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New Interspecific *Brassica* Hybrids with High Levels of Heterosis for Fatty Acids Composition

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Abstract: Winter oilseed rape (*Brassica napus* L.) is the most important oil crop in Europe. Optimizing the profile and quantity of fatty acids in rapeseed is critical for maximizing the value of edible oil. Although the utilization of crop heterosis for hybrid breeding in rapeseed is limited by the relatively narrow genetic basis of adapted germplasm, an up-to-date significant effort has been made to broaden the rapeseed gene pool using different strategies. The present study was aimed to estimate heterosis for oil quality of the newly developed *Brassica* interspecific hybrids, using selected parental lines. For this purpose, five parental genotypes and twenty-two interspecific cross-derived *Brassica* lines were evaluated in a randomized complete block design with three replications in the Greater Poland region during 2009, 2010 and 2011. Generally, the variation among genotypes was evident for most of the tested fatty acids mean values, but the differences between genotypes were not always statistically significant when based on individual fatty acids (FAs). However, the highest number of significant heterosis effects was observed for behenic and lignoceric acids and for *Brassica* hybrid line H1. Based on obtained results it was possible to select one genotype—the hybrid line H5, which is recommended for further inclusion in the breeding programs.

Keywords: *Brassica* hybrids; heterosis; fatty acids; gas chromatography

1. Introduction

Heterosis (hybrid vigor) has been successfully utilized in order to increase the productivity in several crop species such as maize, sugar beet, sunflower, forage crops and grasses. Several authors reported that hybrid vigor is evident also in seed yield of F₁ hybrids for both winter and summer types of oilseed rape *B. napus* [1,2]. Although, in *Brassica oleracea*, heterosis is the most efficient tool providing stimulus to the hybrid vegetable industry, in oilseed rape, heterosis has not been extensively exploited, because of the lack of an effective pollination control system for commercial production of F₁ hybrid seeds [3,4].

Rapeseed is considered as an important source of oil for industrial use, as well as for edible purposes. The objective of modifying oil quality is to develop oils with enhanced nutritional and functional properties. The industrial utilization of rapeseed oil requires a specific fatty acid composition,

e.g., a high oleic acid content [5,6]. This type of fatty acid composition is also of interest as a cooking oil. The range of genetic variability for oleic acid content was thus greatly extended, which created the possibility for selection. The change of the fatty acid composition in interspecific crosses between different *Brassica* species is commonly known [7].

Fatty acids profile plays a key role in the use of *Brassica* oil by humans. Vegetable oilseed because of higher proportion of 16 and 18 carbons unsaturated fatty acids, mostly monounsaturated fatty acids (MUFAs) are utilized principally as a source of edible oil [8–10]. For this purpose, for example, linolenic acid (C18:3) is undesirable, because of reducing the durability of the oil, although it is an essential dietary fatty acid. Moreover, erucic acid (C22:1) contains nearly 50% of total fatty acid, which is undesirable for human consumption, as it is reported to lead to myocardial lipidosis [11]. Although natural forms of rapeseed and mustard contain high levels of erucic acid (over 40% of total fatty acids), levels in rapeseed cultivated for food use are typically below 0.5%.

Heterosis is agronomically important in the use of F_1 hybrid cultivars in many crops and vegetables, especially due to the superior performance, which can appear as biomass, yield and abiotic and biotic stress tolerance.

For obtaining a new hybrid plant, as a first step, some information on the genetic background of important characters should be collected. Next, these details are used to combine desirable traits in a hybrid. Therefore, especially for plant breeders it is essential to have detailed information about the desirable parental combination in hybridization programs, which usually resulted in a high degree of heterotic response. Furthermore, although interspecific hybrid lines do not meet the elite rapeseed standards, they are a valuable source for hybrid breeding due to their large distance from present breeding material and their high heterosis when combined with European rapeseed cultivars. Most heterosis research has been focused on increasing various quality traits in *Brassica* and other crops [12]. According to Pal and Sikka [13] heterosis is a quick, cheap and easy method for increasing crop production. To improve quality and quantity traits in *Brassica* and other crops, heterosis is one of the most effective methods. Owing to heterosis, breeders can increase plant production in a short time by utilization of less input [13].

Therefore, in the present study, an effort was made to identify promising *Brassica* genotypes to be used as a donor parent for the production of low erucic acid canola lines.

In the present studies, heterosis (better-parent) was estimated for the fatty acid composition in F_1 – F_3 generations of *Brassica* interspecific hybrids, using the five parent cross experiment.

2. Materials and Methods

2.1. Plant Material

Plant material for the fatty acids composition analysis consisted of 27 *Brassica* genotypes including five parental genotypes, i.e., *Brassica rapa* ssp. *pekinensis*, *Brassica rapa* ssp. *trilocularis* cv. Yellow Sarson, *B. carinata*, *B. juncea* and the male sterile line of an F_8 generation of *B. napus* (MS8), as well as twenty two interspecific cross-derived *Brassica* lines. The MS8 line was selected from resynthesized oilseed rape (*B. rapa* ssp. *chinensis* × *B. oleracea* var. *gemmifera*) using in vitro cultures of isolated embryos [14] (Table 1). F_1 – F_3 generations of tested lines, as well as parental genotypes, were selected from the rapeseed breeding program of the Department of Genetics and Plant Breeding, Poznań University of Life Sciences (PULS).

Brassica genotypes were tested in the 2009, 2010 and 2011 growing seasons. Field trials were performed in three replicates of a randomized block design at the PULS experimental station Dłoń (51°41'23" N, 17°04'10" E) located 100 km south of Poznań, (Greater Poland, Poland). The field experiment in Dłoń was conducted on typical heavy soil of III quality class of good rye complex [14]. The sums of precipitation during the vegetation season of *Brassica* genotypes in 2009, 2010 and 2011 were respectively: 605.1, 753.9 and 465.5 mm, but the mean temperatures during the vegetation season were respectively, 9.1, 7.9 and 9.8 °C. As shown in Table 2, these three seasons differed slightly in their

climatic conditions. The field trials were arranged in a randomized complete block design with three replicates. Sowing dates were between the 24th and 28th of August. Each genotype was grown in a three-row plot of 9.0 m², with a 0.30 row distance and a sowing density of 60 seeds/m².

Table 1. List of *Brassica* species and interspecific hybrids used as a research material.

