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Estimation of additive and epistatic gene effects of doubled haploid lines of winter oilseed rape (*Brassica napus* L.)

Jan Bocianowski · Kamila Nowosad · Agnieszka Dobrzycka · Joanna Wolko

Abstract In this paper 60 doubled haploid lines of oilseed rape (*Brassica napus* L.) were studied. Genetic parameters as additive and epistasis effects were estimated for nine quantitative traits. The results indicate the importance of both additive and epistasis gene effects of number of branches per plant, number of siliques per plant, number of seeds per silique and silique length in this study.

Keywords Additive effect · Doubled haploid (DH) lines · Epistasis · Oilseed rape

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Introduction

Doubled haploid (DH) lines are used in many studies in rapeseed, because their production is the fastest way to obtain homozygous populations (Cegielska-Taras et al. 2015). DH lines carry a homogeneous DNA, what is useful in phenotyping and genotyping studies (Chen et al. 2007; Radoev et al. 2008). In DHs production only one cycle of recombination takes place, what makes easier to track parents genes in the offspring (Pink et al. 2008).

In order to plan an efficient breeding program, the knowledge of the nature, magnitude of gene effects and their input to the control of metric traits, as well as the association among yield and yield-related traits are required (Bocianowski et al. 2016). To determine the genetic background of quantitative traits in segregating populations of *Brassica napus,* quantitative trait loci (QTLs) analyses were applied (Cai et al. 2014). In the genome of oilseed rape a lot of QTLs and epistatic interactions were discovered, many of which had pleiotropic effects (Udall et al. 2006; Basunanda et al. 2010).

The inheritance of quantitative traits is a very complex issue, because they are the result not only of the activity of multiple individual genes, but also by the interactions between them. In purpose to estimate the parameters components for plants, several genetic statistical models have been developed (Mather and Jinks 1982; Kearsey and Pooni 1996). Gene action of quantitative traits can be evaluated by generation

mean analysis, e.g. parental, F_1, F_2, BC_1. However, more effective estimation of additive and epistasis effects is on the basis of homozygous lines (Bocianowski [2012a], [2013], [2014]; Bocianowski and Nowosad [2015]; Kim et al. [2015]; Monnahan and Kelly [2015]; Ober et al. [2015]).

The purpose of this study was estimation of the parameters connected with the additive and additive-by-additive interaction (epistasis) gene action.

Materials and methods

Plant material

Plant material used in the paper includes 60 DH lines derived from crossing between line 324/2 as a female parent (high oleic acid content, 77.9%) and line 622/3 as a male parent (high oil content, 51.9%; high seed yield).

Field experiments

Field experiment was conducted in Borowo (52°70′N, 16°46′E), Plant Breeding Strzelce Ltd., Co.—IHAR-PIB Group in growing season 2014/15. It had a randomized blocks design with three replications. Each plot contains four rows 2 m long. Distance between rows was 30 cm. The field management followed standard agricultural practice.

Traits evaluated in the field trial were: duration of flowering, flowering time, plant height, number of branches per plant, number of siliques per plant, silique length, number of seeds per silique, 1000 seed weight, and oil content in seeds. More detailed description of phenotyping in Dobrzycka et al. ([2016]).

Statistical analyses

The normality of distribution of the traits was tested using Shapiro–Wilk's normality test and having normally distributed traits, it was assumed that the data followed the multivariate normal distribution. The one-way analysis of variance (ANOVA) was carried out to determine the effects of DH lines on the variability of studied traits. The relationships between observed traits were estimates using Pearson correlation coefficients.

Estimation of the genetic parameters

Estimation of the additive gene effect and additive-by-additive interaction of homozygous loci (epistasis) effect on the basis of phenotypic observations requires identification of groups of extreme DH lines, i.e. lines with the minimal and maximal expression of the observed trait (Choo and Reinbergs [1982]). The group of minimal lines consists of the lines which contain, theoretically, only alleles reducing the value of the trait. Analogously, the group of maximal lines contains the lines which have only alleles increasing the trait value. In this paper we identify the groups of extreme lines using the quantile method (Bocianowski et al. [1999]), in which lines with the mean values smaller (bigger) than 0.03 (0.97) quantile of the empirical distribution of means are assumed as minimal (maximal) lines. The choose the quantiles 0.03 and 0.97 is results of previously study (Bocianowski et al. [1999]). The total additive effect a of all genes controlling the trait and the total additive-by-additive interaction effect aa may be estimated by the formulas (Bocianowski and Krajewski [2009]; Bocianowski [2012b]):

