RELATIONS BETWEEN SULFUR SUPPLY AND GLUCOSINOLATE CONTENT OF 0- AND 00-OILSEED RAPE

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Introduction

Glucosinolates are sulfur containing substances in the secondary plant metabolism, which occurence is typical of Brassica species. In the case of oilseed rape plant breeders succeeded to lower the glucosinolate content up to 80-90% to 15-20 µmol/g dry seed in order to avoid a thyroid effect by use in animal nutrition (MENZEL, 1983). Simultaneously with the introduction of 00-cultivars in agriculture the glucosinolate content has become an important criteria for quality. Therefore it is necessary to know about possible factors influencing the amount of glucosinolates (RÖBBELEN, 1987).
The importance of the sulfur supply on the glucosinolate content has already been stressed by MARQUARD et al. (1968) as well as JOSEFSSON and APPELQVIST (1968); but so far there does not exist an exact quantification of this problem, especially with regard to oilseed rape cultivars poor in glucosinolates. Therefore it has been the aim of the investigations presented to quantify the interactions between sulfur supply on the one hand and glucosinolate content on the other hand.

Materials and Methods

Field surveys and field trials were performed with pure winter oilseed rape cultivars (0-rapeseed cultivars (approx. 100 µmol/g): JET NEUF, MIRANDER; 00-oilseed rape cultivars (approx. 15 µmol/g): RUBIN, CERES, WIEBKE, GLUMANDER). The sites chosen for the experiments showed no growing up of old, single low cultivars except the trial with GLUMANDER in 1986.
Field trials: Raised S-fertilization (0, 50, 100, 150, kg/ha S as K_2SO_4: K-compensation with KCl) in factorial combination with a raised N-fertilization (150, 200, 250 kg/ha N as lime-ammonium-nitrate (26% N); performed on glacial brown earth's: Petersdorf on the Baltic Sea island Fehmarn: 14 % clay, 2.4 % $C_{org.}$, pH $_{(CaCl2)}$ 6.8 and Hohenlieth by Kiel: 12 % clay, 2 % $C_{org.}$, pH $_{(CaCl2)}$ 6.5; size of plots: 50m²; 4-fold repetition.
Pot trials were carried out with spring oilseed rape (0-oilseed rape: NIKLAS; 00-oilseed rape: TOPAS) in sand culture using 5 ltr. MITSCHERLICH-pots (4 plants per pot) in 3-fold repetition. The timing of an optimum sulfur application (150 mg S/pot as K_2SO_4; K-compensation with KCL) was varied; up to this point all pots only received 30 mg S/pot. S-fertilization was done at the following growth stages: germination (seed); start of shooting; shooting; start of flowering of the main brunch ; one week after start of flowering and control with no further S-fertilization. Nutrition with other mineral-elements, lumination, temperature and humidity were adapted to natural conditions as well as the stage of growth.
Sampling: Younger fully differenciated leaves of the upper third were taken at start shooting and seed samples at maturity from field surveys, field and pot trials.
Analytical methods: Sulfur determination in vegetative plant material was conducted by X-ray-fluorescence (SCHNUG, 1984). Determination of glucosinolates in seed: by gaschromatography (THIES, 1978) and by the new X-ray-fluorescence method (SCHNUG and HANEKLAUS, 1987 a-c).
Statistical computation was performed with the SPSS Program System according to NIE et al., 1975.

Results

Relations between the intensity of the sulfur supply and the total glucosinolate content of oilseed rape

The first illustration presents a compilation of the results from field surveys as well as field trials; the corresponding regression equations are put together in table 1. In all cases there is a positive relation between the sulfur content of younger leaves at shooting stage (as a standard of the level of their sulfur supply (SCHNUG et al., 1984)) and the total glucosinolate content of seeds at harvest (fig.1).

Table 1: Regression equations for the relation between total sulfur content of younger, fully differenciated winter oilseed rape leaves at shooting stage and total glucosinolate content of seeds at harvest (Y = total S-content in leaves in ‰; Y = total glucosinolate content of seeds in µmol/g)

cultivar	year	location	n	regression**	r^2(%)
0-cultivars					
Jet-Neuf	1984	Schleswig-Holstein*	52	y = 9.1x + 29.3	53
Mirander	1985	Petersdorf on Fehmarn	48	y = 4.0x + 83.1	50
Mirander	1986	Petersdorf on Fehmarn	48	y = 6.2x + 32.8	50
Jet-Neuf	1986	Hohenlieth by Kiel	48	y = 10.1x + 21.4	60
00-cultivars					
Wiebke	1985	Petersdorf on Fehmarn	48	y = 1.3x + 10.9	39
Glumander	1986	Petersdorf on Fehmarn	48	y = 2.7x + 12.1	52
Ceres	1986	Hohenlieth by Kiel	48	y = 1.4x - 1.5	68
Rubin	1986	Schleswig-Holstein*	54	y = 1.2x - 1.5	49

