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Review

Glucosinolates and their potential role in plant

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Abstract

Glucosinolates are sulfur- and nitrogen-containing plant secondary metabolites common in the Brassicaceae and related plant families. In the plant, they coexist with an endogenous ?-thioglucosidase (EC 3.2.3.1) called myrosinase, though glucosinolates are stored in the vacuoles of so-called S-cells and myrosinase in separate but adjacent cells. Upon plant tissue disruption, glucosinolates are released at the damage site and become hydrolyzed by myrosinase. The chemical nature of the hydrolysis products depends on the structure of the glucosinolate side chain, plant species and reaction conditions. Biosynthesis of glucosinolates comprises three phases: (i) amino acid chain elongation, in which additional methylene groups are inserted into the side chain, (ii) conversion of the amino acid moiety to the glucosinolate core structure, (iii) and subsequent side chain modifications. Glucosinolate pattern differs between species and ecotype as well as between and even within individual plants, depending on developmental stage, tissue and photoperiod. A number of environmental conditions such as light plant, nutritional status, fungal infection, wounding and insect damage can alter the glucosinolate pattern significantly. The change of the glucosinolate profile by several environmental factors has brought forward different theories regarding their potential roles in the plant. However, the most accepted theory is that the glucosinolate-myrosinase system is involved in defense against herbivores and pathogens. This review summarized recent progress in glucosinolate biosynthesis, degradation and organization of the myrosinase-glucosinolate system. Furthermore, current knowledge of the potential role of glucosinolates in the plant, especially in plant defense, is discussed.

INTRODUCTION

Glucosinolates are sulfur- and nitrogen-containing plant secondary metabolites common in the order Capparales, which includes the *Brassicaceae* family with agriculturally important crops, *Brassica* vegetables, and the model plant *Arabidopsis thaliana*. Glucosinolates have a common core structure containing a β-D-thioglucose group linked to a sulfonated aldoxime moiety and a variable side chain derived from amino acids (Figure 1). Glucosinolates can be divided into three classes based on the structure of different amino acid precursors: 1. aliphatic glucosinolates derived from methionine, isoleucine, leucine or valine, 2. aromatic glucosinolates derived from phenylalanine or tyrosine, and 3. indole glucosinolates derived from tryptophan. The biosynthesis of glucosinolates comprises three phases: (*i*) amino acid chain elongation, in which additional methylene groups are inserted into the side chain, (*ii*) conversion of the amino acid moiety to the glucosinolate core structure, (*iii*) and subsequent side chain modifications *(1)*. More than 130 glucosinolates have been identified. Their structural diversity arises

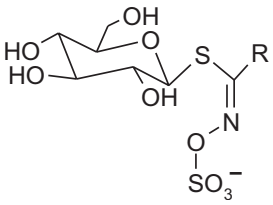


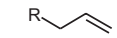
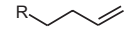
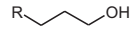
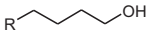
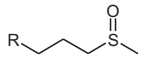
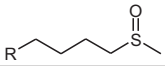
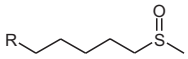
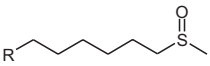
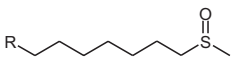
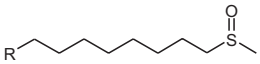
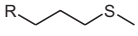
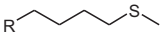
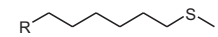
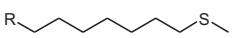
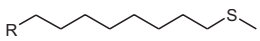
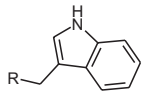
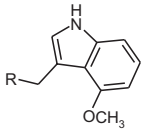
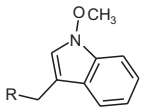
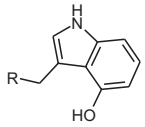
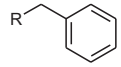
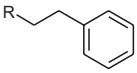
Figure 1. *General structure of glucosinolates. R denotes the variable side chain from amino acids.*

from side chain elongation of the amino acid precursors prior to the formation of the glucosinolate core structure and from a wide range of secondary modifications including oxidation, desaturation, hydroxylation, methoxylation, sulfation and glucosylation (Table 1) *(1, 2)*.

Plants accumulating glucosinolates always possess a ?-thioglucosidase (EC 3.2.3.1) called myrosinase, which catalyzes the hydrolysis of glucosinolates to numerous compounds with diverse biological activities. The enzyme only comes into contact with its glucosinolate substrates if the plant tissues are disrupted as a result of wounding, insect or pathogen attack. The chemical nature of hydrolysis products depends mainly on the structure of the glucosinolate side chain, plant species and reaction conditions *(3, 4)*. Glucosinolates and their hydrolysis products are frequently studied as plant defense system against insects, herbivores and certain microbial pathogens. Besides, they serve as attractants to specialist insects feeding on crucifers *(5)*. Mostly volatile hydrolysis products are responsible for characteristic taste and smell of cruciferous vegetables. In some *Brassica* vegetables such as cauliflower, Brussels sprouts, cabbage and broccoli, glucosinolate degradation products, especially isothiocyanates have been shown to have anticarcinogenic properties *(6)*. However, the presence of degradation products is not always beneficial. For instance, the amount of rape meal that can be used in animal food supplement is restricted due to the goitrogenic effect of 5-vinyloxazolidine-2-thione, the spontaneous cyclization product of 2-hydroxy-3-butenyl glucosinolate which accounts for up to 80% of total glucosinolates in rape seed *(7)*. Diverse biological properties of glucosinolates and their hydrolysis products are the reason why these plant secondary metabolites attract the interest of researchers coming from different research fields. Rapid development of molecular and genetic tools in combination with the availability of new data on the model plant *Arabidopsis thaliana* has greatly enhanced the gain of knowledge in recent years. Nevertheless, there are still many unanswered questions e.g. how glucosinolate diversity and accumulation are regulated in detail. Further research is necessary to allow precise glucosinolate manipulation in order to exploit the potential of these compounds in improving pest resistance, health and nutritional value of crop plants. This review summarizes the recent progress in glucosinolate biosynthesis, degradation and organization. Furthermore, current knowledge on the potential role of the glucosinolate-myrosinase system in plants is discussed.

