VARIATION IN GLUCOSINOLATE CONTENT AND GLUCOSINOLATE PATTERN IN THE DEVELOPMENT OF RAPE

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INTRODUCTION

Cruciferous plants contain glucosinolates which differ in nature and level in seeds and vegetative parts. These compounds are enzymatically hydrolyzed by myrosinase present in the plants and give different products which have in some cases toxic properties and affect the nutritive value of rapeseed meal and fodder rape negatively.
This paper describes a study of the development of glucosinolate (GSL) content and GSL pattern at different stages of the vegetation period of rape with special emphasis on the modification from seed specific to green matter specific GSLes.

MATERIALS AND METHODS

Germination and Seedlings Development

One breeding line (low GSL-content, 00 line) and the F_1 generation of a cross (cross line) between this 00 line and Jet Neuf (high GSL content) as male parent were used. The plants were grown in sand culture with regular sulfur fertilizer. Five samples consisting of twenty plants were taken at each date of sampling.

Table 1. Sampling Dates During Germination

Stage	0	1	2	3	4	5	6	7	8	9
Time after sowing	0h	6h	12h	24h	36h	54h	3d	4d	5d	7d

Two-day-old seedlings and later stages were divided in roots, hypocotyl and cotyledons in addition to whole plant probes.

Further Plant Development

The two lines of the germination experiment and Jet Neuf as male parent were examinated.

Table 2. Definition of Sampling Dates and Growth Stages

Stage No	Time (week)	Definition of Growth Stage	Average Plant Weight (dry matter in g))
1	2	cotyledons	0,0051
2	4	two leaves	0,109
3	6	four leaves	0,611
4	8	six leaves	1,046
5	10	rosette	1,363
6	12	plant height approx.50 cm	6,25
7	15-21	flowering top shoot	12,6

The plants were grown in a greenhouse from october 1987 till summer 1988. Five plants were taken at random each sampling date and treated as described in the following.

Extraction and Chemical Analysis

The plants were dried at 60°C. The myrosinase was inactivated and the GSLs were extracted with boiling methanol (60%). GSLs were isolated on Sephadex A25 micro-columns and desulphated with H1 sulphatase overnight.
HPLC analysis of desulpho-GSLs was conducted using a Knauer Crome-A-scope system with a Nucleosil C18-120mm column, flow rate of 1,5 ml/min and GSLs were detected at 229nm wavelength. The solvent systems employed were (A) water and (B) 20% acetonitrile in water. The programm consisted of a gradient from 95% (A) + 5% (B) to a maximum of 5% (A) + 95% (B).
Different response factors for several GSLs were used: Alkenyl- and sulphinyl GSLs: factor1, indolyl GSLs: factor0,25 and phenyl GSLs: factor0,9.
The calibration of HPLC values were made with defined standard samples of sinigrin and tropaeolin. Duplicate samples were determined for each plant. Analysed GSLs are summarized in table 3.

Table 3. Glucosinolates in Rape

Trivial name	Systematic name	Abbreviation
Alkenyl GSLs		Alk
Sinigrin	allyl-	SIN
Gluconapin	but-3-enyl-	GNA
Glucobrassicanapin	pent-4-enyl-	GBN
Progoitrin	2-hydroxy but-3-enyl-	PRO
Gluconapoleiferin	2-hydroxy pent-4-enyl-	GNL
Glucoalyssin	5-methylsulphinylpentyl-	ALY
Indolyl GSLs		Ind
Glucobrassicin	indol-3-ylmethyl-	GBC
4-Hydroxyglucobrassicin	4-hydroxyindol-3-ylmethyl-	4OH
4-Methoxyglucobrassicin	4-methoxyindol-3-ylmethyl-	4ME
Neoglucobrassicin	1-methoxyindol-3-ylmethyl-	NEO
Gluconasturtiin	phenylethyl-	NAS

RESULTS

Germination and Seedlings Development

Similar trends in GSL contents were observed between the 00 line and the cross line whereby the total GSL content of the cross line was significant higher during the examination period (Fig 1.) Slight decrease of aliphatic GSLs was found with a corresponding reduction of GBN in the 00 line and GNA in both lines (data not shown). Indolyl GSLs increased rapidly during the second and third day as a result of the increasing level of NEO content (Table 4). The other Indolyl GSLs rose constantly after stage 4. The decomposition or alteration of 4OH, amounted to 50% of the seed content on the 7th day. NEO is localized predominantly in roots of the seedlings whereas

GBC and 4OH are largely concentrated in cotyledons (data not shown).

