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REVIEW ARTICLE

THE GLUCOSINOLATES-MYROSINASE SYSTEM: FROM CHEMISTRY, BIOLOGY TO ECOLOGY

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ABSTRACT

Glucosinolates, a well-defined, sulfur-rich class of plant secondary products mainly confined to various crops of the family Brassicaceae, are of prime importance in agriculture and plant biotechnology since the discovery of their role in plant defense against insect herbivory. Till date more than 120 different types of the compound have been reported. The enzyme myrosinase (thioglucosidases), which is stored in specialized plant cells, converts glucosinolates to the several toxic products (e.g., isothiocyanates, thiocyanates, and nitriles). The hydrolysis products have many different biological activities for plants, e.g., as defense compounds as well as attractants. In case of human, they may play several roles as biopesticides, flavor compounds and cancer-preventing agents. In the present article, we try to discuss broadly the biochemistry and the roles of the compounds, their break-down products in the insect-plant relationships and multitrophic interactions. Major focus has been laid on Brassicaceous crop plants, where they are most abundantly found.

INTRODUCTION

Brassica spp. has been one of the most important crops throughout the world, both for human consumptions as well as fodder and green fertilizer. It ranks third as a source of oil production after soybean and palm oil. Brassicaceous crops and the glucosinolates-myrosinase system, their preformed chemical defenses have become a major field of ongoing researches for plant breeders. The so called “mustard oil glucosides” have been a part of human life for thousands of years because of the strong flavours and tastes they impart in these crops. In recent past, these plant secondary metabolites have become a compound of prime importance following the discovery of the roles they play in plant-pest interactions. The “glucosinolates-myrosinase” system is known to play a predominant role in the behaviour of various pests’ complexes. The interaction of insect pests and Brassicaceous crops represents one of the best studied aspects of plant-insect biology. The natural variation of glucosinolates in plant tissues and its impacts on the resistance against insect herbivory illustrates the ecological role of the glucosinolates-myrosinase system. Besides this, they also influence the tastes/flavour and health characteristics of various Brassicaceous crops (Kliebenstein, Kroymann *et al.,* 2005). Moreover these compounds are also known as a cancer prevention agent (Holst and Williamson 2004; Keum, Jeong *et al.,* 2004). With new molecular and gene manipulation techniques emerging, there is a huge promising future in this field of research. Here in the present communication, we present and try to elucidate the relevance of glucosinolates present in these crops in plant-herbivores interactions and the resistance conferred by these compounds to the plants against herbivory.

Glucosinolates

Glucosinolates are a well-studied class of plant secondary metabolites, mainly because of their occurrence in important crops such as cabbage, broccoli, and oilseed rape (Hopkins, van Dam *et al.,* 2009b). There are at least 120 types of different glucosinolates been identified and mostly confined to the Family Brassicaceae (Fahey, Zalcmann *et al.,* 2001) and few other related groups. The various effects of glucosinolates on the quality of both human and animal foods have encouraged interest in their natural biosynthetic pathways, and in the possibility of manipulating glucosinolates levels to produce new and improved commercial varieties. Glucosinolates in *Brassica* spp. has been a major subject of study and there are available various scholarly reviews and literatures by Kjñr (1974, 1976), Fenwick *et al,* (1983), Chew (1988), McDanell *et al,* (1988), Duncan and Milne (1989), Stoewsand (1995) and

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Rosa *et al*, (1997). The glucosinolates-myrosinase system's predominant role is presumably in mediating the interaction of plants with their biotic environment. The hydrolysis products have many different biological activities, e.g., as defense compounds as well as attractants. The content of glucosinolates is of particular interest in the oilseed rape crop. A high content in the seeds impairs the quality because it restricts the possible use of the cake and the meal in animal nutrition. On the other hand, low glucosinolates content in the whole plant seems to be one reason for reducing plant resistance to stress and disease. Hence there has been a considerable interest in manipulating glucosinolates contents and composition in *Brassica* breeding programs. And with the advent of new and improved molecular and genetic tools and technology, the research area in the field is drawing great attentions.

Distribution in the Plant Kingdom

Glucosinolates are mainly confined to the family Brassicaceae. However, there are at least 500 species of non-cruciferous dicotyledonous angiosperms that have been reported to contain one or more of the over 120 known glucosinolates. The plant families, other than Brassicaceae, that accumulate glucosinolates include Capparaceae, Bataceae, Moringaceae, Resedaceae, Tropaoeolaceae, Euphorbiaceae, etc. to name a few (Fahey, Zalcmann *et al.*, 2001). The presence of a particular type of this compound in a group is of high taxonomic importance.

