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Multivariate statistical methods used for evaluation of DH lines of winter oilseed rape on account of various fatty acid compositions

Wielowymiarowe metody statystyczne w ocenie linii DH rzepaku ozimego (*Brassica napus* L.) ze względu na różny skład kwasów tłuszczowych

Key words: *Brassica napus* L., fatty acids, DH lines, MANOVA, winter oilseed rape

This article discusses the application of multivariate statistical methods to evaluation of genotypes taking into account some combinations of variables. These methods were used for the analysis of data from an experiment with 35 genotypes of winter oilseed rape (32 doubled haploid lines, 2 parental forms and standard cv. Kana). The experiment was carried out in a completely randomized block design with three replications. The aim of the study was the evaluation of doubled haploid lines of winter oilseed rape in respect of the requirements concerning five fatty acids: palmitic, stearic, oleic, linoleic and linolenic. For estimation of DH lines in terms of their further usefulness, especially in food, pharmacology, chemical and petrol industry, multivariate analysis of variance (MANOVA) and canonical variates analysis were used.

Słowa kluczowe: *Brassica napus* L., kwasy tłuszczowe, linie DH, MANOVA, rzepak ozimy

W pracy przedstawiono zastosowania statystycznych metod wielowymiarowych do oceny genotypu ze względu na pewne kombinacje obserwowanych zmiennych. Metody te wykorzystano do analizy wyników doświadczenia z 35 genotypami rzepaku ozimego (32 liniami podwojonych haploidów, 2 formami rodzicielskimi oraz odmianą wzorcową Kana), założonego w układzie losowanych bloków w 3 powtórzeniach. Celem doświadczenia była ocena linii podwojonych haploidów (linii DH) rzepaku ze względu na wymagania dotyczące zawartości pięciu kwasów tłuszczowych w oleju (palmitynowego, stearynowego, oleinowego, linolowego i linolenowego). Ponieważ olej rzepakowy znajduje szerokie zastosowanie w przemyśle spożywczym, farmaceutycznym, chemicznym, a ostatnio nawet w produkcji biopaliwa, poszukiwane są odmiany rzepaku, których oleje w zależności od ich wykorzystania winny odznaczać się odpowiednim składem kwasów nasyconych i nienasyconych. Aby spełnić wymagania stawiane tym formom, biorąc pod uwagę różne aspekty ich przeznaczenia, zaproponowano do oceny linii DH zastosowanie wielozmiennej analizy wariancji (MANOVA) oraz innych metod wielozmiennych, w szczególności analizy zmiennych kanonicznych.

Introduction

Rapeseed oil (*Brassica* oils) is an important source of fatty acids. Depending on the composition of fatty acids, the oil is used for different purposes. Rapeseed oil, which is high in oleic acid and essential polyunsaturated fatty acids, has got almost a perfect fatty acid composition as a salad oil. Further oil quality improvements include the development of very high oleic acid/low linolenic acid variety which can be used for frying. Oil with high level of oleic acid is needed for biodisel production (Krzymański 1966, 2000).

Brassica napus oil has high concentration of oleic acid (about 60%) and contains moderate levels of linoleic (about 20%) and linolenic acid (about 10%). This fatty acids composition of vegetable oil is considered by many nutritionists as ideal for human nutrition, and superior to fatty acids composition of other plant oils. Rapeseed oil has also the lowest of all oils saturated fatty acid content of about 7% of total fatty acids, with 4–5% of palmitic acid, and about 2% of stearic acid (Rakow, Raney 2003).

This paper presents a suggestion how to utilise multivariate statistical methods (MANOVA) and other multidimensional methods for estimation as well as for selection of genotypes which meet requirements considering variables (fatty acids) made by industrial, nutritional, chemical or biodisel production. To this end the results of experiments with 32 doubled haploids of winter oilseed rape were used.

Materials and methods

32 doubled haploids (DH) obtained from F_1 hybrids (DH O-120 × DH C-1041) of winter oilseed rape by the use of isolated microspore method (Cegielska-Taras, Szała 1997) at the Plant Breeding and Acclimatization Institute in Poznań as well as two parental forms P_1 and P_2 and standard cultivar Kana made the material for the experiment. Finally, 35 genotypes were studied on the experimental fields at Cerekwica (Polish Academy of Science) in one year 2003.

