Recent advances in glucosinolate research

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Abstract

This contribution will review recent advantages in glucosinolate research based on own research projects and literature data. Glucosinolates were investigated comprehensively in the early 1980s when high glucosinolate contents in rapeseed meal caused problems in animal nutrition and double low oilseed rape varieties were introduced. Since then the focus of research has changed in such way that nowadays metabolism and transport of glucosinolates, ecological relevance of glucosinolates and practical applications in bio-fumigation and use as health promoting substances are the main topics of interest.

Investigations were undertaken to resolve the metabolic background of lower glucosinolate contents in double low oilseed rape varieties in comparison to single lows. Earlier studies have already shown that double low varieties accumulate sulphur in pod walls and it was proposed that a metabolic block exists which led to the accumulation of intermediary products of the glucosinolates biosynthesis which could not be transported into seeds. Recently conducted field trials support this hypothesis. Double low varieties showed distinctly higher total sulphur contents in pod walls at seed maturity than single lows where a higher proportion of sulphur was bound in seeds. During pod development an early accumulation of glucosinolates in seeds took place. Only low glucosinolate contents of <2.5 µmol g^{-1} d.w. could be determined in pod walls of single low varieties. In double low varieties the glucosinolate content in pod walls decreased from more than 30 µmol g^{-1} d.w. at the beginning of pod development to about 1µmol g^{-1} at seed maturity.

Bio-fumigation as a technique to reduce biologically soil-borne infestations with nematodes and fungi is discussed as well as effects of glucosinolates on different insect populations in relation to the sulphur nutritional status.

Health promoting effects of glucosinolates were investigated in experiments with piglets where seeds of *Tropaeolum majus* were fed in different amounts and growth parameters such as animal weight and fodder intake, chemical data like the isothiocyanate content and pH in urine, and microbial investigations of the excrements were recorded. In human nutrition efforts were undertaken to enrich the glucoraphanine and sulphoraphane content in *Brassica oleracea* which are supposedly have a highly anti-carcinogenic potential.

Key words: single/double low oilseed rape, pod wall, biofumigation, health promoting effect

Introduction

In the 1980s and 1990s research in the field of glucosinolates (GSL) focused on the biosynthesis and breeding of varieties, which were low in seed GSLs in order to avoid a thyroid effect when using rapeseed meal in animal nutrition. Besides environmental and agronomic factors, the physiological functions of GSLs were investigated comprehensively (Schnug, 1990, 1993). Since the introduction of double low oilseed rape varieties in the 80´s the physiological background of a lower GSL content in seeds of double low varieties is still unknown. Single low and double low oilseed rape varieties contain similar amounts of total S in vegetative plant parts, while seeds show significantly lower S and GSL contents (Fieldsend and Milford, 1994). There exist opposing theories how these differences can be explained. It is commonly accepted that the seed-GSLs are mainly synthesized in the pod walls (De March et al., 1989). The lower GSL content in double low varieties is explained either by a metabolic block in GSL biosynthesis or by a reduced transport of GSLs or intermediary products of GSL biosynthesis into seeds.

Today the main focus in GSL research is put on application aspects. Efforts are undertaken to take advantage of the biocidal effect of GSLs in agriculture. "Biofumigation" is a term which refers to the release of biocidal compounds from GSL-containing crops during green manure, crop rotation or direct application for example as pellets, which aims at a suppression of soil-borne pests and pathogens (Sarwar et al., 1998; Lazzeri et al., 2004). Additionally research is carried out on the health promoting effects of GSLs and so-called nutraceuticals which contain high contents of health promoting substances (Mithen et al., 2000; Moreno et al., 2006). It is the aim of this contribution to summarize recent advances in GSL research.

Glucosinolates in developing seeds and pod walls of single low and double low oilseed rape varieties

An investigation of the changes in total sulfur (S), sulfate-S and GSL contents of developing seeds and pod walls of a single low and double low winter oilseed rape variety provided new insight into GSL biosynthesis of oilseed rape (Bloem et al., 2007). At the very start of pod development, differences in S accumulation in whole pods were not significant between the two varieties, but already at BBCH 73 a higher proportion of S was bound in seeds of the single low variety (*Jet Neuf*), while in the double low variety (*Ceres*) a higher proportion was bound in pod walls (Fig. 1). Nevertheless it could be shown that no differences in GSL biosynthesis could be observed until BBCH 77. Afterwards a strong increase in seed GSL occurred in the single low variety, while the double low variety remained stable with respect to seed GSLs and free sulfate accumulated in pod walls. As a result the single low variety bonded the same amount of total S in seeds at maturity as the double low variety did in the pod walls.

