

BREEDING OF 00-RAPESEED (BRASSICA NAPUS L.) WITH DIFFERENTIAL  
GLUCOSINOLATE CONTENT IN THE LEAVES

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By experiments in vitro and in vivo it was found that some glucosinolates (gsl) which occur in the greenmatter of rapeseed possess strong antifungal properties and provide resistance against the pathogen Phoma lingam L. (Mithen et al.1987, Peterka 1990).

The possible role as a resistance mechanism and other positive traits like the repellent effect of the leaf-gsl justify any effort to breed rapeseed genotypes combining 00-quality in the seeds with high gsl-contents in the leaves.

Up to now high correlations between the leaf- and seed-gsl content have only been found in rapeseed collections with a wide range in seed-gsl content (Jürges 1982, Stephani 1985). Among rapeseed materials with a low level of seed-gsl the correlation seems to be rather weak (Demes 1989).

In the present investigation quantitative genetic studies were performed to identify the variability of the leaf-gsl content in comparison to that of the seeds. Moreover, quantitative genetic parameters were determined, which are of importance to breed for differential gsl-contents in the greenmatter and in the seeds, respectively. Parallel to our studies regarding the leaf and seed-gsl the response of rapeseed genotypes to Phoma lingam was recorded.

MATERIALS AND METHODS

Sixteen doubledhaploid (dh) lines of winter-rape were crossed in a factorial design. The males and females were taken from 2 different populations of dh-lines, respectively. Both populations were derived by microspore-culture from donor plants with a low gsl-content in the seeds. The 16 parental lines and the 32 F1-hybrids were tested in lattice trials in 1989/90 at 3 locations.

The samples of leaf material for gsl-determination were collected in late autumn and early spring during the vernalisation period. In each replication young leaves of approximately 20 x 25 mm size were taken from the inner part of 20 plants each. For determination of the seed-gsl content only the main stems of the plants were harvested.

The determination of the gsl was performed by HPLC. The samples of greenmatter were dried for 18h at a temperature of 60°C as described by Demes (1989).

Phoma-susceptibility was recorded on single plants by using a 1-9 scale (1 = no Phoma, 9 = highly infected). 40 plants of each replication were dugged out and the main root was cut alongside in order to determine the degree of destruction caused by the fungus.

For statistical analysis the "Plabstat" program provided by Utz (1987) was used. Genetical and environmental effects as well as their variances were separated by using the following biometrical model:

$$X_{fmu} = \mu + g_f + g_m + s_{fm} + u_u + g_{ufu} + g_{umu} + s_{ufmu} + e_{fmu}$$

where:

$X_{mvu}$  = lattice-adjusted mean of a hybrid with the female f and the male m in the environment u

$\mu$  = general mean

$g_f$  = g.c.a.-effect of the female line f

$g_m$  = g.c.a.-effect of the male line m

$s_{fm}$  = s.c.a.-effect of the lines f and m

$u_u$  = effect of the environment u

$g_{ufu}$  = interaction between the g.c.a.-effect of line f and the environment u

$g_{umu}$  = interaction between the g.c.a.-effect of line m and the environment u

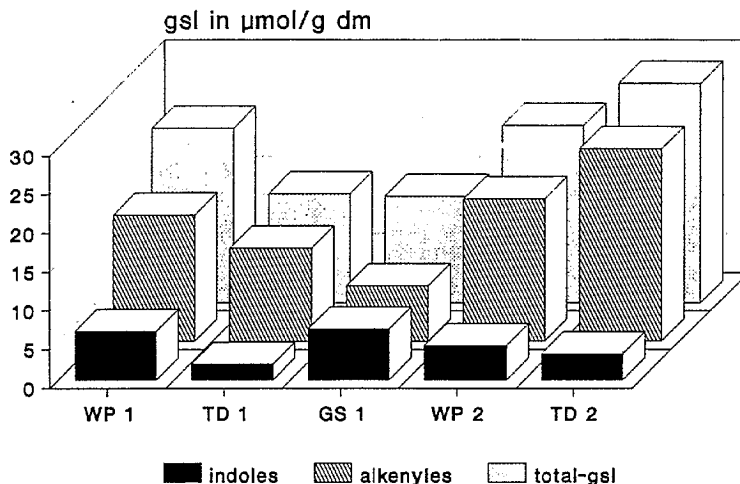
$s_{umvu}$  = interaction between the s.c.a of the lines f and m and the environment u

$e_{mvu}$  = error.

For leaf-gsl content the different samplings and locations were pooled and considered as random environmental effects. The "operative heritability" was calculated as the ratio of the genetic variance to the total phenotypic variance (Strube 1967).

