Heritability, combining ability and heterosis in glucosinolate content in seed of winter rape (*Brassica napus* L.) estimated with diallel crosing between double haploid lines

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Abstract

Six DH lines with very different glucosinolate content were crossed in complete diallel design. Obtained hybrids of F_1 and F_2 generations and parental lines were grown in field trial in complete random block design in replications. Analyses of glucosinolate content and composition were made using gas chromatography of sillyl derivatives of desulphoglucosinolates. Calculation of GCA and SCA were performed according to Griffings method. Analysis of variance showed that the GCA effects for the investigated glucosinolate were statistically very significant. Significant effects of SCA were found for gluconapin and progoitin. SCA for 4-hydroxybrassicin was not significant. Heterosis effects were calculated for pedigrees of individual parents and for hybrids as compared with parent means. Highly significant heterosis effects in F_1 generation lost its significance in F_2 generation. Variance analysis according to Hayman showed very significant additive effects of genes for the examined glucosinolate. Different methods for estimation of heritabilities of glucosinolate contents in seeds were compared. The expected heritabilities were calculated according to Mather in a narrow and wide sense. Realized heritabilities between F_1 and F_2 generations were investigated by calculations of regression coefficient, correlation coefficient and determination coefficient. The best heritability estimator should be determination coefficient because both generations were grown in different conditions during two following years. Good agreement was found for heritabilities in narrow sense (h^2=0.914) and determination coefficient (r^2=0.908) for progoitrin content and slightly worse for gluconapin (h^2=0.868, r^2=0.913). Heritability in a narrow sense according to Mather for 4-hydroxybrassicin was not confirmed by determination coefficient between F_1 and F_2 generations.

Key words: heritability, GCA, SCA, heterosis, variability, alkenyl glukosinolate, indol glucosinolate, DH lines

Introduction

Increase in demand on rapeseed and soybean meals is observed in last years because of withdrawal animal products from fodder production. (Pastuszewska, Raj 2003). In this circumstances the quality of rapeseed meal became a very important problem. The content of glucosinolate in seeds of winter rape is the main factor determining nutritional value of rapeseed meal for use as high protein component of fodder production. Further improvement of rapeseed meal quality is still connected with breeding of new varieties with decreased alkenyl glucosinolate level. This concerns both line varieties and hybrids. (Bartkowiak-Broda 1998; Krzymanski et al.199; Friedt 1999, Raney et al. 2003). Feeding experiments showed that the most detrimental effects to animals have alkenyl glucosinolate - especially progoitrin. Lowering the level just of these seed components should be the next target of breeding works. Indol glucosinolates are less detrimental for animals and some products of its hydrolysis may have positive effects (Rakowska, Ochodzki 1995; Shone et al. 2003; Wang Y et al. 2003).

Better understanding of glucosinolate inheritance in rapeseed should make the breeding more effective and speed up the variety breeding process. Knowledge of genetic background of heterosis and general and specific combining abilities are basis for hybrid breeding. Their occurrence and changes between F_1 and F_2 generation are also important.

Material and Methods

Six DH lines with very different in glucosinolate contents and compositions were crossed in complete diallel design. Obtained hybrids of F_1 generation and parental lines were grown in field trial in complete random block design in four replications. Seeds of F_2 generation were obtained with selfing from F_1 plants. Next autumn the trial with hybrids of F_2 generation and parents were grown in two replications.

Analyses of glucosinolate content and composition were made using gas chromatography of sillyl derivatives of desulphoglucosinolates (Michalski et al. 1995).

Calculation of GCA and SCA were performed according Griffings method (Griffing 1956). Heterosis effects were calculated for pedigrees of individual parents and for hybrids as compared with parent means. Different methods for estimation of heritabilities of glucosinolate contents in seeds were compared.

Study was done for three the most characteristic for rapeseed glucosinolates. They are two alkenyl glucosinolates - gluconapin and progoitrin and one indol glucosinolate 4-hydroxybrasscin. Parental lines and hybrids of F_1 and F_2 generations were very significantly differentiated according contend and composition of these glucosinolates.

Results

Results of chemical analyses and statistical calculations are shown in tables 1 to 7.

