Regulation of glucosinolate biosynthesis in oilseed rape by nutritional factors

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Summary

Among nutritional factors, sulfphur has been found to be the most important parameter governing the glucosinolate content in vegetative and generative tissue of a rapeseed crop, which results in a very close relation between total sulphur concentration in photosynthetic active tissue of oilseed rape and the glucosinolate content. In contrast, there is no direct influence of nitrogen or any other essential mineral nutrient on glucosinolate biosynthesis. All effects following variations of nitrogen or any other nutrients supply are due to shifts in source/sink relations for sulphur containing compounds.

Introduction

Since glucosinolates have gained interest in nutritional sciences, several factors influencing the variability of concentrations in vegetative and generative tissue have been discussed (Buchner, 1988 ; Daun, 1986 ; Kallweit and Schnug, 1988 ; Schnug, 1988b and 1990a). In extended experimental studies on the effect of the nutrition with mineral elements (N, P, S, K, Ca, Mg, Fe, Mn, Zn, Cu, Cl, B, Mo) nutrition on glucosinolate concentrations in generative and vegetative tissue of Brassica species was investigated by Schnug (1988a and unpublished data). These experiments were conducted under field and greenhouse conditions checking basic relations between nutritional status and glucosinolate concentrations as well as the influence of increased fertilizer rate applications over the nutritive range from starvation to surplus.

Although some information from literature exists on the influence of boron (Bible et al., 1981 ; Ju et al., 1982) and magnesium, manganese, copper, zinc compounds on the activity of enzymes involved in the glucosinolate metabolism, significant direct effects of changes in the supply were found only with sulphur. For nitrogen and all other mineral elements, relations between supply and glucosinolate concentrations have been found to be due to indirect effects via modifications in source/sink relationships.

This paper will outline the direct effects of sulphur on glucosinolate biosynthesis and indirect effects of other mineral nutrients exemplary of nitrogen.

Sulphur

Among nutritional factors, sulphur has been found to be the most important factor governing the glucosinolate content in vegetative and generative tissue of a rapeseed crop which results in a very close relation between total sulphur concentration in photosynthetic active tissue of oilseed rape and the glucosinolate content (Schnug, 1990a and 1991a) ; Schnug and Haneklaus, 1993b).

The common understanding of the physiological role of secondary compounds implies that they have no physiological function for the maintenance of life. One of the most exciting findings in recent agroecological research, however, is the detection of the biorecyclisation of glucosinolates through which

they provide the plant with a sulphur storage that can be utilised for the synthesis of primary products in the event of sulphur starvation (Schnug, 1988a). After enzymatic cleavage, glucosinolates yield thiocyanates and sulphate (Srivatava & Hill, 1974), which are precursors for the biosynthesis of amino acids (Fig.1 ; Machev & Schraudolf, 1977 & 1978 ; Marquard et al., 1968.) Myrosinase has been localised in "myrosin cells" called idioblasts (Bones & Iversen, 1985 ; Iversen et al., 1979 ; Lüthy & Matile, 1984 ; Tangstad et al., 1990). However,

myrosinase activity in intact plants results in the occurrence of isothiocyanates among the headspace volatiles of Brassica napus (Seaton et al., 1990 ; Tollsten & Bergström, 1988). The very fast reaction of glucosinolate concentrations in vegetative tissue in response to changes in the sulphur supply (Schnug, 1990a) also contributes to the evidence that in vegetative tissue of Brassica species, glucosinolates and myrosinase are not totally compartmentalised in the vegetative tissue (see also : Höglund et.al., 1991; Iversen, 1970 ; Reiner, 1987).

Fig. 1 Biosynthesis and biorecyclisation of alkenyl-glucosinolates in Brassica napus.


