STUDIES OF WINTER OILSEED RAPE (*Brassica napus* L.) VERY LOW IN ALIPHATIC GLUCOSINOLATE CONTENT

Jan A. KRZYMANSKI, Teresa PIETKA, Krzysztof MICHALSKI, & Krystyna KROTKA

Plant Breeding and Acclimatization Institute (IHAR) - Strzeszynska 36, 60-479 Poznan, Poland, E-mail: krzym@nico.ihar.poznan.pl

ABSTRACT

Glucosinolate level of present double low varieties of oilseed rape (Canola type) is low enough to obtain good body weight gain in animal production. Nevertheless enlarged thyroid gland and changes in its metabolism is usually observed. Glucosinolate split products accumulate in circulating extraction solvent. They are chemically very active so can diminish the value of oil. Therefore breeding for further elimination of aliphatic glucosinolates from rapeseed is desired and purposeful.

Crosses between the best double low lines of winter oilseed rape were made and individual selection were carried on in segregating generations of hybrids with the use of selfing and chemical analyses. Obtained population of 1151 inbred lines (S_2-S_9, F_4-F_{11}) were analyzed on glucosinolate content and composition. These lines despite intensive selection in previous generations and achieved very low aliphatic glucosinolate content still indicate substantial differentiation of this trait.

Histograms for individual and total aliphatic glucosinolates are continuous and asymmetric with longer sloop in direction to higher values. Histogram for 4-hydroxybrassicin is symmetric. This glucosinolate is present only in seed. Selection pressure was not given to this glucosinolate nevertheless its coefficient of variability is lower than for aliphatic glucosinolates after selection, 21 per cent and about 50 per cent respectively.

Investigations made on two generations of lines with very low glucosinolate content show that estimated variability of this trait is still heritable and further selection can be efficient. New lines extremely low in glucosinolate helped to break the strong linkage between the high glucosinolate content and restorer gene for CMS *Ogura*.

KEYWORDS

gluconapin, glucobrassicanapin, progoitrin, napoleiferin, brassicin, 4-hydroxybrassicin

INTRODUCTION

Glucosinolates were the main antinutritive component of oilseed rape (*Brassica napus* L.) (Krzymanski 1970). The research and breeding works decreased aliphatic glucosinolate content to such extend, that the glucosinolate level of modern double low varieties of oilseed rape (Canola type) is low enough to obtain rapeseed meal giving good body weight gain in animal production. Nevertheless enlarged thyroid gland and changes in its metabolism is usually observed. Glucosinolate split products which originate in crushing process are partially volatile and are accumulated in circulating extraction solvent. They are chemically very active so can diminish the value of oil. Non-volatile split products are left in extraction meal (Krzymanski 1993). Therefore breeding for further elimination of aliphatic glucosinolates from rapeseed is desired and purposeful.

Most important glucosinolates of rapeseed are progoitrin, gluconapin, glucobrassicanapin and napoleiferin. Amino acid methionine is precursor of this group called aliphatic glucosinolates. Sinigrin the main glucosinolate of brown and black mustards, occurring sporadically in traces in rapeseed belongs also to this group. Other group of glucosinolates in rapeseed are indol glucosinolates. Amino acid tryptophan is their precursor. 4-hydroxybrassicin is present in seeds and brassicin is present in green parts of plant. Indol glucosinolates are precursors of important plant hormones. Some of their split products can exhibit anti cancer activity (Sorensen 1988, Feldl *et al* 1994). Necessity of elimination of indol glucosinolate content in rapeseed is still not confirmed.

MATERIAL AND METHODS

Crosses between the best own double low lines of winter oilseed rape were made in diallel or factorial design and combinations with the best combining abilities were selected for further breeding works (Krzymanski et al 1994 and 1998). Selection of individual plants for low aliphatic glucosinolate content was carried on in segregating generations of hybrids with the use of selfing and chemical analyses.

Analyses of individual glucosinolate were made by gas chromatography of silyl derivatives. (Thies 1978, Sosulski and Dabrowski 1984, Landerouin *et al*. 1987, Michalski *et al*. 1995). Detector was calibrated using CRM-366 Rapeseed Standard of European Community Bureau of Reference. Obtained results were fully comparable with results of high resolution liquid chromatography when the same standard was used for calibration. Glucosinolate contents were calculated in mM/g of seed.