Genotype's Code	Species or Cross Combination	Genotype's Code	Species or Cross Combination
S1	<i>Brassica napus</i> MS8 line		
S2	<i>Brassica carinata</i>		
S3	<i>Brassica juncea</i>		
S4	<i>Brassica rapa</i> ssp. <i>pekinensis</i>		
S5	<i>Brassica rapa</i> ssp. <i>trilocularis</i> cv. Yellow Sarson		
H1	<i>B. napus</i> × <i>B. carinata</i> (125/1)	H12	<i>B. napus</i> × <i>Brassica rapa</i> ssp. <i>pekinensis</i> (14/1)
H2	<i>B. napus</i> × <i>B. carinata</i> (125/2)	H13	<i>B. napus</i> × <i>Brassica rapa</i> ssp. <i>pekinensis</i> (18/1)
H3	<i>B. napus</i> × <i>B. carinata</i> (126/1)	H14	<i>B. napus</i> × <i>Brassica rapa</i> ssp. <i>pekinensis</i> (19/1)
H4	<i>B. napus</i> × <i>B. carinata</i> (126/2)	H15	<i>B. napus</i> × <i>Brassica rapa</i> ssp. <i>pekinensis</i> (20/1)
H5	<i>B. napus</i> × <i>B. carinata</i> (127/2)	H16	<i>B. napus</i> × <i>Brassica rapa</i> ssp. <i>pekinensis</i> (24/1)
H6	<i>B. napus</i> × <i>B. juncea</i> (55/1)	H17	<i>B. napus</i> × <i>Brassica rapa</i> ssp. <i>trilocularis</i> (2)
H7	<i>B. napus</i> × <i>B. juncea</i> (58/1)	H18	<i>B. napus</i> × <i>Brassica rapa</i> ssp. <i>trilocularis</i> (5)
H8	<i>B. napus</i> × <i>Brassica rapa</i> ssp. <i>pekinensis</i> (7/1)	H19	<i>B. napus</i> × <i>Brassica rapa</i> ssp. <i>trilocularis</i> (6)
H9	<i>B. napus</i> × <i>Brassica rapa</i> ssp. <i>pekinensis</i> (9/1)	H20	<i>B. napus</i> × <i>Brassica rapa</i> ssp. <i>trilocularis</i> (43)
H10	<i>B. napus</i> × <i>Brassica rapa</i> ssp. <i>pekinensis</i> (12/1)	H21	<i>B. napus</i> × <i>Brassica rapa</i> ssp. <i>trilocularis</i> (46)
H11	<i>B. napus</i> × <i>Brassica rapa</i> ssp. <i>pekinensis</i> (13/1)	H22	<i>B. napus</i> × <i>Brassica rapa</i> ssp. <i>trilocularis</i> (47)

Table 2. Meteorological conditions in Dłóń, during the vegetation season of winter oilseed rape in 2009, 2010 and 2011.

Basic Weather Parameters	Dłóń		
	2009	2010	2011
Mean annual temperature (°C)	9.1	7.9	9.8
Sum of precipitation (mm)	605.1	753.9	465.5

2.2. Seeds Sampling and Analysis of Fatty Acids Composition:

For each genotype, 10 single seeds, sampled from three to five plants, were analyzed and the average data were put to statistical analysis. Seeds were harvested at physiological maturity (BBCH 89). Fatty acid content was expressed as per cent of total fatty acid content.

A gas chromatography-flame ionization detection (GC–FID) method was used for direct quantitative analysis of seventeen fatty acids in seeds of analyzed *Brassica* genotypes in the Department of Biochemistry and Food Analysis of PULS. All the measurements were performed using a chromatograph Hewlett-Packard model 5890 series II with a flame ionization detector (FID), equipped with HP INNOWAX capillary columns (30 m × 0.32 mm × 0.15 mm) and a split-splitless injector (Agilent Technologies, Inc., Cheshire, UK). Analyses of fatty acids were done at three replicates, and the arithmetic average was adopted for the results.

Seeds from all the parental materials and hybrids were subjected to analyses of the chosen fatty acid composition (C18:1—oleic acid, C18:2—linoleic acid, C18:3—linolenic acid, C20:0—arachidic acid, C20:1—eicosenoic acid, C22:0—behenic acid, C22:1—erucic acid and C24:0—lignoceric acid). The fatty acid content of the seed oil was measured by gas chromatography of the fatty acid methylesters, according to the protocol described by Momotaz et al. (2000) [15].

2.3. Statistical Analysis

Analysis for parental forms and hybrids were made independently. Firstly, the normality of the distributions of the studied traits were tested using Shapiro-Wilk's normality test [16]. A multivariate analysis of variance (MANOVA) was performed. The canonical variate analysis was applied in order to present a multi-trait assessment of similarity for the tested genotypes (parental forms and/or hybrids) in a lower number of dimensions with the least possible loss of information [17]. This makes it possible to illustrate variation in genotypes in terms of all the observed traits in the graphic form. The Mahalanobis

distance was suggested as a measure of “polytrait” genotypes similarity [18], the significance of which was verified by means of critical value D_α called “the least significant distance” [19]. Mahalanobis distances were calculated. Next, the heterosis effects of all observed traits were tested for each year of study. All the analyses were conducted using the GenStat 18 statistical software package (VSN International Limited, Hemel Hempstead, HP2 4TP, UK).

3. Results and Discussion

Nowadays, selecting high quality rapeseed, with good yield and increased oil content and improved edible oils with a modified fatty acid composition, seems to be one of the most important *B. napus* breeding goals [20]. Furthermore, palmitic, stearic, oleic, linoleic, linolenic, eicosenoic and erucic acids are the most important fatty acids in rapeseed, which determines the flavor and nutritional quality of *B. napus* [21]. However, reports in the literature on the performance of *B. napus* interspecific hybrids, with respect to the potential of heterosis for seed quality traits in rapeseed, are relatively rare [22]. In order to improve breeding efficiency, the selection of desirable genotypes is based mostly on a selected trait, i.e., the fatty acid composition regarded as nutritionally favorable [23]. Therefore, the multivariate approach to evaluating the fatty acid composition of *Brassica* hybrids seed oil can be useful in the assessment and selection of genotypes with the fatty acid composition advantageous from the human nutrition point of view.

The conducted multivariate analysis of variance (MANOVA) made it possible to reject tested hypothesis concerning a lack of average multivariate differences between parental forms ($P < 0.001$). Mean values of eleven prominent fatty acids (FA) (16:0, 16:1, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1, 22:0, 22:1 and 24:0) differ between parental forms (Figure 1, Table 3). Generally, the variation among genotypes was evident for most of the tested fatty acids mean values, but the differences between genotypes were not always statistically significant when based on individual FAs. The highest C22:1 mean value was observed in *B. trilobularis* (61.41%), ranging from 13.10% in *B. napus* MS8 line to 61.41% in *B. trilobularis*, but the minimum C16:1 value was noticed (0.17%) in *B. carinata* and *B. juncea*. Similarly, a low mean value of C24:0 was observed in MS8 line and in *B. juncea*. The most noticeable differences in the fatty acid composition between *B. napus* MS8 line seeds and other parental *Brassica* species were observed (Figure 1). For example, the erucic acid (C22:1) composition of *B. napus* and *B. trilobularis* seeds was 4.24% and 1.76% palmitic acid (C16:0), respectively, 1.69% and 1.03% stearic acid (C18:0), 42.33% and 12.1% oleic acid (C18:1), respectively, 17.56% and 9.8% linoleic acid (C18:2), 8.32% and 5.12% linolenic acid (C18:3), 11.59% and 4.96% eicosenoic acid (C20:1), and 13.1% and 61.41% erucic acid (C22:1).

