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Response of components of doubled haploids seed yield of winter oilseed rape (*Brassica napus* L.) to diverse environments

Reakcja komponentów plonu nasion podwojonych haploidów rzepaku ozimego (*Brassica napus* L.) na zróżnicowane środowiska

Key words: *Brassica napus* L., winter oilseed rape, DH, seed yield components, GE, interaction, stability, adaptation

In this paper the influence of environment on seed yield components of doubled haploid lines (DH) obtained from F_1 hybrids of winter oilseed rape is presented. In order to determine this influence, comparison between components of seed yield of DH lines and a standard cultivar was conducted in the field experiment in six different environments during the period 1999–2003. The material for this experiment constituted randomly selected 32 DH lines obtained from isolated microspores of F_1 hybrids (DH O-120 × DH C-1041) of winter oilseed rape, parental lines DH O-120 (P1), DH C-1041 (P2), as well as F_2 and F_3 hybrids. Performed statistical calculations made it possible to evaluate DH lines as regards different aspects of their environmental interaction and enabled the comparison of individual DH lines with the standard cultivar Kana with respect to their main effects and interactions for the following traits: the length of silique, the number of seeds in silique and thousand seeds weight (TSW). With respect to the length of silique 10 doubled haploids displayed a positive main effect and six of them showed stability. Nine of DH lines exhibited significant interaction with the environment, but for only three lines this interaction can be justified by linear regression. Seven doubled haploids as well as the standard cultivar Kana and a parental line P2 (DH C-1041) showed positive main effect for TSW. The genotype-environment interaction, (GE), concerning this trait was significant for the majority of DH lines. The two DH lines (DH H5-79 and DH H5-729) showed only stability, the TSW for those lines was higher than the mean value for all objects in each environment.

Słowa kluczowe: *Brassica napus* L., rzepak ozimy, DH, komponenty struktury plonu, GE, interakcja, stabilność, adaptacja

Zaprezentowano wyniki badań nad wpływem różnych środowisk na wartość komponentów plonu nasion podwojonych haploidów (DH) rzepaku ozimego. Materiałem do badań były linie DH, losowo wybrane z populacji podwojonych haploidów uzyskanych metodą kultury izolowanych mikrospor mieszańca F_1 (DH O-120 × DH C-1041), linie rodzicielskie: P1 (DH O-120) i P2 (DH C-1041), jak również mieszańce F_2 i F_3. Dla określenia wpływu środowiska przeprowadzono porównanie składników plonu linii DH do standardowej odmiany rzepaku ozimego Kana w doświadczeniu polowym w sześciu latach 1999–2003.

Przedstawione obliczenia statystyczne określały linie DH z punktu widzenia różnych aspektów interakcji z środowiskiem oraz porównanie pojedynczych linii DH z odmianą standardową Kana dla efektów głównych. Ponadto przedstawiono interakcje poszczególnych podwojonych haploidów ze środowiskiem w odniesieniu do cech: długość łuszczyny, liczba nasion w łuszczynie, masa tysiąca nasion (MTN). Dla długości łuszczyn 10 podwojonych haploidów wykazywało pozytywne efekty główne, natomiast sześć z nich wykazywało stabilność w zakresie tej ceche. Dziewięć linii DH wykazywało istotną interakcję ze środowiskiem, lecz tylko trzy z nich można było wytłumaczyć regresją liniową. Siedem podwojonych haploidów, jak również odmiana Kana i linia rodzicielska $P_{2,}$ wykazywało pozytywne efekty główne dla MTN. Genotypowo-środowiskowa interakcja (GE), dotycząca tej cechy okazała się istotna dla większości linii DH. Jedynie dwie linie DH (DH H5-79 i DH H5-729) wykazywały stabilność, MTN dla tych linii była wyższa niż średnia wartość dla wszystkich badanych genotypów w każdym środowisku.