Population of 1151 inbred plants (S_2-S_9 , F_4-F_{11}) harvested in 1996 were used in this research. These plants were analyzed on glucosinolate content and composition and selected for extremely low level of aliphatic glucosinolates. Selected plants were investigated in field trials in 1997/98. These trials were sown with seeds

produced by inbreeding realised by selfing of individual plants. The trials were made in randomized block design in four replications with added standard plots distributed systematically. Interblock variability was reduced with covariance analysis using standard plots.

Initial population of inbred plants, population of selected plants and their progenies were investigated statistically. Variability of individual and total glucosinolate contents were calculated. Heritability was used as a measure of effectiveness of different selection methods. Obtained heritability for individual plant selection with inbreeding was calculated by estimation of selection difference and genetic gain from the means of initial population, population of selected plants and their progeny. Expected heritability for inbred lines selection was calculated from expected values of mean squares in variance analysis of field trials (Allard 1966, Falconer 1960).

RESULTS

Initial population

Investigated lines despite intensive selection in previous generations and achieved very low aliphatic glucosinolate content still indicated substantial differentiation of this trait. Correlation coefficient matrix calculated from 1151 analyses of individual glucosinolates in seed samples of inbred plants shows that very significant linkage exist among the group of aliphatic glucosinolates. Correlations between aliphatic glucosinolates (precursor methionine) and indol (precursor tryptophan) or phenyl (precursor phenylalanine) glucosinolates are very week or not significant.

Table 1. Correlation matrix for glucosinolates in seeds of initial population of 1151 plants

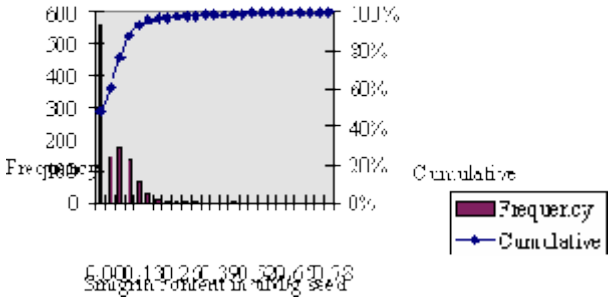
	Glucosinolate	1	2	3	4	5	6	7
1	Sinigrin	1,000						
2	Gluconapin	-0,025	1,000					
3	Glucobrassicanapin	0,048	**0,652**	1,000				
4	Progoitrin	0,045	**0,802**	**0,619**	1,000			
5	Napoleiferin	0,001	**0,190**	**0,225**	**0,266**	1,000		
6	Brassicin	0,093	-0,062	-0,030	-0,096	-0,049	1,000	
7	4-hydroxybrassicin	0,087	0,005	-0,001	-0,032	-0,130	0,093	1,000
8	Sinalbin	-0,025	0,020	0,022	0,023	-0,006	0,019	-0,012

r=0,062 significant at P=0,05 r=0,081 significant at P=0,01

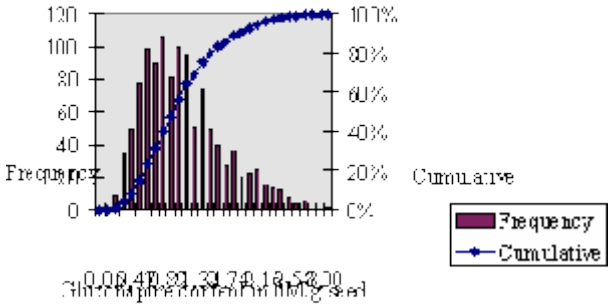
Correlation between two indol glucosinolates brassicin and 4-hydroxybrassicin is significant but lower than by aliphatic glucosinolates. Sinalbin content is not correlated with aliphatic or indol glucosinolates.

Histograms for individual and total aliphatic glucosinolates are continuous and asymmetric with longer sloop in direction to higher values.

