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Glucosinolates: Structure, classification, biosynthesis and functions in higher plants

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ABSTRACT

Objective: To analyze concepts, structure, classification, biosynthesis and functions of glucosinolates (GSLs) in higher plants.

Design/methodology/approach: A search was performed into recent high-impact literature related to glucosinolates (GSLs).

Results: GSLs are secondary metabolites rich in N and S. They are divided into aliphatic, aromatic, and indole GSLs depending on the amino acid from which they arise. The products of their hydrolysis, mediated by thioglucoside glucohydrolase, thioglucosidase or myrosinase enzymes (EC 3.2.1.147), play a role in increasing tolerance to biotic and abiotic stress factors. Furthermore, given their composition, they can serve as a nutrient reservoir under nutrient deficiency conditions.

Limitations on study/implications: GSLs are synthesized only in species of the Capparidaceae, Brassicaceae, Resedaceae, and Moringaceae families.

Findings/conclusions: GSLs are sulfur compounds that can serve as defense mechanisms against biotic and abiotic stress factors and as sources of nutrients in plants, and molecules with important nutraceutical properties in food and human health.

Keywords: secondary metabolites, sulfur, defense compounds, Brassicaceae.

INTRODUCTION

Glucosinolates (GSLs) are sulfur-rich secondary metabolites derived from amino acids (Kamal *et al.*, 2022). They are especially synthesized by species of the Brassicacea family, which includes cultivated plants of global importance for human consumption, such as cauliflower (*Brassica oleracea* var. *botrytis*), cabbage (*Brassica oleracea* L. var. *capitata*), broccoli (*Brassica oleracea* var. *italica*), bok choi (*Brassica rapa* ssp. *chinensis*), radish (*Raphanus sativus*), and Brussels sprouts (*Brassica oleracea* var. *gemmifera*). In plants, GSLs contribute to mitigating

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damage from biotic (pests and diseases) and abiotic (salinity, extreme temperatures, and radiation) stress factors, and in turn, when consumed as food, they promote better health conditions in humans (Nguyen *et al.*, 2020).

Glucosinolates are hydrolyzed by the myrosinase enzyme after the plant perceives a stress signal. After hydrolysis, the resulting compounds comprise isothiocyanates, thiocyanates, epithionitriles, and nitriles (Hanschen and Schreiner, 2017). In species of the Brassicaceae family, the nutrients C, N, and S are used for the synthesis of GSLs (Koroleva *et al.*, 2010; Jeschke *et al.*, 2019), which fulfill important defense functions against biotic and abiotic factors (Feng *et al.*, 2022). At least 130 different structures of GSLs have been identified in species of this plant family (Essoh *et al.*, 2020).

The term glucosinolates refers to the glucosyl ("gluco") moiety, the presence of a sulfate (ate) group, and the property of being a precursor to a mustard oil (sinol). GSLs have been defined as natural substances found in different plants, and participate as part of a defense mechanism against herbivorous insects. Plants of the Brassicaceae family, such as cabbage (Chhajed *et al.*, 2020), mustard (*Brassica nigra*; Blažević *et al.*, 2020), broccoli, Brussels sprouts, cauliflower, kohlrabi (*Brassica* × *napobrassica*), and radish (Marcinkowska and Jeleń, 2020), show these metabolites in the highest concentration. The amount of GSLs varies from one species to another and directly influences the type of plant tissue (Nguyen *et al.*, 2020).

GSLs are responsible for the spiciness of species such as mustard or horseradish. In some cases, they may offer protection against some types of cancer. In particular, the raw consumption of species from the Brassicaceae family offers high bioavailability of isothiocyanates (produced by the myrosinase activity on GSLs). Among the isothiocyanates are benzyl isothiocyanate, phenethyl isothiocyanate, and sulforaphane [1-isothiocyanato-4-(methyl-sulfinyl) butane], which have proven to target proteins related to cell proliferation and homeostasis. The interaction of isothiocyanates with proteins involved in DNA repair inhibits the cell cycle and induces programmed cell death, actions that reduce tumor growth (Soundararajan and Kim, 2018).

GSLs are transported by the phloem and can help the plant defend itself against organisms that feed on phloem products and also acquire the ability to coordinate the synthesis and use of protective resources between different organs (Koroleva *et al.*, 2010). The GSLs that fulfill the defense function of plants are thioglycosides that are derived from their hydrolysis.