Glucosinolates Contents in Plants

There is a great variation in the glucosinolates profile between and within plants. Each plant species containing the compounds has a glucosinolates profile composed of a few limited numbers of the 120 glucosinolates known. This variation in the glucosinolates profile may be attributed to the diversification of species and the emergence of new taxa that can synthesize these compounds (Lazzeri, Curto *et al.*, 2004). Within a species itself, there is a considerable variation in the glucosinolates profile as well. The diversification of the glucosinolates profile might have occurred both during natural as well as artificial selections (Kliebenstein, Kroymann *et al.*, 2005). Glucosinolates and myrosinases occur in almost all plant organs and during all ontogenetic stages of the plant. However, their levels may vary considerably. The glucosinolates content in plants is about 1% of dry weight in some tissues of the *Brassica* vegetables, although the content is highly variable (Farnham, Grusak *et al.*, 2000; Kushad, Brown *et al.*, 1999). In seedlings, their content is maximum in the cotyledons, where as in case of vegetative plant, highest overall concentration can be found in the roots than in the shoots. Young leaf tissues also contain significantly high amount of the compound (Lambdon and Hassall 2005; van Dam, Tytgat *et al.*, 2008). The highest glucosinolates contents are found in reproductive tissues, such as flowers and seeds (Brown, Tokuhisa *et al.*, 2003; van Dam, Tytgat *et al.*, 2008). The concentration can approach 10% in the seeds of some plants, where glucosinolates may represent one-half of the sulphur content of the seeds (Josefsson 1970). There seems to be a tremendous shift in the allocation pattern of the compound during the ontogenetic development of the plant. This reflects the optimal allocation for defense purposes. This may also reflect the adaptation of the plants to protect the most important and valuable organs in terms of the plant fitness (Brown, Tokuhisa *et al.*, 2003). Besides the total glucosinolates levels, there is also a great variation in the glucosinolates composition between different tissues within the same plant. The root and the shoot glucosinolates composition profiles vary and show varying effects against a variety of pests and pathogens. Seed specific glucosinolates are very potent against insect pests such as seed weevils and some fungal pathogens. On the other hand, those glucosinolates which are more prominent in the root tissues, show more potent effects and confer resistance against soil dwelling pests, e.g. Phytophagous nematodes (Potter, Vanstone *et al.*, 2000).

Chemistry

Glucosinolates are plant secondary metabolites that are anionic and rich in sulphur content and that upon hydrolysis by endogenous thioglucosidases called myrosinases produce several different products, e.g., isothiocyanates, thiocyanates, and nitriles (Halkier and Gershenzon 2006). The basic chemical structure of all glucosinolates consists of three building blocks: a β-thioglucose moiety, a sulfonated oxime moiety, and a variable side chain (Lazzeri, Curto *et al.*, 2004). They share a core structure containing a β-D glucopyranose residue linked via a sulfur atom to a (Z)-N-hydroximino sulfate ester, and are distinguished from each other by a variable R group derived from one of several amino acids (Fahey, Zalcmann *et al.*, 2001). Most glucosinolates are assigned to one of the three major types according to the side chain amino acid precursors. About 10% of the known structures of glucosinolates are derived from tryptophan and are known as indole glucosinolates. Aliphatic glucosinolates are derived from metionine, leucine, isoleucine or valine and constitutes 50%, and aromatic glucosinolates (10%) are synthesized from tyrosine and phenylalanine (Halkier and Gershenzon 2006; Muller, Boeve *et al.* 2002).

The R group then undergoes a significant modification from these precursor amino acids. Major modifications are achieved through chain elongation, oxidation, hydroxylation of the side chain (Grubb and Abel 2006; Hartmann 2004) controlled by the *GS-AOP* locus (in *A. thaliana*). Glucosinolates can provide an intact and constitutive resistance against insect herbivores (Kim and Jander 2007). The defensive properties of the compounds are enhanced upon hydrolysis by the enzyme myrosinase. Almost all plants accumulating GS poses a thioglucosidase glucohydrolase activity, the myrosinases that hydrolyzes the glucose moiety on the main skeleton. They are stored in special myrosinase cells found in all plant organs (Rask, Andreasson *et al.*, 2000). When the plant tissue is damaged, for example by a chewing insect, the glucosinolates stored in the vacuole come in contact with the myrosinases. The resulting products are glucose and unstable aglycone that rearrange to form isothiocyanates, nitriles, and other products (Wittstock, Kliebenstein *et al.*, 2003). Hydrolysis in intact plant is hindered as the glucosinolates and the myrosinase are spatially separated or by the inactivation of the myrosinases. The biological activities of the glucosinolates largely depend on the resulting hydrolysis products and the chemical structure of the side chain (Burow, Markert *et al.*, 2006; Lambrix, Reichelt *et al.*, 2001; Wittstock, Kliebenstein *et al.*, 2003). Even a minor structural difference may result in the formation of products with biologically opposing activities. Besides this, the pH and the presence of other associated proteins such as epitiospecifier protein (ESP) and epithiospecifier modifier (ESM1) protein are also important deciding factors for the final products of the glucosinolates–myrosinase reaction (Burow, Markert *et al.*, 2006; Lambrix, Reichelt *et al*., 2001).