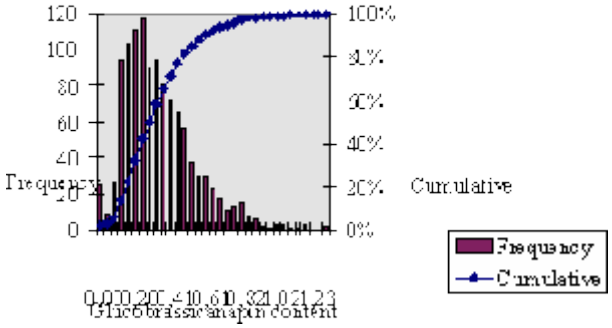
Histogram 1. Distribution of sinigrin content in seeds of individual plants



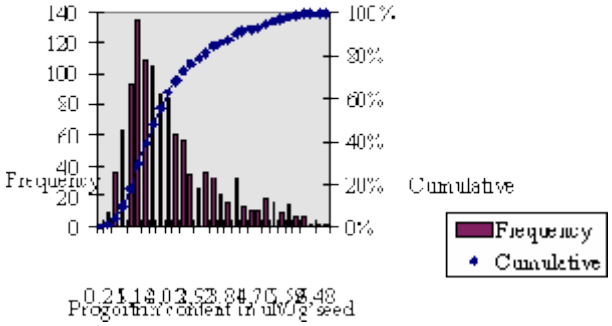
Histogram 2. Distribution of gluconapin in seeds of individual plants



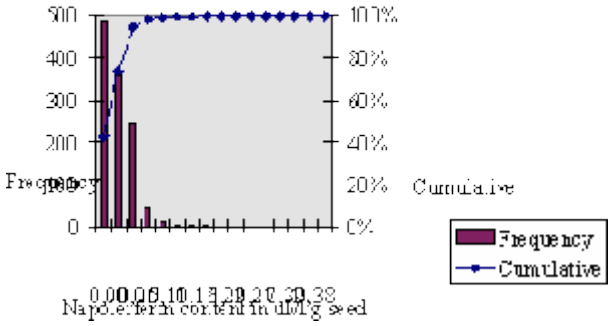
Histogram 3. Distribution of glucobrassicanapin in seeds of individual plants



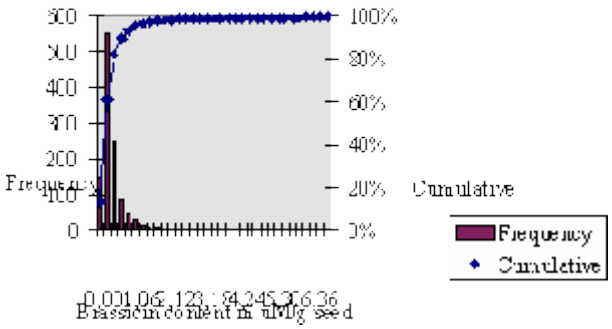
Histogram 4. Distribution of progoitrin in seeds of individual plants



Histogram 5. Distribution of napoleiferin content in seeds of individual plants



Histogram 6. Distribution of brassicin content in seeds of individual plants



Histogram for 4-hydroxybrassicin is symmetric. This glucosinolate is present only in seed. Selection pressure was not given to this glucosinolate nevertheless its coefficient of variability is lower than that for aliphatic glucosinolates after selection, 21 per cent and about 50 per cent respectively (table 2). Very high coefficients of variability for sinigrin and napoleiferin are due their very low contents approximating almost the range of analysis error.

Table 2. Statistical characteristics of initial population of inbred plants of double low winter oilseed rape.

	Sinigrin	Gluconapin	Glucobrassi-canapin	Progoitrin	Napoleiferin	Aliphatic glucosinolate
Average	0,043	1,112	0,329	2,249	0,018	3,752
Standard error	0,002	0,016	0,006	0,039	0,001	0,058
Median	0,010	1,020	0,290	1,860	0,010	3,300
Mode	0,000	0,910	0,190	1,760	0,000	2,790
Kurtosis	40,264	0,483	2,308	0,953	286,677	0,420
Skewness	5,140	0,851	1,261	1,199	13,429	0,981
Coef. of Variability	190,529	49,244	64,124	59,415	203,852	52,358
Range	1,070	3,480	1,350	7,340	0,890	9,470
Minimum	0,000	0,050	0,000	0,250	0,000	0,430
Maximum	1,070	3,530	1,350	7,590	0,890	9,900
	Brassicin	4-hydroxy-brassicin	Indol gluco-sinolates	Glucosino-late total	Sinalbin	
Average	0,333	3,060	3,393	7,144	0,059	
Standard error	0,021	0,019	0,030	0,063	0,004	
Median	0,170	3,040	3,300	6,820	0,000	
Mode	0,000	3,240	3,550	6,790	0,000	
Kurtosis	50,370	1,917	12,214	29,979	212,568	
Skewness	6,579	0,310	2,401	-0,150	17,840	
Coef. of Variability	213,667	21,154	29,625	0,635	3,715	
Range	7,000	6,120	10,090	10,520	0,990	
Minimum	0,000	0,000	0,190	2,440	0,000	
Maximum	7,000	6,120	10,280	12,960	0,990	

