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Estimation of the genetic parameters for fatty acids content in DH lines obtained from winter oilseed rape of F_1 hybrid (DH O-120 × DH C-1041)

Ocena parametrów genetycznych dla zawartości kwasów tłuszczowych w liniach DH rzepaku ozimego uzyskanych z mieszańca F_1 (DH O-120 × DH C-1041)

Key words: *Brassica napus* L., DH lines, fatty acids, genetic parameters, number of genes, winter oilseed rape

Thirty two doubled haploids (DH), hybrids F_2, F_3 as well as standard cv. Kana were objects of three field experiments led in one locality during 1999/2000, 2000/2001, 2001/2002. Doubled haploid lines of winter oilseed rape were obtained from F_1 hybrid (DH O-120 × DH C-1041) using isolated microspore culture. The seeds of these genotypes were analyzed for their fatty acid content using gas chromatography. The content of the following fatty acids: palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$), linolenic acid ($C_{18:3}$) was determined.

On the basis of a population of doubled haploid lines as well as suitable early generations the genetic parameters were estimated. The estimates of additive gene effects [d], dominance effects [h], homozygous × homozygous interaction effects [i] and heterozygous × heterozygous interaction effects [l] were found for individual fatty acid content in every year of study as well as for three years jointly.

The effects of additive genes action calculated for every year separately as well as jointly for three years were significant for all analysed fatty acids. The dominance was significant for the palmitic acid only.

Effects concerning interaction between homozygous loci influenced positively the increase of oleic acid content. The effects connected with non-allelic interaction of heterozygous loci were not significant for all studied acids.

The number of genes or group of linked genes controlling the content of individual fatty acids, which differeciated parental genotypes, were as follows: for stearic acid and palmitic acid one gene, for linoleic and linolenic acids three genes and for oleic acid four.

Słowa kluczowe: *Brassica napus* L., liczba genów, linie DH, kwasy tłuszczowe, parametry genetyczne, rzepak ozimy

Trzydzieści dwa podwojone haploidy (DH), mieszańce F_2, F_3 odmiana wzorcowa Kana były obiektami trzyletnich doświadczeń polowych prowadzonych w jednej miejscowości w sezonach 1999/2000, 2000/2001, 2001/2002. Podwojone haploidy rzepaku ozimego uzyskano metodą kultury izolowanych mikrospor z mieszańca F_1 (DH O-120 × DH C-1041). Każde doświadczenie z tymi samymi 36 genotypami założono w układzie bloków losowych z trzema powtórzeniami.

W oleju nasion badano zawartość pięciu kwasów tłuszczowych: kwasu palmitynowego ($C_{16:0}$), kwasu stearynowego ($C_{18:0}$), oleinowego ($C_{18:1}$), linolowego ($C_{18:2}$) i linolenowego ($C_{18:3}$). W oparciu o wyniki otrzymane dla linii DH oraz odpowiednich pokoleń segregujących oszacowano parametry genetyczne określające efekty addytywnego działania genów [d], dominacji [h] i nieallelicznej interakcji loci homozygotycznych [i] i heterozygotycznych [l] dla zawartości poszczególnych kwasów tłuszczowych w każdym roku badań oraz dla trzech lat łącznie.

Efekty addytywnego działania genów obliczone dla każdego roku oddzielnie jak i średnie efekty wyznaczone dla trzech lat okazały się istotne dla wszystkich analizowanych kwasów tłuszczowych. Dominacja okazała się istotna tylko w przypadku kwasu palmitynowego. Efekty związane z interakcją loci homozygotycznych wpływały na wzrost zawartości kwasu oleinowego. U żadnego z badanych kwasów nie wystąpiły efekty związane z niealleiczną interakcją loci w stanie heterozygotycznym.

Liczba genów lub grup genów ściśle ze sobą sprzężonych kontrolujących zawartość poszczególnych kwasów tłuszczowych, którymi różniły się formy rodzicielskie, wynosiła jeden dla kwasu stearynowego i palmitynowego, trzy — dla linolowego i linolenowego oraz cztery — dla oleinowego.

Introduction

Rapeseed oil is broadly used not only for nutrition, but also in chemical industry as well as in biofuel production. According to Krzymański (2000), one of current tasks in rapeseed breeding is better adaptation of the quality of rapeseed oil to the needs of different kind. The oil after esterification is an important component in the production of biofuels (motor oils). The genotypes with high content of oleic acid can be desirable particularly in this case.