Individual traits are of different importance and have a different share in the joint multivariate variation. A study on the multivariate variation for parental forms also includes an identification of the most important traits in the multivariate variation of parental forms. The analysis of canonical variables is a statistical tool making it possible to solve this problem [24–26]. Results of the analysis of canonical variables for investigated parental forms are presented in Figure 2. The first two canonical variables explained jointly 97.16% total variation between parental forms (Figure 2).

The greatest variation of parental forms in terms of all the traits jointly (measured Mahalanobis distances) was found for MS8 and *B. carinata* (the Mahalanobis distance between them amounted to 143.8). The greatest similarity was found for *B. juncea* and *B. pekinensis* (30.4). Mahalanobis distances between MS8 and other parental forms were statistically significant (Table 4).

Table 3. Fatty acid profile (% of total identified, mean values ± SD) in analyzed *Brassica* genotypes.

Genotypes	C16:0		C16:1		C18:0		C18:1		C18:2		C18:3		C20:0		C20:1		C22:0		C22:1		C24:0	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
S1	4.24	0.10	0.202	0.008	1.692	0.134	42.34	0.37	17.68	0.86	8.46	0.50	0.692	0.044	11.6	0.32	0.39	0.03	13.1	0.50	0.168	0.04
S2	3.658	0.27	0.168	0.016	1.196	0.134	10.42	0.41	20.06	0.46	8.576	0.31	1.068	0.035	7.04	0.36	1.24	0.24	44.84	0.74	1.638	0.14
S3	3.626	0.27	0.172	0.043	1.27	0.210	16.12	0.13	19.26	0.72	17.306	1.81	0.66	0.207	6.2	0.14	0.048	0.01	35.08	1.84	0.188	0.11
S4	2.818	0.04	0.182	0.027	1.318	0.019	17.38	0.35	14.35	0.21	8.88	0.12	1.018	0.036	7.62	0.16	1.206	0.06	43.68	0.29	1.448	0.22
S5	1.758	0.15	0.152	0.008	1.028	0.011	12.1	0.08	9.8	0.12	5.116	0.06	1.106	0.032	4.95	0.08	1.296	0.06	61.44	0.28	0.988	0.02
H1 F1	4.556	0.36	0.216	0.017	1.612	0.106	39.2	3.06	14.9	1.15	7.246	1.15	0.75	0.043	13.18	0.69	0.412	0.03	17.61	1.71	0.2	0.04
H1 F2	4.482	0.17	0.43	0.020	1.74	0.046	36.96	1.37	15.78	1.12	8.348	0.56	0.424	0.015	12.85	0.95	0.096	0.04	18.63	0.90	0.094	0.01
H1 F3	4.519	0.17	0.323	0.016	1.676	0.044	38.08	1.30	15.34	0.75	7.797	0.46	0.581	0.019	13.01	0.71	0.255	0.03	18.11	0.94	0.155	0.03
H2 F1	4.452	0.33	0.184	0.028	1.612	0.167	30.66	0.88	10.38	0.42	4.308	0.29	0.906	0.009	16.2	0.04	0.628	0.11	30.25	0.56	0.256	0.05
H2 F2	4.488	0.45	0.202	0.019	1.694	0.073	30.76	1.06	11.82	0.96	3.944	0.46	0.902	0.016	15.97	0.14	0.578	0.04	29.2	0.14	0.218	0.03
H2 F3	4.458	0.39	0.194	0.018	1.653	0.108	30.86	0.72	11.03	0.89	4.133	0.13	0.905	0.007	16.25	0.18	0.603	0.06	29.47	0.21	0.238	0.03
H3 F1	4.26	0.19	0.184	0.009	1.494	0.070	33.22	0.82	13.57	0.65	6.524	0.76	0.748	0.041	15.71	0.19	0.446	0.01	23.52	0.53	0.224	0.03
H3 F2	4.284	0.19	0.128	0.099	1.504	0.085	33.08	0.46	13.46	0.50	6.372	0.49	0.972	0.018	16	0.33	0.45	0.03	23.37	0.44	0.238	0.02
H3 F3	4.272	0.19	0.157	0.046	1.5	0.076	33.07	0.58	13.51	0.57	6.508	0.53	0.86	0.026	15.79	0.07	0.454	0.01	23.52	0.46	0.232	0.03
H34 F1	4.53	0.05	0.198	0.008	1.608	0.019	36.58	0.34	13.84	0.20	6.666	0.16	0.732	0.004	14.44	0.05	0.382	0.03	20.69	0.29	0.226	0.05
H4 F2	4.482	0.20	0.142	0.111	1.586	0.110	35.29	1.98	13.74	0.37	6.632	0.23	0.972	0.018	15.26	1.21	0.402	0.03	21.1	1.00	0.232	0.02
H4 F3	4.506	0.13	0.171	0.058	1.598	0.061	35.8	0.94	13.78	0.26	6.638	0.19	0.854	0.009	15	0.37	0.392	0.03	20.9	0.66	0.229	0.03
H5 F1	5.888	0.27	0.246	0.015	2.16	0.046	59.67	3.19	10.18	1.63	3.382	0.89	0.83	0.037	8.81	0.40	0.43	0.03	8.04	0.61	0.252	0.12
H5 F2	5.77	0.36	0.238	0.013	2.636	0.319	59.48	1.73	10.41	1.00	3.394	0.62	0.768	0.094	8.61	0.45	0.376	0.06	7.95	0.56	0.218	0.10
H5 H3	5.826	0.22	0.242	0.013	2.334	0.187	59.7	2.15	10.11	1.07	3.394	0.56	0.799	0.034	8.73	0.37	0.404	0.03	8.08	0.44	0.234	0.08
H6 F1	3.626	0.27	0.172	0.043	1.27	0.210	19.01	2.16	13.1	0.59	13.726	0.34	0.66	0.207	6.56	0.54	0.048	0.01	41.58	0.98	0.176	0.08
H6 F2	3.678	0.35	0.278	0.092	1.314	0.255	16.77	0.39	13.92	0.53	13.07	0.47	1.386	0.246	7.43	0.27	0.44	0.11	40.79	0.40	0.886	0.37
H6 F3	3.652	0.12	0.225	0.063	1.292	0.223	16.76	0.51	13.58	0.25	14.394	0.83	1.029	0.157	6.93	0.31	0.243	0.05	41.33	0.28	0.531	0.19
H7 F1	4.208	0.15	0.228	0.036	1.582	0.111	61.31	0.85	18.36	0.73	9.018	0.44	0.628	0.029	2.88	0.37	0.418	0.04	1.19	0.19	0.096	0.04
H7 F2	4.112	0.15	0.332	0.084	1.802	0.619	60.39	0.65	18.1	0.34	8.922	0.19	0.866	0.505	3.78	0.31	0.432	0.14	1.01	0.12	0.2	0.11
H7 F3	4.065	0.31	0.37	0.234	1.736	0.306	51.84	19.63	17.31	2.03	9.919	1.95	0.852	0.337	4.06	1.83	0.407	0.09	9.2	18.17	0.174	0.09
H8 F1	4.048	0.33	0.196	0.015	1.774	0.080	40.12	2.92	14.92	0.54	7.54	1.17	0.774	0.033	13.73	1.43	0.35	0.04	16.48	1.70	0.048	0.07
H8 F2	3.938	0.43	0.196	0.015	1.72	0.162	39.65	4.41	14.31	2.01	7.51	2.30	0.766	0.047	14.8	1.31	0.36	0.05	16.61	1.92	0.092	0.06
H8 F3	3.816	0.21	0.192	0.018	1.612	0.035	37.08	1.49	15.29	0.71	8.254	0.50	0.75	0.027	13.96	1.01	0.376	0.02	18.45	1.47	0.17	0.04
H9 F1	3.586	0.32	0.188	0.026	1.6	0.077	26.65	5.17	12.86	2.16	7.286	0.90	0.81	0.092	16.24	1.07	0.396	0.07	30.13	7.26	0.22	0.05
H9 F2	4.172	0.35	0.246	0.063	1.544	0.064	32.04	1.77	15.05	2.39	6.918	1.59	0.802	0.051	14.09	1.25	0.556	0.14	24.22	2.36	0.238	0.10
H9 F3	4.786	0.34	0.208	0.015	1.76	0.096	31.57	1.02	11.1	0.81	4.638	0.78	0.876	0.038	16.68	0.21	0.472	0.04	27.53	0.60	0.234	0.05
H10 F1	3.55	0.55	0.174	0.025	1.532	0.166	28.75	2.67	11.27	1.88	5.5	1.60	0.89	0.070	13.6	0.55	0.672	0.09	33.59	2.41	0.372	0.07
H10 F2	4.826	0.93	0.214	0.034	1.718	0.234	32.26	2.62	11.45	0.41	4.832	1.04	0.858	0.030	13.9	2.81	0.52	0.08	28.92	1.15	0.284	0.03
H10 F3	5.038	0.67	0.22	0.014	1.928	0.266	35.15	3.07	10.79	3.51	5.806	3.63	0.928	0.110	14.57	1.76	0.548	0.03	24.45	1.68	0.218	0.07
H11 F1	4.156	0.62	0.192	0.024	1.85	0.197	46.91	5.54	15.16	2.34	7.794	2.51	0.768	0.119	11.58	2.17	0.37	0.10	11.06	1.71	0.116	0.17
H11 F2	4.316	0.29	0.21	0.024	1.932	0.123	57.98	3.16	17.47	1.16	7.714	0.84	0.754	0.053	5.1	1.81	0.388	0.06	4.04	1.74	0.036	0.08
H11 F3	4.302	0.48	0.198	0.038	1.918	0.223	61.05	3.26	19.82	1.75	8.258	1.07	0.734	0.101	2.26	0.41	0.404	0.06	1	0.48	0	0.00