Heritability

Individual selection was made on large population of inbred materials. 1151 selfed plants were used as initial population. Exact chemical analyses allowed to select the plants with the lowest glucosinolate level from the lines of good economical value. Only 71 plants were selected so strong selection intensity (6,17 per cent) was applied. Results of this work are given in table 3 and on histograms 8-10.

.

Table 3. Heritability (h^2) calculated from progress in individual plant selection for low glucosinolate content and heritability measures calculated from correlations between two generations:

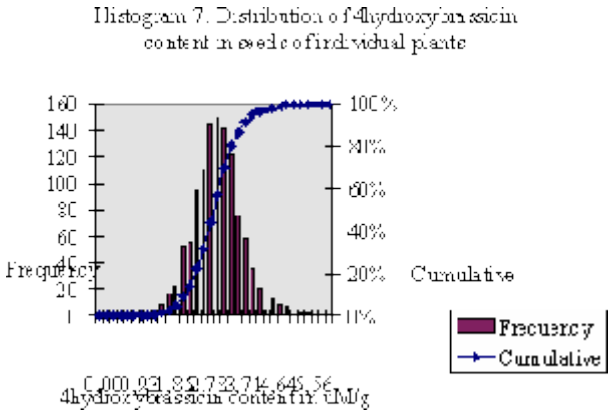
b - coefficient of regression

r - coefficient of correlation

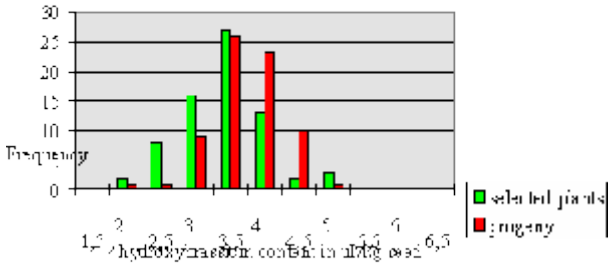
r^2 - coefficient of determination

P - probability of F statistic for b, r and r^2

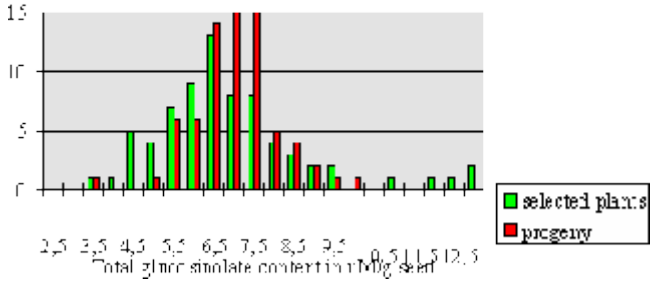
	Average content in seeds (mM/g)		
	Aliphatic glucosinolate	Glucosinolate total	4-hydroxybrassicin
Initial population	3,752	7,144	3,06
Selected plants	2,775	6,704	3,12
Progeny	3,118	6,762	3,482
	Heritability calculation		
Selection difference	0,977	0,44	-0,06
Genetic gain	0,634	0,382	-0,422
Heritability h^2	0,649	0,868	n
b	0,528 ±0,194	0,126 ±0,123	0,155 ±0,204
r	0,548	0,237	0,177
r^2	0,301	0,056	0,031
P	7,42E-7	4,68E-2	1,38E-1



Histogram 8. Comparison of selected plants and their progeny 4hydroxyconcanav.