The experiment was carried out in a randomised complete block design in three replications. The composition of fatty acids in seed oil was estimated with the use of gas chromatography method (Byczyńska, Krzymański, 1969). The contents of five fatty acids: palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) were analysed. Individual fatty acid content was expressed as the percentage of total fatty acids content.

Because the main goal of the experiment was the evaluation of DH lines as regards their further use in some branches of industry, we looked for these varieties of rapeseed whose oil is characterized by desirable composition of saturated and unsaturated fatty acids. Several multivariate statistical methods based on a general model of observation (Caliński, Kaczmarek 1973) were used for evaluation in order to meet the requirements for these forms and taking into consideration various aspects of their deployment.

First of all multivariate analysis of variance (MANOVA) was used along with the testing of general hypothesis about the lack of differences between genotypes considering their main effects for a given variable set (fatty acids). The rejection of the general hypothesis justified verification of particular hypotheses connected with the study of descriminatory power of acids, estimation of the main effects of genotypes and interesting comparisons(contrasts) of DH lines, parental forms and cv. Kana in respect of selected acids jointly and individual acids and their functions. Mahalonobis' distance was suggested as a measure of "polyacid" genotypes similarity, whose significance was verified by means of critical value D_{α} called "the least significant distance". The possibility of graphic distribution of DH lines, parents P_1, P_2 and cv. Kana described by the fatty acids content on a plane, was obtained by the use of the analysis of canonical varieties (Morrison 1976).

Results

Estimations of winter oilseed rape DH lines, conducted according to methods described in MATERIALS AND METHODS section, were carried out for different combinations of fatty acids composition in oil depending on their further uses for industrial purposes. The basis for estimations at the first stage were the results obtained by the use of MANOVA for all five fatty acids. The calculations were done using the data about the content of the five fatty acids in seed oil of 35 genotypes (32 DH lines, 2 parental forms: P_1, P_2, and cv. Kana).

We will describe the course of the analysis which will enable estimation of DH lines concerning the content of fatty acids as well as the analyses in which fatty acid composition was determined by expectations of nutritional, chemical, and biofuel production.

As a result of multivariate analysis of variance for all five fatty acids, conducted in the first stage of calculations, sum of square and products matrices for blocks, genotypes and error and means of content of fatty acids in studied genotypes were calculated. The percentage means of content of five fatty acids in DH lines are presented in Table 1. Above calculations permitted to verify general hypothesis about the lack of difference between genotypes in respect to five fatty acids jointly. The hypothesis was rejected on the level of significance $\alpha = 0.01$ since the value of $F = 20.10$ considerably exceeded the critical value of $F_{0.01} = 1.32$. The subsequent testing of implied hypotheses about discriminatory power of individual fatty acids permitted to describe the share of acids in the rejection of the general hypothesis.

Table 1

The percentage means of five fatty acids in DH lines of winter oilseed rape — *Średnia zawartość procentowa 5 kwasów tłuszczowych w oleju nasion linii DH rzepaku ozimego*

