Changes in the sulphur and glucosinolate content of developing pods and seeds of oilseed rape (*Brassica napus L.*) in relation to different cultivars

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Abstract

Since the introduction of double low oilseed rape varieties in the 1980´s the complete physiological background of a lower glucosinolate content in seeds of double low varieties is still not known. Double low varieties contain virtually the same amount of sulphur in the vegetative plant parts as single lows but the seeds show much lower glucosinolate contents. There are opposing theories how these differences can be explained. It is commonly accepted that seed glucosinolates are synthesised in the pod walls. The lower glucosinolate content in double low varieties might be caused by a metabolic block in the glucosinolate biosynthesis in combination with a reduced transport of glucosinolates into the seeds. It was the aim of this investigation to elucidate this question.

From 1999 to 2001 various field trials were conducted with summer and winter oilseed rape varieties under low and high sulphur supply. The glucosinolate and the total sulphur contents were determined in developing pods of single low and double low oilseed rape varieties. The accumulation pattern of glucosinolates and total sulphur in pod walls and seeds support the hypothesis of a metabolic block in the glucosinolate biosynthesis of double low oilseed rape varieties. Only intact glucosinolates can be transported into the seeds while intermediary products of the glucosinolate biosynthesis were enriched in pod walls of double low varieties where a quick degradation into sulphate may occur.

Keywords: oilseed rape, single low varieties (0), double low varieties (00), glucosinolates, total sulphur, seed development

Zusammenfassung

Veränderung des Gesamtschwefel- und Glucosinolatgehaltes in Schoten und Samen unterschiedlicher Rapssorten während der Schotenentwicklung

Seit Einführung der glucosinolatarmen Rapssorten (Doppelnullsorten) in den achtziger Jahren ist auch bis heute noch nicht vollständig geklärt, wie sich der niedrigere Glucosinolatgehalt in den Samen der Doppelnullsorten erklären lässt. Während Doppelnullsorten annähernd den gleichen Schwefelgehalt in der vegetativen Blattmasse aufweisen wie Einfachnullsorten, zeigen sie einen deutlich niedrigeren Glucosinolatgehalt in den Samen. Es gibt verschiedene Theorien für die Ursachen der Unterschiede in der Glucosinolatakkumulation. Allgemein anerkannt ist, dass die Samenglucosinolate im wesentlichen in der Schotenwand produziert werden und nicht in anderen Pflanzenteilen vom Raps. Daher kann der niedrigere Glucosinolatgehalt in den Samen der Doppelnullsorten auf einem metabolischen Block in der Glucosinolatbiosynthese und einen an intakte Glucosinolate gebundenen Transport in die Samen beruhen.

In den Jahren 1999 bis 2001 wurden Feldversuche mit Doppelnull- und Einfachnullsorten von Sommer- und Winterraps durchgeführt, wobei die Schwefelversorgung variiert wurde. Bestimmt wurde der Gesamtschwefelgehalt sowie der Glucosinolatgehalt in Samen und Schotenwänden von Raps über den Zeitraum der Schotenentwicklung.

Die Akkumulation von Glucosinolaten sowie von Schwefel in Schotenwänden und Samen stützt die These, dass bei Doppelnullsorten ein metabolischer Block in der Glucosinolatbiosynthese vorhanden ist, und dass nur intakte Glucosinolate in die Samen transportiert werden, während sich die Intermediärprodukte in den Schotenwänden der Doppelnullsorten anreichern und dort rasch zu Sulfat degradiert werden können.

Schlüsselwörter: Raps, Einfachnullsorten, Doppelnullsorten, Glucosinolate, Gesamtschwefel, Schotenentwicklung

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1 Introduction

Glucosinolates (GSLs) are sulphur (S) containing compounds of the secondary plant metabolism, which occurrence is typical in *Brassica* species (Figure 1).

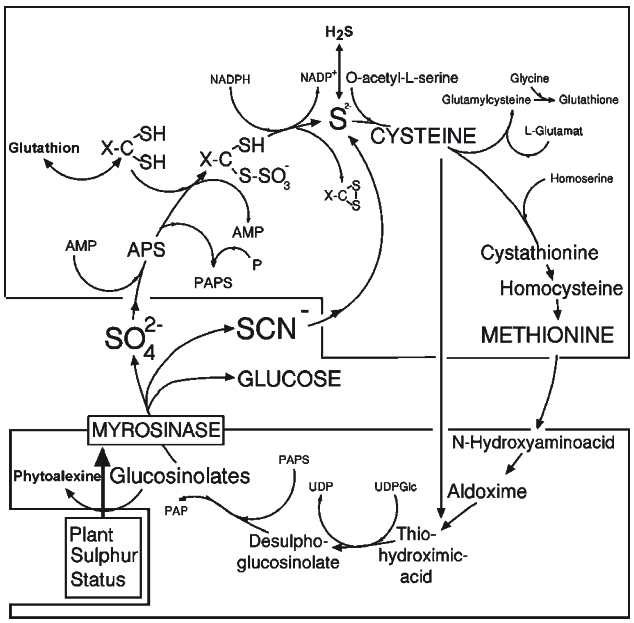

Figure 1:

Biosynthesis and biorecyclisation of alkenyl-glucosinolates in *Brassica napus* (adapted from Schnug, 1993)