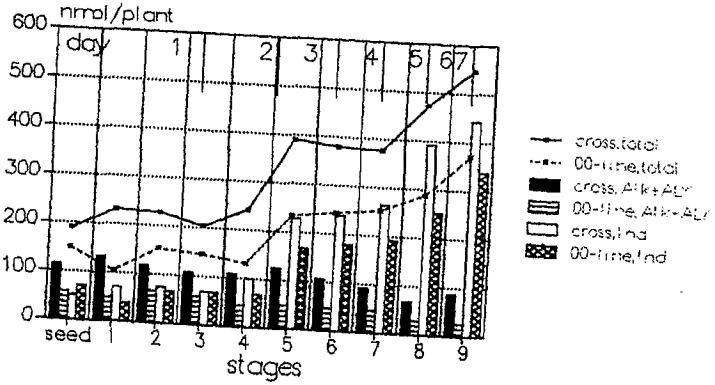

Fig. 1. Total GSL Content of the 00 Line and the Cross Line and their Division in Indolyl GSLs and Aliphatic GSLs

Table. 4: Indolyl GSL Content with Standard Deviations of the Cross Line (nmol/Seedling)

stage	4OH		GBC		4ME		NEO	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
seed	44,4	10,1	3,29	0,57	1,20	0,44	1,30	0,65
1	64,3	8,4	4,40	0,66	1,46	0,36	0,40	0,07
2	69,2	9,1	3,72	0,67	0,84	0,12	0,27	0,04
3	59,6	11,6	5,13	0,63	0,98	0,27	2,71	0,47
4	59,7	4,3	14,0	2,0	1,05	0,39	24,6	0,3
5	53,9	7,5	52,8	4,3	2 84	0,29	120,0	8,0
6	32,6	3,7	40,9	4,5	10,2	1,5	159,3	18,3
7	25,4	6,1	32,6	4,6	20,2	2,2	183,7	22,8
8	31,5	6,6	72,7	17,7	31,7	6,0	257,3	48,9
9	22,5	7,3	113,4	35,4	52,2	16,1	254,2	72,5

Further Plant Development

The analysis of variance showed significant effects on GSL content of stage, breeding line and the interactions between them at significance level of α=0,01. Stage 6 represents the theoretical harvest date for fodder rape. The three lines do not differ significantly (by t-test) at this stage (Fig.2). The expected dominance of high GSL content was only expressed in stage 3 and 4 (Fig. 2) and in the seed stage (Table 5). The total GSL content of the cross line corresponded to the GSL pattern: plants in stages with high GSL content (stage 4) showed patterns like Jet Neuf while those in stages with low GSL content exhibited GSL patterns similar to the 00 line (Fig. 3).

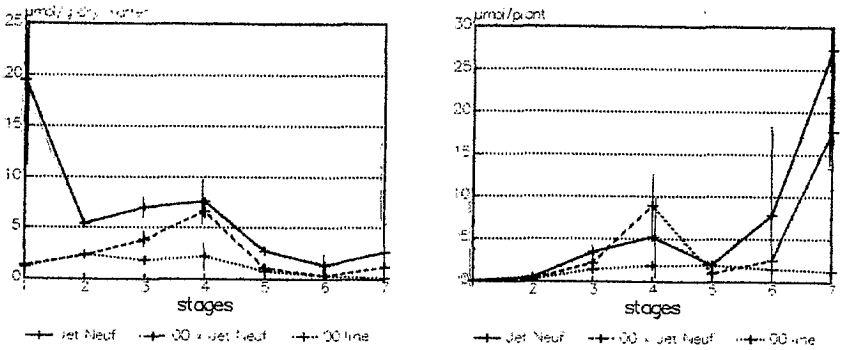

Fig. 2. Sample Means and Standard Deviations of GSL Content of the Seven Investigated Stages for the Two Breeding Lines and Jet Neuf (µmol/g, µmol/Plant)

Table 5. GSL Content of Seeds 87 and Seeds 88 in µmol/g Seed

	Jet Neuf	00 line x Jet Neuf	00 line
seeds 87	61,5	3,9	3,
seeds 88	124,3	63,0	4,69