TABLE 1

Side chain structure of some glucosinolates. R denotes the general structure of glucosinolate.

Glucosinolate	Trivial name	Side chain structure
2-Propenyl	Sinigrin	
3-Butenyl	Gluconapin	
3-Hydroxypropyl	–	
4-Hydroxybutyl	–	
3-Methylsulfinylpropyl	Glucoiberin	
4-Methylsulfinylbutyl	Glucoraphanin	
5-Methylsulfinylpentyl	Glucoalyssin	
6-Methylsulfinylhexyl	Glucohesperin	
7-Methylsulfinylheptyl	Glucoibarin	
8-Methylsulfinyloctyl	Glucohirsutin	
3-Methylthiopropyl	Glucoibervirin	
4-Methylthiobutyl	Glucoerucin	
6-Methylthiohexyl	Glucosquerellin	
7-Methylthioheptyl	–	
8-Methylthiooctyl	–	
Indol-3-ylmethyl	Glucobrassicin	
4-Methoxyindol-3-ylmethyl	4-Methoxyglucobrassicin	
1-Methoxyindol-3-ylmethyl	*Neo*glucobrassicin	
4-Hydroxyindol-3-ylmethyl	4-Hydroxyglucobrassicin	
Benzyl	Glucotropaeolin	
2-Phenylethyl	Gluconastrutiin	

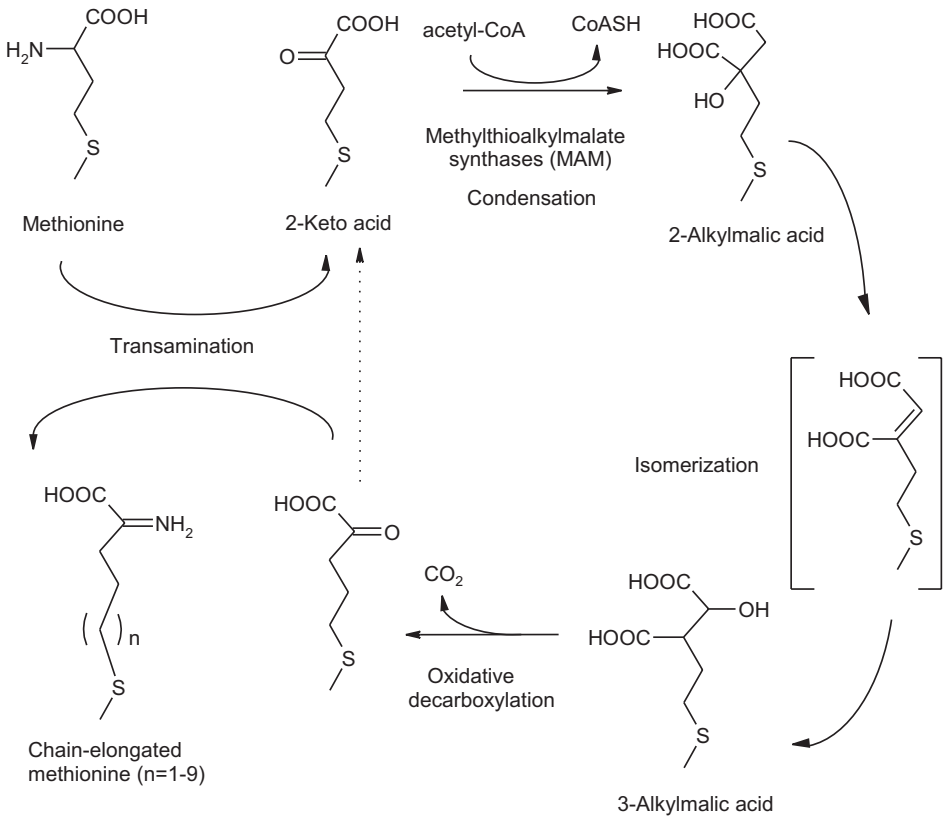


Figure 2. *The chain elongation pathway of methionine in glucosinolate biosynthesis (1).*

Glucosinolate biosynthesis

The biosynthesis of glucosinolates comprises three phases: (*i*) amino acid chain elongation, in which additional methylene groups are inserted into the side chain, (*ii*) conversion of the amino acid moiety to the glucosinolate core structure, and *(iii*) subsequent side chain modifications.

Side chain elongation

The majority of glucosinolates in *A. thaliana* as well as in many other species of the *Brassicaceae* are formed from methionine that has been modified by the sequential addition of 1-9 additional methylene groups to its side chain (Figure 2) *(8)*. The pathway is initiated by transamination of methionine to form the corresponding 2-oxo acid, which is then extended by one methylene group in a three-step cycle consisting of condensation with acetyl-CoA, isomerization and oxidative decarboxylation. The newly formed 2-oxo acid can be transformed to the corresponding methionine derivative or can undergo further cycles of chain elongation. The pathway is similar to the single methylene group incorporation that occurs in leucine biosynthesis *(1)*.

The first step, the condensation reaction, is considered to be critical for side chain variation. Quantitative trait loci (QTL) mapping and fine-scale mapping in *Arabidopsis,* using recombinant inbred lines (RIL) derived from a cross of the ecotypes Landsberg *erecta* (rich in homomethionine-propylglucosinolates) and Columbia (rich in dihomomethionine-butylglucosinolates) identified the *Gsl-elong* locus at chromosome V *(9, 10)*. Based on amino acid similarity to known isopropylmalate synthases (IPMS) which are responsible for condensation reaction in leucine biosynthesis, four *A. thaliana* candidate genes potentially encoding the condensing enzyme of the elongation cycle were identified. Two of these genes are at chromosome I (Atlg74040, At1g8500). Their deduced amino acid sequences share about 90% identity with each other and are more than 60% identical to other plant IPMSs. The other two (At5g23010 and At5g23020) display lower identity to known plant IPMSs but share 85% identity with each other and show an identical intron/exon structure *(10)*. In *A. thaliana* ecotype Columbia the latter two genes were identified as methylthioalkymalate synthases (*MAM*) and have been shown to be responsible for the condensation step of the chain elongation cycle. At5g23010 was designated methylthioalkymalate synthase 1 (*MAM1*) and At5g23020 as methylthioalkymalate synthase-3 (*MAM3*). Both enzymes have similar properties but differ in their substrate specificity. *MAM1* catalyzes the condensation reaction only of the first three elongation cycles, while *MAM3* enzyme is capable to process all six additions of methylene groups *(11, 12)*.