Glucosinolates Biosynthesis

The biosynthesis of glucosinolates can be discussed in three broad headings; (I) Amino acid side chain elongation, (II) Glucone synthesis and (III) Chain modification. First of all, the amino acids are elongated by addition of methylene groups to their side chains. This is followed by reconfiguration of the elongated amino acid to give rise to the glucosinolates core structure. And finally during the side chain modification, the structure so formed goes through a series of modification and transformation to give the final product (Halkier and Gershenzon 2006).

Amino Acid Side Chain Elongation

Chain extension occurs in a reaction cycle that involves transamination of the parent amino acids to give rise to its corresponding oxo-acids. The reaction cycle occurs in three phases. Firstly, condensation of the oxo-acids with acetylCoA takes place, which is catalysed by methylthioalkylmalate synthase (MAM). The product is a substituted 2-malate derivative. This then isomerizes via a 1, 2-hydroxyl shift to a 3-malate derivative. Finally this undergoes an oxidation-decarboxylation to yield a 2-oxo acid with one more methylene group than the starting compound. The resulting chain extended 2-oxo acid can undergo additional chain-elongation cycles, each adding one further methylene group, or, following transamination, can enter the glucosinolate core biosynthetic pathway (Halkier and Gershenzon 2006; Hirai, Klein *et al*., 2005). The genetics of the chain elongation have been studied extensively in *Arabidopsis thaliana* and *Brassica napus.* The locus controlling the chain length of methionine-derived glucosinolates in these plants has been identified (Magrath, Bano *et al.,* 1994). This locus, called the MAM locus comprises of a small tandemly linked gene family encoding the enzymes required for side chain elongation of methionine precursors of aliphatic glucosinolates (Kroymann, Donnerhacke *et al*., 2003). This has also been called as the *GS-Elong* locus. MAM1 has been identified as responsible for chain elongation polymorphism in *A. thaliana* ecotypes (Kroymann, Textor *et al.*, 2001). The gene product is reported to be responsible for condensation reaction of the first two elongation cycles only (Textor, Bartram *et al.,* 2004) and controls the glucosinolates profiles. Two more sequences were identified later, MAM-L (MAM-like) and MAM2 (Field, Cardon *et al.,* 2004; Kroymann, Donnerhacke *et al*. 2003). MAM-L has been reported to provide precursors for aliphatic glucosinolates with long side chain (Field, Cardon *et al.,* 2004) where as MAM1 and MAM2 are responsible for short chain glucosinolates (Kroymann, Textor *et al*., 2001; Textor, Bartram *et al*. 2004). The allelic variation between the MAM alleles has been shown to be responsible for the cause of QTL for glucosinolates and resistance against generalist herbivores in various lines of *A. thaliana* (Kroymann, Donnerhacke *et al.* 2003; Kroymann, Textor *et al*., 2001). This variation may also influence the glucosinolates and the resistance conferred against specialist pests.

Glucone Synthesis

Glucone biosynthesis is initiated by the conversion of protein amino acids (e.g. alanine, methionine, valine, leucine or isoleucine for the aliphatic glucosinolates; phenylalanine or tyrosine for the aromatic glucosinolates and tryptophan for the indole glucosinolates) or chain elongated amino acids (e.g. many of the precursors of aliphatic glucosinolates such as homomethionine, dihomo-methionine, trihomo-methionine) to aldoximes. The core pathway converts the amino acid to an S-alkylthiohydroximate via two consecutive reactions that are catalyzed by structurally specific cytochrome P_{450}, encoded by the CYP79 and CYP83 gene families (Chen, Glawischnig *et al.* 2003; Glawischnig, Hansen *et al.*, 2004). This is followed by the conversion of the aldoximes to thiohydroximic acids that are converted into desulfo-glucosinolates (Grubb, Zipp *et al*., 2004). The final glucosinolates are produced by sulfation by a particular sulfotransferases (Piotrowski, Schemenewitz *et al*., 2004).

Chain Modification

Finally, various secondary side chain modifications and transformations occur to the initially formed parent glucosinolates to give the final product. This includes oxidation, hydroxylation, alkenylation, acylation or esterification (Tokuhisa, Kraker *et al*., 2004). The side chain modification is controlled by a single genetic locus, *GS-AOP* in *Arabidopsis thaliana.* This locus has three alleles, namely *GS-ALK*, *GS-OHP* and *GS-null*, each of which controls the alkenyl side chain, hydroxyalkyl side chain and methylsulfinyl side chain respectively (Hall, McCallum *et al.,* 2001; Kliebenstein, Lambrix *et al.,* 2001). These reactions are of biological as well as biochemical importance as they influence the direction of hydrolysis and their products. Glucosinolates synthesis is controlled in a polygenic manner, i.e. by QTLs. A large number of variable loci control the glucosinolates biosynthesis and their accumulation (Halkier and Gershenzon 2006). There have been four to six QTLs indentified in *B. napus* that control the aliphatic glucosinolates in the seeds(Toroser, Thormann *et al.* 1995; Uzunova, Ecke *et al.,* 1995). Of these *GS-Elong* (MAM) and GS-AOP genes are responsible for the biosynthetic pathway (Halkier and Gershenzon 2006). Allelic variation in the *GS-Elong* locus has been reported to influence the glucosinolates composition and their accumulation in plant tissues. This variation is also known to influence the resistance of the host plant against generalist insect herbivore *Spodoptera exigua*, but not insect specialists (Kroymann, Donnerhacke *et al.,* 2003). More detailed studies have been discussed by Halkier *et al*., 2006 and Kroymann *et al*., 2001, 2003.

