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Inheritance of S-methyl-L-cysteine sulphoxide and thiocyanate contents in forage rape (*Brassica napus* L.)

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Summary. Inheritance of thiocyanate and (+)S-methyl-L-cysteine sulphoxide (SMCO) contents were studied in a diallel cross involving five varieties of forage rape. Although both additive and dominance components were significant for the two characters, the greater mean squares of the additive gene effect indicated its greater importance. Non-allelic gene interaction was detected for thiocyanate only. Narrow sense heritability was relatively high for both characters. The general and specific combining ability effects and heterosis of the individual crosses, identified the potential of the cross 'Nevin' × 'Akela' for production of varieties with low thiocyanate content. Thiocyanate and SMCO contents were positively correlated, and they were not correlated with dry matter yield and its components.

Key words: Forage rape – Thiocyanate – SMCO – Inheritance

Introduction

Forage rape (*Brassica napus* L.) has an important role in British agriculture since it provides fresh herbage for livestock during autumn and early winter. However, there has been a steady decline in the area of forage *Brassica* crops sown in Britain which is largely due to the presence of toxic compounds in the herbage. These compounds are either goitrogenic or cause anaemia (Gosden 1978 a).

When the plant is crushed or eaten, isothiocyanate esters are produced by the action of myrosinase on glucosinolates in the tissue. These esters give rise to products which inhibit synthesis of thyroxine by the thyroid with serious consequences for the regulation of metabolism and growth in the grazing animal.

Anaemia, which can occur in ruminants fed on *Brassica* crops, is the most serious nutritional disorder associated with these crops. It is caused by the presence of (+)S-methyl-L-cysteine sulphoxide (SMCO) in the plant tissue which is metabolised to dimethylsulphide in the rumen. Characteristically, Heinz-Ehrlich bodies appear in the red blood cells after 1–3 weeks feeding and blood haemoglobin falls (Smith 1975).

Since these compounds are detrimental to the feeding value of the crop, it is important to develop cultivars with low concentrations of them. However, there is little information on their inheritance in forage rape. The present paper reports a study of the inheritance of SMCO and thiocyanate contents in a 5×5 diallel cross.

Materials and methods

Plant material

The five parents chosen for the diallel cross-cv. 'Canard', cv. 'Emerald', cv. 'Nevin', cv. 'Windal' and cv. 'Akela'-show a range of SMCO and thiocyanate contents. Appropriate selfs and crosses were made using hand pollination procedures to produce a diallel set of parents and F_1 hybrids. Seeds from the diallel set were sown in experimental plots at the Welsh Plant Breeding Station on 11 July 1979. Three replications were used and in each replication one 4.2 m row was randomly allotted to each parent and F_1 hybrid. The space between rows, and between plants within a row, was 30 cm. Compound fertiliser (20:10:10, $N:P_2O_5:K_2O$) was applied to the plots before sowing and again on 20 September at a rate of 250 kg/ha.

Leaves of the five parents and their 10 F_1 progenies were sampled on 5 November 1979; duplicate samples were taken

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from three plants in each of the three replications. Immediately after harvest the leaf samples were frozen (−18 °C) and stored at this temperature before they were freeze-dried and ground in a hammer-mill with a 0.5 mm screen.

Thiocyanate determination

Thiocyanate ion content of the samples was determined by an automated colorimetric method described by Gosden (1978 b). The ground samples (0.2 g) were extracted at room temperature with 10 ml of buffered enzyme preparation (0.25 g/l ascorbic acid and 0.25 g/l myrosinase dissolved in 0.1 M sodium acetate adjusted to pH 7). After dialysis, the concentration of thiocyanate in the samples was determined colorimetrically (460 nm) using ferric reagent I (320 g ferric nitrate dissolved in 500 ml water and 125 ml nitric acid made up to 1 l and filtered) and ferric reagent II (0.1% mercuric nitrate dissolved in ferric reagent I). The thiocyanate concentration was calculated from the difference between these two reagents.

SMCO determination

SMCO content of the samples was determined by the method described by Gosden (1979). The ground samples (0.1 g) were extracted in 10 ml buffer (0.1 M citric acid, 0.3 M sodium chloride and 0.5 M hydrogen peroxide in water adjusted to pH 1.8 with conc. hydrochloric acid). The filtered sample was loaded onto a short ion exchange column (Amberlite resin CG 120 type II) and SMCO was separated from the common amino acids by elution with a buffer (0.1 M citric acid, 0.3 M sodium chloride and 0.1% brij in water adjusted to pH 2.5 with 40% sodium hydroxide). SMCO was determined colorimetrically (570 nm) after reaction with ninhydrin (20 g ninhydrin dissolved in 1.5 l 2-methoxyethanol added to a mixture of 85 ml acetic acid and 165 g sodium acetate in 1.5 l water).