No DH *Nr DH*	DH lines *Linia DH*	Fatty acids — *Kwasy tłuszczowe*				
		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1	H5 – 8	4.40	1.77	63.77	19.37	8.87
2	H5 – 30	4.15	2.20	64.25	18.62	9.12
3	H5 – 43	4.12	1.97	66.22	17.55	8.70
4	H5 – 71	4.35	1.60	61.35	19.42	11.12
5	H5 – 77	4.30	1.62	62.15	19.40	10.47
6	H5 – 79	4.45	1.80	62.87	19.85	9.15
7	H5 – 85	4.45	1.80	63.80	18.17	9.85
8	H5 – 105	4.57	1.75	62.05	19.80	9.87
9	H5 – 109	4.55	1.77	65.70	16.70	9.40
10	H5 – 114	4.37	1.55	64.80	18.65	8.52
11	H5 – 129	4.17	2.20	68.75	16.15	6.85
12	H5 – 191	4.40	1.90	66.12	16.85	8.75
13	H5 – 202	4.27	1.82	64.15	18.10	9.75
14	H5 – 216	4.62	1.60	60.85	20.22	10.87
15	H5 – 237	4.57	1.72	64.55	19.05	8.52
16	H5 – 238	4.62	1.95	65.00	17.70	8.85
17	H5 – 250	4.32	1.62	63.77	18.90	9.50
18	H5 – 255	4.30	1.62	62.55	20.77	8.87
19	H5 – 261	4.65	1.70	62.95	19.47	9.47
20	H5 – 284	4.35	1.65	62.22	20.67	9.67
21	H5 – 349	4.27	1.97	64.35	18.27	9.10
22	H5 – 396	4.52	1.97	66.17	17.72	7.65
23	H5 – 416	4.20	1.75	62.52	20.45	9.57
24	H5 – 467	4.40	1.50	60.82	21.62	9.65
25	H5 – 493	4.22	1.50	61.67	21.67	9.32
26	H5 – 544	4.40	2.10	64.27	18.42	8.87
27	H5 – 621	4.52	1.87	64.32	19.20	8.55
28	H5 – 729	4.65	1.57	62.75	19.75	9.37
29	H5 – 802	4.42	2.10	63.25	19.60	9.65
30	H5 – 804	4.17	1.47	61.20	20.47	10.27
31	H5 – 876	4.62	1.70	60.07	22.62	9.25
32	H5 – 977	4.47	1.75	62.35	21.30	8.62
33	P_1 O-120	4.52	1.77	65.15	18.32	8.35
34	P_2 C-1041	4.35	1.62	60.02	21.72	10.35
35	cv. Kana	4.60	1.90	63.80	19.15	8.48
Mean — *Średnia*		4.41	1.78	63.45	19.31	9.24

The largest significant differences were caused by the following acids: stearic ($F = 43.89$), linoleic ($F = 41.95$), oleic ($F = 39.48$), linolenic ($F = 17.91$) and palmitic ($F = 6.66$). F-statistic values for all fatty acids — exceeded critical value $F_{0.01} = 1.81$. The analysis of canonical variates was conducted in order to create a graphic image of the arrangement of genotypes by the first two canonical variates (Fig. 1).

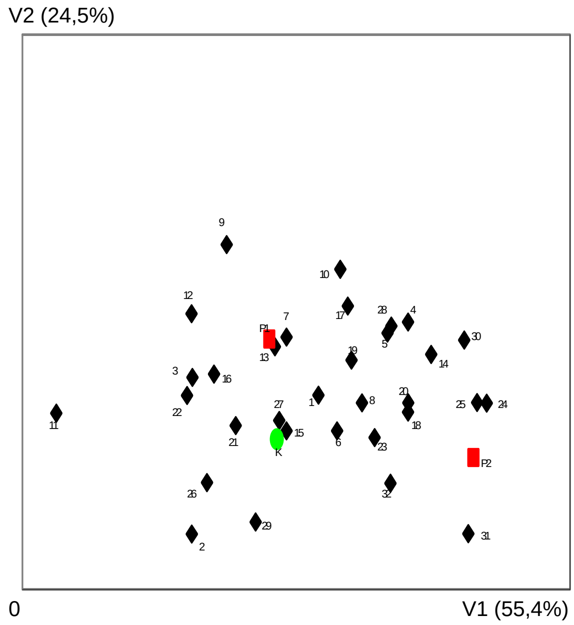

Fig. 1. Configuration of DH lines in the space of two canonical variates V_1 and V_2 calculated for five acids — *Rozmieszczenie linii DH w układzie dwóch pierwszych zmiennych kanonicznych V_1 i V_2 wyznaczonych dla pięciu kwasów tłuszczowych*

The picture was obtained with only a slight loss of information (20,1%) caused by a reduction of 5-dimensional space (defined by five fatty acids) to 2-dimensional space (defined by variates V_1 and V_2). Figure 1 shows that out of all DH lines, lines No: 15, 27, 1, 21, and 6, were the closest to Kana variety (in respect of content of all five fatty acids jointly). Lines No: 7 and 13 were the closest to the parental line P_1. None of the DH lines was similar to the parental line P_2.

The above multivariate analysis ensured that all fatty acids participated in MANOVA and were assigned the same weight. This approach may be considered as a classical one, most commonly used. However, for the estimation of DH lines in reference to their further utilisation it is necessary to impose certain requirements considering the content and proportion of fatty acids. The results of the first stage of the study were used for the estimation of winter oilseed rape DH lines with regard to fatty acids composition demanded for different uses:

nutritional, for chemical industry or for biodisel production. From the point of view of production of universal rapeseed oil, MANOVA was described in the earlier paper by Adamska et al. (2004), therefore in this paper only basic assumptions of the applied approach will be outlined.

