

## INVESTIGATION OF THE RELATIONSHIP OF GENETIC AND PHENOTYPIC DISTANCE OF PARENTAL LINES OF F<sub>1</sub> HYBRIDS WITH YIELDING ABILITY AND HETEROSIS

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### Introduction

Winter oilseed rape (*Brassica napus* L.) in Europe and also in Poland, is an important oilseed crop due to the high nutritional quality of its oil and meal. Recently, attention has been given to its potential as a renewable resource for biofuel production. Therefore a large quantity of seed production is very important for food-, feed- and biofuel producing industry. Major advances in yielding ability have been achieved with hybrid cultivars which became dominant on the market.

According to a numerous studies up to 30% heterosis for seed yield in F<sub>1</sub> hybrid progeny has been reported for winter oilseed rape (Becker 1987; Grant, Beversdorf 1985; Lefort-Buson et al. 1987). However, good exploitation of heterosis phenomenon depends on the selection of parental lines of F<sub>1</sub> hybrids, which is the most important step in hybrid breeding. Knowledge of general (GCA) and specific (SCA) combining ability is helpful in the selection of parental lines but it needs evaluation of yielding ability and heterosis of experimental F<sub>1</sub> hybrids in field trials conducted in several environmental conditions. Because of this reason alternative objective, quick and cheaper methods are investigated for prediction of heterosis effect and yielding ability of F<sub>1</sub> hybrids. The level of genetic diversity between parents has been proposed as a predictor of F<sub>1</sub> yielding ability and heterosis of this trait. Numerous molecular techniques in combination with biometrical methods have opened new possibilities to evaluate input of measurement of genetic distance (GD) of parental lines of F<sub>1</sub> hybrids. However, variable results have been obtained by many authors in the investigations conducted on different plant material. Diers et al. (1996) and Knaak and Ecke (1995) using RFLP markers and Riaz et al. (2001) using SRAP in *B. napus* reported a strong association of heterosis with marker heterozygosity and recommended their use for predicting heterosis. In *B. juncea*, genetic distance was useful for the classification of parental lines into heterotic groups but the absence of direct association of genetic distance with heterosis has been reported by Jain et al. (1994).

Taking this into account, investigations of the relationship between genetic (GD) and phenotypic (PD) distance of parental lines of F<sub>1</sub> hybrids, heterosis effect and yielding ability have been initiated also in our Institute.

### Materials and methods

The plant material in this study consisted of three groups of F<sub>1</sub> hybrids developed using different parental lines. Field trials were performed in a randomised complete block design in four replications, in several environments (Tab. 1).

Statistical analysis was carried out using SERGEN, Statistica and Excel programs. To determine the genetic distance between parental lines of F<sub>1</sub> hybrids CMS *ogura*, analyses of RAPD, AFLP and isozyme markers were performed. Genomic DNA from eight-day-old leaves of 10 plants for each parental line was extracted using a modified CTAB procedure according to Doyle and Doyle (1990). The basic RAPD reaction was performed as described by Williams et al. (1990). The DNA samples were analyzed using Operon Technologies primers (USA). AFLP analysis was performed using 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). DNA was double-digested with *EcoRI* and *MseI* restriction enzymes. Only bands that were repeatedly classified as either intense or medium were included for further analysis. Five isozyme systems were tested, including

isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6 PGD), leucine aminopeptidase (LAP) and phosphoglucoisomerase (PGI). Extraction and electrophoretic separation of enzymatic proteins as well as staining procedures for 5 enzymatic systems were conducted according to methods developed by Shields et al. (1983) and Vallejos (1983). GD for all pairs of parental forms were calculated according to the formula given by Nei and Li (1979).

The phenotypic (Mahalanobis) distances among the parental forms were calculated on the basis of the seed yield and 8 (plant material – A) and 7 (plant material – B) phenotypic traits.

The relationship between seed yield, heterosis, GD and PD were established by correlation coefficient tested at  $\alpha = 0,05$  and  $0,01$ .

Table 1. Hybrids of *B. napus*, their parental lines, crop seasons, locality of field trials and method of investigation of genetic and phenotypic distance

Plant materials	Field trials		Mahalanobis distance / traits	Genetic distance /molecular marker
	crop seasons	localities		
<b>A 14</b> F <sub>1</sub> hybrids: 8 CMS <i>ogu</i> ; 10 paternal lines	2002–2003 2003–2004	2 – Borowo, Zielęcín 2 – Borowo, Zielęcín	* seed yield and 8 traits	isozymes, RAPD, AFLP
<b>B 18</b> F <sub>1</sub> hybrids: 5 CMS <i>ogu</i> ; 5 paternal restorer lines	2003–2004	1 – Borowo	seed yield	RAPD
<b>C 15</b> F <sub>1</sub> hybrids: 3 CMS <i>ogu</i> ; 5 paternal-doubled haploid restorer lines	2006–2007 2007–2008 2008–2009	1 – Borowo 1 – Zielęcín 2 – Małyszyn, Łagiewniki	** seed yield and 7 traits	RAPD, AFLP

\* investigated traits: seed yield, yield components (length of pod, number of seeds in pod, weight of 1000 seeds), beginning and end of flowering, oil content, alkenyl and total glucosinolates content

\*\* investigated traits: seed yield, yield components (number of branches per plant, number of pods per plant, length of pod, number of seeds in pod, weight of 1000 seeds), oil and total glucosinolates content

## Results and Discussion

The correlation coefficients between the seed yield, heterosis effect, genetic distance and the Mahalanobis distance of parental lines of  $F_1$  hybrids (plant material A–C) are shown in Table 2A–2C.

Table 2. Correlation coefficients between genetic distance, phenotypic distance, seed yield of  $F_1$  hybrids and heterosis effect

### A. Plant material A (p.m. A)

Traits	1	2	3	4
1. Genetic distance	1			
2. Phenotypic distance	0,69**	1		
2. Seed Yield	0,74**	0,49	1	
3. Heterosis effect	0,06	0,46	-0,15	1

### B. Plant material B (p.m. B)

Traits	1	2	3
1. Genetic distance	1		
2. Seed Yield	0,27	1	
3. Heterosis effect	0,58**	0,39	1

### C. Plant material C (p.m. C)

Traits	1	2	3	4
1. Genetic distance	1			
2. Phenotypic distance	-0,05	1		
3. Seed yield	-0,02	0,10	1	
4. Heterosis effect	-0,09	-0,43	0,77**	1

\*, \*\* significant at  $\alpha = 0,05$ ,  $\alpha = 0,01$ , respectively

The obtained results revealed statistically significant relationship between genetic distance of parental lines estimated with the use of three types of genetic markers and seed yield of 14  $F_1$  hybrids and with heterosis effect of 18 winter oilseed rape  $F_1$  hybrids (Tab. 2A, 2B). A linear dependence of seed yield of  $F_1$  hybrids (for plant material A) and heterosis effect (for plant material B) on genetic distance are shown respectively in Figures 1 and 2.