STRUCTURE AND CLASSIFICATION OF GLUCOSINOLATES

The structure of glucosinolates consists of a sulfonated aldoxime domain linked to a –D-thioglucose group together with a side chain (aglycone) derived from one or several amino acids (Figure 1; Blažević *et al.*, 2020; Sugiyama *et al.*, 2021).

GSLs contain an oxidized sulfur atom of 3'-phosphoadenosine 5'-phosphosulfate (PAPS), a reduced glutathione atom and, in the case of methionine-derived aliphatic GSLs, a third sulfur atom (Mitreiter and Gigolashvili, 2021).

GSLs are classified as aliphatic, aromatic, and indole GSLs (Kamal *et al.*, 2022), depending on the type of amino acid from which they come (Figure 2).

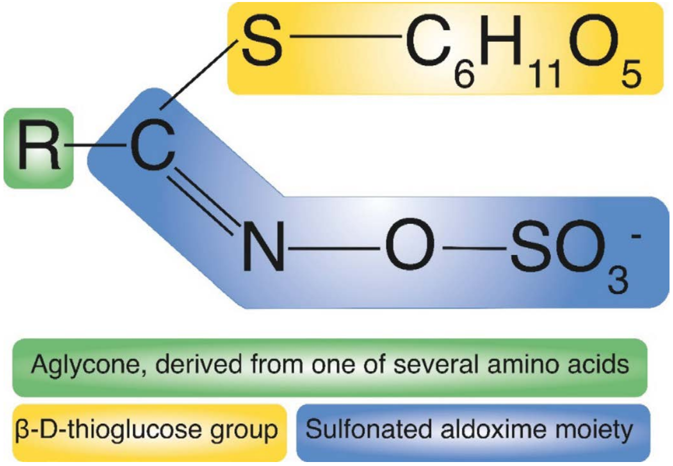

Figure 1. Basic structure of glucosinolates.