Konkludując można stwierdzić, że analiza w sześciu różnych środowiskach dla 32 linii DH rzepaku ozimego, prowadzona dla trzech komponentów struktury plonu: długości łuszczyny, liczby nasion w łuszczynie oraz masy tysiąca nasion, pozwoliła na:

– wyróżnienie grupy najlepszych linii DH w zakresie badanych cech,
– wyróżnienie linii stabilnych dla dwóch cech równocześnie (sześć linii DH) oraz dla jednej cechy (trzy linie),
– wytłumaczenie regresją liniową interakcji ze środowiskiem dla kilku linii DH (Nr 3, 6, 12 i 18) oraz innych linii mało interesujących z punktu widzenia interakcji ze środowiskiem,
– znalezienie wśród badanych genotypów takich linii, które przedstawiają istotną wartość interakcji ze środowiskiem: intensywne linie DH (Nr 3, 6 i 18) oraz ekstensywna linia DH (Nr 12).

Introduction

One of the advantages of an in vitro culture of *Brassica napus* microspores is the production of homozygous lines from haploid embryos and further doubling of chromosomes which is faster than conventional methods of selection of pure lines through self pollination.

In doubled haploid lines (DH) all loci are homozygous and for this reason the expression of all functional genes appears without the gene domination phenomenon and the selection becomes easier because the traits of plants are conspicuous (Cegielska-Taras et al. 1999, Palmer and Keller 1999). The probability of getting DH lines with desirable quality traits as well as the traits which are responsible for seed yield depends on the genotype of the microspores–donor plant. Doubled haploid lines have been widely used in a breeding process of developing a new cultivar (Stringam et al. 1995) or evaluation of homozygous lines as parents of future hybrids (Dewan et al. 1998). The system of doubled haploid lines was compared with traditional breeding methods: pedigree selection for the development of high erucic acid oilseed rape cultivar (Jacobs and McVetty 2005). Successful new genotypes, in particular doubled haploids, must show high performance for various characters. However, their superiority should be reliable over a wide range of environmental conditions (Kaczmarek et al. 2006).

The crossing of DH lines of winter oilseed rape valuable with regard to agronomy traits and subsequent production of new population of doubled haploids from F_1 hybrids seems to be conducive to accumulating desirable alleles in one high yielding DH line which was reported in *Brassica rapa* (Dewan et al. 1998).

The selection of doubled haploids with desirable traits from population of androgenic lines is not easy due to genotype-environment interaction (GE), (Adamska et al. 2004a, Cegielska-Taras et al. 2005). Undoubtedly in the selection of valuable DH lines one should consider the necessity for improvement of agronomic traits such as high seed yield as well as fidelity of yielding estimated through the analysis of genotype-environment interaction.

The research reported in this paper aimed at estimation of the influence of the environment on seed yield components of doubled haploid lines obtained from F_1 hybrids of winter oilseed rape. The estimation was conducted on the basis of the statistical analysis of a series of field experiment trials in six different environments.

Material and methods

The material for this experiment included 32 randomly selected DH lines obtained from the isolated microspores of F_1 hybrids (DH O-120 × DH C-1041) of winter oilseed rape (Cegielska-Taras et al. 1999). The investigation was also conducted on parental lines DH O-120 (P1), DH C-1041 (P2), as well as F_2 and F_3 hybrids and the cultivar of winter oilseed rape Kana – standard (Adamska et al. 2004a, b).

The experiments were performed on the plot (4 m^2) in six environments:
E1 (Poznań 1998/1999), E2 (Cerekwica 1999/2000, E3 (Cerekwica 2000/2001), E4 (Borowo 2001/2002), E5 (Cerekwica 2002/2003) and E6 (Cerekwica 2001/2002). Each of the experiments with the same 37 genotypes was established in a completely randomized block design with three replications.

The following traits concerning the components of seed yield were investigated: the length of silique, the number of seeds per silique and thousand seed weight (TSW).

The data obtained in experiments were analyzed statistically with uni- and multivariate methods which permit to estimate value of DH lines with regard to different aspects of their interaction with environment and to make possible the comparison of each line with the standard cultivar Kana for main effects and interaction effects. Statistical analyses were performed with the computer program SERGEN (Caliński et al. 1998).