Table 3. Cont.

Genotypes	C16:0		C16:1		C18:0		C18:1		C18:2		C18:3		C20:0		C20:1		C22:0		C22:1		C24:0	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
H12 F1	4.032	0.15	0.198	0.004	1.682	0.137	39.83	2.57	16.48	0.93	9.244	0.45	0.72	0.053	12.28	0.72	0.358	0.02	15	1.91	0.158	0.04
H12 F2	4.246	0.53	0.204	0.015	1.96	0.238	47.58	2.93	15.31	1.77	7.164	2.12	0.8	0.090	10.84	2.05	0.376	0.04	11.41	1.80	0.07	0.10
H12 F3	3.878	0.30	0.194	0.009	1.8	0.118	42.12	1.72	14.73	1.54	7.956	1.03	0.754	0.098	12.86	1.09	0.352	0.05	15.19	1.81	0.106	0.02
H13 F1	4.866	0.80	0.272	0.119	1.764	0.202	46.05	6.03	13.04	1.12	6.05	1.53	0.892	0.263	12.54	2.04	0.458	0.11	13.65	2.79	0.188	0.06
H13 F2	5.108	0.98	0.242	0.061	2.056	0.188	53.3	5.05	12.97	1.90	5.714	2.06	0.848	0.086	9.31	1.40	0.452	0.06	9.61	2.47	0.17	0.12
H13 F3	6.118	0.62	0.25	0.012	2.172	0.150	58.94	3.11	9.8	1.31	2.89	0.69	0.952	0.084	8.69	1.02	0.586	0.03	9.04	1.12	0.224	0.09
H14 F1	2.824	0.21	0.146	0.015	1.322	0.164	19.23	3.93	7.82	1.27	3.9	1.39	0.998	0.095	12.18	1.21	0.974	0.08	50.15	4.16	0.334	0.19
H14 F2	3.386	0.12	0.166	0.013	1.61	0.206	30.85	2.59	13.18	0.57	7.838	0.49	0.832	0.075	16.05	0.87	0.434	0.05	25.44	2.04	0.18	0.11
H14 F3	4.226	0.18	0.258	0.077	1.618	0.096	35.2	2.82	13.28	2.08	6.604	1.71	0.924	0.232	15.32	1.46	0.474	0.12	21.9	2.54	0.144	0.11
H15 F1	4.73	0.50	0.21	0.007	1.844	0.106	36.77	2.12	11.06	1.09	4.502	0.98	0.862	0.044	15.98	1.03	0.456	0.09	23.22	1.08	0.166	0.06
H15 F2	4.54	0.67	0.208	0.022	1.856	0.207	37.14	2.67	12.45	2.11	6.24	2.66	0.81	0.072	14.64	1.12	0.41	0.02	21.38	0.30	0.168	0.03
H15 F3	4.656	0.72	0.212	0.026	1.762	0.228	38.01	2.24	12.69	1.92	6.184	2.07	0.776	0.059	14.66	0.79	0.408	0.05	20.29	0.57	0.202	0.09
H16 F1	4.134	0.29	0.228	0.025	1.542	0.058	32.78	2.68	15.8	0.99	8.52	0.77	0.744	0.053	13.7	2.08	0.424	0.11	21.8	2.55	0.238	0.08
H16 F2	4.144	0.38	0.22	0.043	1.716	0.101	36.26	2.13	16.26	1.86	8.064	1.04	0.77	0.045	13.21	1.58	0.432	0.08	18.65	1.23	0.212	0.07
H16 F3	4.766	0.48	0.226	0.015	1.676	0.109	40.89	3.74	15.03	2.11	6.632	1.58	0.776	0.040	13.1	0.13	0.426	0.03	16.2	0.75	0.158	0.04
H17 F1	4.564	0.15	0.2	0.012	1.538	0.023	42.77	3.50	14.72	0.28	7.338	0.61	0.754	0.053	10.91	1.11	0.466	0.15	16.46	5.08	0.174	0.05
H17 F2	4.152	0.52	0.156	0.074	1.46	0.182	36.19	5.34	14.6	2.59	7.222	1.94	0.772	0.090	10.85	3.03	0.578	0.17	23.68	5.85	0.264	0.11