Plant breeders decreased the seed GSL content by 80 - 90 % to 15 - 20 µmol g^{-1} (d.w.) in order to avoid a thyroid effect by use of the meal in animal nutrition. Mature seeds from conventional *Brassica napus* L. cultivars in the 1970´s (0-varieties) accumulated on average 150 µmol g^{-1} aliphatic GSLs (De March et al., 1989). Kondra and Stefansson (1970) and also Lein (1970) made reciprocal crosses with the variety *Bronowski*, a cultivar with only 15 µmol g^{-1} aliphatic GSLs in the seeds. It was the beginning of breeding double low oilseed rape varieties (00-varieties). Single low (0-varieties) and 00-varieties show nearly the same S uptake and contain comparable amounts of S in vegetative plant parts despite of the fact that 00-varieties contain much lower GSL contents in their seeds (Fieldsend and Milford, 1994). Schnug and Haneklaus (1993) observed that 00-varieties showed symptoms of severe S deficiency earlier than 0-varieties and moreover yield effects with decreasing S supply were stronger in 00-varieties. They concluded from these observations that GSLs play a vital role as an intermediary metabolic S storage that can be utilised by the plant for the synthesis of primary metabolites under conditions of S starvation (Figure 1). The physiological background of a lower seed GSL content in 00-varieties compared to 0-varieties is still unknown. Gijzen et al. (1989) developed the hypothesis that seed-GSLs were not synthesised *de novo* in the seeds but somewhere else in the plant and that they were transported to the seeds (Lein, 1970). From grafting studies it has been concluded that the pod (pod plus developing seed) is the main site for the synthesis of GSLs accumulated in the seeds (Lein, 1972) but at least some of the GSLs accumulated in maturing seeds may be synthesised in other plant parts. Alternatively, GSL precursors might be transported from other plant parts for conversion into intact GSLs in pods or seeds (De March et al., 1989) as more GSLs were accumulated in the seeds than were previously synthesised in the pod tissue. De March et al. (1989) concluded from the fact that both low and high GSL-containing lines of *B. napus* showed similar trends in that respect that the lower GSL content in mature seeds of low GSL lines is most likely not the result of reduced transport but of reduced synthesis caused by a genetic block in the biosynthetic pathway as postulated already by Josefsson (1973). The investigations from Fieldsend and Milford (1994) showed that a transport of intact GSLs from vegetative plant parts to the seeds is not likely to occur because the amount of GSLs which had to be translocated from vegetative plant parts was not high enough to explain the increase in seeds of high GSL containing oilseed rape varieties. Secondly, the spectrum of GSLs which was produced in vegetative tissues and which accumulated in pods was different. Thirdly, the leaves of field grown crops started to senesce and were usually dead well before the start of rapid pod and seed growth. They postulated that it is more likely that the pod walls are the primary site of seed GSL biosynthesis despite the fact that only relatively small amounts of GSLs can be measured in pod walls. This could indicate a rapid turnover from the pod walls into the seeds. Alternatively, it has been suggested that the pod wall might be the site of synthesis of GSL precursors which are transported to the seeds and the final steps of GSL biosynthesis, glycosylation and sulphatation, may occur in the seeds (Fieldsend and Milford, 1994). Also Toroser et al. (1995) found no evidence for a transport of GSLs between plant tissues except the transport from pod walls to developing seeds.

2 Materials and methods

Over three years of experimentation not only summer and winter oilseed rape varieties but also 0 and 00-varieties were studied for a better differentiation between genetical and environmental effects (S nutrition). In 1999 five different varieties of summer oilseed rape were grown in Braunschweig on a sandy loam (E 10° 27´, N 52° 18´), two 0 and two 00-varieties and the variety *Bronowski* which was the first oilseed rape variety with a naturally low GSL content and which is the genetically origin of all 00-varieties. The

Table 1:
Experimental design of the oilseed rape field trials from 1999 to 2001

		1999	**2000**	**2001**
		Summer oilseed rape trial	Winter oilseed rape trial	Winter oilseed rape trial
Oilseed rape varieties	single low (0)	*Petranova, Niklas*	*Jet Neuf*	*Jet Neuf*
	double low (00)	*Bronowski, Licosmos,Topas*	*Ceres*	*Ceres*
Sowing date		23.03.99	23.08.99	18.08.00
Seed density	[kg ha^{-1}]	5	5	5
Plot size	[m^2]	18	45	45
S-fertilisation	[kg ha^{-1}]	0/100	100	0
N-fertilisation	[kg ha^{-1}]	130	130	130
Harvesting date		16.08.99	11.07.00	26.07.01
Growth stages of sampling [BBCH Scale]				
73; early pod development		01.07.99		
75; 50 % of the pods reached full size		09.07.99	24.05.00	30.05.01
77; 70 % of the pods reached full size		15.07.99	30.05.00	06.06.01
81; 10 % of the seeds are black		22.07.99	07.06.00	12.06.01
83; 30 % of the seeds are black		29.07.99	14.06.00	19.06.01
85; 50 % of the seeds are black		05.08.99	21.06.00	26.06.01
87; 70 % of the seeds are black		11.08.99	27.06.00	03.07.01
89; Maturity		18.08.99	04.07.00	10.07.01
Number of analysed samples		240	84	126

crops were sown in 18 m^2 plots which were split in two blocks with (100 kg ha^{-1} S as elemental S) and without S fertilisation. All other plant nutrients were sufficiently supplied to warrant optimum growth conditions. The sampling started when first pods were almost fully developed (BBCH 73; Strauß et al., 1994) and was then carried out weekly. At the first two sampling dates it was not possible to separate pod walls and seeds so that whole pods were shock-frozen in liquid nitrogen. At later sampling dates the pods were separated into seeds and pod walls directly during sampling and samples were immediately shock-frozen in liquid nitrogen. Additionally, whole pod samples were taken and prepared in the same way. Around 50 pods per sample were harvested exclusively from the main branch.