Results and discussion

Statistical calculations permitted to evaluate DH lines taking into account different aspects of their environmental interaction and enabled the comparison of individual DH lines with the standard cultivar Kana with respect to their main effects and interactions for the following traits: the length of silique, the number of seeds in silique and thousand seeds weight.

However, in the previous study statistical calculations were presented, which permitted to evaluate DH lines with consideration of different aspects of their environmental interaction and enabled comparison of individual DH lines with standard cultivar Kana with respect to their main effects and interaction as well as for the seeds yield. It was concluded that ,besides the standard cultivar Kana, which yielded the best, four DH lines (H5-43, H5-129, H5-255 and H5-493) were characterized by positive effects, that is, they yielded higher than the average seed yield of all studied genotypes. Interaction with the environment of the four lines was not significant what provides evidence about stability in their yielding (Adamska et al. 2004b).

The studies concerned seed yield components such as: the length of silique, the number of seeds per silique and thousand seeds weight, which are important regarding plant breeding.

Dependence of seeds yield on seed yield components are very useful for selection of genotypes characterized with high yields of seeds. The seed yield and seed yield components are substantially influenced by environment (Wójtowicz 2005).

At the first stage of calculations estimations of main effects of individual genotypes were made. Thus the variation of an average value of particular traits of yield structure of each of the lines from the total average in the environment was estimated. The results are shown in Table 1. Considering the length of the silique ten doubled haploids were characterized by positive main effect and six lines occurred to be stable lines. Nine of the DH lines possessed significant interaction with environment, however only in three cases this may be explained by a linear regression.

For the number of seeds in silique it was found that twelve DH lines exhibited positive significant main effect. Out of these lines ten were stable and in ten lines environmental genotype interaction was observed. The positive main effect was observed for thousand seed weight in nine DHs, the cultivar Kana and parental forms DH C-1041. With respect to this trait the majority of studied doubled haploids showed essential environmental genotype interaction but in eight lines it was possible to explain it by linear regression. Only two lines: DH H5-79 and DH H5-729 were stable while TSW of these lines in each environment was significantly higher than the average value for all of the studied genotypes.

Table 1

Testing of each genotype for main effects and for three traits components of seed yield
Testowanie poszczególnych genotypów dla efektu głównego trzech cech komponentów plonu nasion

No *Nr*	Genotype *Genotyp*	Studied traits — *Badane cechy*					
		length of silique *długość łuszczyny*		number of seeds per silique *liczba nasion w łuszczynie*		thousand seeds weight *masa tysiąca nasion*	
		EME[1]	F[2]	EME[1]	F[2]	EME[1]	F[2]
1	DH H5-8	-0.11	0.35	0.92	1.67	0.03	0.09
2	DH H5-30	-0.62	19.71**	-4.79	20.75**	0.50	12.05**
3	DH H5-43	0.67	15.51**	3.45	25.28**	-0.15	5.17
4	DH H5-71	-0.98	126.02**	-8.16	448.57**	0.75	13.71*
5	DH H5-77	-0.98	62.50**	-6.33	73.57**	0.69	23.30**
6	DH H5-79	0.56	11.89**	-1.34	2.51.	0.31	60.34**
7	DH H5-85	-0.89	57.37**	-2.51	23.81**	0.41	4.51
8	DH H5-105	-1.77	279.98**	-8.91	188.45**	0.94	75.30**
9	DH H5-109	0.55	6.50*	4.18	120.16**	-0.54	79.85**
10	DH H5-114	-0.25	7.85*	3.01	113.65**	-0.34	51.13**
11	DH H5-129	-0.09	4.77	3.42	20.83**	-0.20	3.82
12	DH H5-191	0.05	0.40	3.77	72.48**	-0.19	6.11
13	DH H5-202	0.79	73.50**	2.72	45.12**	-0.19	12.20*
14	DH H5-216	-0.17	3.81	0.58	0.79	-0.59	89.68**
15	DH H5-237	0.02	0.18	1.29	3.81	-0.32	16.24*
16	DH H5-238	0.02	0.07	1.23*	10.25*	-0.15	1.30
17	DH H5-250	-0.40	6.35	-1.07	1.56	0.10	1.92
18	DH H5-255	-2.01	387.43**	-6.74	163.79**	0.40	11.22*
19	DH H5-261	0.96	553.17**	3.01	41.14**	-0.33	4.96
20	DH H5-284	0.91	33.42**	3.02	21.77**	-0.15	5.14
21	DH H5-349	0.52	46.40**	1.10	7.94	-0.42	59.77**
22	DH H5-396	1.58	187.79**	3.83	113.51**	-0.02	0.06
23	DH H5-416	-0.27	17.32**	-3.19	11.80*	0.61	18.37**
24	DH H5-467	-0.10	0.90	0.59	1.28	-0.24	10.99*
25	DH H5-493	0.08	1.65	1.20	2.70	-0.30	9.96*
26	DH H5-544	0.04	0.10	-2.10	15.71*	0.22	4.59
27	DH H5-621	0.46	11.04*	2.79	32.08**	-0.35	11.40*
28	DH H5-729	0.46	3.20	2.02	26.24*	0.16	21.21**
29	DH H5-802	0.46	17.25**	0.99	3.76	-0.43	53.29**
30	DH H5-804	-0.17	1.55	-1.50	1.79	0.28	8.02*
31	DH H5-876	-0.25	16.50**	0.69	5.59	-0.25	6.13
32	DH H5-977	-0.44	27.77**	-0.96	3.90	-0.10	2.06
33	P1 O-120	1.51	53.99**	3.49	48.98**	-0.37	26.89**