The remaining two steps of the chain elongation cycle (isomerization and oxidative decarboxylation) have not

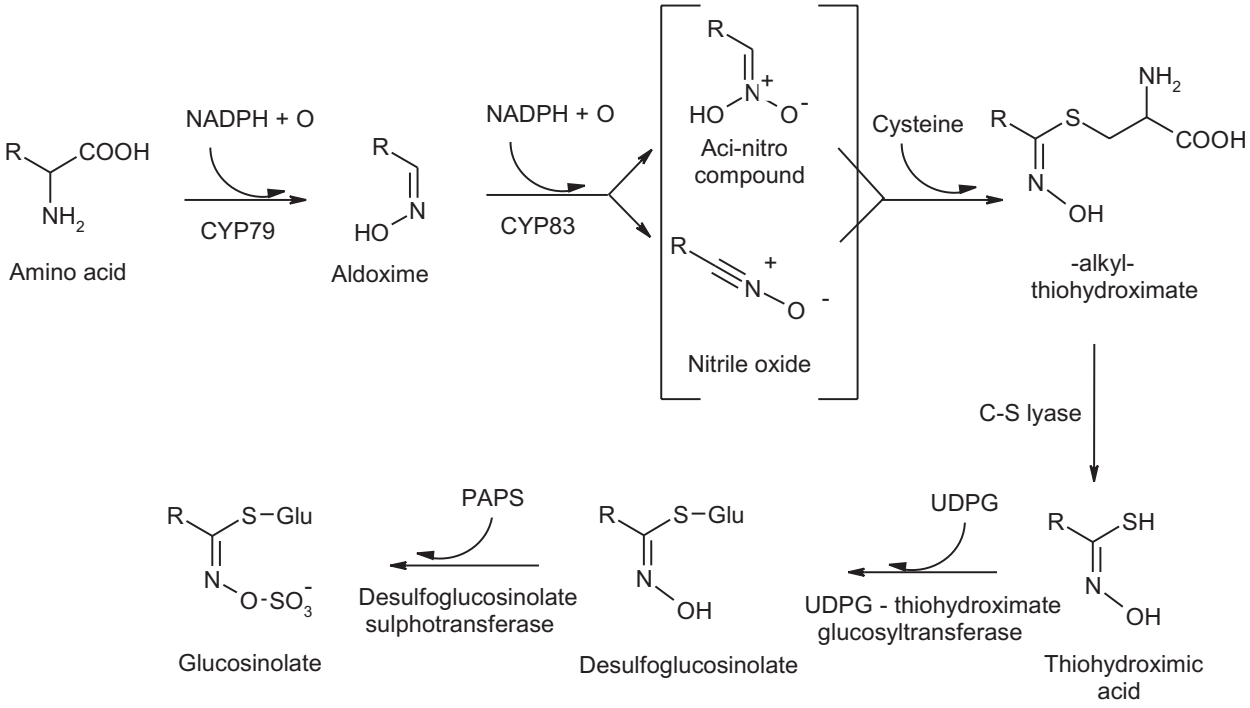


Figure 3. *Biosynthesis of glucosinolate core structure (1).*

been characterized though candidate genes have been suggested based on coexpression analysis *(13)*. The enzymatic steps are presumed to be homologous with the parallel reactions in leucine biosynthesis.

Biosynthesis of glucosinolate core structure

The conversion of the precursor amino acid to the final glucosinolate involves intermediates common to all glucosinolates (Figure 3). The two initial steps of core glucosinolate biosynthetic pathway are catalyzed by cytochrome P450 enzymes belonging to the *CYP79* and *CYP83* families, respectively.

Cytochrome P450 enzymes of the CYP79 family catalyze the conversion of amino acids to corresponding aldoximes. Five of the seven functional *CYP79* homologues found in the *Arabidopsis* genome have been characterized. The substrate of CYP79A2 is phenylalanine *(1),* CYP79B2 and CYP79B3 convert tryptophan to indole-3-acetaldoxime *(14, 15),* and CYP79F1 and CYP79F2 metabolize chain-elongated methionine derivatives *(16, 17, 18)*. CYP79F1 and CYP79F2 differ in their substrate specificity. CYP79F1 is able to metabolize all chain-elongated methionine derivatives, whereas the catalytic activity of CYP79F2 is restricted to pentahomo- and hexahomomethionine *(18)*.

The second step in glucosinolate formation generates an unstable *aci*-nitro intermediate that conjugates with the thiol group of cysteine via the á-carbon atom. This step is catalyzed by members of the CYP83 family. CYP83A1 and CYP83B1 have been identified in *Arabidopsis (16, 19, 20)*. Biochemical characterization of recombinant CYP83A1 and CYP83B1 shows that both enzymes can metabolize all the aldoximes tested. However, CYP83A1 has a high affinity for aliphatic aldoximes, whereas CYP83B1 prefers indole-3-acetaldoxime and aromatic aldoximes as substrates *(20, 21)*.

The remaining steps of glucosinolate biosynthesis involve enzymes that are thought to accommodate nearly all glucosinolate precursors regardless of their side chain. The *S*-alkylthiohydroximates are converted to thiohydroximic acids in a reaction catalyzed by a C–S lyase, which was recently identified using a bioinformatics approach in *A. thaliana (22)*. Enzymes for glycosylation (uridine diphosphate tiohydroximate glucosyltransferase) and sulfation (3'-phosphoadenosine 5'-phosphosulfate: desulfoglucosinolate sulfotransferase) have been characterized in several crucifers and partially purified *(2, 23)*.

Secondary modifications

Following the formation of the basic glucosinolate structure, a wide range of modifications can occur at the side chain as well as at the glucose moiety. These modifications include oxidation, hydroxylation, methoxylation, desaturation, sulfation and glycosylation, and take place in an organ and development-specific pattern *(24, 25)*.