(* field surveys; ** $p > 0.001$)

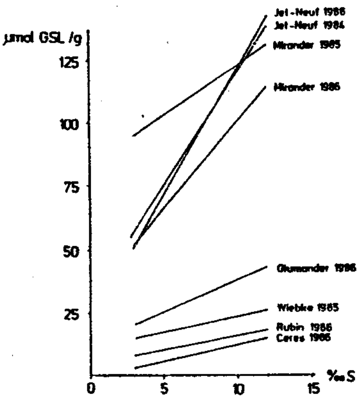

Figure 1: Relations between total sulfur content of younger, fully differenciated winter oilseed rape leaves at shooting stage and the glucosinolate content of seeds at harvest (cp table 1)

On an average the variability of the sulfur supply already explains 50% of the variabilty of the glucosinolate content in seeds at harvest (tab.1). 0-cultivars react more sensitive to a different S-supply, what results in a stronger rise in the glucosinolate content, in comparison with 00-cultivars. Therefore in the trial with GLUMANDER (1986) the rise in the glucosinolate content corresponds to the extent of old, 0-oilsed rape plants (approx. 10%) among the sawn 00-plants (fig.1).
Only the 0-oilseed rape cultivars show different relations between sulfur- and glucosino-

late content: so the cultivar JET NEUF showed a much more steeper rise of the regression function than the cultivar MIRANDER did (tab.1).
On the average of all trials the glucosinolate content in seeds increased up to 7.5 µmol/g with regard to 0-oilseed rape cultivars respectively 1.5 µmol/g dry seed in 00- cultivars, if the sulfur content of leaves increases by 0.1% S.

Influence of temporal variations in sulfur supply on the total glucosinolate content of oilseed rape

The striking difference between the different years is the varying level of the glucosinolate content (fig. 1). So the glucosinolate content of the variety MIRANDER alters 30 µmol/g between 1985 and 1986 on Fehmarn. The reasonable explanation for this occurence reveal the results from the experiments with an altered S-supply (fig. 2).

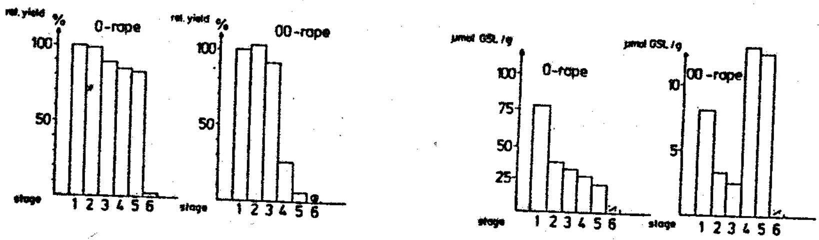

Figure 2: Relative yield of seeds and total glucosinolate content of spring oilseed rape in dependence of the optimum timing of sulfur supply (0-oilseed rape: NIKLAS; 00-oilseed rape: TOPAS; fertilization of 30 mg S per 4 plants, additional 150 mg S per 4 plants at the following stages: 1.: germination (seed), 2.: start of shooting, 3.: shooting, 4.: start of flowering of the main brunch, 5.: one week after start of flowering, 6.: without additional S-supply; 100% yield = 15.7 g seeds/pot)

These trials reveal that a scanty sulfur supply until shooting has no immense influence on yield of oilseed rape, but a later timing leads to drastic losses of yields (fig. 2.). The glucosinolate content is in so far influenced as variations with an insufficient S-supply in comparison with those that received the fully S-amount at seed show a lower glucosinolate content by equal yield level (fig. 2: stage 2 and 3).

Discussion

The sulfur supply of oilseed rape is one of the most important environmental factors influencing the glucosinolate content of rape seeds (JOSEFSSON and APPELQVIST, 1968; JOSEFSSON, 1970). The quantification of this interaction has become of special interest as in future only 00-oilseed rape will be cultivated and for this reason the economic success will depend on quality as well as quantity of oilseed rape seeds.
The results presented reveal that the glucosinolate content is in proper proportion to the S-status of the vegetative plant material (fig. 1) and moreover 39-68% of the variabilty of this feature accounts for the different sulfur status of the plants (table 1). Therefore a high sulfur supply may result in glucosinolate contents that exceed those of the employed seed, vice versa a scanty sulfur supply may produce glucosinolate contents which are much lower than the original value (fig. 1). The latter phenomenon can be attributed to an increase in the activity of the glucosinolate splitting enzyme myrosinase by sulfur deficiency (UNDERHILL, 1980). This enzyme not only cuts glucose, but severs in a second step sulfate from the glucosinolate molecule, which then again is available for the sulfur metabolism of the plant. One important evidence for this mechanism is the fact that in all trials shown in fig. 1 no significant change in the protein fraction was found. This process of an enzymatic remobilisation of sulfate leads to the conclusion that glucosinolates, among others, present a reserve fraction for sulfur in plant metabolism.