Myrosinases: The Glucosinolates Degrading Enzymes

Chemically, thioglucohydrolase, myrosinases (as more commonly known by its trivial name) are the enzyme responsible for the breakdown of glucosinolates. These enzymes are known to be found in all tissues of glucosinolates containing plants. It is also reported in some fungi, bacteria and insects (Rask, Andreasson *et al.,* 2000). The activity of these enzymes in plants is dependent on the type of species, cultivar and the plant organ in which they contain. Highest activity is usually found in seeds and seedlings (Bones 1990) which can be correlated with the protection of the most important organs, i.e. the reproductive organs. Studies in *Sinapis alba* L. have revealed that the myrosinases enzymes are encoded by gene families. There exist at least two main groups of myrosinases gene families, namely MA and MB.

There has been shown that there exist three to four MA genes and about ten MB genes (Xue, Jorgensen *et al.,* 1995). In *Arabidopsis thaliana,* the presence of three myrosinases genes has been suggested (Xue, Lenman *et al.,* 1992). The enzymes are stored in special idioblast termed as the myrosin cells and is found in all plant organs (Husebye, Chadchawan *et al.,* 2002). First reported in 1884 by Heinricher, these cells showed deviant histochemical staining characteristics and differed both in size and morphology from adjacent cells. Myrosin cells occur as scattered cells in roots, stems, leaves, petioles, seeds, and seedlings. Myrosin cells in longitudinal sections from cortex tissue in hypocotyls appear rectangular or cuboid, while in roots they are elongated in the direction of the organ axis. The main organelles in the myrosin cells are the spherical myrosin grains containing homogeneous electron-dense material (Rask, Andreasson *et al.,* 2000). The enzymes come in contact with the glucosinolates stored in vacuole only when the plant tissue is damaged, for example by chewing insect (Hopkins, van Dam *et al.,* 2009a). The enzymes are known to exist in several isoforms in different species which differ in their subunits molecular masses (Bjorkman and Janson 1972; Buchwaldt, Larsen *et al.,* 1986; Lenman, Rodin *et al.,* 1990).

Glucosinolates: Importance To Humans

Glucosinolates have long been drawing attentions of plant breeders because of their presence in Brassicaceous crops and the importance of these crops to human. There is a considerable interest in manipulating the glucosinolates contents and composition in *Brassica* breeding programs. These substances and their hydrolysis products are responsible for the characteristic sharp or bitter tastes and flavours of these crops. In the past years, glucosinolates have assumed major agricultural significance due to the increasing importance of rapeseed crops in which they contain. Major efforts are focussed towards the manipulation of the compounds in seeds as oil meal of *Brassica* origin is a good source protein and high glucosinolates in seeds renders their use as livestock feeds (Hopkins, van Dam *et al.,* 2009a). Moreover these compounds are known to confer resistance to the plants against a variety of herbivores.

Biological Effects

The glucosinolates-myrosinase system has been actively investigated as a feature of plant defense system for years, but there are still many gaps in our knowledge. The activation of glucosinolates upon plant damage and the biological properties of their hydrolysis products have long suggested that the major function of these compounds in plants is to defend against herbivores and pathogens (Halkier and Gershenzon 2006). A large numbers of studies conducted have shown that glucosinolates exhibit toxicity, growth inhibition, or feeding deterrence to a wide range of potential plant enemies, including mammals, birds, insects, molluscs, aquatic invertebrates, nematodes, bacteria, and fungi (Buskov, Serra *et al.,* 2002; Lazzeri, Curto *et al.,* 2004). The very compound may also serve as attractant for adapted herbivores. Many insect herbivores have come to specialize on glucosinolates-containing plants and often use these compounds as cues for feeding or oviposition (Gabrys and Tjallingii 2002; Halkier and Gershenzon 2006; Hopkins, van Dam *et al.* 2009a). Food crops that contain very low amounts of glucosinolates (e.g. canola) have been developed, because the use of glucosinolates-containing crops as primary food source for animals was shown to have negative effects. The glucosinolate sinigrin, among others, was shown to be responsible for the bitterness of cooked cauliflower as well as in Brussels sprouts. On the other hand, plants producing large amounts of glucosinolates are also desirable, because substances derived from these can serve as natural pesticides and are under investigation in the prevention of cancer (with sulforaphane in broccoli being the best known example). Consumers of higher levels of Brassica vegetables, particularly those of the genus Brassica (broccoli, Brussels sprouts and cabbage), may benefit from a lower risk of cancer at a variety of organ sites (Holst and Williamson 2004). Brassica vegetables contain high concentrations of glucosinolates that can be hydrolyzed by the plant enzyme, myrosinase, or intestinal microflora to isothiocyanates, potent inducers of cytoprotective enzymes and inhibitors of carcinogenesis.