No *Nr*	Genotype *Genotyp*	Studied traits — *Badane cechy*					
		length of silique *długość łuszczyny*		number of seeds per silique *liczba nasion w łuszczynie*		thousand seeds weight *masa tysiąca nasion*	
		EME[1]	F[2]	EME[1]	F[2]	EME[1]	F[2]
34	P2 C-1041	1.65	67.76**	-6.56	40.33**	0.66	21.83**
35	F2	1.01	58.09**	3.79	74.57**	-0.06	0.66
36	F3	0.47	21.99**	2.59	7.74*	0.05	0.26
37	cv. Kana	0.02	0.24	0.57	4.56	-0.42	13.57*
Critical value *Wartość krytyczna*		$F_{0.05}$ $F_{0.01}$	6.61 16.26	2.23 3.06			

*, ** significant at P = 0.05 and P = 0.01, respectively
[1] EME = estimated main effect, [2] F = F statistics for main effect
EME = ocena efektu głównego; F = statystyka efektu głównego

For further and more detailed analysis there were chosen these lines whose estimate of the main effect for at least one trait, was positive and significant. These conditions were fulfilled by the following DH lines Nos.: 3, 6, 11, 12, 16, 20, 22, 28 and 30. In addition DH lines No 18 and No 25 were used. Although they do not match the mentioned criteria, they showed positive and higher significant seeds yield (Adamska at al. 2004a, b). The doubled haploid no 13 was also included because this line was very good as regards two traits: the length of silique and the number of seeds per siliqe; but with respect to TSW significantly differed from the total average value at the level P = 0.05. This DH line was characterized by positive although not significant main effect connected with seed yield (Adamska et al. 2004a, b). Table 2 shows average values and F statistic value for interaction with the environment for all mentioned above DH lines and the standard cultivar Kana. Among twelve chosen DH lines, eight were stable with regard to the length of silique, eight with regard to the number seeds per siliique and five with regard to TSW.

The analysis of the results presented in Table 2 reveals that the best DH lines with regard to the yield components are DH lines no: 3, 20 and 22. These doubled haploids exhibited significantly higher mean value for the length of silique and the number of seeds in silique and TSW did not differ significantly from the total mean value. Beneficial values for the two traits were characteristic of the lines DH no 6 (for the length of the silique and TSW) and DH no 13 (for the length of silique and the number of seeds in silique). The DH no 13 possessed lower total average value of TSW. The DH lines no: 11 and 12 were noteworthy because the total mean value for the number of seeds per silique were higher than the mean value of all studied lines whereas the value of the other two traits were on the level of the total mean value.