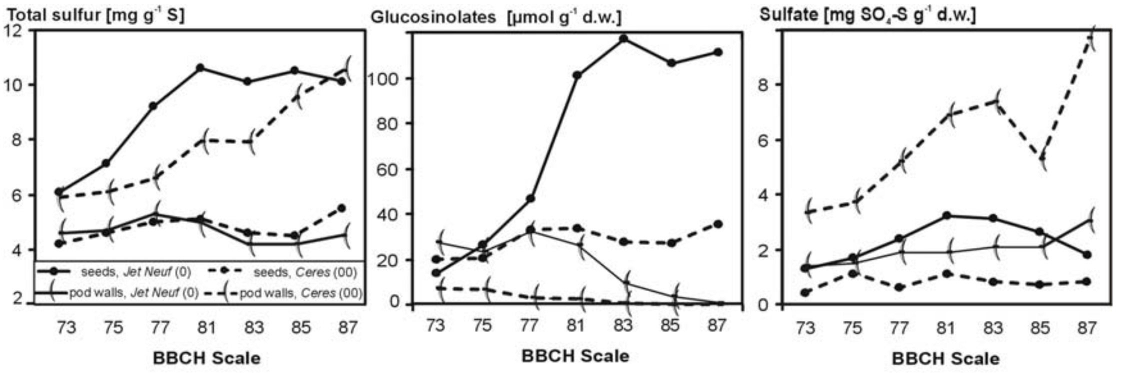

Figure 1. Changes in total sulfur, sulfate-S and total GSL content in developing seeds and pod walls of a single low and a double low oilseed rape variety (data from Bloem et al., 2007; BBCH scale according to Strauss et al., 1994).

Time-course experiments have shown that an increase in seed GSLs in developing pods was associated with a decrease in the GSL content of the pod walls what is an indication that the pod walls are the main sites of GSL biosynthesis (De March et al., 1989, Fig. 1). It was Josefsson who observed already in 1971 that the low GSL content in the Polish spring rape cultivar *Bronowski* was not caused by a reduced S uptake and he could show in radio-tracer experiments the existence of a metabolic block in GSL biosynthesis. Zhao et al. (1993) concluded from the increasing sulfate content in pod walls of double low oilseed rape varieties that a metabolic block in the GSL synthesis is responsible for the low GSL content of double low rapeseed varieties. Also Bloem et al. (2007) found increasing sulfate contents in the pod walls of double low varieties during pod development but this increase was consistent during the whole period of pod development, including the very early stages of pod development (BBCH 75-81). The fact that at the beginning of seed development the single and double low winter oilseed rape varieties behave similar in accumulating GSLs in seeds, which are most likely translocated from the pod walls into the seeds, makes it difficult to explain the phenomenon of different GSL contents in seeds of single and double low varieties. It is most likely that the transport of GSLs is no problem but that less intact GSLs are produced in double low varieties later in pod development. Moreover double low oilseed rape varieties seem to be less efficient in using S, which is indicated by lower S contents in vegetative tissues and pods in double low summer rape varieties early in pod development compared to single low varieties (Bloem et al., 2007). Most likely there is more than a single explanation for the lower GSL contents in seeds of double low varieties. 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. In general, a decrease of the GSL content after harvest is assumed when seeds are air-dried. When whole pods during pod development are, however freeze-dried and subsequently separated into pod walls and seeds in order to prevent GSL losses, Bloem et al. (2007) could show that this procedure delivered a very high variation of the GSL content, 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 the sampling procedure. So, an increased sulfate content in the pod walls of double low varieties might be a result of break-down from intermediary products of the GSL biosynthesis and glucosinolates, or free sulfate might have accumulated.

Application of glucosinolate research

Today most research in the field of GSLs concentrates on their use in agriculture, human nutrition and health. Next a brief overview of recent advances in this kind of GSL research are highlighted.