Table 1. Glucosinolate contents (μM/g of seed) and composition (%) in seeds of parental lines.

DH line	Glucosinolate									
	Gluconapin		Progoitrin		4-hydroxy- brassicin		Total of alkenyl glucosinolate		Total of glucosinolate	
	μM/g	%	μM/g	%	μM/g	%	μM/g	%	μM/g	%
H1-112	0.5	10.9	0.6	13.0	3.4	73.9	1.2	26.1	4.6	100
H5-771	0.3	6.8	0.4	9.1	3.7	84.1	0.8	18.2	4.4	100
H5-925	0.3	6.7	0.4	8.9	3.8	84.4	0.8	17.8	4.5	100
W-86	1.2	22.2	3.3	61.1	0.7	13.0	4.8	88.9	5.4	100
174-26	14.7	33.6	24.5	56.1	3.1	7.1	40.5	92.7	43.7	100
174-99	12.2	25.9	26.7	56.7	5.5	11.7	41.6	88.3	47.1	100

Table 2. Analysis of variance according to Hayman for glucosinolate content in seeds from plants of F_1 and F_2 generations of hybrids between DH lines of winter oilseed rape.

Source of variation	Degree of freedom	F_1			F_2		
		Sum square	Mean square	F statistic	Sum square	Mean square	F statistic
Gluconapin							
Dominance	15	102.66	6.84	8.48***	44.65	2.98	3.54**
Unidirection	1	20.69	20.69	25.64***	0.02	0.02	0.03
Asymmetry	5	36.42	7.28	9.03***	5.06	1.01	1.20
Residue	9	45.55			39.56		
Additivity	5	755.10	151.02	187.15***	394.41	78.88	93.77***
Progoitrin							
Dominance	15	117.70	7.85	4.57***	131.87	8.79	3.97***
Unidirection	1	3.84	3.84	2.24	11.20	11.20	5.06*
Asymmetry	5	17.96	3.59	2.09	7.98	1.60	0.72
Residue	9	95.89			112.69		
Additivity	5	1530.75	306.15	178.41***	1435.12	287.02	129.65***
4-hydroxybrassicin							
Dominance	15	4.04	0.27	2.00*	1.69	0.11	0.81
Unidirection	1	1.11	1.11	8.22**	0.02	0.02	0.14
Asymmetry	5	1.54	0.31	2.29	1.08	0.22	1.56
Residue	9	1.39			0.59		
Additivity	5	14.14	2.83	20.97**	7.10	1.42	10.24***

*** - significant at $\alpha = 0.001$ ** - significant at $\alpha = 0.01$ * - significant at $\alpha = 0.05$

Table 3. Characteristics of lines and of diallel crosses between DH lines - glucosinolate content in seeds of F_1 and F_2 generations

Means	Gluconapin		Progoitrin		4-hydroxybrassicin	
	F_1	F_2	F_1	F_2	F_1	F_2
Total	6.85	4.83	8.87	8.55	3.58	3.60
Parents	5.15	4.78	8.14	9.8	3.19	3.55
Hybrids	7.18	4.85	9.01	8.3	3.66	3.61
Means of line progeny						
H1-112	5.61	3.95	6.25	6.28	3.41	3.57
H5-771	4.08	2.76	4.69	4.52	3.73	3.82
H5-925	4.58	3.14	5.90	5.37	3.59	3.59
W-86	5.12	3.46	7.09	6.39	2.94	2.96
174-26	10.46	7.51	13.68	13.41	4.11	3.91
174-99	12.15	8.20	15.99	14.68	3.93	3.79

Table 4 Estimation of heterosis effects for progenies in relation to parent lines for individual glucosinolate content in seeds of F_1 and F_2 generations.

No.	Line	Gluconapin		Progoitrin		4-hydroxybrassicin	
		F_1	F_2	F_1	F_2	F_1	F_2
1	H1-112	3.53***	1.82	4.79***	2.84	0.31	0.08
2	H5-771	3.36***	1.94	3.89**	3.16	0.14	0.36
3	H5-925	3.63***	1.97*	4.90***	3.49*	0.13	0.10
4	W-86	2.25*	0.90	2.32	0.98	2.55***	0.01
5	174-26	-0.81	-3.73***	-5.79***	-10.39***	-0.26	-0.04
6	174-99	0.25	-2.47*	-4.85***	-9.05***	-0.05	-0.12

Table 5. Genetics parameter according to Mather for glucosinolate content in seeds harvested from plants of diallel hybrids of F_1 generations between DH lines.