In 2000 and 2001, two different varieties of winter oilseed rape (*Ceres* and *Jet Neuf*) were grown. In 2000, the plots received a S fertilisation of 100 kg ha^{-1} while in 2001 no S was applied. In table 1 relevant field parameters are summarised.

Chemical analysis:

S_t: The total S content of seeds was determined by microwave digestion: 500 mg of ground seed or pod wall material was digested for 20 minutes in a microwave oven using 5 ml of a mixture of HNO_3 (65 %) and H_2O_2 (35 %) (4:1, vv). After cooling, samples were diluted with bi-distilled water to a final volume of 25 ml, filtered and measured by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, Spectro Flame M120S from Spectro).

GSL: Glucosinolates in seeds and pod walls were determined according to the EU method L170/28 (Anon, 1990). The desulphoglucosinolates were measured by HPLC with UV detection at 229 nm using acetonitrile (20 %) and water as eluents with a flow of 1.5 mL min^{-1} and a Hypersil C18 (ODS, 250 x 4.6 mm, 5µm, Phenomenex) column for separating desulphoglucosinolates.

3 Results

In general, degradation of GSLs after sampling is possible as long as pod walls and seeds are photosynthetically active and samples are not immediately shock-frozen. The dissection of seeds and pod walls was extremly time-consuming, especially during early pod development so that only a limited number of samples could be investigated. Additionally, whole pods were sampled and immediately shock-frozen in liquid nitrogen in order to prevent GSL losses. These whole pod samples were freeze-dried and hereafter separated into pod walls and seeds in the laboratory. Despite time-consuming sampling in the field and possible breakdown of GSLs higher contents were found in the field samples than in samples which were separated after conditioning in the laboratory. Especially during

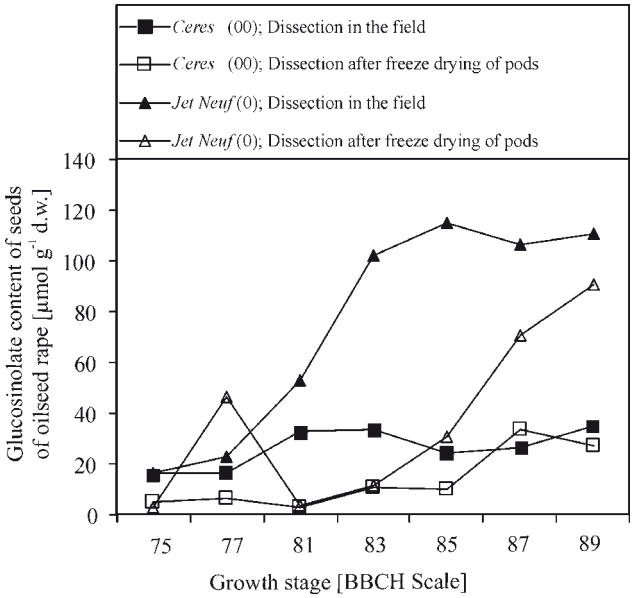

Figure 2:

Comparison of the seed glucosinolate content of field dissected seeds and pod walls of oilseed rape and samples which were dissected after freeze-drying of whole pods in the laboratory (BBCH scale according to Strauß et al., 1994)

early pod development distinctly higher GSL contents were measured (Figure 2). The samples which were separated in the field reflected much better the process of GSL accumulation in the seeds of oilseed rape and there was less variation between repetitions. A particulary high standard deviation in the seed GSL content of up to 60 µmol g^{-1} was determined for the 00-variety during early pod development when the pods were dissected in the laboratory after freeze-drying of the sample whereas a maximum standard deviation of up to 10 µmol g^{-1} was determined in the field and for the 0-variety. Though the reason for this divergence can not be explained at the moment, samples collected and prepared in the field seem to be more suitable to follow up differences between cultivars in relation to the S supply so that results presented exclusively refer to these samples.

Next, the results of the summer and winter oilseed rape varieties are shown separately because of basic differences in growth and pod development. In the discussion time-dependent changes in the GSL content are shown and compared for all investigated varieties.

Summer oilseed rape varieties

Samples from summer oilseed rape varieties were taken from very early pod development (BBCH 73) onwards when a separation into seeds and pod walls was not jet possible without mashing the seeds. Therefore whole pods were sampled and analysed at start of the experiment (Table 2). In table 2 the changes in the total S and GSL content in developing seeds and pod walls of different summer rape varieties are presented. The total S content in leaves and pods at early pod development (about 30 % of the pods had achieved full size), and seeds and pod walls at harvest are shown in figure 3. The results reveal basic differences in total S accumulation and GSL biosynthesis between different summer oilseed rape varieties (Table 2, Figure 3).