The preferable fatty acids composition for this purpose has the highest level of oleic acid, optimum ratio of linoleic to linolenic acids and low level of saturated fatty acids: palmitic and stearic. For selection of DH lines with such content and proportion of fatty acids variables y_1, y_2, .., y_5, described by fatty acids such as: palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) were transformed into new variables s_1, s_2 and s_3.

$$\begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \\ y_5 \end{bmatrix} = \begin{bmatrix} C_{16:0} \\ C_{18:0} \\ C_{18:1} \\ C_{18:2} \\ C_{18:3} \end{bmatrix} \Rightarrow \begin{bmatrix} C_{18:1} \to \max \\ C_{18:2} - 2 \times C_{18:3} \to 0 \\ C_{18:1} + C_{18:2} + C_{18:3} - C_{16:0} - C_{18:0} \to \max \end{bmatrix} = \begin{bmatrix} s_1 \\ s_2 \\ s_3 \end{bmatrix}$$

Further analysis with the use of various multivariable methods was conducted on such variables as s_1, s_2 and s_3.

We will also assess DH lines taking into account fatty acids composition in oil intended for chemical industry. For such use the content of linoleic and linolenic acids should be the highest possible. Hence, the estimation of the doubled haploid lines is reduced to one variable only:

$$x = y_4 + y_5 = C_{18:2} + C_{18:3} \to \max,$$

for which the analysis of variance was conducted.

The general hypothesis of no difference between genotypes with respect to variable x was rejected at the significance level $\alpha = 0.01$ ($F = 42.34$ at $F_{0.01} = 1.84$). It means that the estimates of the main effects of individual genotypes are considerably differentiated. Table 2 shows genotypes ordered from the highest value to the lowest value. The results shown in Table 2 allow for identification of DH lines with positive estimates of the main effects. Besides a parental form P_2, these are the following lines No: 31, 24, 14, 25, 30, 4, 20, 23, 32, 5, 8, 18, and 29. The results of testing of comparisons of chosen lines with parental form P_2 allow to find lines (not significantly different from P_2), which can be used for paint and varnish production. These are lines No: 31, 24, 14, and 25.

If oil from oilseed rape is to be used for production of biofuel and lubricant, oil should contain maximal amount of one oleic acid ($C_{18:1}$), as well as minimal amount of linolenic acid ($C_{18:3}$). In order to estimate DH lines with respect to the purposes mentioned above, it may be reasonable to transform initial variables y_i (i = 1, 2, ..., 5) into two new ones, z_1 and z_2.

Table 2

Estimates of main effects of DH lines for the variable x = $C_{18:2} + C_{18:3}$ and the F-statistic values

Ocena efektów głównych dla zmiennej x = $C_{18:2} + C_{18:3}$ wraz z wartościami statystyki F

No. DH *Nr linii DH*	DH line *Linia DH*	Estimates of main effects for variable x *Ocena efektu głównego dla zmiennej x*	F-statistic values *Wartość statystyki F*
P_2	P_2 C-1041	3.63	129.88**
31	H5 – 876	3.43	115.58**
24	H5 – 467	2.81	77.68**
14	H5 – 216	2.63	68.04**
25	H5 – 493	2.53	62.82**
30	H5 – 804	2.27	50.68**
4	H5 – 71	2.06	41.90**
20	H5 – 284	1.86	33.96**
23	H5 – 416	1.52	22.83**
32	H5 – 977	1.42	19.85**
5	H5 – 77	1.37	18.44**
8	H5 – 105	1.16	13.31**
18	H5 – 255	1.14	12.73**
29	H5 – 802	0.72	5.18*
28	H5 – 729	0.60	3.51
6	H5 – 79	0.47	2.16
19	H5 – 261	0.42	1.71
17	H5 – 250	-0.15	0.22
1	H5 – 8	-0.30	0.91
7	H5 – 85	-0.54	2.82
13	H5 – 202	-0.72	5.03*
2	H5 – 30	-0.82	6.59*
27	H5 – 621	-0.82	6.59*
K	cv. Kana	-0.94	8.82**
15	H5 – 237	1.00	9.81**
21	H5 – 349	-1.20	14.27**
26	H5 – 544	-1.28	16.16**
10	H5 – 109	-1.41	19.56**
P_1	P_1 O-120	-1.92	36.45**
16	H5 – 238	-2.05	41.48**
3	H5 – 43	-2.36	54.90**
9	H5 – 109	2.52	62.31**
`12	H5 – 191	-3.03	90.40**
22	H5 – 396	-3.26	104.74**
11	H5 – 129	-5.71	320.48**