H17 F3	4.654	0.16	0.196	0.015	1.596	0.127	43.64	1.64	13.95	1.87	6.79	1.78	0.784	0.117	11.01	0.89	0.48	0.19	16.64	5.48	0.164	0.04
H18 F1	3.046	0.28	0.172	0.024	1.338	0.100	25.37	1.63	13.14	2.53	7.57	1.93	0.832	0.054	12.5	0.92	0.708	0.08	34.82	3.69	0.406	0.07
H18 F2	3.042	0.22	0.176	0.026	1.308	0.134	23.82	2.67	12.48	1.84	7.474	1.74	0.848	0.078	11.98	0.92	0.76	0.03	37.51	2.49	0.432	0.14
H18 F3	3.058	0.28	0.172	0.024	1.34	0.100	25.63	2.00	13.22	2.55	7.64	1.98	0.826	0.056	12.51	0.92	0.7	0.08	34.4	4.20	0.4	0.08
H19 F1	3.13	0.17	0.154	0.009	1.414	0.029	23.46	6.13	9.26	2.02	4.396	1.68	0.94	0.085	12.82	1.10	0.868	0.15	43.03	8.51	0.372	0.05
H19 F2	5.762	1.28	0.246	0.061	1.942	0.320	54.17	4.65	12.59	3.65	5.396	3.35	0.888	0.189	8.63	0.98	0.52	0.15	9.45	0.62	0.234	0.13
H19 F3	3.022	0.19	0.148	0.013	1.378	0.071	20.84	0.61	8.5	0.38	3.946	0.70	0.958	0.046	13.08	0.52	0.92	0.04	46.63	0.66	0.402	0.03
H20 F1	3.078	0.28	0.17	0.023	1.348	0.104	27.48	2.31	12.46	0.65	7.496	1.90	0.824	0.061	12.73	0.79	0.688	0.10	33.24	4.03	0.39	0.07
H20 F2	3.134	0.25	0.178	0.026	1.336	0.156	26.01	6.37	12.82	2.14	7.602	1.84	0.832	0.085	11.68	0.93	0.714	0.10	35.12	7.55	0.412	0.16
H20 F3	3.03	0.29	0.168	0.024	1.33	0.100	24.95	1.39	12.73	2.59	7.248	1.82	0.834	0.054	12.46	0.92	0.722	0.09	36.02	3.39	0.414	0.06
H21 F1	4.468	0.33	0.196	0.015	1.508	0.056	42.21	3.82	13.85	0.41	7.658	0.64	0.742	0.030	12.1	0.79	0.43	0.08	16.52	4.94	0.198	0.10
H21 F2	4.09	0.57	0.148	0.069	1.486	0.146	35.22	6.54	13.56	1.52	6.882	1.65	0.79	0.075	11.91	2.90	0.552	0.17	25.04	6.82	0.262	0.11
H21 F3	4.476	0.31	0.196	0.015	1.55	0.032	42.36	4.40	14.29	1.11	7.33	0.62	0.766	0.078	11.34	0.26	0.466	0.15	16.96	6.21	0.162	0.05
H22 F1	4.432	0.41	0.194	0.018	1.514	0.062	42.9	6.59	14.23	0.49	7.238	0.76	0.752	0.055	10.27	1.72	0.44	0.12	17.72	7.41	0.182	0.07
H22 F2	4.28	0.66	0.19	0.023	1.482	0.215	38.87	6.26	13.74	0.73	7.504	1.59	0.764	0.074	10.78	2.91	0.558	0.16	21.57	5.32	0.194	0.03
H22 F3	4.388	0.39	0.24	0.096	1.268	0.603	41.57	5.31	13.38	1.25	8.304	2.68	0.788	0.048	11.86	0.71	0.464	0.08	17.38	7.11	0.234	0.10
ANOVA F	17.27		5.15		11.51		54.03		14.55		15.47		6.32		48		33.04		56		38.81	
LSD _{0.001}	0.9		0.1		0.4		8.15		3.14		2.939		0.2		2.51		0.2		7.91		0.2	

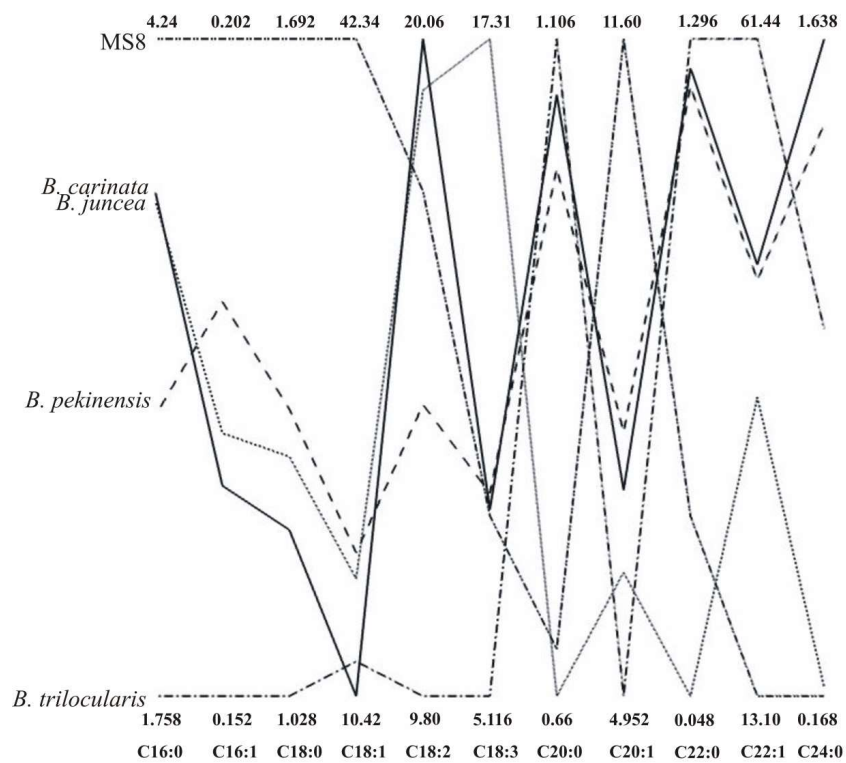


Figure 1. Parallel coordinate plots (PCPs) for five parental forms and 11 traits.