Effects on Humans and other Mammals

The effects of the hydrolysis products on vertebrates are highly variable. Glucosinolates are well known for their toxic effects (mainly as goitrogens) in both humans and animals at high doses. One of the predominant rapeseed glucosinolates, 2-hydroxy-3-butenyl glucosinolate, forms an oxazolidine-2-thione upon hydrolysis that causes goitre and has other harmful effects on animal nutrition (Griffiths, Birch *et al.,* 1998). Glucosinolates rich diets may also be linked to growth depression, poor palatability, liver lesions and necrosis, etc (Nishie and Daxenbichler 1980; Nishie and Daxenbichler 1982). The role of these defense compounds is highly complicated and varies a lot. In contrast, at subtoxic doses, their hydrolytic and metabolic products act as chemoprotective agents against chemically-induced carcinogens by blocking the initiation of tumours in a variety of rodent tissues, such as the liver, colon, mammary gland, pancreas, etc. They exhibit their effect by inducing Phase I and Phase II enzymes, inhibiting the enzyme activation, modifying the steroid hormone metabolism and protecting against oxidative damages (Holst and Williamson 2004; Keum, Jeong *et al,.* 2004).

Glucosinolates-Myrosinase System: Relations With The Biotic Environment

Plants and insects have co-existed for millions of years since the earliest form of land plants and insects came into existence. They have evolved a series of relationships which affect the organisms at all levels, from basic biochemistry to population genetics (Glawischnig, Hansen *et al.,* 2004). Some of these relations between these two phyla can be mutually beneficial, such as pollination. But the most common interaction involves insect predation of plants, and development of plant defenses against herbivorous insects. In these long standing relationships, there are diverse strategies deployed by plants in attempt to resist or evade their insect herbivores. These may involve both preformed mechanisms as well as induced mechanisms. Other cases of interaction may be indirect, for example, multitrophic interactions. Moreover plant products may also provide insects with various types of token stimuli, feeding and oviposition stimuli. Glucosinolates present a classical example of preformed plant secondary metabolites affecting these plant-insect interactions. The most important role of these compounds in plant-insect interaction is the resistance they confer to the host plant against a variety of insect pests. Besides this, they may also act as attractants for other insects. Even they are present as a constitutive defense mechanism, their contents and concentrations are variable between and within the plant tissues and highly influenced by both biotic and abiotic factors including insect damage. There is a great variation in the effects of glucosinolates towards a variety of insect pests. The compounds may act as deterrents for a class of insects called as the generalists where as they may act as attractants for insect specialists.

Glucosinolates as Defense Compounds

Glucosinolates can also serve as defense compounds for the plants against a variety of pathogens. The isothiocyanates formed as a result of reaction between the glucosinolates and myrosinase are frequently responsible for the defensive activity of the parent glucosinolates. This can be demonstrated by the non preference by a variety of insect herbivores for plants with high glucosinolates contents. The negative effects of the product of the glucosinolates-myrosinase system on insect generalists are broad and quite complicated as generalist insects, except for few, are usually not well equipped to cope with the hydrolysis products viz. nitriles and epithionitriles generated in the presence of epithiospecifier (ESP) protein from alkyl and alkenyl glucosinolates, respectively (Kliebenstein, Kroymann *et al.,* 2005). Isothiocyanates are formed spontaneously in the absence of these protein factors, as in the case of the generalist insects. Either these compounds may have adverse effect on the biology of the insects, i.e. antibiosis (Painter 1941) or the plant products may result in non-preference or avoidance of the plants by the insects i.e. antixenosis (Kogan and Ortman 1978).

In addition to insect pests, glucosinolates have been demonstrated to have broad negative effects on other pathogens and vertebrate herbivores. A characteristic, specialised insect fauna are found on glucosinolates-containing plants, including familiar butterflies such as Large White, Small White, Orange Tips, but also certain aphids, moths, saw flies, flea beetles, etc. The biochemical basis of these specialisations is being unravelled. Herbivores that are specialized on glucosinolates accumulating host plants have certain mechanisms to overcome the toxicity of their hosts.

These insect specialists are physiologically equipped to cope up with the toxic nature of glucosinolates and its hydrolysis products. The strategies may include detoxification, excretion and sequestration. *Myzus persicae*, a specialist on *Brassica* spp. is known to excrete glucosinolates in its honeydew (Hirai, Klein *et al.,* 2005). Other insect specialists are known that redirect the glucosinolates breakdown pathway from the normal course and metabolize them to non toxic derivatives. The Whites and Orange tips all possess the so-called nitrile specifier protein (NSP), which diverts glucosinolate hydrolysis toward nitriles rather than reactive isothiocyanates (Wittstock, Kliebenstein *et al.,* 2003). These nitriles are then excreted in their frass. In case of *Pieris rapae*, the cabbage White butterfly, a protein factor similar to EPS is secreted in the guts which readily redirects the hydrolysis of glucosinolates by myrosinase towards the formation of nitriles and epithionitriles. These compounds are less toxic and are excreted in the faeces (Wittstock, Kliebenstein *et al.,* 2003). In contrast, the Diamondback Moth (*Plutella xylostella*) possesses a completely different protein, glucosinolate sulphatase, which desulphates glucosinolates, thereby making them unfit for degradation to toxic products by myrosinase.