Based on genetic studies, a model for the side-chain modification of aliphatic glucosinolates, which are most extensively modified, has been proposed *(26)*. Three genetic loci have been shown to be involved. The *Gsl-oxid* locus controls the oxidation of methylthio- to methylsulfinylalkylglucosinolates. With the flavin-monooxygenase GS-OX1, the appropriate S-oxygenating enzyme has been identified recently *(27)*. The *Gsl-alk* locus controls the removal of the methylsulfinyl residue and the introduction of a double bond and, the *Gsl-oh* locus is responsible for the hydroxylation of butenylglucosinolate. In *Arabidopsis,* an additional locus termed *Gsl-ohp* controls the conversion of methylsulfinylpropyl- to hydroxypropylglucosinolate *(11)*. Kliebenstein et al. identified three potential genes, *AOP1*, *AOP2* and *AOP3*, which all encode 2-oxoglutarate-dependent dioxygenases. No function was assigned to *AOP1*. Heterologous expression of genes in *E. coli* showed that AOP2 catalyzes the conversion of 3-methylsulfinylpropyl- and 4-methylsulfinylbutylglucosinolate to the corresponding alkenylglucosinolates, while AOP3 can convert 3-methylsulfinylpropyl- to 3-hydroxypropylglucosinolate *(28, 29)*.

Glucosinolate degradation

The plants which are able to synthesize glucosinolates always also possess a ?-thioglucosidase known as myrosinase (EC 3.2.3.1). The loss of cellular integrity as a result of wounding, insect or pathogen attack activates the binary glucosinolate-myrosine system and leads to the generation of thioglucose, sulfate and an unstable intermediate which rearranges spontaneously into several degradation products *(3, 4)*. Chemical conditions such as pH, availability of ferrous ions and presence of myrosinase-interacting proteins determine the final composition of the product mix which can include isothiocyanates, oxozolidine-2-thiones, nitriles, epithionitriles, and thiocyanates (Figure 4).

Hydrolysis at neutral conditions typically results in the formation of isothiocyanates. If a hydroxyl group at the C-2 of the glucosinolate side chain is present, the isothiocyanates formed are unstable and cyclize to oxazolidine-2-thiones. At acidic pH and in the presence of Fe^{2+} ions, the formation of nitriles occurs *in vitro (30)*, while *in vivo* a protein factor such as epithiospecifier protein (ESP) is involved *(31, 32)*. In the presence of ESP, glucosinolates with a terminal double bond in their side chain have an epithionitrile as hydrolysis product. Thiocyanates are exclusively formed from benzyl-, allyl-, and 4-methylsulfinylbutyl-glucosinolates *(33)*. Thiocyanate-forming protein (TFP) has been shown to be involved in their formation *(34)*. Indole glucosinolate breakdown products differ from others due to the instability of the initially formed isothiocyanates at neutral or slightly acidic pH resulting in the production of indole-methanols, ascorbic acid conjugates, and oligomeric mixtures *(35)*.

Myrosinase activity is carried out by a group of isoenzymes present in all *Brassicaceae* species examined and is also found in 14 other plant families. Myrosinase is a dimeric protein with a molecular weight in the range of 62–75 kDa *per* subunit. Purified and characterized enzymes have shown to be highly glycosylated and are characterized by varying degrees of ascorbic acid activation. Distribution of myrosinase isoenzymes seems to be both organ-specific and species-specific *(3, 4)*. Most myrosinases hydrolyze multiple glucosinolate substrates, but some are highly specific *(32, 36)*. The substrate specificity can be affected by associated factors like epithiospecifier protein, myrosinase binding protein, myrosinase binding protein-related protein, and myrosinase associated proteins, although the role of these proteins has not yet been clarified *(4, 31, 37, 38, 39)*.

Enzymes with myrosinase activity have also been found in fungi, such as *Aspergillus sydowi (40)* and *Aspergillus*

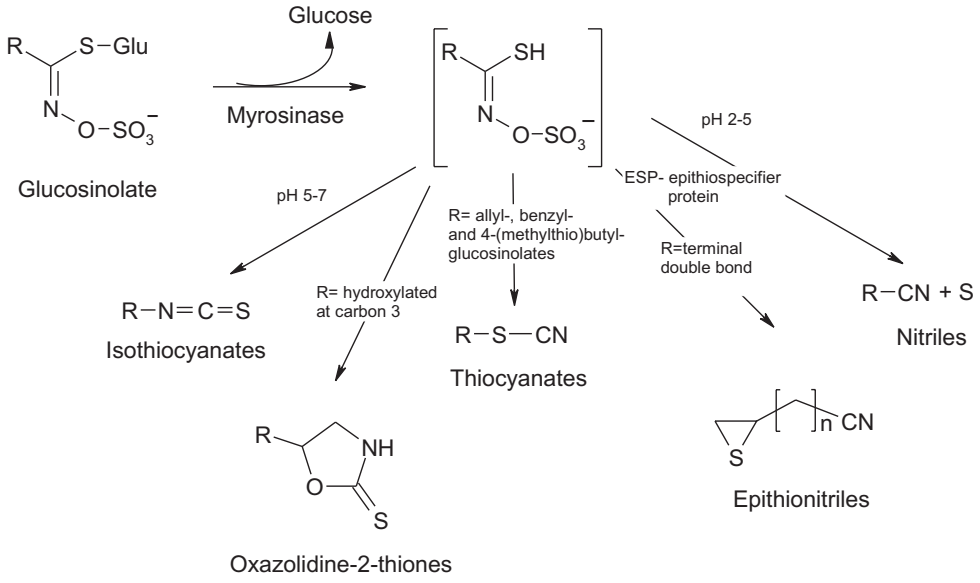


Figure 4. *Structure of possible glucosinolate degradation products (1).*

niger (41), in the intestinal bacteria *Enterobacter cloacae (42)* and *Paracolobactrum aerogenoides (43),* in mammalian tissues *(44)* and in the cruciferous aphids *Brevicoryne brassicae* and *Lipaphis erisimi (45, 46)*.

Distribution of glucosinolates and organization of myrosinase-glucosinolate system

Glucosinolates are known to be regulated both developmentally and environmentally in various organs and tissues. Even though more than 120 glucosinolates have been identified, plants contain only some of these compounds in greater quantity. Glucosinolate pattern differs between species and ecotypes as well as between and within individual plants, depending on developmental stage, tissue and photoperiod. For instance, Brown et al. carried out a detailed study regarding the accumulation of glucosinolates in the Columbia ecotype at different stages during its life cycle and reported significant differences among organs in concentration and composition. The highest concentration was found in reproductive organs such as seeds and siliques, followed by young leaves, while senescing rosette leaves contained the lowest concentration of glucosinolates. Intermediate concentrations were found in leaves, stem and root *(25)*. The high accumulation of glucosinolates in seeds is not connected with a corresponding high level of associated biosynthesis, suggesting the involvement of transport processes *(47)*. Evidence for long distant transport via phloem comes from a study by Chen et al. They demonstrated the translocation of radiolabeled *p*-hydroxybenzyl glucosinolates from leaves to seeds, either exogenously applied or *de novo* synthesized *(48)*.