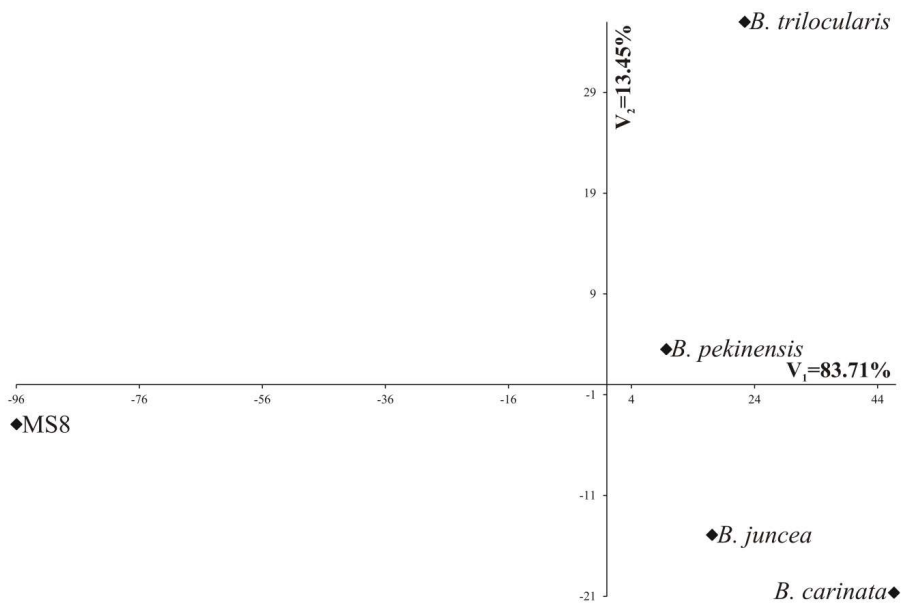


Figure 2. Location of *Brassica* parental forms in the system of the first two canonical variables.

Table 4. Mahalanobis distances for parental forms.

Parental Form	MS8	<i>B. carinata</i>	<i>B. juncea</i>	<i>B. pekinensis</i>	<i>B. trilocularis</i>
MS8	-				
<i>B. carinata</i>	143.8	-			
<i>B. juncea</i>	115.0	39.4	-		
<i>B. pekinensis</i>	106.1	44.7	30.4	-	
<i>B. trilocularis</i>	125.0	62.6	53.2	36.3	-

$D_{0.05} = 73.80.$

Generally, 458 significant heterosis effects were obtained: 148 in F₁, 151 in F₂ and 159 in F₃ (Table 5). The number of the significant heterosis effects for individual hybrids ranged from eight (for H18) to 28 (for H1) (Table 5). Considering observed traits, the lowest number of statistically significant heterosis effects was obtained for C16:1 (14), however, the highest was for C22:0 and C24:0 (62) (Table 3). The obtained results indicated statistically significant heterosis effects in all three generations for 122 out of 242 cases. In 61 cases, we observed non-significant heterosis effects in each of the three generations. Statistically significant heterosis effects for individual hybrid had the same sign in particular generations, except H6 for C22:0, H11 for C20:1, H14 for C16:0, C18:1, C22:0 and C22:1, as well as H19 for C18:1 and C22:1 (Table 4). The hybrid H5 is recommended for further inclusion in the breeding programs because it has the highest positively heterosis effects for C18:1, and negatively for C18:2, C18:3 and C22:1 in all three generations.

Table 5. Heterosis effects for particular hybrids in three generations.