Schnug, 1992
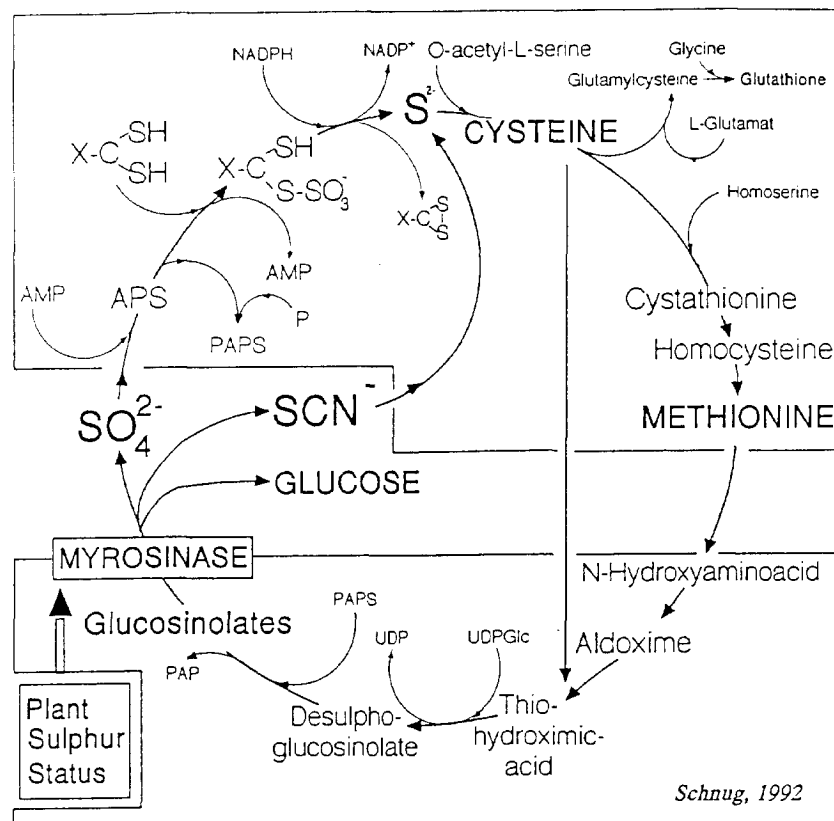
A proposed way for the regulation of the glucosinolate/myrosinase system, which also explains why compartmentalisation of glucosinolate and myrosinase in vegetative tissue is not necessary, is given in Fig. 2. The activity of the enzyme myrosinase is governed by the concentrations on a mmol level (Horbowicz & Bakowski, 1988 ; Reiner, 1987) and thus, in the range where increasing ascorbate concentrations are related to decreasing myrosinase activities (Björkman, 1976). In Brassica seeds, however, ascorbate concentrations are too low to inhibit myrosinase activity. The fact that total glucosinolate concentrations in seeds and the

pattern of individual glucosinolates in seeds are very stable within time (Schnug & Haneklaus, 1988 & 1991 ; Schnug et al., 1992) proves that there is no myrosinase activity outside the myrosin cells in seeds.

Again, the ascorbic acid concentrations are dependent upon the regeneration of dehydro ascorbate in the ascorbate-glutathione cycle (box in middle of Fig. 2 ; Halliwell & Gutteridge, 1989). The link with the sulphur nutritional status is evident from a strong dependency of glutathione levels on the external sulphur supply (upper box in Fig. 2 ; de Kok et al., 1981).