Since the tissue-level location of glucosinolate accumulation has a major influence on its hydrolysis to bioactive products, it is important to determine the site of biosynthesis to better understand the role of glucosinolates in the plant. Present data indicate that glucosinolate biosynthesis does not occur in the cells in which they are stored. Koroleva et al. report that glucosinolate rich S cells of the *Arabidopsis* flower stalk are situated between the phloem of every vascular bundle and the endodermis *(49)*. The promoter-GUS (*â*-glucuronidase) studies performed with glucosinolate biosynthetic genes (CYP79F2, CYP79F1 and CYP79B2) now have not yet shown transcript accumulation in S cells *(15, 17, 18)*. Even though there are some differences reported in expression pattern for CYP79F1 and CYP79F2, probably due to differences in the promoter-GUS constructs used, both studies support the vascular tissue as the site of glucosinolate biosynthesis *(17, 18)*. Subcellular localization of glucosinolate biosynthetic enzymes CYP79F2 and CYP79F1 with green fluorescent protein (GFP) showed that both enzymes were localized in the endoplasmic reticulum *(17)*. Methionine side-chain elongation is believed to be localized in chloroplasts due to predicted targeting sequences at the *N*-terminus of MAM proteins. This suggested localization was supported by the fact that MAM synthase activities were successfully detected in a chloroplast preparation of *Eruca sativa (50)*. A chloroplast preparation of *A. thaliana* failed to show any MAM activity *(11)*, while a more recent study localized MAM3 in chloroplasts, suggesting that this organelle is the site of methionine side-chain elongation *(12)*.

Cellular organization of the myrosinase-glucosinolate system is still unclear and has been subject of intensive research for a long time. The question is how to keep myrosinases separate from glucosinolates to prevent the production of toxic products in an intact plant? At the same time the plant needs to allow both partners to rapidly get into direct contact after damage has occurred. A potential solution has been proposed in the »mustard oil bomb« hypothesis by Lüthy and Matile *(51)*. Myrosinases are localized to specific cells named myrosin cells or myrosin idioblasts, scattered throughout most plant tissues in a frequency range from 2% to 5% *(52)*. Myrosin cells contain protein rich vacuolar-type structures called myrosin grains where myrosinase is localized *(53, 54)*. In the *Arabidopsis* lines with myrosinase promoter-GUS fusions, the GUS stain occurred in idioblast cells of the phloem parenchyma in leaves, stem and inflorescences, as well as in guard cells *(55, 56, 57)*. Glucosinolates have been reported to be localized in vacuoles in non specific cells together with ascorbic acid which is able to modulate myrosinase activity (i.e. inhibition at higher concentration and activation at lower concentration) *(23, 58, 59)*. The only evidence that glucosinolates are stored in a certain type of cells comes from Koroleva *et al. (49)*. They have described a group of sulfur rich cells (»S-cells«) in *Arabidopsis* flower stalk suited between vascular bundles and endodermis with a high concentration of glucosinolates (>100 mM). Taken together, the myrosinase enzyme is in *Arabidopsis* flower stalk localized in sperate cells in direct neighborhood to S-cells *(56)*. In some other *Brassica* spp., myrosine cells were found outside the vascular system in aleurone-type cells in seeds and seedlings of *B. juncea (55, 57)*.

Potential role of the glucosinolate-myrosinase system in the plant

A number of environmental conditions such as light *(60)*, nutritional status of the plant *(61, 62),* fungal infection, wounding and insect damage *(2)* can alter the glucosinolate pattern significantly. A change of the glucosinolate profile by several environmental factors has brought forward different theories regarding their potential roles in the plant. For instance some studies using several glucosinolate biosynthesis mutants suggested that glucosinolate metabolism might play a role in plant development *(17, 20, 22, 63, 64)*. However, the most accepted theory is that the glucosinolate-myrosinase system is involved in defense against herbivores and pathogens.

Nutritional status of the plant and its impact on glucosinolate-myrosinase system

Brassica species require a high amount of sulfur. It has been proposed that glucosinolates may act as a sulfur

storage pool which can be mobilized through hydrolysis by myrosinases *(65)*. Glucosinolates also contain nitrogen and the glucosinolate-myrosinase system can be considered to be a sink for both nutrients. It was expected that fertilizers would have an influence on this system in *Brassica* crops *(4)*. Zhao *et al.* showed a clear influence of both nitrogen and sulfur supply on glucosinolates in *B. napus (66)*. Their results suggest that an increase in nitrogen supply favors the hydrolysis step converting 3-butenyl to (2*R*)-2-hydroxy-3-butenyl glucosinolates. Aliphatic glucosinolates show a greater sensitivity to sulfur deficiency than indole glucosinolates due to the already sulfur containing precursor methionine. Several studies have shown that increased sulfur availability increased the glucosinolate content *(61, 62),* whereas a decreasing sulfur supply resulted in a decrease of free sulfate and glucosinolates while at the same time myrosinase activity increased *(65, 67)*. Later studies demonstrated that sulfate was the main storage compound in the vegetative tissue *(68)* and sulfur measurements in *B. napus* showed that glucosinolates contained only a small portion of the crop's total sulfur *(69)*. More recently, a combination of metabolite and transcript profiling revealed coordinated repression of most glucosinolate pathway genes in response to sulfate limitation *(70)*. Taken all together, the relation between sulfur metabolism and the glucosinolate-myrosinase system exists, but it is not likely that glucosinolates are a major source of recyclable sulfur.