* the estimate of main effect significant at 0.05 level — *ocena efektu głównego istotna na poziomie 0,05*

** the estimate of main effect significant at 0.01 level — *ocena efektu głównego istotna na poziomie 0,01*

$$\begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \\ y_5 \end{bmatrix} = \begin{bmatrix} C_{16:0} \\ C_{18:0} \\ C_{18:1} \\ C_{18:2} \\ C_{18:3} \end{bmatrix} \Rightarrow \begin{bmatrix} C_{18:1} \Rightarrow \max \\ C_{18:3} \Rightarrow \max \end{bmatrix} \Rightarrow \begin{bmatrix} C_{18:1} \to \max \\ C_{16:0} + C_{18:0} + C_{18:1} + C_{18:2} - C_{18:3} \to \max \end{bmatrix} = \begin{bmatrix} z_1 \\ z_2 \end{bmatrix}$$

In the above transformation, the replacement of linolenic acid ($C_{18:3}$), whose content should be the lowest possible (min), by the difference between acids [$C_{16:0} + C_{18:0} + C_{18:1} + C_{18:2}$] and $C_{18:3}$ favourable for lines when the difference is the largest possible, was meant to aid interpretation of results of the analysis.

It should be noticed that using interpretation variables y_i (i = 1, 2, ..., 5) for new variables z_1 and z_2, DH lines should be the best when the values of both variables are the highest.

Table 3 shows the estimates of main effects of DH lines for the variables z_1 and z_2 and their significance and F statistic value for the two variables jointly. It is interesting to observe that line No 11 ranks first. This line is characterized by the highest values of z_1 and z_2 variables and by the highest value of F statistic. To the second group belong lines No: 22, 3, and 12, which differ significantly from line No 11, but also significantly exceed any other DH lines. This conclusion is confirmed by Mahalanobis' distances, obtained between line No 11 and each of the remaining DH lines. The estimates are shown in the last column of Table 3. Also the graphic image of the distribution of DH lines presented on a plane (Fig. 2.)

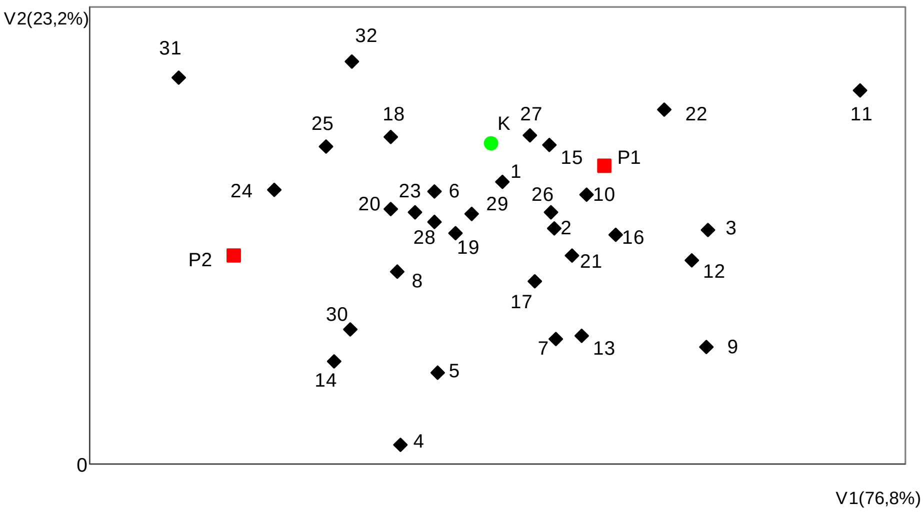

Fig. 2. Configuration of DH lines in the space of two canonical varieties calculated for the fatty acid functions z_1 and z_2 — *Rozmieszczenie linii DH w układzie dwóch zmiennych kanonicznych wyznaczonych dla funkcji kwasów tłuszczowych z_1 i z_2*

Table 3

Estimates of main effects of DH lines and the F-statistic values for variables z_1 and z_2 jointly