Table 2

Average value of selected DH lines and test of their interaction with environment for three traits of yield components

Średnie wartości plonu wyselekcjonowanych linii oraz testowanie interakcji ze środowiskiem dla trzech cech struktury plonu

No *Nr*	Genotype *Genotype*	Studied traits — *Badane cechy*							
		seeds yield *plon nasion*		length of silique *długość łuszczyny*		Number of seeds per silique *liczba nasion w łuszczynie*		thousand seeds weight *masa tysiąca nasion*	
		average *średnia*	F-statistic for interaction *statystyka F dla interakcji*	average *średnia*	F-statistic for interaction *statystyka F dla interakcji*	average *średnia*	F-statistic for interaction *statystyka F dla interakcji*	average *średnia*	F-statistic for interaction *statystyka F dla interakcji*
3	DH H5-43	1.38	1,63	8.97	3.14*	28.1	2.22	4.45	1.56
11	DH H5-129	1.41	0,68	8.21	0.19	28.1	2.64*	4.40	4.05**
12	DH H5-191	1.27	2,57**	8.35	0.76	28.5	0.92	4.41	2.26*
13	DH H5-202	1.29	3,42**	9.09	0.93	27.4	0.77	4.41	1.16
18	DH H5-255	1.34	1,37	6.27	1.15	17.9	1.30	5.00	5.34**
22	DH H5-396	1.27	1,59	9.88	1.45	28.5	0.61	4.58	2.38*
25	DH H5-493	1.31	0,76	8.38	0.43	25.9	2.52*	4.30	3.39**
	cv. Kana	1.61	2,30*	8.32	0.29	25.3	0.34	4.18	4.99**
Total average *Średnia całkowita*		1.36		8.43	–	26.3	–	4.47	–
Critical value *Krytyczna wartość*		$F_{0.05}$ $F_{0.01}$	2.23 3.06		2.23 3.06		2.23 3.06		2.23 3.06

*, ** significant at P = 0.05 and P = 0.01 respectively — *kontrasty istotne dla P = 0.05 i P = 0.01*

The experiments which were conducted with doubled haploids of winter oilseed rape in six environments permitted to determine objectively the interaction of each line with these environments. The interaction of DH lines with the environment occurred to be significant with respect to each of the three studied traits of seed yield components: the length of silique — F = 1.79, the number of seeds per silique — F = 1.89, TSW — F = 4.04 with the critical value $F_{0.05}$ = 1.22 and $F_{0.01}$ = 1.33. The presented above value of F statistic refutes a general hypothesis about the lack of interaction between DH line and environments and explicitly shows great influence of the environment on the value of thousand seeds weight of DH lines. It is confirmed by the value of F statistic for selected DH lines presented in Table 2. With respect to the other traits DH lines are mostly stable (Table 2). The explanation of interaction effects of DH lines in relation to environmental effects by linear regression was successful for several doubled haploids.

Similarly, the interaction of DH line No 3 with the environment for to the number of seeds in a silique may be explained by regression..

The coefficient of regression is positive (= 1.35) and significant at P = 0.1 and the coefficient of determination is 56.8%. On the condition that the value of F statistic for regression is relatively high F = 5.26, the value of F statistic for deviates from regression turned out not significant (F = 1.20 in the presence $F_{0.05}$ = 2.39). It means that the regression line in Fig. 1 illustrates very well the dependence of the interaction of DH line no 3 on environmental effects. Both DH lines: no 6 and no 3 can be recognized as intensive lines with respect to the length of silique and the number of seeds per silique. Considering TSW, the line DH No 18 was intensive; the coefficient of regression was equal to 0.54 (Fig. 2). A different type of dependency between interaction effects and environmental effects was observed in DH line No 12 for TSW (Fig. 3). The interaction of this line with the environment can be also explained by linear regression, however, the course of the linear regression is described by negative regression coefficient equal to –0.32. For line DH No 12 the coefficient of determination was 58,8%, the value of F statistic for regression 5.71, and for deviation from regression 1.16. This line can be recognized as extensive with regard to TSW. It means it is a line with thousand seeds weight higher in the environments generally considered to be less favourable for this trait and lower in more favourable environments.