Glucosinolate metabolism in growth regulation

Indole glucosinolates have been proposed as precursors for the plant hormone indole-3-acetic acid (IAA). The indole glucosinolate are supposedly hydrolyzed to indole acetonitrile (IAN), which could be hydrolyzed further to IAA by nitrilase *(71, 72)*. Recently, several studies have provided evidence for a link between indole glucosinolates and IAA. Indole-3-acetaldoxime, the first intermediate in the indole glucosinolate biosynthesis and a product of the reaction catalyzed by CYP79B2 and CYP79B3, was found to be a precursor of IAA and is considered to be the branch point between the two metabolic pathways *(20)*. Plants that overexpressed *CYP79B2* displayed elevated levels of indole glucosinolates and IAA, while *Arabidopsis cyp79B2 cyp79B3* double mutants were strongly deficient in indole glucosinolates and partially deficient in IAA, suggesting the existence of another pathway for IAA production *(73)*. In contrast to, this *Arabidopsis* mutants for the genes involved in the late steps of biosynthesis of indole glucosinolates all display high levels of IAA and a corresponding dwarf phenotype (including adventitious root). Those mutants further demonstrate that disruption of the conversion of indole-3-acetaldoxime to indole glucosinolates causes increased flux into IAA *(20, 22)*. Based on structural similarity, it was also found that the indole phytoalexin camelexin is synthesized directly from indole-3-acetaldoxime *(74)*. Thus, in *Arabidopsis* indole-3-acetaldoxime represents a key metabolic branching point at which the flow of indole-3-acetaldoxime into the biosynthetic pathways of indole glucosinolates, camalexin and IAA must be tightly regulated (Figure 5).

Analysis of an *Arabidopsis* CYP79F1 knockout mutant showed that abolishing the formation of short-chain methionine-derived glucosinolates is accompanied by increased levels of IAA and cytokinin *(17, 63, 64)*. Although there is no evidence for CYP79F1 involvement in hormone metabolism, several explanations have been proposed. It is likely that the accumulation of high levels of short-chain elongated methionine and derivatives perturbs the methionine metabolism. Amongst others, methionine is the precursor for *S*-adenosylmethionine which is involved in many biosynthetic processes (i.e. ethylene biosynthesis, *trans*-methylation and regulation of cytokinin oxidase) *(18)*.

It has also been reported that unilateral blue light promotes myrosinase activity in the illuminated side of *Raphanus sativa* hypocotyls, leading to the degradation of 4-methylthio-3-butenyl glucosinolates into natural growth inhibitors, the raphanusanins, which were involved in phototropic growth while the levels of IAA were uninfected *(60)*. Other researchers doubted the existence of natural raphanusanins and claimed them to be artifacts of the extraction method *(75)*. So, the possible interaction between glucosinolate metabolism, especially aliphatics, and plant development is still unclear and opens a field for further research.

The role of the glucosinolate-myrosinase system in plant-insect/herbivore interactions

Glucosinolates and their hydrolysis products clearly play a role as mediators in plant-insect interactions. In general, plant-feeding insects can be classified as generalists or specialists. The role of the glucosinolate-myrosinase system consequently differs, i.e. glucosinolates can serve as general poison and deterrent for generalists while at the same time they can attract and stimulate feeding and egg laying of insects which are specialists on cruciferous plants.

Several studies have reported that glucosinolates exhibit growth inhibition or feeding deterrence to a wide range of general herbivores such as birds, slugs and generalist insects *(26, 76)*. It was also found that plants respond to herbivore or insect damage by systematically accumulating higher levels of glucosinolates and thus presumably increasing their resistance *(77)*. Usually it is the indole glucosinolates which become induced. Mewis et al. studied the glucosinolate response in *A. thaliana* to phloem-feeding aphids, the generalist *Myzus persicae* and the specialist *Brevicoryne brassicae*, and to a generalist caterpillar species *Spodoptera exigua* Hubner. They report an increase in short-chain aliphatic methylsulfinyl glucosinolates for all three insect species as well as in long-chain 8-methylsulfinyloctyl glucosinolate (8MSOO) which increased only in response to *S. exigua*. Surprisingly, indole glucosinolates were not significantly affected *(78)*. In a field experiment using lines of *Brassica napus* which differed in their glucosinolate content,

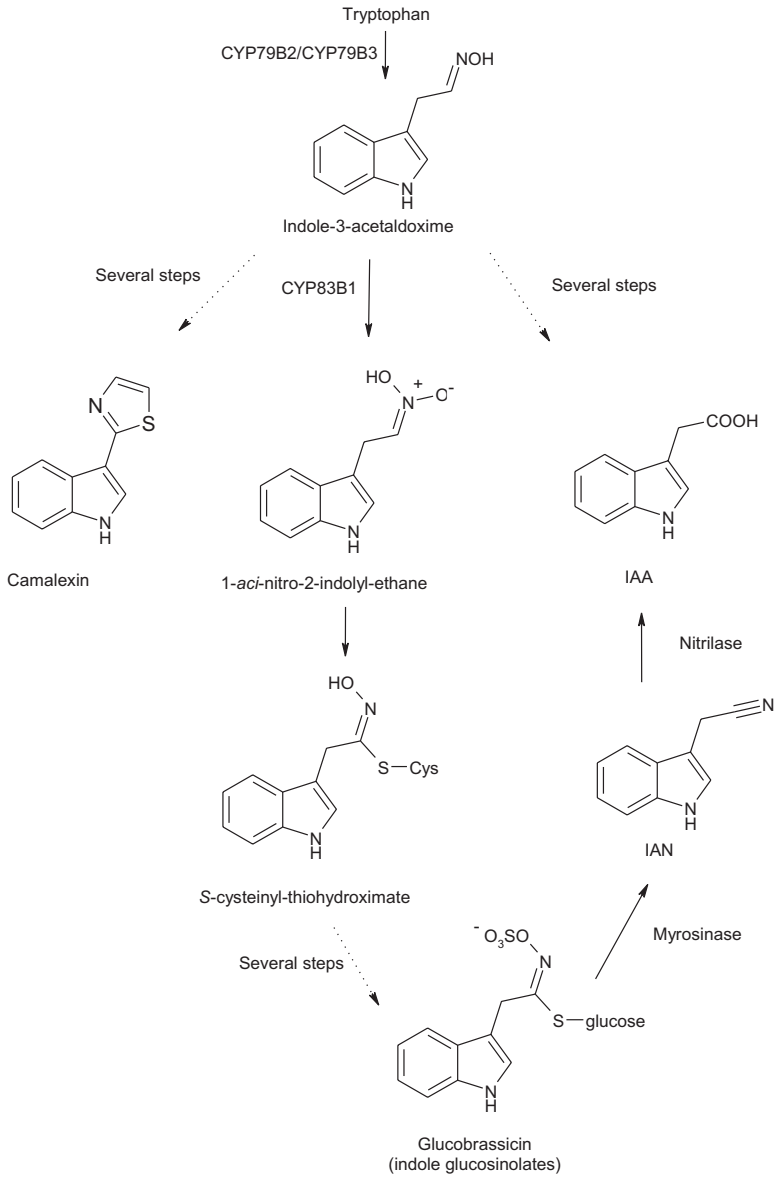


Figure 5. Indole-3-acetaldoxime represents a key metabolic branching point between indole glucosinolates, camalexin and IAA biosynthesis *(20, 74)*