Biofumigation is a term which refers to the release of biocidal, fungicidal and nematicidal compounds from GSL-containing crops during green manure, crop rotation or direct application, for example as pellets, which suppress soil-borne pests and pathogens (Sarwar et al., 1998; Lazzeri et al., 2004). Research shows that the GSL-myrosinase system may provide a natural alternative for methyl bromide soil fumigation (Lazzeri et al., 2004). GSLs have no biocidal action, but their hydrolysis products, the isothiocyanates (ITCs) show biocidal action against a broad range of bacteria, fungi and nematodes in the soil (Buskov et al., 2002; Rosa et al., 1997). Because of this anti-microbial activity, GSL-containing crops have been tested as bio-fumigants. So, the incorporation of plant residues into the soil resulted in the release of biocidal ITCs (Brown and Morra, 1997). Many ITCs showed, however, a highly biocidal action in laboratory trials, while the results from field experiments often failed to show significant effects. The reason is that only a low proportion of the GSLs is transferred into ITCs (Gimsing and Kirkegaard, 2006). Calculated ITC-release efficiency accounted for 26% and 56% for high GSL rape and mustard, respectively (Gimsing and Kirkegaard, 2006). Morra and Kirkegaard (2002) could show that the greatest improvement in the use of *Brassica* biofumigants would be to develop methods to increase cell disruption and thereby maximizing GSL hydrolysis and ITC release.

The release of ITCs depends on soil pH: in soils with a pH above 5.5 ITCs will be the predominant product of GSL breakdown (Borek et al., 1996) while at lower pH other products like nitriles are released. Other soil parameters which affect the release of ITCs from GSLs are soil moisture, the organic matter and clay content as ITCs can be adsorbed to soil particles and organic matter (Matthiessen and Shackleton, 2005).

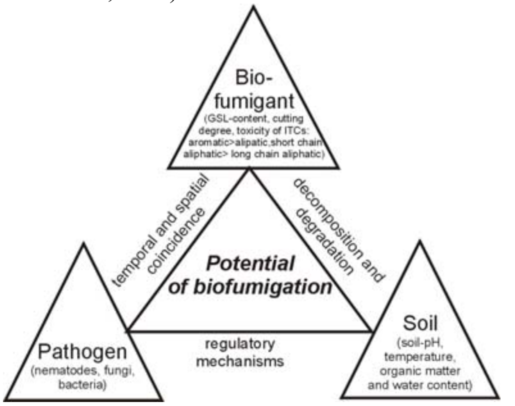

Figure 2. Factors affecting the potential of biofumigation of green manure, glucosinolate-containing crops in crop rotation and application of glucosinolate-containing products, respectively.

For a high potential of biofumigation the biofumigant needs to have a high GSL content, needs to be finely dispersed in the soil and needs to release ITCs of high toxicity. Aromatic ITCs are more toxic than aliphatic ITCs; from aliphatic ITCs those with short side-chains are much more toxic than those with longer aliphatic side chains (Manici et al., 1997; Sarwar et al. 1998). The activity of aromatic ITCs is restricted because they need to be in direct contact with the pathogen as they have a lower volatility than aliphatic ITCs (Matthiessen and Shackleton, 2005). Also important for the biofumigation potential is the coincidence of pathogen attack and the release of ITCs (Fig. 2). Gimsing and Kirkegaard (2006) could show that the concentration of GSLs and the corresponding ITCs declined rapidly during the first 4 days after incorporation of pulverized GSL-containing plant material although both could be detected in the soil for up to 8 days after incorporation. The highest levels of soil ITCs were determined in the soil immediately (30 minutes) after pulverization and incorporation of mustard with a high GSL content into the moist soil. Thus, a coincidence of ITC release and pathogen attack is not very likely if GSL-containing crops are grown in the crop rotation or as green manure. It seems to be more promising to develop a functional bio-fertilizer, with high concentrations of the most biocidal ITC-releasing GSLs and coatings which will warrant a continuous release of GSLs and ITCs and which will prevent adsorption by clay minerals or organic matter.