This enzyme in the gut of the insect larvae competes with the myrosinase leading to conversion of glucosinolates to less toxic compounds than nitriles and isothiocyanates. The enzyme cleaves the sulphate residue from the glucosinolates core structure to give an end product that is no longer a substrate for the myrosinase (Miles, del Campo *et al.,* 2005). The sulfatase enzyme has also been reported from generalist insect, *Helix pomotia* (Thies 1979). There are also certain insect species that completely bypass the myrosinase hydrolysis. These insects (specialised sawflies and aphids) sequester glucosinolates (Muller, Agerbirk *et al.,* 2001). Several of these insects have evolved their own myrosinase and use a bipartite glucosinolates-myrosinase system for their own defense (Jones, Winge *et al.,* 2002). This sequestration is shown to deter the predators such as birds, lizards, and ants. In specialised aphids, a distinct animal-myrosinase is found in muscle tissue, leading to degradation of sequestered glucosinolates upon aphid tissue destruction (Bridges, Jones *et al.,* 2002). The enzyme is apparently stored separately from the glucosinolates in the aphid's body and when the aphid is damaged or killed by a predator, forms isothiocyanates that serve for their own defense as well as to warn the colony.

This diverse panel of biochemical solutions to the same plant chemical plays a key role in current attempts to understand the evolution of plant-insect relationships (Wheat, Vogel *et al.,* 2007). Moreover, there are also some behavioural adaptations of some insects to cope with the glucosinolates of the host plants. This relies on the fact that there is a great variation in the distribution and accumulation of the compounds within a single plant itself. This variation provides both challenge as well as opportunity for a variety of specialist and generalist insects (Hopkins, van Dam *et al.,* 2009a). There may be also variation in the concentration of glucosinolates in a single leaf. This provides the insects scope to modify their feeding behaviour according to the conditions. *Peiris brassicae* was found to feed preferably on flower tissues which are rich in glucosinolates content than on ordinary leaf tissues (Smallegange, van Loon *et al.,* 2007). Apart from the potential negative effects, even for specialists, a number of adapted insect species may feed on plant parts with high glucosinolates concentrations. This behavioural adaptation may presumably enhance the survival of the insects as they gain better protection against natural enemy attack. Moreover, this modification in the feeding behaviour also avoids competition with other insects and is known to be more advantageous in nutrition to the feeding insects (Hopkins, van Dam *et al.*, 2009a).

Glucosinolates as Oviposition and Feeding Stimuli

Besides their role as defense compounds, glucosinolates present yet another major role in plant-pests interactions, as a classical case of token stimuli for oviposition and feeding stimuli for specialist insects (Hopkins, van Dam *et al.,* 2009a; Schoonhoven, van Loon *et al.*, 2005.). These compounds acts as potent oviposition and feeding stimulants for a range of insect species in the Coleoptera, Lepidoptera and Diptera that are specialized on Brassicaceous plants (Hopkins, van Dam *et al.,* 2009a). This can be asserted by their restricted occurrence in certain plant taxa and the occurrence of the cognate chemoreceptors in the insects specialized to feed on them. In *D. radicum*, which represents a well-studied example, a thia-triaza-fluorene compound was found to be 100 times more powerful than the most stimulatory glucosinolates for oviposition (Hurter, Ramp *et al.*, 1999; Roessingh, Stadler *et al.*, 1997). This compound was reported to stimulate a neuron in tarsal sensilla other than the glucosinolate-sensitive neurons (Marazzi, Patrian *et al.*, 2004). Whereas for the diamondback moth, *Plutella xylostella*, a range of glucosinolates differentially stimulated oviposition on artificial substrates (Reed, Pivnick *et al.*, 1989). These findings provide a clear cut picture of glucosinolates acting as oviposition stimuli of a variety of insect specialists. Although the active compounds have not been identified, there are evidences that the glucosinolates hydrolysis products may also serve as oviposition stimulants (Renwick, Haribal *et al.*, 2006).