Hybrid	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1	C24:0
H1 F1	1 **	0.03	0.2	13 ***	-3.98 ***	-1.272	-0.1 *	3.9 ***	-0.4 ***	-11 ***	-0.7 ***
H1 F2	1 *	0.25 ***	0.3 **	11 ***	-3.09 ***	-0.17	-0.5 ***	3.5 ***	-0.7 ***	-10 ***	-0.8 ***
H1 F3	1 *	0.14 ***	0.2 *	12 ***	-3.53 ***	-0.721	-0.3 ***	3.7 ***	-0.6 ***	-11 ***	-0.7 ***
H2 F1	1 *	0	0.2	4 *	-8.49 ***	-4.21 ***	0	6.9 ***	-0.2 ***	1	-0.6 ***
H2 F2	1 *	0.02	0.2 *	4 *	-7.05 ***	-4.574 ***	0	6.7 ***	-0.2 ***	0	-0.7 ***
H2 F3	1 *	0.01	0.2 *	4 *	-7.84 ***	-4.385 ***	0	6.9 ***	-0.2 ***	1	-0.7 ***
H3 F1	0	0	0	7 **	-5.3 ***	-1.994 *	-0.1 *	6.4 ***	-0.4 ***	-5 **	-0.7 ***
H3 F2	0	-0.06 *	0.1	7 **	-5.41 ***	-2.146 **	0.1	6.7 ***	-0.4 ***	-6 **	-0.7 ***
H3 F3	0	-0.03	0.1	7 **	-5.36 ***	-2.01 **	0	6.5 ***	-0.4 ***	-5 **	-0.7 ***
H4 F1	1 *	0.01	0.2	10 ***	-5.03 ***	-1.852 *	-0.1 *	5.1 ***	-0.4 ***	-8 ***	-0.7 ***
H4 F2	1 *	-0.04	0.1	9 ***	-5.13 ***	-1.886 *	0.1	5.9 ***	-0.4 ***	-8 ***	-0.7 ***
H4 F3	1 *	-0.01	0.2	9 ***	-5.09 ***	-1.88 *	0	5.7 ***	-0.4 ***	-8 ***	-0.7 ***
H5 F1	2 ***	0.06 *	0.7 ***	33 ***	-8.7 ***	-5.136 ***	0	-0.5	-0.4 ***	-21 ***	-0.7 ***
H5 F2	2 ***	0.05	1.2 ***	33 ***	-8.46 ***	-5.124 ***	-0.1	-0.7	-0.4 ***	-21 ***	-0.7 ***
H5 F3	2 ***	0.06 *	0.9 ***	33 ***	-8.77 ***	-5.124 ***	-0.1	-0.6	-0.4 ***	-21 ***	-0.7 ***
H6 F1	0	-0.02	-0.2 *	-10 ***	-5.37 ***	0.843	0	-2.3 ***	-0.2 ***	17 ***	0
H6 F2	0	0.09 ***	-0.2	-12 ***	-4.55 ***	0.187	0.7 ***	-1.5 *	0.2 ***	17 ***	0.7 ***
H6 F3	0	0.04	-0.2	-12 ***	-4.89 ***	1.511 *	0.4 ***	-2 **	0	17 ***	0.4 ***
H7 F1	0	0.04	0.1	32 ***	-0.11	-3.865 ***	0	-6 ***	0.2 ***	-23 ***	-0.1
H7 F2	0	0.15 ***	0.3 **	31 ***	-0.37	-3.961 ***	0.2 **	-5.1 ***	0.2 ***	-23 ***	0
H7 F3	0	0.18 ***	0.3 *	23 ***	-1.16	-2.964 ***	0.2 **	-4.8 ***	0.2 ***	-15 ***	0
H8 F1	1 *	0	0.3 **	10 ***	-1.1	-1.13	-0.1	4.1 ***	-0.4 ***	-12 ***	-0.8 ***
H8 F2	0	0	0.2 *	10 ***	-1.7 *	-1.16	-0.1	5.2 ***	-0.4 ***	-12 ***	-0.7 ***
H8 F3	0	0	0.1	7 ***	-0.73	-0.416	-0.1	4.4 ***	-0.4 ***	-10 ***	-0.6 ***
H9 F1	0	0	0.1	-3	-3.15 ***	-1.384	0	6.6 ***	-0.4 ***	2	-0.6 ***
H9 F2	1 **	0.05 *	0	2	-0.97	-1.752 *	-0.1	4.5 ***	-0.2 ***	-4	-0.6 ***
H9 F3	1 ***	0.02	0.3 *	2	-4.92 ***	-4.032 ***	0	7.1 ***	-0.3 ***	-1	-0.6 ***
H10 F1	0	-0.02	0	-1	-4.75 ***	-3.17 ***	0	4 ***	-0.1 **	5 *	-0.4 ***
H10 F2	1 ***	0.02	0.2 *	2	-4.56 ***	-3.838 ***	0	4.3 ***	-0.3 ***	1	-0.5 ***
H10 F3	2 ***	0.03	0.4 ***	5 *	-5.23 ***	-2.864 ***	0.1	5 ***	-0.3 ***	-4	-0.6 ***
H11 F1	1 **	0	0.3 ***	17 ***	-0.85	-0.876	-0.1	2 **	-0.4 ***	-17 ***	-0.7 ***
H11 F2	1 ***	0.02	0.4 ***	28 ***	1.46	-0.956	-0.1	-4.5 ***	-0.4 ***	-24 ***	-0.8 ***
H11 F3	1 ***	0.01	0.4 ***	31 ***	3.8 ***	-0.412	-0.1	-7.3 ***	-0.4 ***	-27 ***	-0.8 ***
H12 F1	1 *	0.01	0.2	10 ***	0.47	0.574	-0.1*	2.7 ***	-0.4 ***	-13 ***	-0.7 ***
H12 F2	1 **	0.01	0.5 ***	18 ***	-0.71	-1.506	-0.1	1.2	-0.4 ***	-17 ***	-0.7 ***
H12 F3	0	0	0.3 **	12 ***	-1.29	-0.714	-0.1	3.3 ***	-0.4 ***	-13 ***	-0.7 ***
H13 F1	1 ***	0.08 **	0.3 *	16 ***	-2.98 ***	-2.62 **	0	2.9 ***	-0.3 ***	-15 ***	-0.6 ***
H13 F2	2 ***	0.05	0.6 ***	23 ***	-3.04 ***	-2.956 ***	0	-0.3	-0.3 ***	-19 ***	-0.6 ***
H13 F3	3 ***	0.06 *	0.7 ***	29 ***	-6.21 ***	-5.78 ***	0.1	-0.9	-0.2 ***	-19 ***	-0.6 ***
H14 F1	-1 **	-0.05	-0.2	-11 ***	-8.2 ***	-4.77 ***	0.1*	2.6 ***	0.2 ***	22 ***	-0.5 ***
H14 F2	0	-0.03	0.1	1	-2.83 ***	-0.832	0	6.4 ***	-0.4 ***	-3	-0.6 ***
H14 F3	1 **	0.07 *	0.1	5 *	-2.74 ***	-2.066 **	0.1	5.7 ***	-0.3 ***	-6 **	-0.7 ***
H15 F1	1 ***	0.02	0.3 ***	7 **	-4.95 ***	-4.168 ***	0	6.4 ***	-0.3 ***	-5 *	-0.6 ***
H15 F2	1 ***	0.02	0.4 ***	7 ***	-3.57 ***	-2.43 **	0	5 ***	-0.4 ***	-7 **	-0.6 ***
H15 F3	1 ***	0.02	0.3 *	8 ***	-3.33 ***	-2.486 **	-0.1	5.1 ***	-0.4 ***	-8 ***	-0.6 ***
H16 F1	1 **	0.04	0	3	-0.21	-0.15	-0.1	4.1 ***	-0.4 ***	-7 **	-0.6 ***
H16 F2	1 **	0.03	0.2 *	6 **	0.24	-0.606	-0.1	3.6 ***	-0.4 ***	-10 ***	-0.6 ***
H16 F3	1 ***	0.03	0.2	11 ***	-0.98	-2.038 **	-0.1	3.5 ***	-0.4 ***	-12 ***	-0.7 ***

Table 5. Cont.