The purpose of the work described in this paper was the evaluation of genetic parameters controlling the content of fatty acids in seeds oil and also the calculation of the number of genes or group of linked genes controlling the contents of these acids.

Materials and methods

Thirty two doubled haploids (DH), hybrids of F_2 and F_3 generations and cv. Kana were the objects of three field experiments led in one locality Cerekwica (Polish Academy of Science) in years: 1999/2000, 2000/2001, 2001/2002. Doubled haploid lines of winter oilseed rape were obtained from F_1 hybrid (DH O-120 × DH C-1041) by the use of isolated microspore culture (Cegielska-Taras, Szała 1997).

Each experiment with the same genotypes was carried out in a randomized complete block design in three replications. A detailed description of the experiment and the obtained results according to yield and oil content were presented by Adamska et al. (2001, 2002).

The composition of fatty acids in seed oil was estimated by gas chromatography method (Byczyńska, Krzymański 1969). The contents of five fatty

acids: palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$) oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$), and linolenic acid ($C_{18:3}$) were analysed. The individual fatty acid content was expressed as the percentage of total fatty acid content.

Information on genetic control of the content of the above fatty acids may be available by estimation of genetic parameters. Genetic parameters were calculated on the basis of doubled haploid line population and two early hybrid generations F_2, F_3 using the method elaborated by Surma et al. (1997). The formulas given in this method permit to find estimates of additive gene action [d] and homozygote with homozygote interaction [i] as well as effects of dominance [h] and heterozygote with heterozygote interaction [l]. Estimators of the parameters are as follows (Surma et al. 1997):

$$m = \bar{L}$$

$$[d] = \frac{1}{2}(\bar{L}_{max} - \bar{L}_{min})$$

$$[i] = \frac{1}{2}(\bar{L}_{max} + \bar{L}_{min}) - \bar{L}$$

$$[h] = 8F_3 - 2F_2 - 6\bar{L}$$

$$[l] = 8\bar{L} + 8F_2 - 16F_3$$

The F statistic for corresponding contrast of DH lines and F_2, F_3 hybrid generation was used for testing the hypothesis of no additive, no dominance and no non-allelic interaction effects. Variance analysis for studied fatty acids was described in an early paper (Adamska et al. 2001).

The method proposed by Kaczmarek et al. (1988) was applied for estimation of the number of genes on the basis of DH lines. This method permits to estimate the number of genes or group of linked genes taking into account also effects of non-allelic interaction.

Results and discusion

The preliminary information about the genetic differentiation of studied 32 doubled haploid lines (DH) of winter oilseed rape was described by Adamska et al. 2001. The genetic parameters determining the yield components and the oil content in winter oilseed rape, estimated on the basis of DH lines population and F_1, F_2 and F_3 hybrid generations, were given by Adamska et al. (2002).

The general profile of genotypes in relation to five fatty acids content is illustrated in Table 1. Average contents of five fatty acids in individual years of experiment for DH line, for hybrids of F_2 and F_3 generations as well as standard cv. Kana were presented.

Table 1

The values of L_{max}, L_{min} DH lines and F_2 and F_3 hybrid generations, standard cv. Kana for fatty acids in per cent of total fatty acids — *Wartości L_{max} i L_{min} linii DH, mieszańca F_2 i F_3 oraz odmiany Kana dla kwasów tłuszczowych w procentach całkowitej zawartości kwasów tłuszczowych*

Fatty acids *Kwasy*	Years *Lata*	Standard *Wzorzec* Kana	L_{mean} *$L_{średnie}$*	L_{max}	L_{min}	F_2	F_3
$C_{16:0}$	2000	5.13	5.23	5.33	4.83	5.20	5.33
	2001	5.03	4.93	5.30	4.70	5.20	5.47
	2002	4.40	4.43	4.60	4.36	4.50	4.50
	mean *średnia*	4.86	4.87	5.08	4.64	4.97	5.10
$C_{18:0}$	2000	1.47	1.37	1.60	1.04	1.47	1.20
	2001	2.03	1.80	1.97	1.60	1.77	1.70
	2002	1.73	1.60	2.10	1.30	1.60	1.63
	mean *średnia*	1.74	1.58	1.88	1.30	1.61	1.51
$C_{18:1}$	2000	60.70	59.20	63.40	57.40	58.70	59.33
	2001	60.07	58.90	64.80	56.30	59.37	57.70
	2002	65.27	64.27	68.10	63.70	63.90	64.47
	mean *średnia*	62.01	60.79	65.43	59.13	60.66	60.50
$C_{18:2}$	2000	21.33	22.00	23.70	20.50	22.80	21.50
	2001	20.80	21.80	23.10	20.40	21.60	22.73
	2002	17.97	18.63	21.26	17.13	18.40	18.53
	mean *średnia*	20.03	20.81	22.69	19.34	20.93	20.92
$C_{18:3}$	2000	10.57	11.40	12.40	10.20	11.10	11.20
	2001	10.10	10.03	10.91	7.99	10.50	11.03
	2002	9.43	9.90	11.33	8.47	9.90	9.67
	mean *średnia*	10.03	10.44	11.54	8.88	10.50	10.63