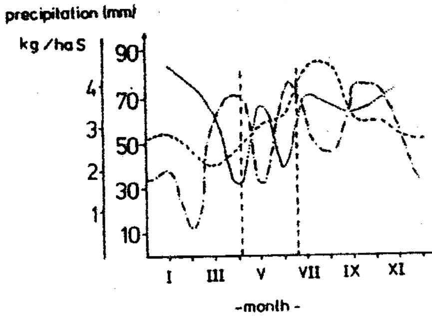

Figure 3: Monthly averages of amount of precipitation and atmospheric sulfur deposition in Petersdorf on Fehmarn in 1985 and 1986 (____ = longterm grand mean ; ···· = 1985; -·-· = 1986; vertical lines mark out the main growing season)

The logical connection between the results from fig. 1 and 2 could be seen in a time dependant variability of the sulfur supply: The natural sulfur supply in Schleswig-Holstein is mainly based on precipitation, the deposited S-amount being proportional to the quantity of precipitation (SCHNUG and HOLZ, 1987).
As the distribution of precipitations varies between different years, S-supply and demand may differ greatly. The trial Petersdorf (Fehmarn) is a good example of the differing distribution of precipitations between 1985 and 1986 (fig. 3). In 1986 the maximum of precipitations during maximum growth delayed 14 days in comparison with 1985. Referring to the results presented above this seems to be the reason for the lower glucosionlate contents in 1986 (fig. 1).
Differences in the sulfur supply of agricultural soils are based on local (e.g. distance to SO_2-emittands (SAUERBECK, 1983) and technical factors of production (e.g. use of manure or sulfur containing fertilizers) as well as amount and distribution of precipitation (SCHNUG and HOLZ, 1987) Therefore the variability of the glucosinolate content of 00-oilseed rape, as it could be observed so far under controlled cultivation of 00-oilseed rape (RÖBBELEN, 1987), can be explained to a great extend by the extraordinary high regional variability of the sulfur supply of soils in the FRG.
The yield limiting S-content of young oilseed rape leaves at shooting stage amounts to 0.65% S (SCHNUG et al., 1984), whereas the glucosinolate content increases with higher S-contents (fig 1.; cp MARQUARD et al.,1968). Sulfur-fertilization on areas with an insufficient sulfur supply requires an exact diagnosis of the nutritional S-status of the vegetative plant material in order to adapt the amount of a necessary sulfur-fertilization to the diverse criteria of yield niveau on the one hand and quality on the other hand.
The fact the that glucosinolate content of 0-oilseed rape increases much stronger with sulfur fertilization than 00-oilseed rape does, is important for those areas where many old, 0-oilseed rape plants grow up among the 00-oilseed rape plants sawn, because in this case the critical value for a sufficient quality can be exeeded easily. In contrast, on areas with 00-oilseed rape throughout a sulfur fertilization of 75 kg/haS, as it is necessary to raise the sulfur content in leaves from 0.5 to 0.65% S, lifts up the glucosinolate content in seeds only 2.4 µmol /g . This means that there is no serious risk for the quality of the harvest if the glucosinolate content of the seed is guaranteed to be less than 17 µmol/g by the plant breeder.

Summary

The influence of amount and timing of a various sulfur supply on the total glucosinolate content of 0- and 00-oilseed rape was investigated by means of field surveys as well as field and pot trials.
In all trials the total glucosinolate content in seeds at harvest corresponded to the level of S-supply, measured as the total S-content of younger fully differenciated leaves at start shooting. The variability of the sulfur supply already explaines about 50 % of

the variability of the glucosinolate content in seeds. A raised sulfur supply results in a higher raise of the glucosinolate content of 0-oilseed rape than 00-oilseed rape. An elevation of the sulfur content in leaves of 0.1% on average brought about an increase in the glucosinolate content of seeds of 7.5 µmol/g for 0-oilseed rape respectively 1.5 µmol/g for 00-oilseed rape. A temporal delay of sulfur application until shooting stage resulted in 70% lower glucosinolate contents in both, 0- and 00-oilseed rape in comparison with an optimum S-supply at germination (seed) at an equal yield level.
Therefore the sulfur supply is the most important environmental factor with regard to the glucosinolate content in oilseed rape, which regional and technical variability immensely influences the production of a high quality of oilseed rape.

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