SULPHUR METABOLISM IN OILSEED RAPE PLANTS WITH PARTICULAR REFERENCE TO DOUBLE LOW VARIETIES

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INTRODUCTION

Due to the high protein content of seeds which contain high amounts of sulphur (S) containing aminoacids oilseed rape is a crop with an extraordinarily high S demand. One of the most impressive observations during the introduction of double low oilseed rape varieties into practical cropping was that a direct relationship exists between the genetically predetermined glucosinolate content and sensitivity of varieties to S deficiency in that double low varieties show symptoms of severe S deficiency earlier than single lows and moreover the reaction of yields to a decreasing S supply is stronger in double low varieties. Although there is still a lot of research work needed to improve the knowledge of biochemical details, this contribution will try to introduce to that fascinating field of plant physiology and present an overview concerning the recent understanding of the role of S in the oilseed rape crop.

METABOLISM OF PRIMARY SULPHUR COMPOUNDS

The first step of the S metabolism in higher plants is characterised by the activation of sulphate in the reaction with adenosine-phosphates and the reductive incorporation into organic compounds. Catalysed by a sulphurylase system sulphate reacts with ATP (adenosine 5'-triphosphate) to APS (adenosylphosphosulphate) which again is activated by phosporylation to PAPS (3'-phosphoadenosine 5'phosphosulphate). PAPS plays an important role during the biosynthesis of sulpholipides and sulphateesters like glucosinolates. Following this initial steps APS transfers the oxidised S to a H-carrying tripeptide (probably glutathione) from which, catalysed by ferredoxin, sulphide is released. Liberating acetate acetylserine reacts with the sulphide to prone cysteine. Cysteine is the first stable organic compound in which the S taken up by roots or leaves appears in the plant metabolism. All reduced S containing compounds in the primary (e.g. methionine or gluthathione) and the so called 'secondary' (e.g. glucosinolates or alliins) metabolism at least derive from cysteine.

Increasing the S supply in the growth media results in a linear increase of the total S concentrations in vegetative parts of *Brassica napus*. When growing four rapeseed plants in 5 kg substrate (closed system) according to Schnug (1988) the total S concentration in fully differentiated leaves at beginning of stem elongation (Y) is a closely correlated ($r = 0.981$) function of the amount of sulphate-S applied to the seeds (X) described by the algorithm: $Y = 0.14*X+0.71$. This relationship is linear between sulphur concentrations of 0.1 and 1 % total sulphur in the leaves. This linearity casts

substantial doubts on the evidence for a feed back of higher S concentrations to S uptake (Cram, 1990) in *Brassica* species. Data from field experiments or pot experiments carried out in open systems sometimes show no or a decreased S uptake with increasing S supply (e.g. Evans et al. 1991) and might lead to the idea of such a feed back. However, this effect is related to factors affecting the transport of fertilizer S into and throughout the active root zone and not to a decreasing uptake of S by the roots due to a feed back from high S concentrations in plant tissue.

An increase in the total S concentrations in vegetative tissue of *Brassica* corresponds with increasing amounts of S containing aminoacids only up to the threshold below which visible symptoms of S deficiency can be expected (fig . 1; and Schnug, 1990). According to Schnug 1988 symptoms S of deficiency will appear in rapeseed plants when the total S concentration in fully differentiated leaves drop below 0.3% in single- and 0.35% S in double low varieties respectively.

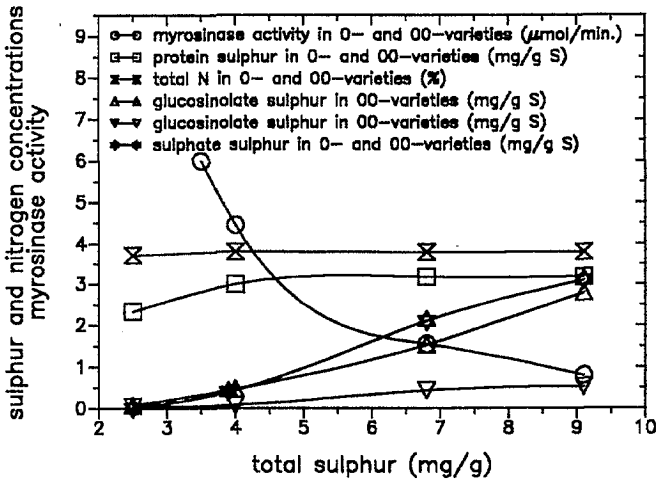

Figure 1. Relations between total S and individual S compounds and myrosinase activity in younger, fully differentiated leaves of single- and double-low oilseed rape (Schnug, 1988).