The genetic parameters were estimated on the basis of studied population of DH lines and early F_2 and F_3 generations. The estimated additive gene effect [d], the effects of dominance [h] and the non-allelic interaction homozygous loci [i] and heterozygous loci [l] for the content of individual fatty acids in each year of investigations as well as for three years jointly are presented in Table 2.

Effects of additive gene action calculated for each year of the experiment as well as for the three years jointly were significant for all analysed fatty acids.

Effects of additive gene action were essential for the oleic acid content, but non-allelic interaction of homozygous loci [i] was also significant in two years out of three and influenced the increase of content of this acid in oil (Table 1).

The effects connected with non-allelic interaction of heterozygous loci [l] did not appear for studied fatty acids.

The domination was significant for the palmitic acid only (Table 2). Effects connected with interaction of homozygous loci influenced positively the growth of oleic acid content.

Table 2

Estimation and results of testing the genetic parameters for fatty acids
Ocena i wyniki testowania parametrów genetycznych dla kwasów tłuszczowych

Fatty acids *Kwasy tłuszczowe*	Years *Lata*	No of genes *Liczba genów* k	Genetic parameters — *Parametry genetyczne*			
			d	h	i	l
$C_{16:0}$	2000	1	0.250**	0.864	-0.150	-1.872
	2001	1	0.300**	3.736**	0.067	-6.400*
	2002	1	0.116	0.400	0.050	-0.528
	mean *średnia*	1	0.222**	1.666*	-0.011	-2.933
$C_{18:0}$	2000	1	0.283**	-1.536	-0.050	3.472
	2001	1	0.185*	-0.736	-0.015	1.328
	2002	2	0.400**	0.264	0.100	-0.528
	mean *średnia*	1	0.289**	-0.669	0.012	1.424
$C_{18:1}$	2000	4	3.000**	2.064	1.200	6.128
	2001	4	4.250**	-10.560	1.650**	22.928
	2002	4	2.200**	2.336	1.633**	-6.128
	mean *średnia*	4	3.150**	-2.040	1.494**	3.557
$C_{18:2}$	2000	2	1.600**	-5.600	0.100	14.400
	2001	4	1.350**	7.864	-0.050	-16.528
	2002	3	2.065**	-0.335	0.567	-0.272
	mean *średnia*	3	1.672**	0.643	0.205	-0.800
$C_{18:3}$	2000	2	1.100**	-1.000	-0.100	0.800
	2001	2	1.459**	2.264	-0.583	-5.872
	2002	4	1.433**	-1.864	0.000	3.728
	mean *średnia*	3	1.327**	-0.200	-0.228	-0.448

* significant at the $\alpha = 0.05$ level — *istotne na poziomie $\alpha = 0,05$*
** significant at the $\alpha = 0.01$ level — *istotne na poziomie $\alpha = 0,01$*

The number of genes or group of linked genes controlling the contents of individual fatty acids, were: one for stearic and palmitic acid, three — for linoleic and linolenic acids, and four — for oleic acid (Table 2).

The obtained results show that most probably it will be possible to change the profile of fatty acids content in seed oil according to industry expectations. Particularly, this is evident in the case of oleic acid content, where the effect linked with additive genes action and joint action of homozygous loci make it possible to breed the oilseed rape characterized by higher level of oleic acid. Such oil is suitable for biofuel production. Also significant effects of additive genes of linoleic and linolenic acid content, make selection of oilseed rape forms feasible. It is true for lower as well as higher content of these acids in seed oil .

Conclusions

1. Effects of additive gene action calculated for each year of investigations as well as for three years jointly were significant for all analysed fatty acids, whereas non-allelic interaction of homozygous loci took place only in the content of oleic acid.
2. The dominace effect was non-significant for content of all studied fatty acids except for palmitic acid. Non-allelic interaction of heterozygous loci were non-significant.
3. The number of the genes or group linked genes controlling the content of unsaturated acids was higher (3–4) than the number of the genes controlling the content of saturated acids (1).

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