

Influence of yellow-seeded parent on DH populations of *Brassica napus* obtained from reciprocal crosses between black- and yellow- seeded DH line

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Introduction

Nowadays, in the world increased the value of oilseed rape not only as an oil plant but as a protein plant as well. Protein of the seeds is characterized of very beneficial (balanced) amino acids content. However nutritive value of the meal of black seeded oilseed rape is limited by the high level of fibre, which is resulted low digestibility. Therefore development of yellow-seeded genotypes has been one of the major objectives of oilseed rape breeders in quality breeding programs. The value of the yellow-seed trait have was recognized in lowering meal fibre level and thereby significantly increasing its feed value (Bell, 1993).

The production of doubled haploids (DH) in microspore culture of winter oilseed rape has been broadly applied in breeding. Owing to this method it is possible to obtain a great number of completely homozygous DH lines. When F₁ hybrids are obtained from carefully selected parents, it is possible to expect a very advantageous segregation and recombination in gametes and to obtain DH lines valuable for agriculture. In case of a great number of DH lines population and a little number of seeds only unreplicated experiment with replicated standard gives a possibility to estimate all genotypes.

Materials and methods

Two population of doubled haploid lines obtained from F₁ hybrids of the reciprocal crosses yellow-seeded DH Z-114 and black-seeded DH H₂-26 were studied. Using the microspore culture method 176 lines were received: 98 DH lines from F₁ hybrid DH Z-114 x DH H₂-26 (ZH population) and 78 DH lines from hybrid DH H₂-26 x DH Z-114 (HZ population). The value of genetic distance between parental components, estimated on the basis of 20 RAPD markers, was 0.7569.

Doubled haploids were examined in an unreplicated field trial with regularly distributed standards of two parental lines in every eight objects. Seed coat colour of was determined with spectrophotometer Color Flex on a scale from 0 (black) to 5 (yellow). Light colour DH lines were compared with yellow parent with regard to the studied traits. Fat content was determined with magnetic resonance (NMR). To determine protein and fibre content, spectrophotometric analysis in near infrared with Infratec 1255 was applied.

Preliminary estimation was performed independently for 8 biochemical traits (per cent of fat, content of: protein, acid detergent fibre (ADF) and neutral detergent fibre (NDF), per cent of: oleic, linoleic, linolenic acids in oil and glucosinolates content (GLS) and seeds colour. Analysis of variance enables statistical characteristics for each of the two DH populations (HZ and ZH), as well as for parental populations (H₂-26, Z-114). The comparison of each studied DH line with a better parent allows to distinguish DH lines with advantageous and significant effects of transgression for studied traits.

Results and discussion

The statistical characteristics of doubled haploid populations and parental populations are shown in Table 1. Preliminary estimation of DH line populations ZH and HZ showed large differentiation in the value as well as of biochemical traits and seeds colour. The largest variability was found in the value of seeds colour. The coefficient of variation for this trait was 98.48% in ZH population and 87.48% in HZ population.

Table 1 Statistical characteristics of DH lines populations (ZH and HZ) and parental populations (Z and H) of oilseed rape for studied traits

Trait	Population	Number	Mean	Standard deviation	Coefficient of variation (%)	Min.	Max.
Protein content (% of dry matter)	ZH	98	21.77	1.64	7.53	18.8	25.5
	HZ	78	22.16	1.84	8.33	16.0	26.2
	H	27	21.34	1.15	5.40	19.8	23.8
	Z	27	21.46	1.43	6.66	18.2	23.5
NDF content (% of dry matter)	ZH	98	24.00	2.52	10.49	18.6	28.3
	HZ	78	23.09	2.89	12.50	18.1	29.2
	H	27	26.37	0.78	2.97	25.0	27.9
	Z	27	19.88	1.10	5.53	17.6	22.0
ADF content (% of dry matter)	ZH	98	12.85	2.29	17.85	8.8	18.1
	HZ	78	12.20	2.45	20.05	8.2	17.2
	H	27	15.20	0.59	3.88	14.2	16.1
	Z	27	9.15	0.44	4.84	8.4	10.2
Oleic acid content (%)	ZH	98	61.88	2.57	4.15	52.2	68.3
	HZ	78	62.37	2.71	4.35	56.2	68.3
	H	27	62.03	1.28	2.07	58.1	64.5
	Z	27	60.34	1.00	1.65	58.2	61.6
Linoleic acid content (%)	ZH	98	21.50	1.81	8.40	16.5	26.0
	HZ	78	21.44	2.33	10.85	17.1	27.7
	H	27	20.75	0.77	3.73	19.1	23.1
	Z	27	24.06	0.87	3.61	21.3	25.5
Linolenic acid content (%)	ZH	98	9.63	1.24	12.87	6.5	12.3
	HZ	78	9.32	1.08	11.56	6.5	12.0
	H	27	10.09	0.70	6.97	8.6	11.5
	Z	27	8.76	0.86	9.86	7.8	10.9
Fat content (%)	ZH	98	46.82	2.73	5.82	36.1	51.4
	HZ	78	46.92	2.61	5.57	38.1	51.9
	H	27	49.35	1.30	2.63	46.2	52.4
	Z	27	44.35	1.30	2.92	42.2	48.0
Total GLS content ($\mu\text{mol g}^{-1}$ of seeds)	ZH	98	10.32	2.96	28.69	3.9	18.5
	HZ	78	10.90	3.08	28.21	4.8	21.6
	H	27	10.66	1.76	16.54	6.6	12.8
	Z	27	8.12	1.41	17.42	5.3	10.1
Seeds colour (scale 0-5)	ZH	98	1.46	1.32	90.48	0.13	4.55
	HZ	78	1.81	1.59	87.48	0.01	5.21
	H	27	0.44	0.07	15.49	0.26	0.58
	Z	27	5.13	0.26	5.07	4.37	5.55