Ocena efektów głównych linii DH i wartości statystyki F dla zmiennych z_1 i z_2 łącznie

No. *Nr*	DH line *Linia DH*	Estimate of main effect for variable — *Ocena efektu głównego dla zmiennej*		F-statistic value for z_1 and z_2 jointly *Wartość statystyki F dla z_1 i z_2 łącznie*	Mahalanobis distance between DH No 11 and the rest *Odległość Mahalanobisa między DH No 11 i pozostałymi*
		z_1	z_2		
11	H5 – 129	5.30**	4.72**	159.50**	–
22	H5 – 396	2.72**	3.04**	84.26**	4.42
3	H5 – 43	3.17**	1.47*	69.44**	4.52
12	H5 – 191	2.67**	0.82*	54.38**	5.08
9	H5 – 105	2.25**	-0.38	50.54**	6.42
P_1	P_1	1.70**	1.72**	16.66**	6.02
16	H5 – 236	1.55**	0.72	16.15**	6.35
10	H5 – 114	1.35**	1.14**	10.40**	6.59
15	H5 – 237	1.10**	1.67**	9.49**	7.10
27	H5 – 621	0.87**	1.67**	8.71**	7.53
21	H5 – 349	0.90**	0.07	7.64**	7.44
K	cv. Kana	0.35	1.27**	5.74**	8.41
26	H5 – 544	0.82**	0.62	4.97*	7.47
2	H5 – 30	0.80*	0.39	4.24*	7.52
29	H5 – 802	-0.20	0.02	0.43	9.18
19	H5 – 262	-0.50	-0.41	1.41	9.67
17	H5 – 250	0.32	-0.58	1.65	8.42
1	H5 – 8	0.32	0.74	1.73	8.33
28	H5 – 729	-0.70	-0.36	1.85	10.02
6	H5 – 79	-0.58	0.12	2.05	9.86
17	H5 – 250	0.32	-0.58	1.65	8.42
23	H5 – 416	-0.93	-0.36	4.33*	10.41
20	H5 – 284	-1.23**	-0.48	10.53**	10.93
8	H5 – 105	-1.40**	-1.41**	11.21**	11.18
7	H5 – 85	0.35	-1.33**	15.75**	8.68
18	H5 – 255	-0.90**	.0.67	16.52**	10.63
13	H5 – 202	0.70	-1.11**	19.48**	8.18
5	H5 – 77	-1.30**	-2.71**	22.85**	11.30
30	H5 – 804	-2.25**	-2.66	30.99**	12.64
25	H5 – 493	-1.78**	0.04	32.45**	12.11
32	H5 – 977	-1.10**	1.54**	42.10**	11.47
14	H5 – 216	-2.60**	-3.28**	43.41**	13.27
24	H5 – 467	-2.63**	-1.01*	48.85**	13.40
4	H5 – 71	-2.10**	-4.11**	52.65**	12.87
31	H5 – 876	-3.38**	0.07	57.02**	15.34
P_2	P_2	-3.43**	-2.33**	69.32**	14.60
Critical values — *Wartości krytyczne*				$F_{0.05} = 3.09$ $F_{0.01} = 4.82$	$D_{0.05} = 1.77$ $D_{0.01} = 2.21$

* the estimate of main effect significant at 0.05 level — *ocena efektu głównego istotna na poziomie 0,05*

** the estimate of main effect significant at 0.01 level — *ocena efektu głównego istotna na poziomie 0,01* in the plane array of two canonical varieties V_1 and V_2 agrees with the results obtained in the earlier verification of hypotheses connected with the significance of the main effects of DH lines and significance of individual Mahalanobis' distances. It turned out that Mahalanobis' distance between DH line No 11 and DH line No 22, which is the closest line to the line No 11, is highly significant and significantly exceeds the smallest significant distance $D_{0.01}$.

Conclusion

Multivariate methods discussed in this article may be useful for the evaluation and selection of doubled haploids of winter oilseed rape with fatty acids composition advantageous from the point of view of industry. The weighted approach of MANOVA is noteworthy. Both the results of testing of interesting hypotheses, one — and multivariate, and the use of Mahalanobis' distance as a measure of „polyacid" similarities of DH lines, prove to be very useful. The results of these calculations may be supplemented by graphic images of lines situated on a plane due to the application of analysis of canonical variables.

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