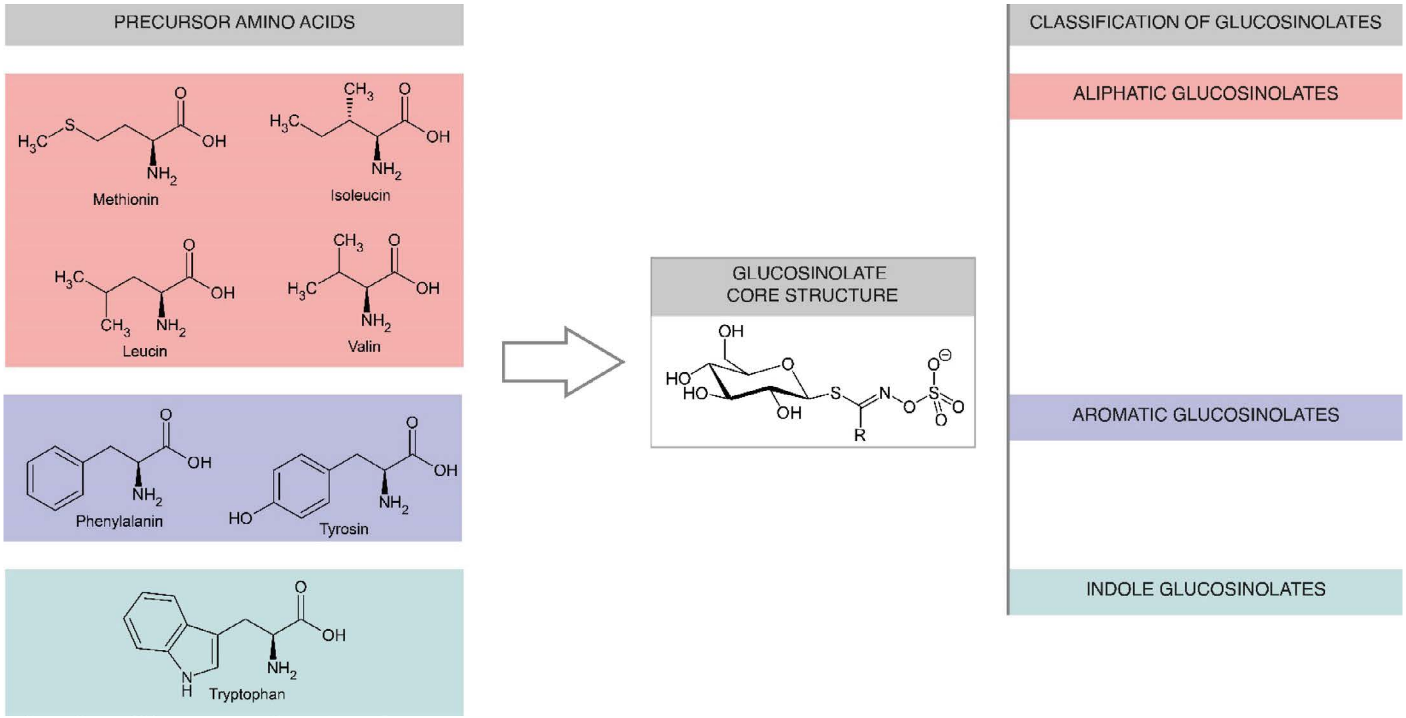

Figure 2. Classification of glucosinolates (GSLs) according to the type of precursor amino acid. Aliphatic GSLs are derived from methionine, isoleucine, leucine, or valine. Aromatic GSLs are derived from phenylalanine or tyrosine. Indole GSLs are derived from tryptophan.

GSLs are biologically inactive (Urbancsok *et al.*, 2018) and are degraded by the thioglucoside glucohydrolase, thioglucosidase, or myrosinase enzyme (EC 3.2.1.147), which hydrolyzes the glucose residue (Mitreiter and Gigolashvili, 2021). after cell damage, thus obtaining degradation products such as: glucose, sulfate and, depending on the chemical structure (Aghajanzadeh *et al.*, 2014), isothiocyanates, thiocyanates, epithionitriles, and nitriles (Bones and Rossiter, 2006) that function as defense substances

(Sugiyama *et al.*, 2021). The general reaction catalyzed by thioglucoside glucohydrolase is shown in Figure 3.

The final products of the degradation of GSLs depend on factors such as pH, availability of ferrous ions, and proteins that interact with the thioglucoside glucohydrolase enzyme (Martínez-Ballesta *et al.*, 2013).

The storage of GSLs and thioglucoside glucohydrolase enzymes is spatially distinct. Therefore, they only interact after the plant has faced some kind of stress. Specialized cell types can act as different storage locations: S-cells for GSLs and myrosin cells for classical myrosinases (Mitreiter and Gigolashvili, 2021). S-cells contain up to 40% of the total sulfur of *Arabidopsis thaliana* flower stem tissue (Koroleva *et al.*, 2010).

BIOSYNTHESIS OF GLUCOSINOLATES

The biosynthesis of glucosinolates occurs mainly in the leaves, from where they are transported to other organs of the plant. Their biosynthesis in different organs is more active in young growth stages and less so in mature stages (Feng *et al.*, 2022).

The biosynthesis of GSLs consists of three stages (Figure 4): I) chain elongation in which a methylene group is inserted into the side chain of aliphatic amino acids; II) the metabolic reconfiguration of the rest of the amino acids to produce the central structure; and III) the modification of the core structure to produce GSLs with various aglycone structures (Nguyen *et al.*, 2020).

The first stage begins with a deamination of the amino acids by branched-chain amino acid aminotransferase (BCAT) that transforms them into 2-oxoacids, which condense with acetyl-coenzyme A by the action of the methylthioalkylmalate synthases enzyme (MAMs) and thus forms a 2-malate derivative. This last compound is isomerized to a 3-malate derivative by isopropylmalate isomerase (IPMI). This is followed by decarboxylation by the isopropylmalate dehydrogenase enzyme (IPMDH) and produces an elongated 2-oxoacid intermediate that can undergo transamination to provide extended amino acids for the next stage or re-enter the transformation cycle for further elongation (Nguyen *et al.*, 2020; Figure 4).