### RESULTS

The mean values of the tested genotypes for the total-, alkenyle-, and indole-gsl content at different samplings and locations are given in Fig. 1, which illustrates the dependence of the leaf-gsl on external conditions. The variable proportion of the indoles of the total-gsl content indicates that there are evident differences in the gsl-pattern, in addition to the variability of the total-gsl content.



**Fig. 1:** Means over all genotypes for total-, alkenyle- and indole-gsl content at 3 locations (TD, GS and WP) at different sampling dates (1 and 2)

In table 1 the estimated values of components of variance for the total-, alkenyle-, and indole-gsl contents in the leaves are summarized. Very similar results were obtained in variance analysis of seed-gsl.

**Table 1:** Estimates of variance components and "operative heritability" ( $h^2$ ) for the total-, alkenyle- and indole-gsl content in the leaves

variance component	gsl-content		
	total	alkenyles	indoles
g.c.a. females(F)	0.27	1.52*	0.02
g.c.a. males(M)	53.04***1)	53.07***	0.31
s.c.a.	2.02**	0.62	0.21
environment(U)	34.21***	40.37***	4.24***
F x U	4.07***	1.66**	0.96
M x U	10.85***	9.90***	0.76**
F x M x U	0.76	1.56**	0.62
error(rest)	19.65	9.44	7.04
$h^2$	0.97	0.99	0.65

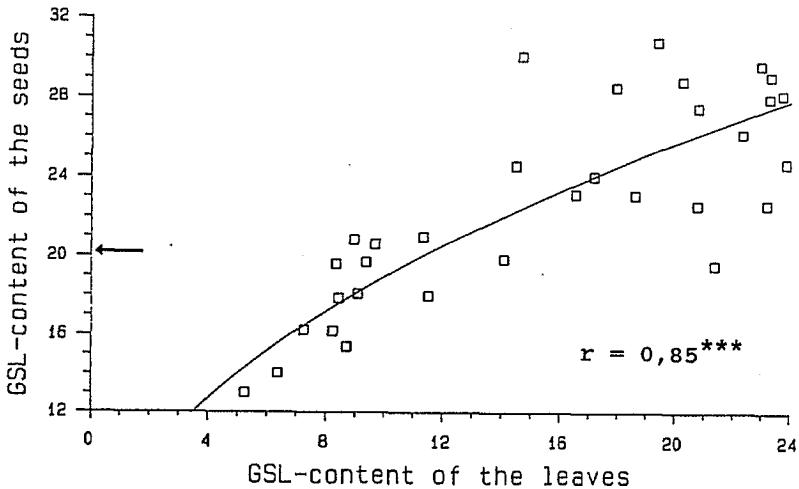
1) \*, \*\*, \*\*\*: significant at  $p = 0.05, 0.01, 0.001$

For the indole-gsl-content in the seeds and in the leaves rather small general combining ability(g.c.a.)- and specific

combining ability(s.c.a.)-effects were found. Whereas pronounced environmental effects have been observed, the genotype x environment-interactions were of less significance. Contrary to the indoles, very high g.c.a.-effects were present for the alkenyles. In comparison to the g.c.a.-effects the s.c.a.-effects for the alkenyles particularly for the seed-gsl could be neglected. The variance component for the g.c.a of the males was much larger than that of the females. This was caused by a larger variation of the gsl-content among the males (data not shown). For the alkenyle-gsl the environmental effect is of high importance, but the interactions of genotypes with the environments are rather small again.

For the total leaf-gsl content the results were similar to those of the alkenyle-gsl, because the proportion of alkenyles of the total gsl-content is much larger than that of the indoles. Moreover, the variation of the alkenyles was found to be larger than that of the indole-gsl.

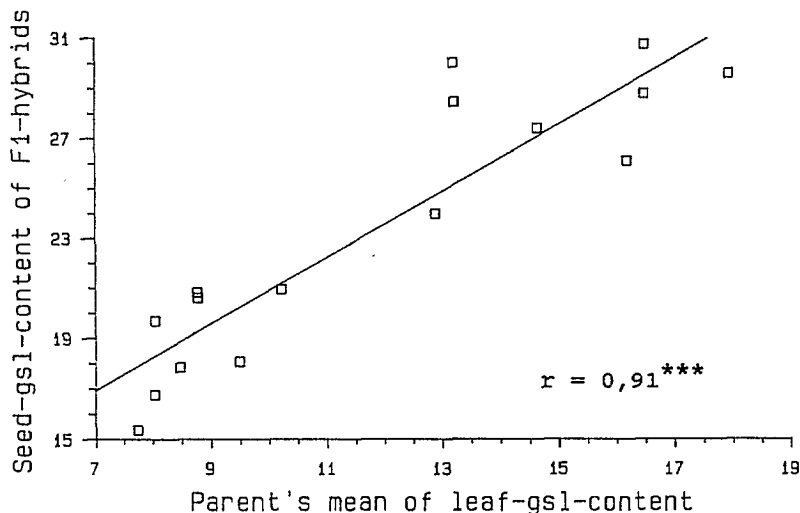
Although there was a stronger environmental effect observed for leaf-gsl content than for seed-gsl, the "operative heritability" of leaf-gsl content was found to be comparable to that of the seed-gsl.