Hybrid	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1	C24:0
H17 F1	2 ***	0.02	0.2	16 ***	0.99	0.55	-0.1*	2.6 ***	-0.4***	-21 ***	-0.4***
H17 F2	1 ***	-0.02	0.1	9 ***	0.86	0.434	-0.1*	2.6 ***	-0.3***	-14 ***	-0.3***
H17 F3	2 ***	0.02	0.2 *	16 ***	0.21	0.002	-0.1	2.7 ***	-0.4***	-21 ***	-0.4***
H18 F1	0	0	0	-2	-0.6	0.782	-0.1	4.2 ***	-0.1 **	-2	-0.2 **
H18 F2	0	0	-0.1	-3	-1.25	0.686	-0.1	3.7 ***	-0.1	0	-0.1 **
H18 F3	0	0	0	-2	-0.52	0.852	-0.1	4.2 ***	-0.1 **	-3	-0.2***
H19 F1	0	-0.02	0.1	-4	-4.48***	-2.392**	0	4.5 ***	0	6 **	-0.2***
H19 F2	3 ***	0.07 *	0.6 ***	27 ***	-1.15	-1.392	0	0.4	-0.3***	-28 ***	-0.3***
H19 F3	0	-0.03	0	-6 **	-5.24***	-2.842***	0.1	4.8 ***	0.1	9 ***	-0.2***
H20 F1	0	-0.01	0	0	-1.28	0.708	-0.1	4.5 ***	-0.2 **	-4	-0.2***
H20 F2	0	0	0	-1	-0.91	0.814	-0.1	3.4 ***	-0.1 **	-2	-0.2 **
H20 F3	0	-0.01	0	-2	-1.01	0.46	-0.1	4.2 ***	-0.1 *	-1	-0.2 **
H21 F1	1 ***	0.02	0.1	15 ***	0.12	0.87	-0.2 *	3.8 ***	-0.4***	-21 ***	-0.4***
H21 F2	1 ***	-0.03	0.1	8 ***	-0.18	0.094	-0.1	3.6 ***	-0.3***	-12 ***	-0.3***
H21 F3	1 ***	0.02	0.2	15 ***	0.56	0.542	-0.1 *	3.1 ***	-0.4***	-20 ***	-0.4***
H22 F1	1 ***	0.02	0.2	16 ***	0.49	0.45	-0.1 *	2 **	-0.4***	-20 ***	-0.4***
H22 F2	1 ***	0.01	0.1	12 ***	0	0.716	-0.1 *	2.5 ***	-0.3***	-16 ***	-0.4***
H22 F3	1 ***	0.06 *	-0.1	14 ***	-0.36	1.516 *	-0.1	3.6 ***	-0.4***	-20 ***	-0.3***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Individual traits are of different importance and have a different share in the joint multivariate variation. A study on the multivariate variation for treatments also includes identification of the most important traits in the multivariate variation of treatments. Canonical variables analysis (CVA) is a statistical tool making it possible to solve this problem [27,28]. The results of the analysis of canonical variables for investigated hybrids analyzed in three years are presented in Figure 3. The first two canonical variables explained jointly 66.37% total variation between hybrids. The first canonical variable accounted for 38.97% of the total variation, while the second canonical variable accounted for 27.40% (Figure 3).

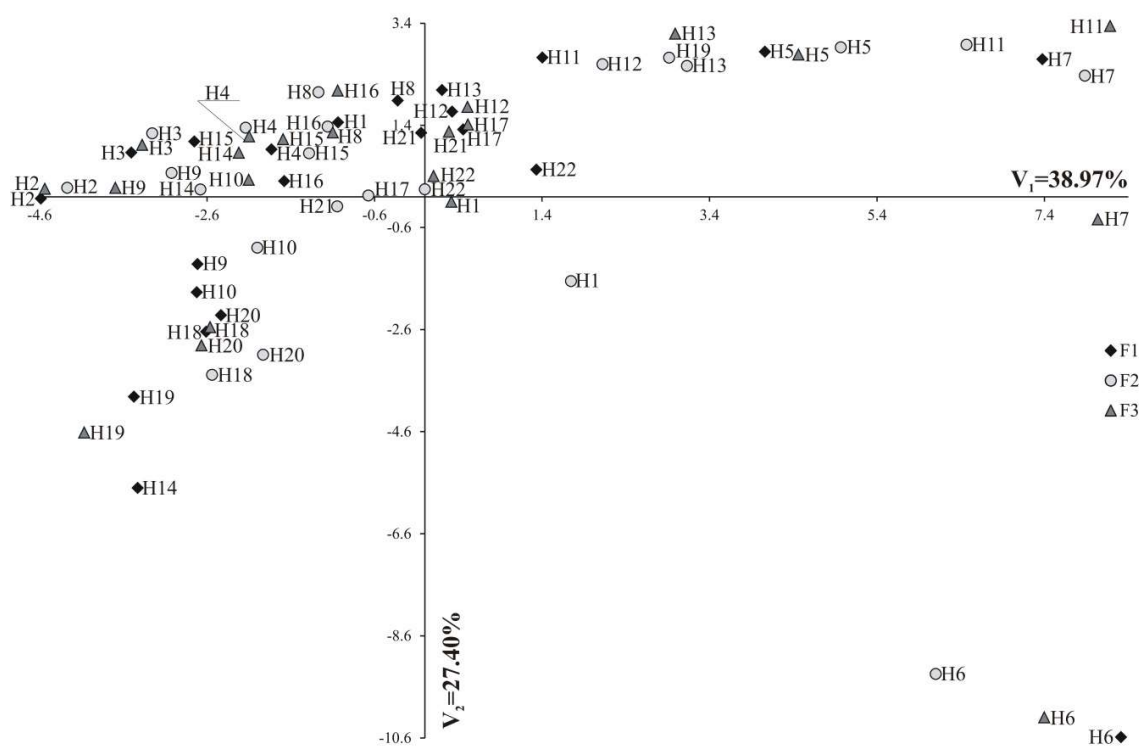


Figure 3. Location of Brassica hybrids in the system of the first two canonical variables.

The most significant, positive, linear relationship between the first canonical variables was found for C16:0, C16:1, C18:0, C18:1, C18:2 and C18:3, while it was negative for C20:1, C22:0 and C22:1. The second canonical variable was significantly positively correlated with C16:0,18:1 and C18:2, while it was negatively correlated with C18:3, C20:0, C22:1 and C24:0. The greatest variation in terms of all the 11 fatty acids jointly (measured Mahalanobis distances) was found for H3 in F₂ and H6 in F₁ (the Mahalanobis distance between them amounted to 19.919). The greatest similarity was found for H18 in F₁ and H18 in F₃ (0.150). According to the literature data, canonical variable analysis and Mahalanobis distances are statistical tools, which may be confirmed by their extensive application by breeders and geneticists [29–34].

Our results fully confirmed that assumption and proved that in order to identify the best *Brassica* genotypes in respect of heterosis for fatty acids requirements, the multivariate analysis of variance conducted for the eleven analyzed acids is a very useful statistical method of high importance.

Some authors reported that, most probably, it will soon be possible to change the profile of fatty acids content in *Brassica* seed oil, according to industry expectations [35,36].

Moreover, interspecific hybridization between appropriate *Brassica* species have a great potential in creating lines with a modified fatty acid composition [37,38]. In particular, as our results showed, the development of lines with a high oleic acid content seems promising. According to the magnitude of the predicted breeding values in this study, it is possible to select desirable breeding materials (hybrid line H5), which show the proper spectrum of the fatty acids in oil.

4. Conclusions

All analyzed parental genotypes as well as *Brassica* hybrid lines show statistically significant multivariate diversity for eleven fatty acids. The greatest variation of parental forms in terms of all the traits jointly was found for *B. napus* MS8 line and *B. carinata*. These two genotypes would be the best as parental forms in future breeding programs.

Analysis of canonical variables is a good tool for multivariate analysis of relationships among genotypes. The first two canonical variables explained jointly 97.16% and 66.37% total variation between parental forms and hybrids, respectively.

The highest number of significant heterosis effects was observed for behenic and lignoceric acids and for *Brassica* hybrid line H1.

The hybrid line H5 is recommended for further inclusion in the breeding programs, because it has the highest positively heterosis effect for oleic acid, and negatively for linoleic, linolenic and erucic acids in all three generations.

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