5115</td>
<td>IZO: 0.2513, RAPD: 0.5719, AFLP: 0.3680</td>
<td>0.4424, 0.4361</td>
</tr>
<tr>
<td>MR 226</td>
<td>32.40</td>
<td>21,7654</td>
<td>IZO: 0.0572, RAPD: 0.5958, AFLP: 0.4183</td>
<td>0.4836, 0.4677</td>
</tr>
<tr>
<td>MR 289</td>
<td>36.01</td>
<td>9,5472</td>
<td>IZO: 0.9445, RAPD: 0.4463, AFLP: 0.3970</td>
<td>0.4159, 0.4283</td>
</tr>
<tr>
<td>MR 320</td>
<td>31.58</td>
<td>12,0172</td>
<td>IZO: 0.4925, RAPD: 0.4055, AFLP: 0.3399</td>
<td>0.3649, 0.3685</td>
</tr>
<tr>
<td>MR 390</td>
<td>30.65</td>
<td>5,1315</td>
<td>IZO: 0.5878, RAPD: 0.3102, AFLP: 0.3804</td>
<td>0.3525, 0.3588</td>
</tr>
<tr>
<td>Mean</td>
<td>38.27</td>
<td>17,8363</td>
<td>IZO: 0.4665, RAPD: 0.4956, AFLP: 0.4253</td>
<td>0.4510, 0.4574</td>
</tr>
</tbody>
</table>

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 F₁ seed yield (B).
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 F₁ 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 Thensg (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

<table>
<thead>
<tr>
<th>Marker</th>
<th>Yield</th>
<th>Distance Mahalanobis</th>
<th>IZO</th>
<th>RAPD</th>
<th>AFLP</th>
<th>RAPD+AFLP</th>
<th>IZO+RAPD+AFLP</th>
</tr>
</thead>
<tbody>
<tr>
<td>IZO</td>
<td>1</td>
<td>1</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAPD</td>
<td>-0.171</td>
<td>-0.595*</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFLP</td>
<td>0.419</td>
<td>0.572*</td>
<td>-0.467</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAPD+AFLP</td>
<td>0.601*</td>
<td>0.611*</td>
<td>-0.270</td>
<td>0.469</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IZO+RAPD+AFLP</td>
<td>0.604*</td>
<td>0.688**</td>
<td>-0.415</td>
<td>0.822**</td>
<td>0.888**</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>IZO+RAPD+AFLP</td>
<td>0.741**</td>
<td>0.692**</td>
<td>-0.425</td>
<td>0.741**</td>
<td>0.852**</td>
<td>0.936**</td>
<td>1</td>
</tr>
</tbody>
</table>

* significant at the level α=0.05  **significant at the level α=0.01

### Conclusion

Positive correlations between genetic distance of parental lines of F₁ 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