All varieties showed a sufficient S supply when S was fertilised with S contents in the leaves above the critical value of 6.5 mg S g^{-1} d.w. for winter oilseed rape varieties

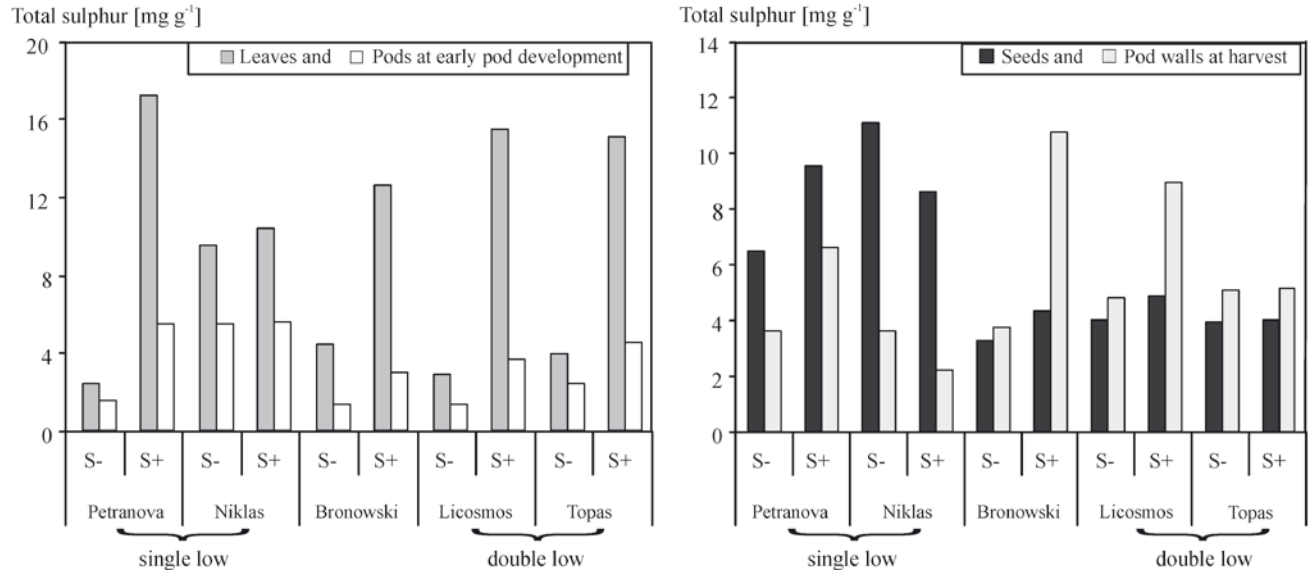

Figure 3:

Total sulphur content in leaves and pods at early pod development (BBCH 73) and in seeds and pod walls at harvest (BBCH 89) in five different varieties of summer oilseed rape under different sulphur supply (S- no S-application, S+ 100 kg ha^{-1} S)

Table 2:
Changes in the total sulphur and glucosinolate content in developing seeds and pod walls of different summer oilseed rape varieties

	Without S fertilisation				With S fertilisation (100 kg ha^{-1} S)			
	Total sulphur [mg g^{-1} S]		Total GSL [µmol g^{-1}]		Total sulphur [mg g^{-1} S]		Total GSL [µmol g^{-1}]	
Growth stage	Seed	Pod wall	Seed	Pod wall	Seed	Pod wall	Seed	Pod wall
Single low varieties								
Petranova								
BBCH 73		1.5*		1.3*		5.5*		10.0*
BBCH 75		1.9*		1.6*		3.8*		2.0*
BBCH 77	4.3	1.7	18.1	4.4	6.4	3.7	36.0	6.9
BBCH 81	5.2	1.9	14.7	1.0	6.5	4.0	16.5	1.3
BBCH 83	5.7	2.3	30.5	1.7	6.6	4.2	35.8	3.2
BBCH 85	5.9	2.5	15.7	1.7	8.2	3.6	32.6	4.3
BBCH 87	5.6	2.1	23.9	0.7	6.4	2.7	37.4	0.9
BBCH 89	6.5	3.6	36.6	1.1	9.5	6.6	48.8	0.6
Niklas								
BBCH 73		5.5*		17.1*		5.6*		23.7*
BBCH 75		6.0*		6.3*		5.5*		11.6*
BBCH 77	9.2	4.9	60.8	34.0	7.7	4.7	55.9	24.1
BBCH 81	10.0	4.4	32.4	16.5	9.1	4.0	28.5	18.3
BBCH 83	10.1	3.3	85.0	9.0	9.0	2.2	78.2	3.7
BBCH 85	9.8	2.9	76.8	1.8	9.8	2.5	76.1	0.9
BBCH 87	10.1	2.6	74.8	1.6	9.5	1.9	81.6	2.1
BBCH 89	11.1	3.6	73.4	1.0	8.6	2.2	60.4	0.4
Double low varieties								
Bronowski								
BBCH 73		1.4*		0.3*		3.1*		1.1*
BBCH 75		1.8*		0.5*		3.9*		0.7*
BBCH 77		2.5*		1.4*		4.3*		1.3*
BBCH 81	3.2	3.2	2.3	1.0	4.1	6.2	5.5	0.6
BBCH 83	3.3	3.3	4.8	1.1	4.2	5.4	9.5	1.2
BBCH 85	3.5	4.8	5.1	1.1	4.5	8.6	7.4	1.3
BBCH 87	3.2	2.7	6.3	1.3	3.7	7.0	8.4	1.1
BBCH 89	3.3	3.8	2.9	0.1	4.3	10.7	6.9	0.1
Licosmos								
BBCH 73		1.4*		0.5*		3.6*		2.2*
BBCH 75		1.8*		0.4*		4.0*		2.7*
BBCH 77	2.7	2.0	5.1	1.2	4.0	4.7	9.6	2.5
BBCH 81	2.7	2.1	1.1	0.3	4.2	4.9	5.2	0.8
BBCH 83	3.8	3.9	5.8	1.2	3.6	4.6	3.0	1.5
BBCH 85	3.6	4.1	8.3	0.9	4.2	5.6	8.4	1.5
BBCH 87	3.3	5.7	3.9	1.5	3.6	5.4	5.6	1.9
BBCH 89	4.0	4.8	6.3	0.5	4.9	9.0	7.6	0.5
Topas								
BBCH 73		2.5*		0.3*		4.6*		1.9*
BBCH 75		2.0*		0.5*		3.9*		0.9*
BBCH 77		2.3*	3.2	1.5		4.3*	4.0	1.1
BBCH 81	3.3	3.3	1.5	0.2	3.7	4.3	1.5	0.1
BBCH 83	3.4	3.9	7.5	0.5	3.9	5.5	11.1	0.5
BBCH 85	3.4	4.1	5.3	0.5	4.1	7.1	6.4	0.4
BBCH 87	3.7	6.1	4.8	1.1	4.1	7.4	6.6	1.0
BBCH 89	4.0	5.1	6.9	0.3	4.0	5.2	9.3	0.3