Genetic analysis

Diallel analyses based on the methods proposed by Hayman (1954); Jinks (1954) and Jinks and Hayman (1953) were carried out. Analyses of variance of half diallel tables were done according to Jones' (1965) modification of Hayman's (1954) analysis. General and specific combining ability effects were estimated following model 1 and method 4 of Griffing (1956). The formulae suggested by Miller et al. (1958) were adopted for calculating the phenotypic and genotypic correlation coefficients. Heterosis over mid-parent (MP) and better parent (BP) was calculated based on the better parent containing the lower content of SMCO and thiocyanate.

Results and discussion

Analyses of variance showed that differences among the 15 genotypes in the diallel were highly significant for both SMCO and thiocyanate contents. Further analyses of variance of these data to obtain estimates of the significance of the different genotypic variance components are presented in Table 1. Both additive (GCA) and dominance (SCA) components were significant for thiocyanate and SMCO contents. Comparatively greater GCA mean squares indicated that the additive gene effect was more important than the dominance effect for both characters. Of the three components of SCA, only b_2 was significant for both characters suggesting asymmetrical distribution of genes in the parents.

The non-significant regression coefficient of Wr on Vr (b) indicated non-allelic gene interaction in thiocyanate content, whereas for SMCO content b was significantly different from zero but not from unity, which demonstrated the absence of non-allelic gene interaction (Table 2).

Values of Vr/Wr greater than 1 demonstrated overdominance for the expression of both thiocyanate and SMCO (Table 2). The broad sense heritabilities of thiocyanate and SMCO were 91% and 88% and the narrow sense heritabilities were 65% and 51%, respectively. The (Wr+Vr) values were quite variable in both characters (Table 3) suggesting varying degrees of dominance in the parents. The correlation coefficients between (Wr+Vr) and parental values were positive but non-significant (Table 3).

General combining ability effects (gca) of the five parents together with the parental mean values are shown in Table 3. 'Akela', 'Windal' and 'Canard' had good gca effects for both thiocyanate and SMCO. Non-significant correlation coefficients between gca

Table 1. Mean squares from Hayman's analysis of variance for 5×5 diallel experiment of forage rape (*B. napus*)

Item	d.f.	Thiocyanate	SMCO
GCA	4	76.08***	0.0126***
SCA	10	30.95*	0.0092**
b_1	1	1.61	0.0099
b_2	4	58.89**	0.0180**
b_3	5	14.46	0.0020
Block (B)	2	122.61***	0.0058
B×GCA	8	18.43	0.0036
B×SCA	20	7.89	0.0027
$B \times b_1$	2	11.96	0.0032
$B \times b_2$	8	6.91	0.0020
$B \times b_3$	10	7.86	0.0032
Block interactions	28	10.90	0.0030
Bartlett's X^2	4	NS	NS

* $P<0.05$; ** $P<0.01$; *** $P<0.001$
NS = Non-significant

Table 2. Regression coefficient (b) of Wr (array covariance) on Vr (array variance), Vr/Wr and heritability in the broad sense (Hb) and narrow sense (Hn) in a 5×5 diallel experiment of forage rape (*B. napus*)

Character	b±SE	Vr/Wr	Hb	Hn
Thiocyanate	0.280±0.246	2.22	90.76	64.51
SMCO	1.835±0.325	2.36	87.90	50.81

Table 3. Estimates of general combining ability effects (gca), parental performance, parental order of dominance (Wr+Vr) and correlation coefficient (r) of parental mean with gca and (Wr+Vr) in 5×5 rape diallel experiment

Parents	Thiocyanate			SMCO		
	gca	Parental mean	(Wr+Vr)	gca	Parental mean	(Wr+Vr)
'Canard'	−1.24	9.77	11.64	−0.006	0.372	0.0020
'Emerald'	4.81	13.85	15.07	0.047	0.373	0.0068
'Nevin'	−0.10	19.78	38.12	−0.007	0.538	0.0040
'Windal'	−1.54	8.62	10.02	−0.021	0.372	0.0014
'Akela'	−1.93	16.73	12.20	−0.012	0.535	0.0044
SE	0.98			0.016		
r	0.132		0.776	−0.325		0.206

Table 4. Estimates of specific combining ability effects (sca), and heterosis over mid-parent (MP) and better parent (BP)[a] for thiocyanate and SMCO