**Fig.2:** Regression of the alkenyle-gsl content in the seed on that of the leaf (gsl in  $\mu\text{mol/g dm}$ ). The arrow marks the average seed-gsl content of the cultivars 'Arabella', 'Ceres' and 'Lirabon'

Figure 2 shows the regression of alkenyle-gsl content in seed on that of the leaves. This relationship was much closer for

genotypes with a seed-gsl content below  $20 \mu\text{mol/g dm}$  than for genotypes with a higher content. A comparison of the seed-gsl level of the tested rapeseed collection with the average content of the 00-varieties 'Arabella', 'Ceres' and 'Lirabon' indicates that the seed-gsl level of the rapeseed material used in this study is comparable to that of modern cultivars.



**Fig.3:** Regression of the alkenyle-gsl content of the hybrids on the midparent value for alkenyle-gsl in the leaves (gsl in  $\mu\text{mol/g dm}$ )

Due to the pronounced variance components for the g.c.a-effects of the gsl-content in the leaf and the seed it is possible to predict the hybrid-performance regarding the gsl-content based on the parent's g.c.a.. Moreover, the seed-gsl content of the hybrids can be predicted on the basis of the midparent leaf-gsl content (figure 3).

Contrary to expectation, no relationship between Phoma-reaction and leaf-gsl content could be observed ( $r = -0.17$ ).

#### DISCUSSION

The correlation between leaf- and seed-gsl content estimated in this study is rather high in comparison to the results of other authors (Jürges 1982, Stephani 1985, Demes 1989), although the level of seed-gsl content was relatively low in our study.

Due to physiological reasons, such a close relationship can be expected, because the storage of gsl in the seeds results from a synthesis in the greenmatter of the respective maternal plant (Kondra and Stefansson 1970).

As a main reason for weak correlations between the seed- and leaf-gsl content, Demes (1989) discussed the dependence of the leaf-gsl content on environmental effects and growing stage, which both can cause a high instability of the leaf-gsl and therefore handicap an exact comparison of the genotypes regarding their leaf-gsl content.

The very high "operative heritability" for the leaf-gsl content found in this study indicates that we succeeded in minimizing the influence of the instability of leaf-gsl content by using an appropriate sampling method.

The low correlation between the leaf-gsl content and the Phoma-susceptibility may not be interpreted in the way, that the gsl have generally no effect on Phoma-infection. However, it is likely that the level of leaf-gsl content in the tested material was generally too low to affect the fungus. Obviously, in material with a very low gsl-content resistance mechanisms other than gsl play a more important role.

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#### REFERENCES

- DEMES, H. (1989). Untersuchungen über Glucosinolat-, Nitrat- und Protein-Gehalt in der Raps-Grünmasse in Abhängigkeit von Genotyp, Umwelt und Entwicklungsstadium. Dissertation Univ. Giessen, FRG
- JÜRGES, K. (1982). Möglichkeiten einer Auslese auf Glucosinolat-Armut in der Grünmasse von Brassica napus und B. campestris. Z. Pflanzenzüchtg. 89, 74-87
- KONDRA, Z.P. and STEFANSSON, B.R. (1970). Inheritance of the major glucosinolates in rapeseed (Brassica napus L.) meal. Can.J.Plant Sci. 50, 643-647
- MITHEN, R.F., LEWIS, B.G and FENWICK, G.R (1987). In vitro activity of glucosinolates and their products against Leptosphaeria maculans. Trans.Br.mycol.Soc.87 (3), 433-440
- PETERKA, S. (1990). Grundlagen der Resistenz von Brassica spp. gegenüber Leptosphaeria maculans, Dissertation Univ. Giessen, FRG
- STEPHANI, V. (1985). Selektion auf Gehalt und Zusammensetzung der Glucosinolate in der Grünmasse von Raps (Brassica napus L.). Dissertation Univ. Göttingen, FRG
- STRUBE, H.G. (1967). Merkmalskorrelationen bei Hybridmais und ihre Bedeutung für die Selektion. Dissertation Univ. Hohenheim
- UTZ, H.F. (1987). A computer program for statistical analysis of plant breeding experiments. Uni Hohenheim, FRG