*whole pods as separation into pod walls and seeds was not feasible

(Haneklaus et al., 2006) (Figure 3). 0 and 00-varieties did not differ significantly in the total S content of leaves. Without S fertilisation, all varieties except *Niklas* showed symptoms of S deficiency whereby the S content in the leaves varied between 2.4 to 4.5 mg S g^{-1}.

The S content of the pods increased with S fertilisation, too. Here, the pods of 0-varieties showed a higher S content than 00-varieties (Figure 3). At this early stage of development 0-varieties had already a higher potential to accumulate S in pods. This difference between 0 and 00-varieties was also reflected in the total S content of pod walls and seeds at harvest (Figure 3). In general, S fertilisation increased the total S content in seeds and pod walls. The 0-varieties contained much more total S in seeds with about 10 mg g^{-1} and less S in pod walls with at maximum 6 mg g^{-1}, while the 00-varieties accumulated total S in their pod walls with values of up to 11 mg g^{-1}. For the 0-variety *Niklas* no differences in the total S content were determined in relation to S fertilisation during early pod development as well as at harvest. The 00-variety *Topas* showed a S response with an increasing S content in leaves with S fertilisation at early pod development but at harvest no more differences in the S content could be determined in relation to S nutrition (Figure 3).

Not only for total S, but also for the GSL content a clear difference between 0 and 00-varieties was found already very early during pod development. A much higher accumulation of GSLs of up to 24 µmol g^{-1} in pods of 0-varieties was determined (Figure 4). Additionally the variety *Niklas* which developed the highest seed GSL content had also a higher GSL content in leaves of about 5.8 µmol g^{-1}. In addition higher GSL contents in the S fertilised plots were found despite the fact that there were no differences

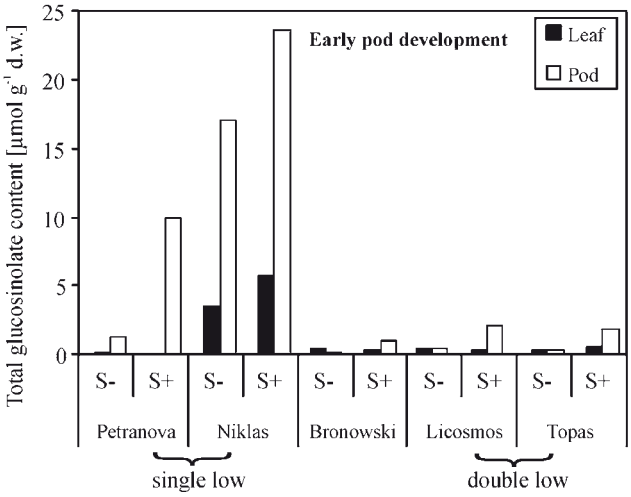

Figure 4:

Total glucosinolate contents in leaves and pods of different summer oilseed rape varieties at the start of pod development (BBCH 73)

Table 3:

Changes in the total S and glucosinolate content in developing seeds and pod walls of the winter oilseed rape varieties *Jet Neuf* (0-variety) and *Ceres* (00-variety)