The role of glucosinolates as oviposition stimuli has been a controversial issue as how the glucosinolates in an intact tissue is perceived by the insects. As a feeding stimulants, relatively a very little known about exact identity of these cues (Hopkins, van Dam *et al.*, 2009a). In the flea beetle genus *Phyllotreta* (Coleoptera: Chrysomelidae), several species are specialized feeders on Brassicaceae and are stimulated by glucosinolates, but *Phyllotreta armoraciae* uses both flavonoid glucosides and glucosinolates as token stimuli (Nielsen, Larsen *et al.*, 1979). The feeding stimulating roles of glucosinolates would seem to be more relevant in consideration of piercing-sucking insect pests. These insects accept or reject a plant on the basis of mechanical and chemical cues located at the level of individual plant cell types. Such token stimuli seem to be involved in case of the Brassicaceae-specialist aphid *Brevicoryne brassicae.* These aphids strongly prefer to feed on glucosinolates rich inflorescence stems to feeding on leaves (Hopkins, van Dam *et al.*, 2009a) which are an adaptation to avoid competition with other insects occupying similar niche. A better understanding of such interactions would be promising and more of such studies need to be done to confirm as how these interactions actually occurs in the nature.

Glucosinolates in Multitrophic Interactions

Although not clearly known, the glucosinolates contents in the host plants also influence the higher trophic levels significantly. The role of glucosinolates and their hydrolysis products in multitrophic interactions has received tremendous attention in the recent years. A number of specialist insects are known to sequester glucosinolates. *Athalia rosae* is reported to sequester ingested glucosinolates in its haemolymph, although 80% is rapidly excreted. Upon an attack by predators, these insects easily bleed haemolymph containing the sequestered glucosinolates. This defense response provides these insects protection against a variety of predators. Another insect species, *Murgantia histriontica*, a bug, also sequester glucosinolates, at a level 30 times more in their body tissues than in their guts. The bugs were found to be rejected by two species of bird predators, most likely due to their glucosinolate-based deterrence (Hopkins, van Dam *et al.*, 2009a). *Brevicoryne brassicae* and *Lipaphis erysimi*, both specialist aphids present a special case of sequestration. Both aphids sequester the compound in a concentration 15-20 times higher in their haemolymph than the concentration found in the leaf tissues. The insects also have their own myrosinase in their nonflight muscles, thus separating the substrate and the enzyme spatially (Francis, Lognay *et al.*, 2001; Kazana, Pope *et al.*, 2007). In contrary, generalist aphid *Myzus persicae* excretes glucosinolates in their honey dew (Hopkins, van Dam *et al.*, 2009a).

When aphids are attacked by the predators, the sequestered glucosinolates and aphid myrosinase come together, mix and volatile isothiocyanates and nitriles are released hydrolytically, conferring a toxic effect on the predator (Francis, Lognay *et al.*, 2001), thus serving an effective defensive function against further attack of the aphid colony (Kazana, Pope *et al.*, 2007). Glucosinolates and their hydrolysis products are also known to influence the behaviours of parasitoids of the insects feeding on the plants. The volatiles produced as a result of insects damage and glucosinolates breakdown may attract a variety of parasitoids. In studies with *P. rapae* and their parasitoids *Cotesia rubecula*, the plant population and their glucosinolates profile on which they were reared was found to affect the herbivore performance. Affects were also seen on the adult size of the parasitoids, giving a clear picture that the parasitoids performance in affected by the diet quality of the herbivores. A similar case is also seen in the interaction between the generalist *Mamestra brassicae* and its endoparasitoid, *Microplitis mediator*. Plant population with varying glucosinolates profile have dramatic effects on the survival of *M. mediator* which is directly reflected on the fitness and performance of its endoparasitoids (Hopkins, van Dam *et al.*, 2009a).

Concluding Remarks and Future Directions

Insect Arthropods represent the largest group of organisms in the biosphere. Hence their studies provide huge prospects in modern day's agricultural practice. Plants are subjected to attacks by a variety of herbivore pests and pathogens. The responses of plants towards herbivory can be direct defenses, indirect defenses, and tolerance. Secondary metabolites (including glucosinolates) are the most well known classes of direct preformed constitutive defense mechanisms. The induction of the glucosinolates-myrosinase system as a result of herbivory and pathogen attacks may vary to a great extent. The presence of these compounds in Brassicaceous crops has provided significant research area due to the high economic values of these crops. Moreover, their occurrence in *Arabidopsis thaliana*, the model plant system for major molecular and genetic researches, has provided a key innovation in the research field of the plant-insect interactions. An integrative approach to elucidate the interaction between plants and its biotic environment will provide helpful informations with potential application in ecological and agricultural systems. Arrival of new and advanced technologies in genomics, proteomics, metabolomics, lipidomics and bioinformatics allows us to tackle these complicated biological issues more efficiently. High throughput and transgenic techniques will also bring improved crop plants with enhanced resistance using the identified targets for agriculture. Besides their role in plant-herbivore interactions, glucosinolates are also known to act as chemoprotective agents against chemically-induced carcinogens. Unravelling the basic mechanisms and biology as how these compounds and their hydrolysis products act in preventing cancers may provide huge prospects in future pharmaceuticals and medical research.

REFERENCES

Bjorkman R., Janson J.C. 1972. Studies on Myrosinase I. Purification and characterization of myrosinase from White mustard seeds (*Sinapis alba* L.). *Biochem Biophys Acta* 276; 508-518.