	Thiocyanate				SMCO			
	sca	Mean content (mg/100 g)	Heterosis (%)		sca	Mean content (mg/100 g)	Heterosis (%)	
			MP	BP			MP	BP
'Canard'		9.77				0.372		
'Emerald'		13.83				0.373		
'Nevin'		19.78				0.538		
'Windal'		8.62				0.372		
'Akela'		16.72				0.535		
C×E	1.06	17.98	52.37**	84.03**	−0.009	0.501	34.50**	34.68*
C×N	−2.06	9.95	−32.68*	1.84	−0.028	0.428	−5.93	15.05
C×W	0.35	10.93	18.70	26.77	0.033	0.475	27.69*	27.69*
C×A	0.64	10.82	−18.34	10.75	0.004	0.455	0.33	22.31
E×N	2.32	20.37	21.24	47.36*	0.009	0.518	13.72	38.87**
E×W	−2.76	13.85	23.33	60.67*	−0.011	0.484	29.93**	30.11*
E×A	−0.63	15.60	2.09	12.80	0.011	0.515	13.44	38.07**
N×W	1.08	12.78	−10.00	48.26*	0.006	0.447	−1.76	20.16
N×A	−1.35	9.96	−45.40**	−40.41*	0.014	0.464	−13.51	−13.27
W×A	1.33	11.20	−11.67	29.93	−0.028	0.408	−10.03	9.68
SE	1.35				0.023			

[a] For these characters, better parent has the lower contents of thiocyanate or SMCO

* $P<0.05$; ** $P<0.01$

and parental values indicated that selection of parents for hybridization on the basis of parental content of these compounds may not be reliable.

Specific combining ability effects (sca) of thiocyanate and SMCO contents of the 10 F_1 hybrids are shown in Table 4. For thiocyanate content, the crosses 'Canard'×'Nevin', 'Emerald'×'Windal' and 'Nevin'×'Akela' had high negative sca. Four crosses showed negative, but non-significant sca for SMCO content.

Analysis of thiocyanate and SMCO contents in the F_1 progenies for heterosis are presented in Table 4. For thiocyanate content, the cross 'Canard'×'Emerald' showed the most undesirable response with significantly higher content than the mid parent and better parent values while three other crosses only showed higher contents than the better parent. Two crosses showed lower thiocyanate contents than the mid parent value but only one of these ('Nevin'×'Akela') showed heterosis over its better parent. However, the two parents in this cross had high thiocyanate contents and even with significant heterosis the absolute concentration in the F_1 (9.96 mg/100 g) was only comparable with those in the best cultivars used in this experiment (8.62 and 9.77 mg/100 g for 'Windal' and 'Canard', respectively).

Undesirable increases in SMCO content were also found over mid parent values (three crosses) and better parent values (five crosses). No cross had significantly

Table 5. Phenotypic (P) and genotypic (G) correlation coefficients of thiocyanate and SMCO with yield and components of yield in 5×5 rape diallel experiment (calculated from five parents and ten F_1s)

		No. of leaves	Plant height	Fresh wt			Dry wt			SMCO
				Leaf	Stem	Total	Leaf	Stem	Total	
Thiocyanate	P	0.011	−0.047	−0.045	−0.151	−0.093	−0.164	−0.254	−0.220	0.533**
	G	0.120	−0.147	−0.025	−0.145	−0.045	−0.145	−0.268	−0.210	0.584**
SMCO	P	0.217	−0.141	0.251	−0.153	0.175	0.260	−0.182	0.145	–
	G	0.198	−0.120	0.354	−0.168	0.168	0.326	−0.148	0.175	–

** $P < 0.01$

lower SMCO content than either the mid parent or better parent although 'Nevin'×'Akela' did show some reduction in SMCO content compared with 'Akela', its better parent. As with thiocyanate, the parents of this cross had higher SMCO contents than the other cultivars and their F_1 progeny had appreciably higher content than either the lower cultivars or the F_1 progenies from certain crosses between these lower cultivars. Since none of the hybrids was lower than the best cultivar for thiocyanate and SMCO contents, their potential is that of breeding material for selection rather than for direct use as F_1 hybrid strains.

The absence of any improvement compared with the best cultivars used in this study may indicate that rigorous selection for lower levels will be necessary to achieve significant improvement. In fact the scope for improvement may not be very great since levels of thiocyanate and SMCO in these forage rapes are appreciably lower than those reported in other *Brassica* crops, for example kale (Bradshaw and Borzucki 1981). Allowing for variation due to age and type of tissue, time of harvest and cultivar, the thiocyanate contents in the present experiment (0.009–0.020% of dry matter) compared favourably with those for kale (0.016–0.033%). Similarly, the SMCO contents for rape in the present experiment (0.37–0.54%) were much lower than those for kale (0.8–1.0%).

Johnston and Gosden (1975) have shown that thiocyanate producing glucosinolates in kale are subject to genetic-control and can be altered readily by plant breeding procedures. Similarly, Ellerström and Josefsson (1967) have selected successfully in rape for low content of specific thioglucosides.

Phenotypic and genotypic correlation coefficients, in the parental and F_1 hybrids, for thiocyanate and SMCO with yield and components of yield are shown in Table 5. Thiocyanate and SMCO contents were positively correlated with each other but neither character was positively correlated with any of the yield components. These correlations indicate that selection for low concentrations of both thiocyanate and SMCO can be made simultaneously and without any undesirable reduction of yield.

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