Fig. 2. Regulation of the biorecyclisation of glucosinolates

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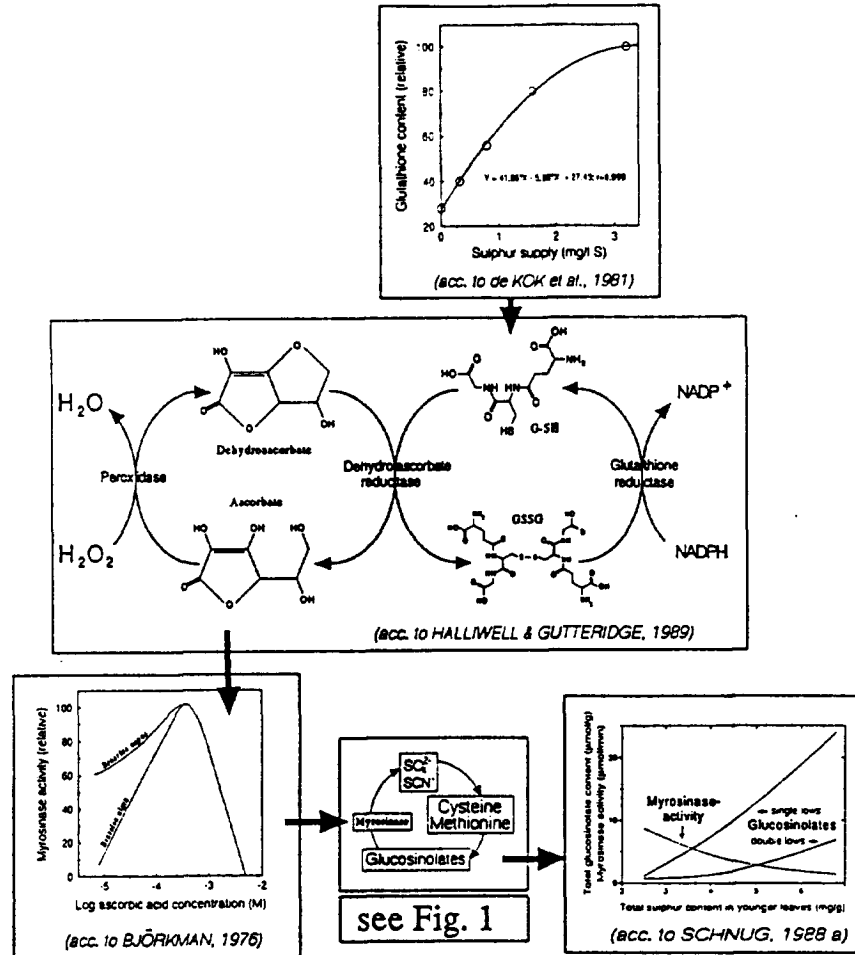
This model for regulation explains some of the glucosinolate related phenomena affecting plant resistance against pests and diseases (Ernst, 1990; Schnug & Ceynowa, 1990). The biologically active part of glucosinolates is the aglucone which is released from the molecule by enzyme activity. Wounding or infestation of vegetative tissue of Brassica napus results in higher oxidation potential in the tissue causing increased consumption of ascorbate (Halliwell & Gutteridge, 1989), followed by increased myrosinase activity resulting again in a decrease of the amount of intact alkenyl-glucosinolates (Koritsas et al., 1991).

The sulphur supply dependent on the biorecyclisation of glucosinolates is a fundamental part of the ecological survival strategy of Brassica species since the dissemination of these plants relies on a large number of small seeds with high concentrations of sulphur containing amino acids. Thus, these plants have an extraordinary high demand for sulphur during seed production. The above ground biomass of a rapeseed crop yielding at 4 t/ha of seed takes up 40 kg /ha sulphur in the

first month after the start of growing (Schnug, 1988 ; Schnug & Haneklaus, 1993b ; Zhao et al., 1993b). This is more than three times the amount of a high yielding cereal crop or the actual average annual supply by atmospheric deposition in the areas of Northern Europe (Schnug & Evans, 1992 ; Schnug, 1991b).

The poor performance of low glucosinolate containing Brassica napus breeds (Schnug, 1990a & 1991a) in low sulphur input agroecosystems -evident from visible symptoms of severe sulphur starvation being the most widespread nutrient deficiency in Brassica species (Haneklaus & Schnug, 1992c) - is an impressive proof of the importance of the glucosinolate biorecyclisation in affecting plant vigour.

The physiological background to this phenomenon is the fact that in the alkenyl- and sulphinyl-glucosinolate pathway of double low cultivar, a metabolic block exists prior to the formation of the aldoxim (Fig. 1.). The myrosinase system has no access to these intermediary compounds and a remobilisation of the sulphur into basic compounds is disabled.