In the second stage (Figure 4) there is an oxidation of the amino acid into aldoximes. This oxidation is catalyzed by three enzyme systems [cytochrome-P450 (CYP79) dependent monooxygenase, flavin-containing monooxygenase, and peroxidase]. The participation of each enzymatic system depends on the nature of the amino acid precursors. Cytochrome monooxygenases CYP83 activate the aldoxime resulting from oxidation of the amino acid to the corresponding thiohydroxymate. The activated aldoxime is conjugated with reduced

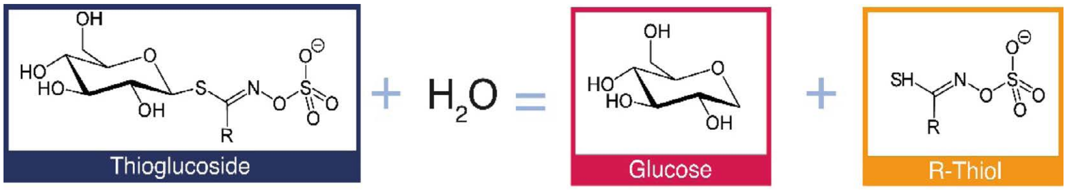

Figure 3. Representation of the reaction catalyzed by thioglucoside glucohydrolase (EC 3.2.1.147). R-thiol varies depending on the chemical structure, and can be isothiocyanates, thiocyanates, epithionitriles, and nitriles.

glutathione (GSH), which donates S to produce the intermediate thiohydroxymate. The S-alkyl-thiohidoximate intermediate formed is cleaved by the activity of a C-S lyase enzyme: SUR1 to form thiohydroxymates. These thiohydroxymates are transformed by the UDP-glucose:thiohydroxymic acid S-glucosyltransferases (S-GT) and desulfoglucosinolate sulfotransferases enzymes to produce the core structure of GSLs with the corresponding side chains (Nguyen *et al.*, 2020).

In the third stage, chemical transformations of the GSLs side chains occur through enzyme-catalyzed oxidations, eliminations, alkylations, and esterifications (Figure 4). These modifications contribute to the structural diversity of GSLs (Nguyen *et al.*, 2020).

FUNCTIONS OF GSLs IN PLANTS

GSLs are widely synthesized in species of the Capparidaceae, Brassicaceae, Resedaceae, and Moringaceae families (Lockwood, 1988), although most studies have been done on species of the Brassicaceae family.

When the species of the Brassicaceae family suffer an attack, the GSLs are hydrolyzed by thioglucoside glucohydrolases or myrosinases enzymes into different defense products, including isothiocyanates, which are the most characterized. Isothiocyanates are toxic to insect pests and disease-causing pathogenic microorganisms. However, when synthesized excessively, these compounds can be harmful to the plant, as they can cause stomatal closure, alter microtubules in the cytoskeleton, deplete reduced glutathione (GSH), inhibit root growth or induce cell death (Ting *et al.*, 2020).

GSLs act as excellent defense mechanisms against generalist herbivores, but are less effective against specialist herbivores (Schweizeir *et al.*, 2013). In addition, these sulfur compounds can also be toxic to microbial pathogens both in the soil and in the aerial part of the plant (Mitreiter and Gigolashvili, 2021).