Histogram 10. Comparison of selected plants and their progeny - total gluco anolates



Unexpected high heritability was obtained with aliphatic and with total glucosinolates but not with 4-hydroxybrassicin. To confirm these results heritabilities for selection of inbred lines based on field trials made in four replications were calculated. Expected values of mean squares from variance analysis were used for this purpose. Results are as follow:

- total glucosinolates h^2=0,483 (P=1,74E-4)

- aliphatic glucosinolates h^2=0,750 (P=4,87E-15)

- 4-hydroxybrassicin h^2=0,226 (P=8,51E-2)

Obtained results refer to selected new population with extremely low glucosinolate content but more precise evaluation in trial give still the possibility for further aliphatic glucosinolate elimination. Heritability of 4-hydroxybrassicin also estimated in replicated trial is still low, significant only at P=8,5% level. To increase the effectiveness of selection for this glucosinolate it is necessary to find wider genetic variability or to improve precision and repeatability of 4-hydroxybrassicin analyses. This glucosinolate is very labile and it is partially lost by preparing the sample to chromatography.

Characteristics of new selected population is given in table 4.

Table 4. Comparison of initial and selected new population

	Total glucosinolate		Aliphatic glucosinolate		4-hydroxybrassicin	
Populations	initial	new	initial	new	initial	new
Mean	7,144	6,762	3,752	3,118	3,060	3,482
Error of mean	0,063	0,127	0,058	0,111	0,019	0,062
Coefficient of variability	30,0	15,9	52,3	30,0	21,1	14,9
Range	10,52	6,575	9,47	4,15	6,12	2,675
Minimum	2,44	3,425	0,43	1,35	0	1,9
Maximum	12,96	10	9,9	5,5	6,12	4,575

CONCLUSIONS

Investigations made on two generations of lines with very low glucosinolate content show that estimated variability of this trait is still heritable and further selection can be efficient.
New lines extremely low in glucosinolate helped to break the strong linkage between the high glucosinolate content and restorer gene for CMS *Ogura*.

REFERENCES

Allard R.W.: Principles of Plant Breeding - London 1966

Falconer D.S.: Introduction to Quantitative Genetics - Edinburgh 1960

Krzymanski J. 1970. Genetyczne mozliwosci ulepszania skladu chemicznego nasion rzepaku ozimego. (Genetic possibilities of improvement of chemical composition of winter oilseed rape (*Brassica napus)* seeds.). Hodowla Roslin Aklimatyzacja i Nasiennictwo. 14:95-133.

Krzymanski J. 1993. Mozliwosci pelniejszego wykorzystania wartosci rzepaku podwojnie ulepszonego (Possibilities to take the full advantages of the quality of double low oilseed rape). Postepy Nauk Rolniczych. 6:161-166.

Krzymanski J., Pietka T., Krotka K. 1994. Zdolnosc kombinacyjna i heterozja mieszancow diallelicznych rzepaku ozimego podwojnie ulepszonego II. Pokolenia F_1 i F_2. (Combining ability and heterosis in diallel crosses of double low winter oilseed rape II. F_1 and F_2 generations). Rosliny Oleiste (Oilseed Crops). 15:21-32.

Krzymanski J., Pietka T., Krotka K., Michalski K. 1998. Wspolzaleznosc miedzy plonem nasion a zawartoscia glukozynolanow u pokolenia F_1 mieszancow rzepaku ozimego podwojnie ulepszonego (*Brassica napus l.*)(Relationship between seed

yield and glucosinolate content in F_1 hybrid generation of double low winter oilseed rape (*Brassica napus* L.). Rosliny Oleiste (Oilseed Crops) 19:389-398.

Landerouin A., Quinsac A., Ribaillier D. 1987. Optimization of silylation reactions of desulphoglucosinolates before gas chromatography. World Crops 13: 26-37.

Michalski K., Kolodziej K., Krzymanski J. 1995. Quantitative analysis of glucosinolates in seeds of oilseed rape. Effect of sample preparation on analytical results. Proceedings of 9th Inter national Rapeseed Congress, Cambridge, UK, 4-7.07.1995, 3: 911-913.

Sosulski F.W., Dabrowski K.J. 1984. Determination of glucosinolates in canola meal and protein products by desulfatation and capillary gas-liquid chromatography. Journal of Agriculture and Food Chemistry . 32: 1172-1175.

Thies W. 1978. Quantitative analysis of glucosinolates after their enzymatic desulfatation on ion exchange columns. Proceedings of 5th Int. Rapeseed Conference., Malmo. 1:136-139.