Growth stage	Without S fertilisation				With S fertilisation (100 kg ha^{-1} S)			
	Total sulphur [mg S g^{-1} TS]		Total GSL [µmol g^{-1} TS]		Total sulphur [mg S g^{-1} TS]		Total GSL [µmol g^{-1} TS]	
BBCH	Seed	Pod wall	Seed	Pod wall	Seed	Pod wall	Seed	Pod wall
Single low variety								
Jet Neuf								
75	3.8	2.9	4.0	6.8	6.1	4.6	13.9	27.7
77	4.7	3.2	7.8	15.6	7.1	4.7	26.2	23.2
81	5.6	3.0	7.6	9.8	9.2	5.3	46.3	32.3
83	6.6	2.7	21.1	9.7	10.6	5.0	101.1	26.3
85	7.1	2.6	61.4	7.4	10.1	4.2	117.2	9.3
87	7.7	3.4	73.7	2.2	10.5	4.2	106.4	3.3
89	9.4	4.2	77.3	0.5	10.1	4.5	111.3	0.6
Double low variety								
Ceres								
75	3.0	3.6	1.8	3.0	4.2	5.9	20.0	7.2
77	3.5	4.2	2.4	5.2	4.6	6.1	20.2	6.7
81	3.8	4.1	6.2	4.9	5.0	6.6	33.1	2.9
83	4.3	3.8	15.3	3.2	5.1	7.9	33.7	2.2
85	4.4	3.3	27.2	2.0	4.6	7.9	27.2	0.3
87	4.8	5.1	28.6	0.6	4.5	9.5	26.8	0.2
89	4.4	5.7	24.0	0.3	5.5	10.5	35.4	0.2

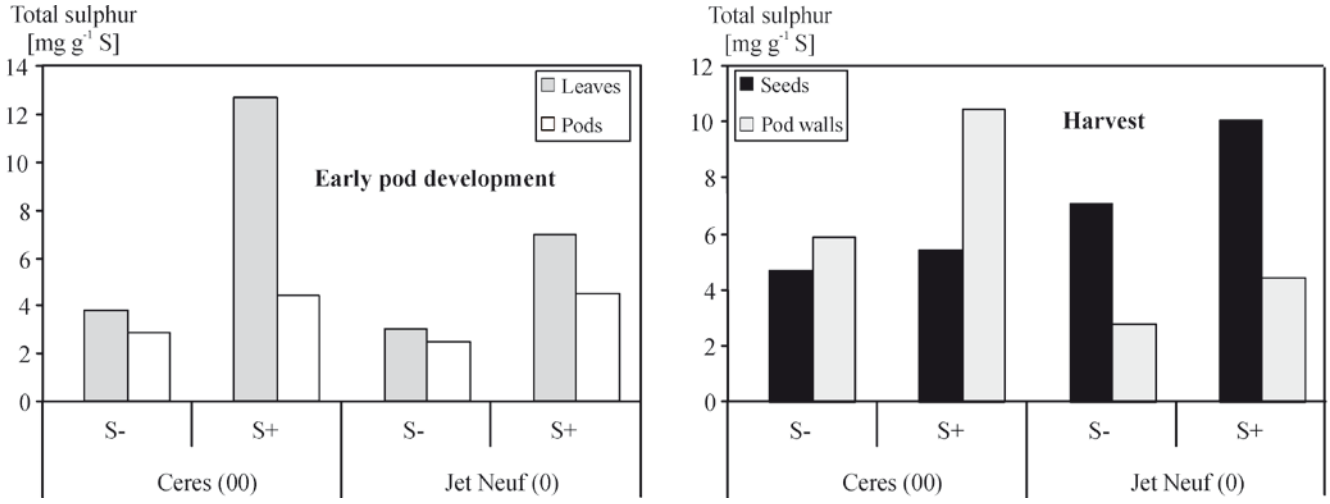

Figure 5:
Total S content in leaves and pods at early pod development (BBCH 75) and in seeds and pod walls at maturity (BBCH 89) of two varieties of winter oilseed rape in relation to the S supply

in the total S content at early pod development (Figure 3).

The 0-varieties accumulated S and also GSLs very fast during pod development (Table 2). The variety *Niklas* showed also very high GSL contents of up to 34 µmol g^{-1} in the pod walls during early pod development and the content decreased fast during maturation to a value of only 1 µmol g^{-1}. The 00-varieties had always very low GSL contents in the pod walls with values of ≤ 2.5 µmol g^{-1}.

Winter oilseed rape varieties

The winter oilseed rape varieties showed a different pattern of S and GSL accumulation in pod walls and seeds compared to summer varieties. In table 3 the accumulation of total S and GSLs is shown during pod development. Compared to the summer rape varieties winter oilseed rape accumulated GSLs much slower, but more consistently.

The winter oilseed rape varieties showed comparable results to the summer varieties in accumulating total S in the seeds at maturity but a slightly different result was found at early pod development (Figure 5): At an early stage of pod development the 00-variety *Ceres* responded much more to S fertilisation with highly increasing S contents especially in the leaf material. In contrast to the summer oilseed rape varieties the winter varieties showed no difference in total S accumulation in whole pods during early pod development. At harvest the 0-variety *Jet Neuf* accumulated total S in the seeds while the 00-variety *Ceres* accumulated S in the pod walls.

S fertilisation increased the total S content in both varieties but while *Jet Neuf* (0) accumulated 10.1 mg S g^{-1} (d.w.) in the seeds *Ceres* (00) accumulated the same amount, 10.5 mg S g^{-1} (d.w.) in the pod walls. Additionally, the GSL content of both varieties increased with S fertilisation. The major GSLs in the seeds and pod walls of both varieties were progoitrin (50 - 90 % of the total GSL content), gluconapin (5 - 40 %) and glucobrassicanapin (< 10 %) which account together for more that 90 % of the total GSL content (data not shown).