Giamoustaris and Mithen (1995) found that increasing levels of glucosinolates resulted in a decrease of damage by generalist herbivores. The opposite was true for *Brassicaceae* specialists. Furthermore, it was found that a decrease in the side chain length of aliphatic glucosinolates and in the extent of hydroxylation increased the feeding amount by *Psylliodes chrysocephala,* suggesting that this *Brassicaceae* specialist is more responsive to particular types of glucosinolates than to the others *(76)*.

It has been recognized and accepted that glucosinolates serve as cues for feeding and oviposition of many insect herbivores which have become specialists on glucosinolate-containing plant *(79, 80)*. Volatile hydrolysis products may serve as a signal for attraction from a distance, whereas intact glucosinolates might act as contact cues for feeding or oviposition stimulation. Behavioral experiments were confirmed by electrophysiological investigations in which receptor organs or cells responded directly to glucosinolates or their hydrolysis products *(80, 81)*. Volatiles produced by glucosinolates can also attract natural enemies of herbivores such as parasitoids and provide indirect protection of the plant *(82, 83)*.

Herbivores that specialize on glucosinolate-containing plants have a mechanism to overcome the toxicity of glucosinolates and their hydrolysis products. For instance, *Plutella xylostella* possesses its own sulfatase gut enzyme that removes the sulfate moiety from the glucosinolate structure. The resulting desulfoglucosinolate does not serve as a substrate for myrosinase anymore and passes through the insect's digestive tract *(84)*. *Pieris rape* uses nitrile-specifying protein to direct hydrolysis toward nitriles which are less toxic than the usually produced

isothiocyanates *(5)*. Another way of specialist herbivore protection is the possibility to sequester glucosinolates in their own tissues without harm and exploit them in self-protection. Several examples of glucosinolate-sequestering insects have been recently described, including the harlequin bug, *Murgantia bistronica (85),* the sawfly, *Athalia rosae (86)* and the aphids, *Brevicoryne brassicae* and *Lipopbis erysimi (87)*. To ensure the normal function of glucosinolates in defense, myrosinase should be present and therefore glucosinolate-sequestering insects contain endogenous myrosinase or rely on the myrosinase activity present in the guts of their enemies. The aphid *Brevicoryne brassicae* has been reported to possess its own myrosinase *(88)* which produces isothiocyanates from sequestered glucosinolates when the aphid is damaged or killed. Interestingly, these isothiocyanates even serve as alarm signal to other members of the colony *(87)*.

The role of the glucosinolate-myrosinase system in plant/pathogen interactions

The role of glucosinolates in defense against pathogens is less clear than that for herbivores. There are many reports demonstrating the toxicity of glucosinolate hydrolysis products to bacteria and fungi *in vitro (89, 90),* but only a few *in vivo* studies were able to correlate glucosinolates with pathogen resistance *(91)*.

Brassica crops were recognized as »break crops« due to glucosinolates and their hydrolysis products which show inhibitory effects on soil borne pathogens *(92, 93)*. Field experiments showed that wheat grown after Indian mustard and canola gives greater yield than if grown after wheat *(94)*. Also, it was reported that the concentration of isothiocyanates achievable in the soil from the breakdown of *Brassica* tissue was sufficient to control pathogenic fungi *(92)*. In addition the dominant soil fungal species found near glucosinolate-containing crops differ from the normal species found elsewhere and show increased tolerance to isothiocyanates *(95)*. The hydrolysis products of indole glucosinolates were demonstrated to stimulate growth of certain ectomycrorrhizal species *(96)*.

When the relative antifungal activity of several isothiocyanate breakdown products from different glucosinolates was compared, it was found that aromatic isothiocyanates were more toxic than aliphatic ones and that the fungal toxicity of aliphatic isothiocyanates decreased with increasing length of the side chain *(97, 98)*. Tiernes et al. studied the antimicrobial role of crude aqueous extracts from *Arabidopsis*. In one of the fractions, 4-methylsulphinylbutyl isothiocyanate was identified as a major compound with a broad spectrum of antimicrobial activity. A wide range of the fungi and bacteria tested showed 50% inhibition *in vitro* at a concentration lower than 350 ìM of 4-methylsulphinylbutyl isothiocyanate *(99)*. When they tested the resistance of an *Arabidopsis MAM1* mutant which after damage exhibits a lower amount of 4-methylsulphinylbutyl isothiocyanate, only *Fusarium oxysporum* was found to be significantly more aggressive than on wild-type plants, suggesting that glucosinolate-derived isothiocyanates might play a role in the protection of *Arabidopsis* against particular pathogens *(99)*.

Development of clubroot disease of the *Brassicaceae*, caused by the obligate biotrophic *Plasmodiphora brassicae*, was related to an increase of auxin and cytokinins resulting in increased cell division and cell elongation *(100, 101)*. The high IAA content was attributed to the conversion of indole glucosinolates to IAN by myrosinase, and further conversion by nitrilase to IAA *(102, 103)*. Ludwig-Müller *et al.* (1999) investigated several *Arabidopsis* mutants altered in glucosinolate levels. They could not draw a general conclusion about the role of indole glucosinolates in the clubroot disease development. However, almost all mutants as well as Columbia wild type showed an increase of indole-3-methylglucosinolate, and some of the mutants showed less susceptibility to the pathogen. In this study, the levels of certain aliphatic glucosinolates, 2-hydroxy-3-butenyl and 3-butenyl, were also increased after infection in both wild type Columbia and some mutants, although the authors did not discuss that point *(103)*.