Pharmacological relevance of GSL-containing crops: GSLs have long been known for their anti-nutritive effects in animal nutrition. In contrast, the breakdown products of GSLs are responsible for the flavor of *Brassica* vegetables and there is increasing evidence that they act as anti-carcinogens in human aliments (Verhoeven et al., 1997; Hecht, 1999; Mithen et al., 2000). Sulforaphane, an ITC in broccoli, is an efficient inducer of Phase II enzymes which help to detoxify carcinogens (Talalay et al., 1995). Allyl ITC´s blocked the growth of cancer cells *in vitro* (Musk et al., 1995), phenylethyl ITC induced cell death (Huang et al., 1998) and sinigrin suppressed proliferation, increased apoptosis and reduced carcinogenesis in rats after treatment with a carcinogen (Smith et al., 1998). The most important reaction of ITCs is probably the modulation of the activities of Phase I and II enzymes which catalyze a variety of hydrolytic, oxidation or reduction reactions (Phase I), which products are then available for conjugation reactions (Phase II) and excretion (Mithen et al., 2000). The fact that GSLs are the principal source of anti-carcinogenic activity in *Brassica* vegetables provides a strong motive for the manipulation of GSL levels in vegetables for human consumption (Mithen et al., 2000).

Parameters influencing the GSL content are S fertilization, light intensity, temperature, growth stage of the crop, plant part, interactions with pathogens and genetic variability (Rosa et al., 1997). Just recently it could be shown that applying controlled post-harvest abiotic stress to harvested plant parts of nasturtium enhanced significantly the glucotropaeolin (GTL) content, which is an important criteria for the phytopharmaceutical value of the raw material. Drying leaves of nasturtium at 40°C in a ventilated oven increased the GTL from about 40 to > 200 % compared to freeze-dried leaf material (Bloem et al., 2006).

GSLs can be also an interesting alternative to recently banned in-feed-antibiotics in animal nutrition as ITCs may unfold anti-microbial effects. GTL from nasturtium is used against urinary tract infections of humans. Just recently a first experiment was conducted with weaning piglets to investigate the effect of different doses of GTL on feed intake, growth performance, intestinal micro-biota and benzyl-ITC concentrations in the urine. For the first time it could be shown that supplementation of animal feed with nasturtium was well-tolerated, had no influence on feed intake and growth rate of the piglets. Benzyl-ITC was found in the urine of those piglets which received nasturtium. With higher rates of supplementation also the ITC excretion increased but the total value depended very much on the amount of urine, which was excreted by the piglets (unpublished data).

Research in GSL is of prime practical relevance and recent advances in GSL research are expected to contribute substantially to food quality and sustainable agricultural production.

References

Bloem E, Haneklaus S, Schnug E (2006) Comparative effects of sulfur and nitrogen fertilization and post-harvest processing parameters on the glucotropaeolin content of *Tropaeolum majus* L. J. Sci. Food Agric. (in press).

Bloem E, Haneklaus S, Schnug E (2007) Changes in the glucosinolate and total sulphur content of developing seeds and pods of single low and double low summer and winter oilseed rape varieties (*Brassica napus* L.) J Plant Nutr. (in prep).

Borek V, Morra MJ, McCaffrey JP (1996) Myrosinase activity in soil extracts. Soil Sci. Soc. Am. J. 60:1792-1797.

Brown PD, Morra MJ (1997) Control of soil-borne plant pests using glucosinolate-containing plants. Adv. Agron. 61: 167-231.

Buskov S, Serra B, Rosa E, Sorensen H, Sorensen JC (2002) Effects of intact glucosinolates and products produced from glucosinolates in myrosinase-catalyzed hydrolysis on the potato cyst nematode (*Globodera rostochiensis* Cv. Woll). J. Agric. Food Chem. 50: 690-695.

De March G, McGregor DI, Séguin-Shwartz G (1989) Glucosinolate content of maturing pods and seeds of high and low glucosinolates summer rape. Can. J. Plant Sci. 69: 929-932.

Fieldsend J, Milford GFJ (1994) Changes in glucosinolates during crop development in single- and double-low genotypes of winter oilseed rape (*Brassica napus*): I. Production and distribution in vegetative tissues and developing pods during development and potential role in the recycling of sulphur within the crop. Ann. Appl. Biol. 124: 531-542.