$$\hat{a} = \frac{1}{2}\left(\overline{L}_{\max} - \overline{L}_{\min}\right) \tag{1}$$

and

$$\hat{aa} = \frac{1}{2}\left(\overline{L}_{\max} + \overline{L}_{\min}\right) - \overline{L}, \tag{2}$$

where $\overline{L}_{\min}$ and $\overline{L}_{\max}$ denote the means for the groups of minimal and maximal DH lines, respectively, and $\overline{L}$ denotes the mean for all DH lines. The test statistics to verified hypotheses about genetic parameters different than zero are given by

$$F_a = \frac{MS_a}{MS_e} \text{ and } F_{aa} = \frac{MS_{aa}}{MS_e},$$

where MS_a denote mean square for parameter a, MS_{aa}—mean square for epistasis aa, MS_e—mean square for residual.

Results and discussion

All studied traits have a normal distribution. Analysis of variance indicates that the main effects of DH lines was significant ($P < 0.001$) for all the traits of study.

Table 1 shows a correlation matrix for the observed traits. Plant height was correlated with flowering time and oil content in seeds; number of branches per plant was correlated with number of siliques per plant, flowering time and oil content in seeds; silique length was correlated with number of seeds per silique, flowering time and oil content in seeds. All statistically significant correlation coefficients were positive (Table 1). Many important traits are positively or negatively correlated, because they are controlled by some of the same genes or because they are developmentally or structurally related.

Estimates of additive gene action effects based on DH lines were significantly larger than zero only for plant height, number of branches per plant, number of siliques per plant, number of seeds per silique, duration of flowering and silique length (Table 2). Estimates of epistatic effects were statistically significant for number of branches per plant, number of siliques per plant, number of seeds per silique, 1000 seed weight and silique length (Table 2).

Table 1 The Pearson correlation coefficients matrix for the nine traits

Trait	Flowering time	Plant height	Number of branches per plant	Number of siliques per plant	Silique length	Number of seeds per silique	1000 seed weight	Oil content in seeds
Duration of flowering	−0.230	0.061	−0.05	0.088	−0.15	−0.073	−0.050	0.130
Flowering time		0.416***	0.257*	0.196	0.332**	0.194	−0.150	0.203
Plant height			0.069	0.083	0.223	0.051	0.240	0.382**
Number of branches per plant				0.689***	0.115	−0.006	−0.190	0.342**
Number of siliques per plant					0.188	−0.098	−0.240	0.201
Silique length						0.338**	−0.090	0.405**
Number of seeds per silique							−0.156	0.104
1000 seed weight								0.122

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table 2 Estimates of additive and epistasis effects for observed traits of doubled haploid lines

Trait	Estimates of		Mean value
	Additive effect, a	Epistasis effect, aa	
Duration of flowering	2.75*	0.400	24.35
Flowering time	4.00	1.533	117.47
Plant height	18.35*	−0.450	146.85
Number of branches per plant	3.50**	0.552**	10.17
Number of siliques per plant	197.95***	29.157**	434.84
Silique length	14.86**	3.204**	59.29
Number of seeds per silique	4.86**	0.797**	14.80
1000 seed weight	0.53	0.108*	5.28
Oil content in seeds	3.67	0.438	43.28

* $P < 0.05$

** $P < 0.01$

Estimation of genetic parameters has a significant role in winter oilseed rape breeding. The result indicates the importance of both additive and epistasis gene effects of number of branches per plant, number of siliques per plant, number of seeds per silique and silique length in this study. The presence of epistasis has important implication for any breeding program. Statistically significant epistasis effect and non-significant additive effect for 1000 seed weight means that this trait was probably determined by genes with small individual effects but strong gene-by-gene interaction effects. Confounding epistatic effects in models suggested that inheritance of these traits is complex and polygenic. Epistasis has been reported for many traits in a number of crops: barley (Bocianowski et al. 2016); maize (Li et al. 2016); rice (Matsubara et al. 2015), and wheat (Jaiswal et al. 2016).

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