Above this content the increasing supply only increases the content of free sulphate and glucosinolate S (fig. 1).

METABOLISM OF GLUCOSINOLATES

Glucosinolates are typical S containing products occurring in all *Brassica* species. The biosynthesis of glucosinolates is briefly described in the following chapter, for references of certain steps one may refer to Schnug, 1990 b. Starting up with an α-aminoacid (eg. methionine in case of alkenyl-, thio-, sulfinyl- and sulfonylglucosinolates; tryptophan in case of indolglucosinolates) the first stable products in this pathway are hydroxylated amino acids, which are the precursors of aldoximes. In the next step the thiol group (-SH) of cysteine is transferred to aldoxime, synthesising a thiohydroximic acid. Catalysed by thiohydroximate-glucosyltransferase the addition of ß-glucose leads to desulfoglucosinolates. After transfer of sulphate from PAPS by a sulfotransferase glucosinolates derive. From this basic structure different glucosino-

lates are derived by action of specific enzymes through elongation and hydroxylation of the side chains.

In double low varieties the low glucosinolate content appears to be induced by a metabolic block before synthesis of 5'-methylthiopentaldoxime. However, the chemical structure of the remaining intermediary products are unknown so far. Sulphate uptake, amino acid pattern and myrosinase activity remained unaffected by genetic differences (fig. 1).

De novo synthesis of glucosinolates take part in all vegetative tissues of *Brassica* species, but not in the seed itself. All alkenylglucosinolates occurring in *Brassica* seeds are synthesised in the pod walls and then transported into the seeds. This can be explained by the fact that also aminoacids and proteins needed to establish the seedling are also provided by the pod wall (Norton and Harris, 1975) and S containing aminoacids are again the precursors of glucosinolates. The transport of glucosinolates is related to the physiological activity of the pod and thus directly related to dry matter accumulation in the seed.

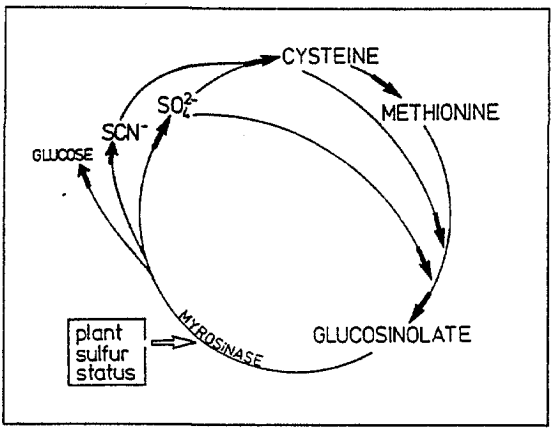

Figure 2. Biochemical pathway for the remobilisation of glucosinolate bound sulphur in *Brassica* species (schematic).

The transport rates, however, depend upon the amounts of glucosinolates provided by the pod wall tissue. During very early stages of development the rates are fairly low because there is a strong sink for S in aminoacid synthesis in order to keep up with the demands of the establishing seedling. This probably leads to increased myrosinase activity in the tissue which again keeps the amount of intact glucosinolates (this mechanism is explained further down) and thus the transport rates into the seed down. After the seed is established and this sink disappears glucosinolates remain stable and their transport rates are proportional to dry matter accumulations in the seed (Bilsborrow, 1991).

The mechanism of the glucosinolate transport into seeds is obviously specific for intact glucosinolates, so that intermediate products, occurring in *Brassica* species with a genetically low glucosinolate content can't enter seeds. Thus pods of double low varieties contain higher sulphur concentrations than pods of single low varieties, whereas the total S uptake remains the same in both variety types (Schnug, 1988 & 1989).