Gimsing al., Kirkegaard JA (2006) Glucosinolate and isothiocyanate concentration in soils following incorporation of *Brassica* biofumigants. Soil Biol. Biochem. 38: 2255-2264.

Hecht SS (1999) Chemoprevention in cancer by isothiocyanates, modifiers of carcinogen metabolism. J. Nutr. 129: 768S-774S.

Huang C, Ma WY, Li J, Hecht SS, Don Z (1998) Essential role of p53 in phenylethyl isothiocyanate-induced apoptosis. Cancer Res. 58: 4102-4106.

Lazzeri L, Leoni O, Manici LM (2004) Biocidal plant dried pellets for biofumigation. Indust. Crops Prod. 20: 59-65.

Josefsson E (1971) Studies of the biochemical background to differences in glucosinolates content in *Brassica napus* L. I. Glucosinolate content in relation to general chemical composition. Physiol. Plant. 24: 150-159.

Manici LM, Lazzeri L, Palmieri S (1997) In vitro fungitoxic activity of some glucosinolates and their enzyme-derived products toward plant pathogenic fungi. J. Agric. Food Chem. 45, 2768-2773.

Matthiessen JN, Shackleton MA (2005) Biofumigation: environmental impacts on the biological activity of divers pure and plant derived isothiocyanates. Pest Managem. Sci. 61 (11): 1043-1051.

Mithen RF, Dekker M, Verkerk R, Rabot S, Johnson IT (2000) The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods. J. Sci. Food Agric. 80: 967-984.

Moreno DA, Carvajal M, Lopez-Berenguer C, Garcia-Viguera C (2006) Chemical and biological characterisation of nutraceutical compounds of broccoli. J. Pharmaceut. Biomed. Anal. 41: 1508-1522.

Morra MJ, Kirkegaard JA (2002) Isothiocyanate release from soil-incorporated *Brassica* tissues. Soil Biol. Biochem. 34: 1683-1690

Musk SR, Stephenson P, Smith TK,Stening P, Fyfe D, Johnson IT (1995) Selective toxicity of compounds naturally present in food toward the transformed phenotype of human colorectal cell line HT29. Nutr. Cancer 24: 289-298.

Rosa E, Heaney RK, Fenwick GR, Portas CAM (1997) Glucosinolates in crop plants. Hortic. Rev. 19: 99-215.

Sarwar M, Kirkegaard JA, Wong PTW, Desmarchelier JM (1998) Biofumigation potential of brassicas - III. In vitro toxicity of isothiocyanates to soil-borne fungal pathogens. Plant and Soil 201: 103-112.

Schnug E (1990) Glucosinolates – fundamental, environmental and agricultural aspects. In: Sulfur nutrition and sulfur assimilation in higher plants, (Rennenberg et al. eds.), The Hague: SPB Acad. Publ. pp. 97-106.

Schnug E (1993) Physiological functions and environmental relevance of sulfur-containing secondary metabolites. In: Sulfur nutrition and assimilation in higher plants. (De Kok et al. eds.) The Hague: SPB Acad. Publ. pp.179-192.

Smith TK, Lund EK, Johnson IT (1998) Inhibition of dimethylhydrazine-induced aberrant crypt foci and induction of apoptosis in rat colon following oral administration of the glucosinolate sinigrin. Carcinogenesis 19: 267-273.

Strauss R, Bleiholder H, van den Boom T, Buhr L, Hack H, Hess M, Klose R, Meier U, Weber E (1994) Einheitliche Codierung der phaenologischen Entwicklungsstadien mono- und dikotyler Pflanzen. Ciba-Geigy AG, Basel, 56 pp.

Talalay P, Fahey JW, Holtzclaw WD, Prestera T, Zhang Y (1995) Chemoprotection against cancer by phase 2 enzyme induction. Toxicol. Lett. 82/83: 173-179.

Verhoeven DT, Verhagen H, Goldbohm RA, van den Brandt PA, van Poppel GA (1997) Review of mechanisms underlying anticarcinogenicity by brassica vegetables. Chem. Biol. Interact. 103: 79-129.

Zhao FJ, Evans EJ, Bilsborrow PE,Syers JK (1993) Sulphur uptake and distribution in double and single low varieties of oilseed rape (*Brassica napus* L.). Plant and Soil 150: 69-76.