Among the studied biochemical characters the highest value of variability coefficients was noted for GLS and ADF content, and the lowest value of variability coefficients was for fat and oleic acid content. The direction of crossing did not influence on DH populations: ZH and HZ, considering the average values of biochemical traits (Table 1). However, it distinctly influenced on seeds colour in first generation. The seed coat colour is determined by the genotype of the plant on which it develops because testa is maternal tissue (Henderson and Pauls, 1991). Seeds colour of F_1 hybrid of Z-114 x H₂-26 was yellow but seeds colour of F_1 hybrid of H₂-26 x Z-114 was black. However average value of seeds colour in the HZ population was a little higher (1.81), that in ZH population (1.46). Moreover, in HZ population twelve yellow-seeded DH lines were found, but in ZH population - only four (Table 3).

Table 2 DH lines with positive transgression effects in the populations ZH and HZ

DH lines	Mean	Effect of transgression		DH lines	Mean	Effect of transgression	
		Estimate	F-test			Estimate	F-test
Protein content (% of dry matter)							
ZH-6	25.2	3.75	7.24**	HZ-7	24.6	3.12	5.02*
ZH-25	25.6	4.17	8.95**	HZ-10	25.8	4.31	9.56**
ZH-29	24.7	3.30	5.59*	HZ-26	25.1	3.66	6.88*
ZH-31	24.7	3.24	5.43*	HZ-44	25.9	4.42	10.04**
ZH-32	24.8	3.32	5.66*	HZ-47	24.3	2.85	4.16*
ZH-69	24.5	3.06	4.80*	HZ-50	24.4	3.00	4.61*
ZH-70	25.3	3.90	7.80**	HZ-54	26.2	4.80	11.82**
ZH-80	24.5	3.03	4.71*	HZ-58	24.3	2.82	4.07*
ZH-104	24.8	3.30	5.22*				
ZH-115	24.5	3.00	4.31*				
Oleic acid content (% of total fatty acids)							
ZH-7	65.4	3.42	9.13**	HZ-2	64.4	2.32	4.20*
ZH-24	64.9	2.82	6.21*	HZ-5	64.6	2.92	6.65*
ZH-25	68.1	6.02	28.26**	HZ-9	66.8	4.72	17.37**
ZH-48	64.7	2.64	5.43*	HZ-25	64.4	2.34	4.26*
ZH-53	64.8	2.74	5.84*	HZ-30	64.4	2.34	4.26*
ZH-70	65.2	3.21	8.05*	HZ-32	68.2	6.14	29.36**
ZH-79	65.9	3.84	11.48**	HZ-33	65.4	3.44	9.21**
ZH-80	64.4	2.34	4.26*	HZ-34	65.6	3.54	9.76**
ZH-88	64.4	2.34	4.26*	HZ-42	65.4	3.34	8.69**
ZH-91	64.7	2.64	5.43*	HZ-44	65.8	3.74	10.89**
ZH-92	68.0	5.94	27.48**	HZ-45	65.2	3.12	7.68**
ZH-96	66.4	4.44	15.35**	HZ-48	67.3	5.31	22.00**
ZH-102	65.5	2.94	6.26*	HZ-49	65.7	3.71	10.75**
ZH-106	67.5	5.54	22.22**	HZ-54	65.5	3.51	9.62**
ZH-115	65.3	3.24	7.60*	HZ-65	67.3	5.31	22.00**
				HZ-69	64.7	2.64	5.43*
				HZ-75	68.0	5.94	27.48**
				HZ-76	65.3	3.24	8.17**
Linoleic acid content (% of total fatty acids)							
				HZ-41	27.75	3.69	21.51**
				HZ-63	26.78	2.73	11.72**
Linolenic acid content (% of total fatty acids)							
ZH-100	6.96	-1.80	4.35*	HZ-32	6.56	-2.20	6.99*
ZH-106	6.56	-2.20	6.50*				
Total glucosinolates content ($\mu\text{mol g}^{-1}$ of seeds)							
ZH-59	5.2	-2.93	5.10*	HZ-71	5.0	-3.11	5.73*
ZH-100	3.7	-4.48	11.04**				
ZH-115	4.0	-4.08	9.16**				