In the case of generative parts of Brassica napus, these intermediary compounds are enriched in the pod walls during seed development, because they are not transported from the place of synthesis, which is the pod wall, into the seed. Later, as a consequence of senescence, these intermediates are susceptible for an oxidative cleavage which also results in enrichments of sulphate as an unspecific secondary process (Table 1). As an artifact, this sulphate has been misinterpreted as the final product of the glucosinolate pathway in double lows (Zhao et al., 1993 a & b) secondary process. However, the clear difference in the utilisation efficiency between single- and double low varieities of Brassica napus (Schnug & Haneklaus, 1993, a & b) proves that sulphate cannot be the final product (Zhao et al., 1993a) of the disordered biochemical pathway of double lows. The sulphate found in mature pod walls is not usable for the organism as seed filling has finished and local protein biosynthesis is stopped at that stage of development. Under field conditions, this sulphate is often leached from the senescent pod material. In these cases, even lower total sulphur concentrations may be found in pod walls of double lows (Luedeke, 1990).

Because the glucosinolate metabolism is connected to the biosynthesis of amino acids, and all precursors for protein biosynthesis in the seeds are provided by the pod wall, there is no de novo synthesis of at least alkenyl glucosinolates in the seeds. Therefore, the transport of glucosinolates into the seeds is related to the physiological activity of the pod and thus directly related to dry matter accumulation in the seed. Thus, the total glucosinolate content of the seeds is governed at least by two major factors : the sulphur nutritional status of the photosynthetic active pod wall tissue and the dry matter production rate.

Table 1. Sulphur fractions in mature single- and double low Brassica napus seeds and podwalls (75 days after flowering) as influenced by the sulphur nutritional level (Schnug, 1988a).

| S-supply (mg S/pot) je Gef.)	---- S E E D S ----		-------------- P O D W A L L S --------------				sulphur in seeds and podwalls
	protein-sulphur (%)	glucosi-nolates (mol/g)	total sulphur (%)	SO4- sulphur (%)	glucosi-nolates (mol/g)	inter-mediary sulphur	
			Single Low Variety NIKLAS				
60	0.205	36.7	0.08	<0.01	<0.5	<0.01	0.57
120	0.257	61.5	0.13	0.02	2.1	<0.01	0.86
220	0.293	96.9	0.17	0.03	4.7	<0.01	1.18
280	0.292	119.9	0.19	0.06	5.3	<0.01	1.34
			Double Low Variety TOPAS				
60	0.203	6.6	0.30	<0.01	<0.5	0.23	0.57
120	0.253	13.3	0.47	0.07	0.9	0.29	0.87
220	0.293	20.8	0.71	0.14	1.4	0.46	1.13
280	0.296	24.8	0.83	0.17	2.2	0.55	1.28
LSD 5%	0.012	3.4	0.08	0.03	0.5		

Nitrogen and other essential mineral nutrients

Compared to the specific effects of sulphur on the glucosinolate content, the influence of variations in the supply with all other mineral nutrients is due to a change of the source/sink relationships for metabolised sulphur.

The way in which nitrogen and any other mineral nutrient act on the total glucosinolate content in dependent upon the nutritional nitrogen and sulphur status of the crop at the beginning of a nutritional trial (Table 2) and should be clearly evaluated before statements are made concerning experimental results (Janzen and Bettany, 1984).