Among all studies, there were few investigating both changes of glucosinolates in inoculated leaves (= local response), and in non-inoculated leaves (= systematic response) *(103, 104)*. Doughty *et al.* (1991) studied the glucosinolate response after *Alternaria brassicae* infection of two *Brassica napus* cultivars, Bienvenu (low in erucic acid) and Cobra (low in erucic acid and glucosinolates). Different cultivars showed different glucosinolate changes after inoculation. Local induction was more obvious in the Bienvenu cultivar, aliphatic glucosinolates accumulated rapidly but declined later while indole and aromatic glucosinolates accumulated at a slower rate and reached a maximum after 16 days. In a similar manner an induction of indole and aromatic glucosinolates were found in the Cobra cultivar. A systemic response with a threefold increase of aliphatic glucosinolates was found only in the Cobra cultivar 5 days after inoculation *(104)*. Li *et al.* (1999) studied both local and systemic changes in glucosinolate pattern after *Sclerotinia sclerotiorum* inoculation of several *Brassica napus* cultivars. In the same way, as previously reported, different cultivars showed different glucosinolate pattern both locally and systemically. Changes were mostly due to the increase of indole and aromatic glucosinolates, although an increase of aliphatic glucosinolates was also reported *(91)*.

In analogy to herbivores, certain pathogens may have evolved to be able to tolerate and detoxify glucosinolates. For example, high levels of 4-pentenyl glucosinolate did not correlate with resistance against *Leptoshaeria maculans (105),* perhaps due to the existence of a hydratase which is able to degrade nitriles to the less toxic formamides *(106)*.

Glucosinolates and defense signal molecules

During their lifetime, plants have to react to various threats coming from the environment, including micro-

bial pathogens and herbivores. As a plant is unable to escape an attack by moving to a more favorable environment, plants have evolved a broad range of defense mechanisms. The main signal molecules, recognized by the plant, are derivatives of jasmonic acid, salicylic acid and ethylene which mediate the plant response resulting in the activation of distinct sets of defense genes *(107, 108)*. Several studies indicated changes in glucosinolate pattern after treatment with signal molecules providing another indication for a defense related role of glucosinolates. Previous studies showed that exogenous JA application usually elicits dramatic increase of indole glucosinolates while the aliphatic ones remain unchanged *(109, 110)*. For instance, the treatment of white mustard and oilseed rape with methyl jasmonate (MeJa) accumulated indole-3-ylmethyl glucosinolate *(109)*. In other studies, oilseed rape and *Arabidopsis* treated with MeJA were shown to accumulate both indole-3-ylmethyl glucosinolate and *N*-methoxyindole-3-ylmethyl glucosinolate *(104)*. By contrast, SA application to the roots increased all classes of glucosinolates in the shoots of oilseed rape, with aromatic glucosinolate levels increasing more than those of indole and aliphatic ones *(111)*.

Mikkelsen et al. studied glucosinolate content in response to either a single or a combination of treatments with MeJA, 2,6-dichloro-isonicotinic acid (INA) as functional homologue of SA and 1-aminocyclopropane-1-carboxylate (ACC) as ethylene precursor in *A. thaliana (112)*. The results obtained confirmed the accumulation of indole glucosinolates after MeJA treatment, e.g., the concentration of all indole glucosinolates increased upon treatment with MeJA. Treatment with INA or ACC induced 4-methoxyindole-3-ylmethyl glucosinolate levels. Treatments with both MeJA and ACC induced indole-3-ylmethyl and *N*-methoxyindole-3-ylmethyl glucosinolate levels while accumulation of 4-methoxyindole-3-ylmethyl glucosinolate was absent. Changes of aliphatic glucosinolate levels were also noticed. Upon MeJA treatment the levels of 5-methylsulfinylpentyl, 8-methylthiooctyl and 8-methylsulfinyloctyl glucosinolates increased significantly while the levels of 8-methylsulfinyloctyl glucosinolates also increased after INA, ACC and both MeJA and ACC treatments *(112)*.

Kliebenstein et al. report altered glucosinolate accumulation upon MeJA and SA treatment of different ecotypes, suggesting different responses in different *A. thaliana* cultivars. After treatment of the Landsberg ecotype Ler with MeJA and SA, four different glucosinolate response patterns were identified which were not seen in the ecotype Columbia Col-0. The first pattern is characterized by a MeJA induction and includes the 3-hydroxypropyl, indole-3-ylmethyl and 1-methoxyindole-3-ylmethyl glucosinolates. The MeJA induction maximized within 24 hours, after which the levels of 3-hydroxypropyl glucosinolate returned to control levels, while indole-3-ylmethyl glucosinolate levels remained elevated. The second response pattern was the induction of 4-methoxyindole-3-ylmethyl glucosinolate as the only glucosinolate induced exclusively by SA but not by MeJA. The third response pattern was a synergistic action of both MeJA and SA which resulted in the accumulation of 8-methylsulfinyloctyl glucosinolate after 48 hours. The last response included 8-methylthiooctyl glucosinolate that showed no regulation by any of the compounds tested *(113)*.

A recent interesting study has monitored the response of glucosinolates in *Brassica olearacea* and *Brassica nigra* after the application of JA and SA either on shoots or on roots. There were changes in all classes of glucosinolates, but those changes differed depending on the treatment (root or shoot application of signal molecules) as well as on the species *(114)*. For example, *B. olearacea* after JA treatment of either shoot or root or both showed increased levels of all indole and some aliphatic glucosinolates in shoots. In roots, a similar increase was observed in case of aliphatic glucosinolates while indoles were not affected. The treatment of root and both root and shoot with SA induced some aliphatic glucosinolates only in shoot, while the treatment with both SA and JA induced both aliphatic and indole glucosinolates also in shoots *(114)*.

From all the glucosinolate literature present, it is evident that the overall defense response of a plant is a rather complex process which considerably varies depending on environmental conditions and the species involved and therefore we are far from a complete understanding of glucosinolate function in plant defense. Numerous studies have shown that glucosinolates and their hydrolysis products have an impact on herbivores and pathogens; however, many questions about the mechanism of glucosinolate induction, the signaling pathways involved and the plant's potential benefit from glucosinolate accumulation remain to be solved.

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