Parameter	Glukonapin	Progoitrin	4-hydroxybrassicin
D	24.47	80.23	1.62
F	-21.28	-20.00	0.84
H(1)	16.39	13.51	0.39
H(2)	12.07	12.26	0.27
h^2	11.49	2.13	0.62
Mean degree of dominance	0.818	0.410	0.49
Number of gene group showed dominance	1	0	2
Product of frequency in loci (u*v) showed dominance	0.184	0.227	0.17
Ratio of dominant to recessive allel number	0.306	0.534	3.277
Narrow sense heritability	0.868	0.914	0.689
Broad sense heritability	0.972	0.969	0.793

Table 6. Comparison of different heritability estimation of glucosinolate content in seeds of winter oilseed rape from diallel crosses of DH lines.

Parameter	glukonapin	progoitrin	4-hydroxybrassicin
Expected heritability according to Mather			
Narrow sense	0.868	0.914	0.69
Broad sense	0.972	0.969	0.79
Realized heritability between F_1 and F_2 generations			
Regression coefficient	0.685	0.934	0.467
Correlation coefficient	0.956	0.953	0.635
Determination coefficient	0.913	0.908	0.403

Table 7- Heritability for combining abilities between F_1 and F_2 generations

Parameter	glukonapin	progoitrin	4-hydroxybrassicin
General combining ability			
Correlation coefficient	0.997**	0.997**	0.993**
Specific combining ability			
Correlation coefficient	0.682**	0.547*	-0.327
Reciprocal effects			
Correlation coefficient	0.514*	0.423	0.104

Discussion

Analysis of variance for F_1 and F_2 generations showed that the GCA effects of parental lines for investigated glucosinolate were statistically very significant. Significant effects of SCA were found for gluconapin and progoitrin. SCA for 4-hydroxybrassicin was not significant. Differences between reciprocal crosses were also not significant (table 2). Calculated results for combining abilities for alkenyl glucosinolates suggested that more important in controlling these glucosinolate content are genes with additive effects. This conclusion is confirmed by genetic parameters calculated according Mather method presented in table 5. Also high correlation coefficients for alkenyl glucosinolate GCA and SCA between two generations may suggest the same conclusion.

4-hydroxybrassicin performed differently as compared with alkenyl glucosinolate. It looks so that this indol glucosinolate content is mainly influenced by non-heritable factors.

Data presented in tables 3 and 4 concern heterosis effects on glucosinolate contents in seeds. Highly significant and mainly positive heterosis effects observed in F_1 generation lost its significance in F_2 generation. This observation can be explained by disappearing of non-additive effects in F_2 generation. Inversely the results obtained for F_2 generation should be more useful for pedigree breeding.

Different methods for estimation of heritabilities of glucosinolate contents in seeds were compared. The expected heritabilities were calculated according Mather in narrow and in wide sense. Realised heritabilities between F_1 and F_2 generations were investigated by calculations of regression coefficient, correlation coefficient and determination coefficient. The best heritability estimation should be determination coefficient, because the hybrid generations were grown in different conditions in two following years. Good agreement was found for heritabilities in narrow sense (h^2=0.914) and determination coefficient (r^2=0.908) for progoitrin content. A little worse for gluconapin (h^2=0.868, r^2=0.913). Heritability in narrow sense according Mather for 4- hydroksybrassicin was to high and was not confirmed by determination coefficient between F_1 and F_2 generations.

Conclusions

Alkenyl glucosinolate content in seeds and its composition were controlled genetically on additive manner with partial heterosis mainly in direction to higher content. This heterosis effect was diminished in F_2 generation. High heritability was find for these compounds.

Variability in 4-hydroxybrassicin was mainly conditioned by non-heritable factors and its heritability was much lower.

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