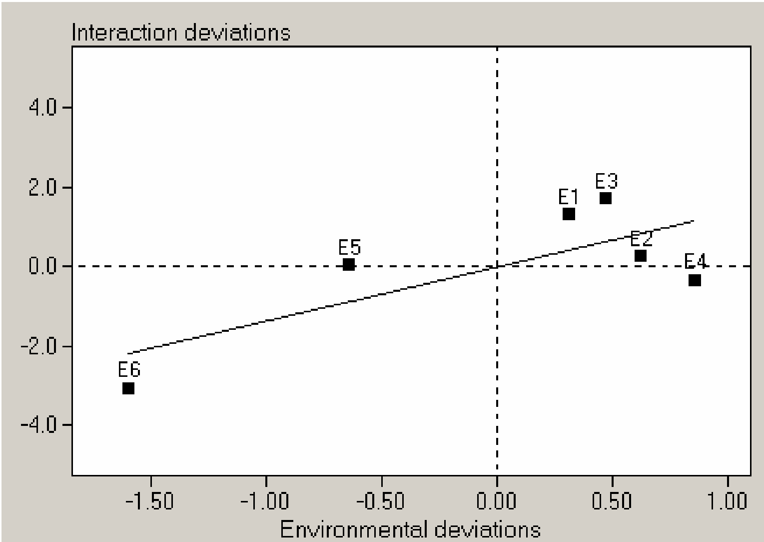

Fig. 1. Regression of interaction effects of DH line No 3 on environment for the number of seeds in silique — *Regresja efektów interakcyjnych ze środowiskiem linii DH Nr 3 pod względem liczby nasion w łuszczynie*

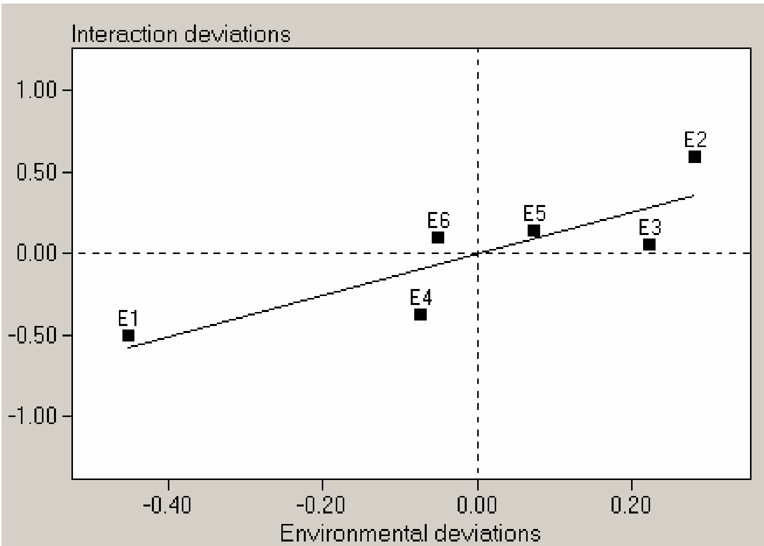

Fig. 2. Regression of interaction effects of DH line No 18 on environment for thousand seeds weight (TSW) — *Regresja efektów interakcyjnych ze środowiskiem linii DH Nr 18 pod względem masy tysiąca nasion*