The thioglucosid glucohydrolase 'myrosinase' hydrolyses the ß-glucosidic link between glucose and reduced S atom. The

aglucone decomposes in weakly acid conditions to sulphate, and isothiocyanate results from Lossen-rearrangement. Myrosinase occurs in tissues, either isolated in idioblasts, stomatal guard cells or associated to cisterns of the endoplasmatic reticulum or mitochondria. Whereas isolated in cellular structures the myrosinase activity depends on mechanical injury of the plant tissue, in all other cases an endogenous regulation of myrosinase activity is possible. However, there is surely more comprehensive research work needed to improve the understanding of the localisation of substrate and enzyme as well as the mechanisms governing the spatial and biochemical turnover. One important factor regulating the enzyme activity is the S status of the plants: a decreasing sulfur supply to the plant results in a decrease in free sulfate and glucosinolate concentrations and an increase in myrosinase activity (fig. 1). This implies that the increase in myrosinase activity during sulfur stress has the function of a remobilization of sulfate sulfur from glucosinolates because sulfate and isothiocyanates can be utilised as S sources in the primary metabolism of the plants (fig. 2).

According to this the role of glucosinolates in plant metabolism seems not to be restricted to biological interactions (Schnug and Ceynowa, 1990), but they also act as a vital storage for S. The importance of this function is stressed by the fact that there is only a slow turnover of sulphate in leaf vacuoles which limits the retranslocation of sulphate under sulphur stress (Bell et al., 1990).

Figure 2. Double low variety (left, showing S deficiency symptoms) and a single low volunteer plant (right, healthy appearance from a sulphur deficient oilseed rape field in Northern Germany.

One of the most impressive observations during the introduction of double low oilseed rape varieties into practical cropping was, that there exists a direct relationship between the genetically predetermined glucosinolate content and sensitivity of varieties to S deficiency in the way that double low varieties react more sensitively to S starvation in the fields (fig. 3) and also the reaction of yields to a low S supply is stronger in double low varieties (Schnug, 1988 & 1989).

The S nutritional status of a rapeseed crop has been found to be the most important factor governing the glucosinolate content in vegetative and generative tissue. Comprehensive studies have shown that the importance of the S nutritional status for the glucosinolate content of rapeseeds is approxi-

mately three times higher than varietal effects (Schnug, 1989). Thus very close linear relations between total sulfur concentration in younger fully differentiated leaves of *Brassica* napus (as an indicator of the nutritional status (Haneklaus and Schnug, 1991); X in % total S) and glucosinolate content (Y in µmol/g total glucosinolate) exist: younger leaves: single low varieties: Y=5.63*X-11.3 r=0.929; double low varieties: Y=0.94*X-0.70 r=0.914; seeds: single low varieties: Y=8.3*X+37 r=0.747; double low varieties: Y=1.2*X+12 r=0.701 (Schnug, 1988).

Compared to the distinct effects of S on the glucosinolate content reports about interactions with nitrogen (N) are quite different (e.g. Bilsborrow et al., 1991; Janzen and Bettany, 1984; Schnug, 1988). The way in which N effects the total glucosinolate content is, however, dependand upon the nutritional N and S status of the crop at the beginning of a fertilizer trial (tab. 1) and should be clearly evaluated before statements are made concerning experimental.

Table 1. Effects of increasing N applications on the total glucosinolate content in rapeseeds depending upon N and S status of the plants (for further details refer to the text).

	nitrogen status: insufficient	nitrogen status: sufficient
insufficient S status	A:↓; B:=	=
sufficient S status	↑	=

N fertilization to in which N and S is insufficient will lead to decreasing glucosinolate content because the demand of an increasing sink due to increasing numbers of seeds can't be meet by the limited S source (tab. 1 'A') except in those cases in which the extent of the root system is increased by the enhanced N supply and thus the capacity of the S source is enlarged (tab. 1 'B'). Increasing glucosinolate concentrations in the seeds can be expected after N applications to N demanding crops grown without S starvation due to increased biosynthesis of S containing aminoacids which are again precursors of glucosinolates (see above). In case of a crop already supplied sufficiently with N there is no evidence for any specific N/S interactions on the glucosinolate content.

CONCLUSION

Glucosinolates in oilseed rape should not only be seen as secondary compounds which enable the plant to 'communicate' with it's environment. Moreover they are an essential source of S to meet the high S demand which can be utilised more effectively by the plant than sulphate. By breeding for lowered glucosinolate content in double lows, the efficient use of S by *Brassica* species has been lowered along with their natural vigour. This is supported by the fact that the feature of low glucosinolate content was found only in the variety 'Bronowsky' which shows less efficient use of S and reduced strength. It would be interesting to confirm that 'Bronowsky' was originally found in an environmen with a significantly

higher S balance. It should be stressed that the less efficient use of S by double low varieties has increased the problems of S nutrition in a crop which is already receiving reduced atmospheric S inputs into soils.

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