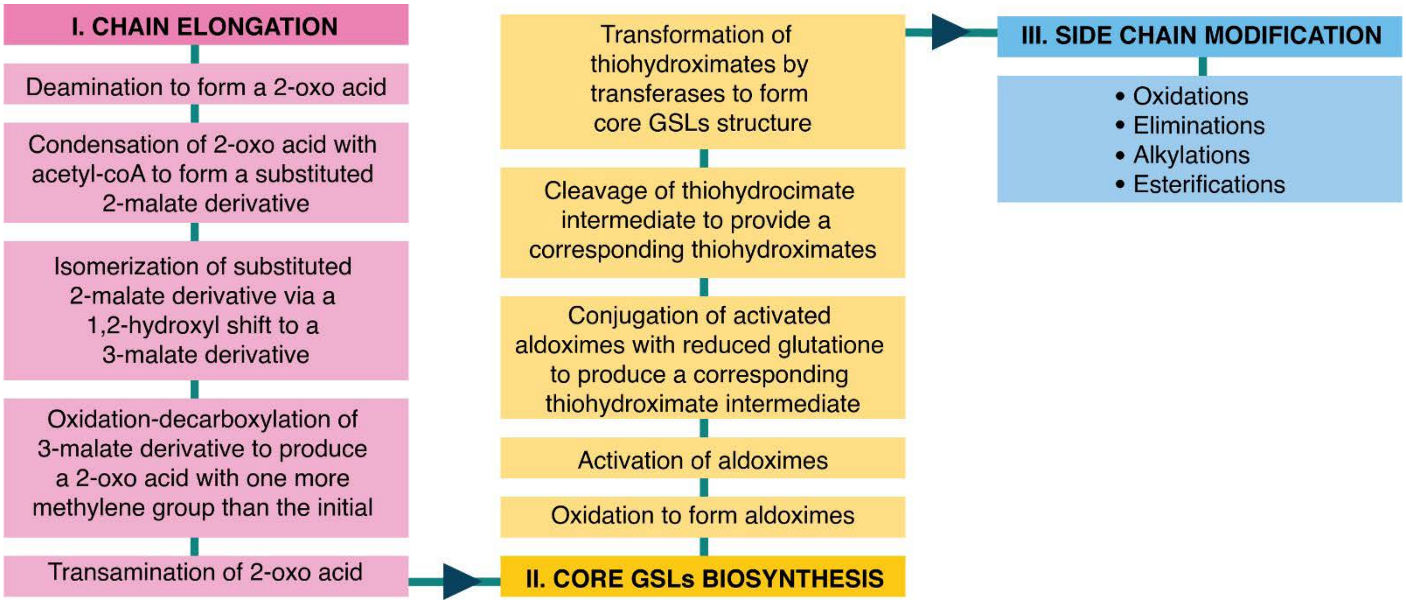

Figure 4. Biosynthesis stages of glucosinolates (GSLs) (Nguyen *et al.*, 2020).

To produce crops with a greater amount of desirable compounds, several strategies can be followed. The first is to select species, genotypes, or cultivars that contain a genetically determined higher level of phytochemicals (Bouargalne *et al.*, 2022; Zhan *et al.*, 2022). The second is to manipulate the growth factors and environmental conditions for plant cultivation (Trejo-Téllez *et al.*, 2019; Šamec *et al.*, 2021). A third alternative is the use of genetic engineering, metabolic engineering, and genome editing (Miao *et al.*, 2021).

In adverse environmental conditions such as drought, salinity, extreme temperatures, and excessive exposure to UV radiation, plants activate defense mechanisms that include the accumulation of specialized metabolites or phytochemicals (Šamec *et al.*, 2021). These natural plant defense mechanisms can be stimulated during the cultivation of certain species, which triggers greater production of desirable compounds.

Eustressors are biological, physical, or chemical stressors that activate signaling pathways that lead to increased content of bioactive compounds. Salinity is considered a chemical stress factor that affects the physical quality and chemical composition of various plant products (Rouphael *et al.*, 2018).

By increasing the level of salinity in crops of species of the Brassicaceae family, a concomitant rise in the content of bioactive compounds can be observed, at the expense of their growth and yield (Santander *et al.*, 2022).

Salinity differentially affects the metabolism of GSLs in plants, which depends on environmental conditions such as temperature and radiation, nutritional management, type of GSL synthesized, and the genotype of the plant (Rios *et al.*, 2020).

In *Brassica oleracea* L. var. *italica* exposed to 40 and 80 mM NaCl for two weeks, an increase in the content of GSLs was observed, the same as in *Brassica rapa* L. exposed to 20, 40, and 60 mM NaCl for five days (Steinbrenner *et al.*, 2012).

In species of the Brassicaceae family, GSLs can represent up to 30% of the total sulfur concentrations (Falk *et al.*, 2007; Sugiyama *et al.*, 2021). This means that GSLs can be nutrient reservoirs, which under nutrient deficiency can be hydrolyzed by myrosinase enzymes, so that sulfur is reallocated to primary metabolites such as cysteine (Sugiyama *et al.*, 2021). Thus, under stress conditions, these secondary metabolites can be degraded for the formation of other molecules.

Given the importance of different species of the Brassicaceae family in human nutrition, it is important to highlight that GSLs can contribute to improving health as these compounds have shown protective properties against the incidence of cancer and cardiovascular diseases (Traka, 2016).

CONCLUSIONS

GSLs are secondary metabolites rich in N and S; they are mainly synthesized by plant species of the Brassicaceae family. By the type of amino acid from which GSLs come, they are divided into aliphatic, aromatic, and indole GSLs. The products of their hydrolysis mediated by myrosinase enzymes play a role in increasing tolerance to biotic and abiotic stress factors. In addition, given their composition, they can serve as a nutrient reservoir under deficiency conditions. Finally, GSLs have nutritional functions and can contribute to improving human health.

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