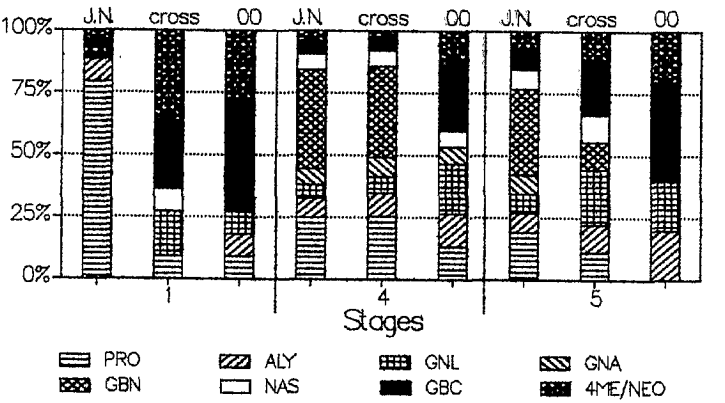

Fig. 3. GSL Pattern of Jet Neuf (J.N.), the Cross Line (cross) and the 00 Line (00) at Stage 1,4 and 5

GBN increases rapidly in concentration over the first eight weeks (Stage 4) in Jet Neuf and the cross line, whereas it was not detectable in the 00 line. Typical for greenmass with high GSL content was also a high content of PRO and a low GNL concentration. The 00 lines were characterized by their high concentration of 4 OH.
The differences between seed GSL pattern of aliphatic GSLs and greenmass GSL pattern have been pointed out in Table 6. They are also characteristic between high and low GSL types.

Table 6. Aliphatic GSL Pattern of Seed and Greenmass (Stage 4) in % of Aliphatic GSL Content

	PRO	ALY	GNL	GNA	GBN
seed J.N.	60,4	-	4,3	25,1	10,2
seed 00	62	-	14	19	5
stage 4 J.N.	30	9	6	8	45
stage 4 00	25	25	38	12	-

DISCUSSION

The biosynthesis of Indolyl GSLs in germinating plants started at the second day. The dramatic increase can not be explained by a conversion of 4OH, so the start of the plant genotype expression can be assumed. Mc Gregor (1988) also found an increase of GBC in the cotyledons of seedlings grown in light at the second day. In this work no differences between the crossed and selfed plants could be detected concerning the relative Indolyl GSL content. Regarding the AlkenylGSL content an incomplete dominance (PRO,GNA) or overdominance (GBN) was expected (Kondra and Stefansson, 1970). The plants' own alkenyl GSL biosynthesis began between the fourth and the sixth week. Buchner (1988) determined the beginning of alkenyl biosynthesis at week two till five after sowing. The GSL content of the cross increased to the same level of the paternal parent in stage 4 after eight weeks. After this stage a degradation of GSLs occured in all three lines. There was no significant difference between the lines in stage 5 (µmol/plant) or in stage 6 (µmol/g) respectively. This result should be considered, if breeders intend to select low GSL fodder rapes by measuring the seed GSL content.
The Alkenyl GSL pattern of the different vegetation stages and seeds depended on the total GSL content of the lines:

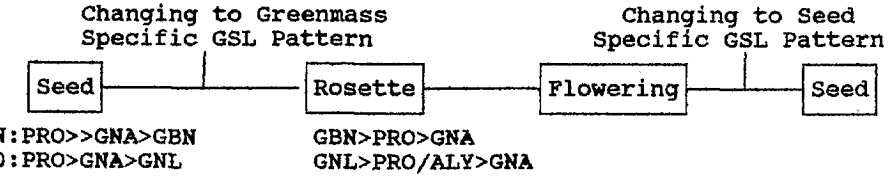

Fig. 4. Conversion of Seed Specific- and Greenmass Specific GSL Patterns

Macfarlane Smith and Griffiths (1988) analyzed three forage rapes. One also lacked GBN and GNL at all stages of greenmass.

The other two are resynthesized rapes. One of them contained GBN and GNL and the other only GBN in the greenmass. No seed GSL content was given, so their results could not be integrated in the system above. Two other fodder rape strains with low GSL content, which were investigated together in this trial (data not shown), showed also a high content of GNL in the greenmass but no GBN. Further investigations on this point are planned.

CONCLUSIONS

One cultivar with high GSL content , one with low GSL content and the F1 between them grown in a glasshouse were investigated throughout the vegetation period. The time of switching the maternal determinated GSL pattern to the pattern represented by their own genotype was determined. The Indolyl GSL biosynthesis started at the second day after sowing whereas the aliphatic GSL were produced after the sixth week.
Between seed/greenmass and low-GSL-lines/high-GSL-lines differences in GSL pattern were found. The seed GSL pattern was dominated by Progoitrin, wheras greenmatter of low GSL lines contained Gluconapoleiferin and greenmatter of high GSL lines contained Glucobrassicanapin.
No correlation was found between the seed GSL content and the GSL content in greenmass of rosette stage until begin of flowering.

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