4 Discussion

Josefsson (1971a) determined that the low GSL content in the Polish summer oilseed rape cultivar *Bronowski* was not caused by a reduced S uptake and he could show the existence of a metabolic block in the GSL biosynthesis by radiotracer experiments (Josefsson, 1971b, 1973). Additionally, Josefsson (1973) showed that the low GSL level in seeds of *Bronowski* was associated with a large accumulation of S in the pod walls. Different possible explanations for the lower GSL content in seeds of 00-varieties of oilseed rape were discussed. The pod wall has been identified as the main site of glucosinolate biosynthesis by Lein (1972). Therefore lower GSL contents in seeds of 00-varieties can be understood as a combination of a blocked formation of GSLs at some intermediate stage in combination with a specific transporter which only accepts intact GSLs, leaving incomplete GSL-intermediates behind causing an increase of total S in the pod walls. Simultaneously increasing sulphate contents observed in pod walls only support the fragility of the intermediates and their strong vulnerability to degradation.

It was the aim of the presented experiments to further elucidate this problem by following up changes in the S and GSL metabolism of summer and winter oilseed rape varieties, 0 and 00-cultivars in relation to the S supply. It

was difficult to investigate GSL accumulation in seeds during the early stages of seed development because of the difficulty to separate seeds from pod walls. However, very early in seed development (BBCH 73), when it was not possible to dissect seeds from pod walls the 0-varieties of summer oilseed rape had already accumulated total S and also larger amounts of GSLs in the pods compared to the 00-summer varieties and also higher amounts of S compared to the winter oilseed rape varieties (Figure 3, 4). For the winter oilseed rape varieties a separation into seeds and pod walls was easier to carry out because seeds were bigger and the seed coat was harder. The 0 and 00 winter varieties showed comparable GSL contents in the seeds until BBCH 77 and with S deficiency even longer until BBCH 83. Then a sharp increase in the seed GSL content was observed for the 0-variety *Jet Neuf*, while the 00-variety had already reached the maximum GSL content.

The differences in S and GSL metabolism between 0 and 00 summer and winter varieties rely among others on morphogenetic differences during pod development. In figure 6 the relative changes in GSL and S content from pod development (BBCH 73) to harvest (BBCH 89) were calculated on a daily basis for all oilseed rape varieties to reflect the main differences in the S metabolism of the investigated varieties. This procedure assumes a linear relationship between S uptake and metabolisation in the plant. In fact, the relationship was curve-linear (Figure 2) so that values are only indicative and reflect basic differences between cultivars. Changes during pod development as determined for the different cultivars (Table 2, 3) can not be followed up in this way. In figure 6, data for the S-unfertilised plots are shown to outline genetic differences between varieties in accumulating S and GSL as S fertilisation is known to increase total S and GSL contents (Schnug, 1997). Varieties such as *Topas* and *Bronowski* had already accumulated the whole amount of S in the seeds when pod development started so that no further changes were measured during the experimental time.

When comparing the relative daily changes in the GSL content the 00 winter variety *Ceres* showed a similar S metabolism in accumulating GSLs in seeds and pod walls like the 0 summer variety *Petranova*. In comparison the 0 winter variety *Jet Neuf* showed a very different S metabolism with a high incorporation rate of GSLs into seeds. The same result was found for changes in the total S content of seeds and pod walls. Again *Jet Neuf* showed a higher capacity to incorporate total S into seeds. The relative daily changes in the total S content were similar for the 00-variety *Ceres* and the 00-varieties *Bronowski*, *Licosmos* and *Topas* with a preferential incorporation of S into the pod walls. *Niklas* was the only 0-variety where a high decrease of GSLs was observed in pod walls which was also accompanied by a high reduction of total S in the pod walls

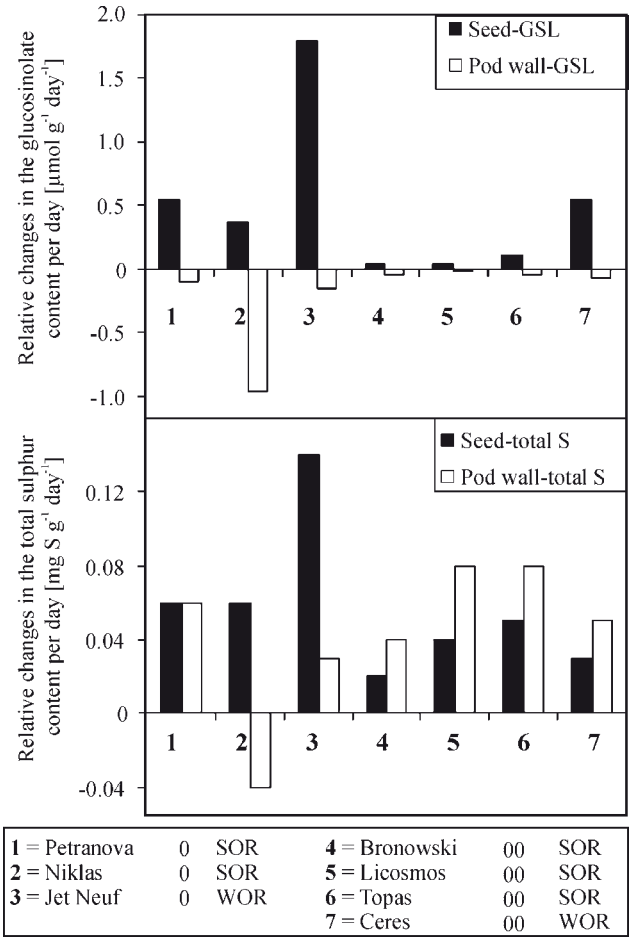

Figure 6:

Relative daily changes in the total glucosinolate and sulphur content of seeds and pod walls of different varieties of summer and winter oilseed rape during pod development (data from plots which were not fertilised with sulphur, SOR = summer oilseed rape, WOR = winter oilseed rape)

over time. The relative daily changes in the GSL and total S content (Figure 6) show very clear differences in the S metabolism of the investigated oilseed rape varieties which are related to their genetic origin. 0-varieties accumulated S in the form of GSLs in the seeds while in 00-varieties S was accumulated in pod walls.