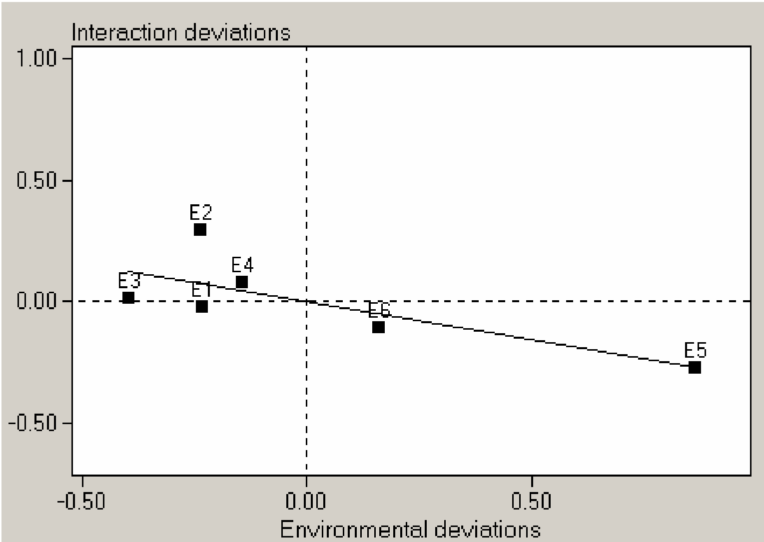

Fig. 3. Regression of interaction effects of DH line No 12 on environment for thousand seed weight (TSW) — *Regresja efektów interakcyjnych ze środowiskiem linii DH Nr 12 pod względem masy tysiąca nasion*

Individual DH lines may be evaluated using the results of testing of contrasts between them, which are of interest. In our experiment it is interesting to compare each from the chosen DH lines with standard cultivar Kana. The results of analysis of these contrasts are presented in Table 3. It may be concluded from them that DH lines of the highest positive contrast with Kana are lines: 22, 20, 13, 3, 6 and 28, with regard to main effects for length of silique, lines 22, 12, 3, 11, 20, 13 and 28, with regard to main effects for number of seeds per silique and all DH lines, except for line No 25, with regard to thousand seeds weight. The DH lines No. 3, 13, 20, 22 and 28 are significantly better than cv. Kana for all studied traits.

As a basis for breeding, novel genetic variation has been created by different means including haploids technique such as microspore culture. Statistical elaboration of important agronomic traits of DH lines obtained from F_1 hybrid of winter oilseed allows the selection of doubled haploid rape characterized with suitable traits under different environmental conditions.

Table 3

Estimates of the contrasts between DH lines and standard cultivar Kana and results of their significant tests — *Ocena kontrastów pomiędzy liniami DH oraz odmianą Kana i wynik ich istotności*

No *Nr*	DH line *Linia DH*	Estimate of contrasts for trait — *Ocena kontrastów dla cechy*		
		length of silique *długość łuszczyny*	number of seeds per silique *liczba nasion w łuszczynie*	thousand seeds weigh *masa tysiąca nasion*
3	DH H5-43	0.64**	2.88**	0.28*
6	DH H5-79	0.53**	-1.91*	0.73**
11	DH H5-129	-0.12	2.84**	0.22*
12	DH H5-191	0.03	3.20**	0.23*
13	DH H5-202	0.77**	2.14*	0.23*
16	DH H5-238	0.00	0.66	0.27*
18	DH H5-255	-2.05**	-7.31	0.82**
20	DH H5-284	0.88**	2.44**	0.27*
22	DH H5-396	1.56**	3.26**	0.40*
25	DH H5-493	0.06	0.63	0.12
28	DH H5-729	0.44*	1.44*	0.58**
30	DH H5-804	-0.20	-2.08*	0.70**

*, ** contrast significant at P = 0.05 and P = 0.01 respectively
kontrasty istotne dla P=0,05 i P=0,01

Conclusions

The analysis of six experiments in different environments with 32 DH lines of winter oilseed rape conducted for three components of seed yield: the length of silique, the number of seeds per silique and thousand seeds weight makes it possible to:

- distinguish the group of the best DH lines with respect to all studied traits;
- distinguish DH lines stable for two traits simultaneously (six DH lines) and to one trait only (three DH lines);
- explain the interaction with environments of several DH lines (No 3, 12, and 18) and other lines less interesting in respect to environmental effects by linear regression;
- find among the DH lines which showed significant interaction with the environment intensive DH lines (No 3 and 18) and extensive DH line (No 12).

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