The following explanations are given for nitrogen but are also valid for other mineral nutrients. Nitrogen fertilisation to plants which are insufficiently supplied with nitrogen and sulphur will lead to decreasing glucosinolate content, because the demand of an increasing sink due to increasing numbers of seeds produced by more nitrogen cannot be satisfied by a limited sulphur source, except in those cases in which the extent of the root system is increased by an enhanced nitrogen supply and the capacity of the sulphur source is enlarged by this way. Fig. 3 demonstrates this, outlined by the results of a pot experiment with limited nitrogen and sulphur supply.
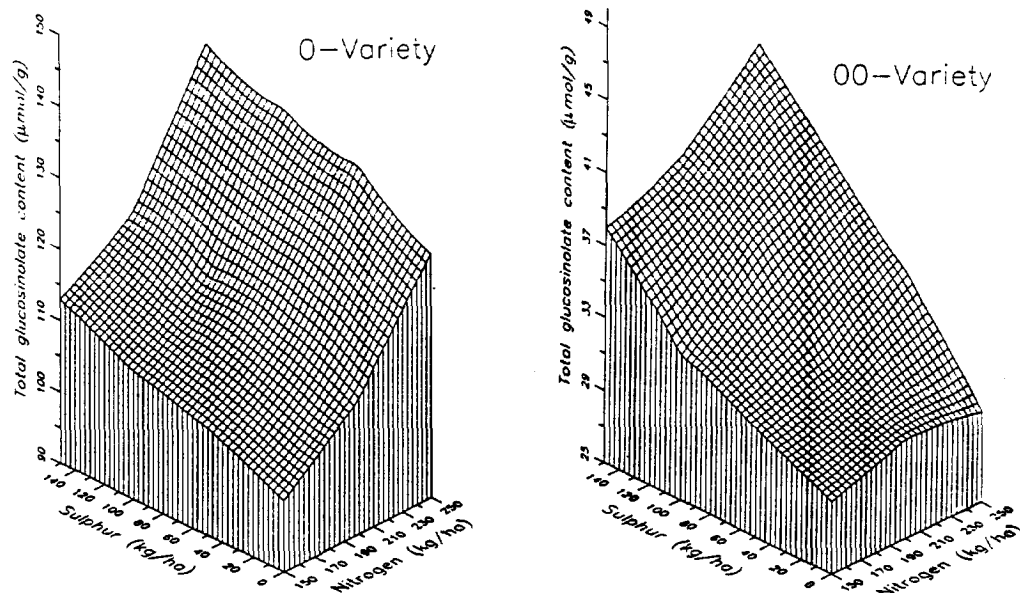
Fig. 3. Total glucosinolate concentrations in single low oilseed rape seeds (NIKLAS) as influenced by nitrogen and sulphur supplies in a pot trial (Schnug, 1988).
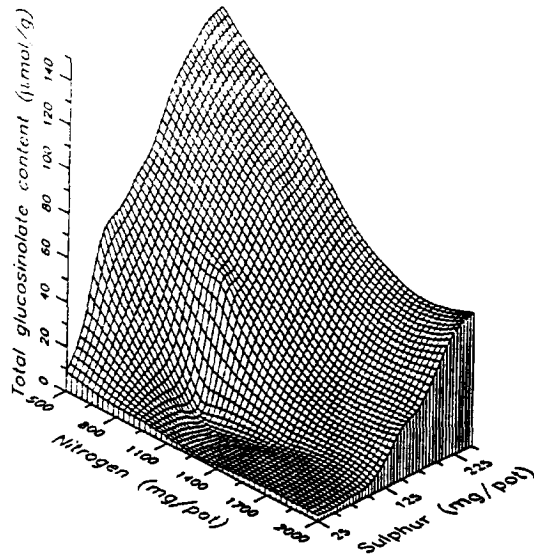
In comparison with pot trials under field conditions, the capacity of the sulphur source is enlarged by an extended root system and the glucosinolate content increases after enhanced mineral nutrient supply (Fig. 4). For single low cultivars, this relationship is much stronger than for double lows due to the biorecyclisation of glucosinolates in the range of low sulphur supply.

In the case of a crop already supplied sufficiently with mineral nutrients, there is no evidence for any specific N/S interactions on the glucosinolate content.

Fig. 4. Total glucosinolate concentrations in single- and double low oilseed rapeseeds (MIRANDER and WIEBKE) as influenced by nitrogen and sulphur supply in a field trial conducted on a brown earth in Northern Germany (Schnug, 1988).

Conclusions

The nutritional status of Brassica crops explains a significant part of the variability observed in total glucosinolate concentration of the seeds (Schnug, 1989). Among essential mineral elements, sulphur has been found to be the most important parameter governing the glucosinolate content in vegetative and generative tissues of a rapeseed crop. In contrast, there is no direct influence of nitrogen or any other essential mineral nutrient on glucosinolate biosynthesis. All effects following variations in

nitrogen or any other nutrient supplies are due to shifts in source/sink relations for sulphur containing compounds.

As the optimisation of mineral nutrition is primarily focused on a maximised utilisation of fertiliser nutrients in the frame of monetary values for inputs and seed yield and the seed glucosinolate concentrations are of no real commercial importance, there is little change to maintain glucosinolate concentrations by fertiliser practices.

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