In this study the S supply was varied because of the observation that 00-varieties react earlier to S deficiency than 0-varieties of oilseed rape (Haneklaus and Schnug, 1992) so that differences in the GSL boiosynthesis were expected. The winter varieties showed no symptoms of severe S deficiency but the S contents of the leaves indicated moderate S deficiency when no S was fertilised. The summer varieties that received no S showed visual symptoms of S deficiency but no differences in the expression of S deficiency between 0- and 00-varieties were found. Generally S fertilisation increased the total S content in leaves and pods and also the GSL content in seeds and pod walls. S

fertilisation did not increase the GSL content of seeds of 00-varieties significantly. Such effect was observed, however, under controlled growth conditions and in a great number of field trials (Schnug, 1989).

The fact that under conditions of S starvation 00-varieties react earlier with symptoms of S deficiency than 0-varieties (Haneklaus and Schnug, 1992) suggests that a metabolic block exists in the GSL biosynthesis of 00-varieties which is located prior to the aldoxim synthesis. Intermediary products which are enriched in the pod walls of 00-varieties can not be remobilised by the myrosinase system in a situation of S starvation (Schnug and Haneklaus, 1993) while intact GSLs of 0-varieties can be remobilised. Later these intermediates are metabolised as a consequence of senescence and the sulphate content increased because inorganic sulphate within cell vacuoles is unavailable for phloem transport (Cram, 1990). The immobilisation of sulphate would explain the high accumulation of S in 00-varieties during senescence (Table 2, 3). Also Zhao et al. (1993) could show that the increase of S in the pod walls of 00-variety did not result from a restricted translocation of GSLs since exogenously supplied allylglucosinolates (Sinigrin) were translocated rapidly from the pod walls to the seeds in the 0 as well as in the 00-oilseed rape varieties.

The fact that in the beginning of seed development the 0 and 00-varieties of winter oilseed rape reacted similarly in accumulating GSLs in the seeds which are most likely translocated from the pod walls into the seeds make it difficult to explain the phenomenon of different GSL contents in seeds of 0 and 00-varieties later on during pod development.

High GSL contents in the pod walls of more than 30 µmol g^{-1} (d.w.) were only determined in the 0-winter variety when S was no limiting factor (Table 3) and they were still high at BBCH 83 when the seeds had nearly realised their final GSL content. Under conditions of S deficiency both the seed and pod wall GSL content was highly affected, especially in the 0-variety of winter oilseed rape. With lower GSL contents in the pod walls the seeds were also able to accumulate GSL but it took a longer time and the final GSL content in the 0 winter variety was lower than with S fertilisation.

Moreover 00-varieties of oilseed rape seem to use S less efficiently which is reflected by the lower S contents in vegetative tissue and pods of 00 summer rape varieties compared to 0-varieties in early pod development.

The GSL content is a highly variable parameter which is not only influenced by environmental, agronomic and genetic factors, but to a great extent also by the post-harvest treatment (Bloem et al., 2007). In general, a decrease of the GSL content of green plant parts after harvest is assumed but the data in figure 2 show that higher GSL contents were measured when the pods were separated directly in the field. Freeze-drying of whole pods with subsequent separation led to changes in the GSL content and to a high variation, which could not be explained by seed development. It proved to be necessary to separate seeds from pod walls directly in the field to produce representative results. This procedure is particularly time-consuming during early pod development. In any case, the question remains open which apparently measured changes in S metabolites have been caused by different sampling procedures.

5 Conclusion

In the present study it was demonstrated that differences existed between summer and winter oilseed rape varieties with respect to the pattern of GSL accumulation in 0 and 00-varieties. The 0-varieties of summer oilseed rape showed a higher potential to accumulate GSLs very early in pod development while in case of the investigated winter cultivars the 00-varieties accumulated GSLs over a longer period of pod development. Early in pod development there were no differences in GSL accumulation between 0 and 00 winter varieties and there seemed to be no difference in GSL transport from pod walls to seeds and also no difference in the metabolism of GSLs. Later during pod development the 0-variety was still accumulating GSLs in seeds while the 00-variety had stopped to further accumulate GSLs in seeds but was accumulating S in pod walls. Therefore the difference in GSL accumulation pattern of 0- and 00-varieties is probably related to the developmental stage of the pods. Moreover the different investigated summer and winter oilseed rape varieties showed a different accumulation pattern for GSLs where the 00 winter variety *Ceres* reacted more similar to the 0 summer varieties.

S fertilisation had a strong influence on the GSL content in seeds and the S accumulation in pod walls. As many different factors are affecting the GSL content in pods it would be necessary to investigate also precursors of the GSL biosynthesis in pod walls and seeds to elucidate the question if the increase in total S in pod walls of 00-varieties is caused by an accumulation of anorganic sulphate or if an increasing sulphate content is caused by a degradation of intermediary products of the GSL biosynthesis which are not sequestered to intact GSLs.

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