The doubled haploid lines obtained from F_1 hybrids were studied with regard to the occurrence of transgression effects, positive for protein, oleic acid, linoleic acid and fat content and negative for content acid detergent fibre, neutral detergent fibre, linolenic acid and glucosinolates. The obtained results are demonstrated in Table 2. Among 98 DH lines of ZH population twenty four lines revealed advantageous effects of transgression (24.5%). But in the HZ population which consisted of 78 DH lines advantageous effects of transgression occurred in twenty seven lines (34,6%). In both populations, the highest number of transgressive segregants was observed for oleic acid in oil and protein content. Average values of protein and oleic acid content of parental lines were similar. The frequency of occurrence of transgressive lines depends on genetic differentiation of parental components, but at simultaneously with phenotypic similarity (Rieseberg et al., 1999). In the case of the other traits, none or a few transgressive lines were detected.

Selected DH lines with light seed colour (>4) were compared with yellow-seeded parent Z-114 with regard to the studied traits, Table 3. The DH lines: ZH-70 and HZ-54 with significant higher protein content as well as oleic acid and per cent of fat than parental line DH Z114 have been worthy of notice.

Table 3 Estimation and results of comparison testing between light seed colour DH lines and their parental yellow-seeded line Z-114 for biochemical traits

DH line	Content of							
	Protein (% of d.m.)	NDF (% of d.m.)	ADF (% of d.m.)	C18:1 (%)	C18:2 (%)	C18:3 (%)	Fat (%)	GLS ($\mu\text{mol g}^{-1}$ of seeds)
ZH-68	2.13	1.12	0.4	-1.5	0.3	1.1	0.78	2.4
ZH-70	3.90**	-0.78	0.6	4.9**	-4.8**	-0.6	4.18**	4.8**
ZH-76	-0.34	1.02	0.7	-2.7*	-1.9*	1.9*	4.78**	2.4
ZH-81	-1.22	1.74	0.6	-0.2	-1.3	-0.1	2.41	4.0**
HZ-6	0.37	-0.35	0.3	-3.1	-0.4	3.5**	1.42	5.6**
HZ-10	4.31**	-1.95	-0.1	-0.2	-1.3	1.4	-3.38**	1.2
HZ-24	0.88	-0.69	1.2*	-3.1**	0.1	2.7**	-1.21	8.7**
HZ-41	1.99	-0.39	0.1	-4.3**	3.7**	0.9	3.28**	2.7*
HZ-47	2.85*	-0.68	0.7	1.6	-1.6	-0.1	3.58**	2.0
HZ-48	-5.37**	-0.18	0.7	7.0**	-6.1**	-1.2	0.58	2.9*
HZ-49	2.25	1.22	1.1*	5.4**	-4.6**	-0.5	2.68**	2.1
HZ-54	4.80**	-0.78	0.1	5.2**	-5.8**	0.7	5.28**	4.4**
HZ-58	2.82*	0.22	-0.1	1.9	-1.1	-1.0	-5.42**	5.3**
HZ-66	1.67	2.74**	0.6	3.3**	-2.3**	-1.2	3.81**	3.2*
HZ-68	2.72	0.84	0.0	-0.7	-0.8	0.2	0.01	0.5
HZ-78	2.52	-1.12	-0.3	2.7*	-2.0**	-0.7	1.41	0.6
Z-114	21.46	19.88	9.15	60.34	24.06	8.76	44.35	8.12

Conclusion

The large number of segregants characterized of different seeds colour and better values of biochemical traits have been obtained from a crossing of yellow seeded DH lines with black seeded DH H₂-26 and by the use of doubled haploids method. Application of the statistical method enabled the selection of DH lines with positive transgressive effects on the basis of unreplicated experiment.