Bones A.M. 1990. Distribution of β-thioglucosidase activity in intact plants, cell and tissue cultures and regenerant plants of *Brassica napus* L. *Exp Bot* 41; 737–744.

Bridges M., Jones A.M.E., Bones A., Hodgson C., Cole R., Bartlet E. 2002. Spatial organization of the glucosinolate-myrosinase system in Brassica specialist aphids is similar to that of the host plant. *Proc R Soc Lond B Biol Sci* 269; 187–191.

Brown P.D., Tokuhisa J.G., Reichelt M., Gershenzon J. 2003. Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*. *Phytochemistry* 62; 471–81.

Buchwaldt L., Larsen L.M., Ploger A., Sorensen H.J. 1986. Fast liquid chromatography isolation and characterization of plant myrosinase, β-thioglucoside glucohydrolase isoenzymes. *Chromatography* 363; 71-80.

Burow M., Markert J., Gershenzon J., Wittstock U. 2006. Comparative biochemical characterization of nitrile-forming proteins from plants and insects that alter myrosinase-catalysed hydrolysis of glucosinolates. *FEBS J.* 273; 2432–46.

Buskov S., Serra B, Rosa E., Soerensen H., Soerensen J.C. 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; 690–95.

Chen S., Glawischnig E. 2003. CYP79F1 and CYP79F2 have distinct functions in the biosynthesis of aliphatic glucosinolates in Arabidopsis. *Plant J,* 33; 923-937.

Fahey J.W., Zalcmann A.T., Talalay P. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 56; 5–51.

Farnham M.W., Grusak M.A., Wang M. 2000. Calcium and Magnesium Concentration of Inbred and Hybrid Broccoli Heads. *J. Amer. Soc. Hort. Sci.* 125; 344–349.

Field B., Cardon G., Traka M., Botterman J., Vancanneyt G., Mithen R. 2004. Glucosinolate and amino acid biosynthesis in *Arabidopsis*. *Plant Physiol.* 135; 828–39.

Francis F., Lognay G., Wathelet J.P., Haubruge E. 2001. Effects of allelochemicals from first (Brassicaceae) and second (*Myzus persicae* and *Brevicoryne brassicae*) trophic levels on *Adalia bipunctata*. *J. Chem. Ecol.* 27; 243–56.

Gabrys B., Tjallingii W.F. 2002. The role of sinigrin in host plant recognition by aphids during initial plant penetration. *Entomol. Exp. Appl.* 104; 89–93.

Glawischnig E., Hansen B.G., Olsen C.E., Halkier B.A. 2004. Camalexin is synthesized from indole-3-acetaldoxime, a key branching point between primary and secondary metabolism in *Arabidopsis. Proc Natl Acad Sci* 101; 8245-8250.

Griffiths D.W., Birch A.N.E., Hillman J.R. 1998. Antinutritional compounds in the Brassicaceae—Analysis, biosynthesis, chemistry and dietary effects. *J. Hort. Sci. Biotech.* 73; 1–18.

.Grubb C.D., Abel S. 2006) Glucosinolate metabolism and its control. *Trends Plant Sci.* 11; 89–100.

Grubb C.D., Zipp B.J., Ludwig-Muller J., Masuno M.N., Molinski T.F., Abel S. 2004. Arabidopsis glucosyltransferase UGT74B1 functions in glucosinolate biosynthesis and auxin homeostasis. *Plant J* 40; 893-903.

Halkier B.A., Gershenzon J. 2006. Biology and biochemistry of glucosinolates. *Annu. Rev. Plant Biol.* 57; 303-33.

Hall C., McCallum D., Prescott A., Mithen R. 2001. Biochemical genetics of glucosinolate modification in Arabidopsis and Brassica. *Theor Appl Genet* 102; 369-374.

Hartmann T. 2004. Plant-derived secondary metabolites as defensive chemicals in herbivorous insects: a case study in chemical ecology. *Planta* 219; 1-4.

Hirai M.Y., Klein M., Fujikawa Y., Yano M., Goodenowe D.B. 2005. Elucidation of gene-to-gene and metabolite-to-gene networks in *Arabidopsis* by integration of metabolomics and transcriptomics. *J. Biol. Chem.* 280; 25590–95.

Holst B., Williamson G. 2004. A critical review of the bioavailability of glucosinolates and related compounds. *Nat. Prod. Rep.* 21; 425–47.

Hopkins R.J., van Dam N.M., van Loon J.J. 2009a. Role of glucosinolates in insect-plant relationships and multitrophic interactions. *Annu Rev Entomol* 54; 57-83.

Hopkins R.J., van Dam N.M., van Loon J.J.A. 2009b. Role of Glucosinolates in Insect-Plant Relationships and Multitrophic Interactions. *Annu. Rev. Entomol.* 54; 57–83.

Hurter J., Ramp T., Patrian B., Stadler E., Roessingh P. 1999. Oviposition stimulants for the cabbage root fly: isolation from cabbage leaves. *Phytochemistry* 51; 377–82.

Husebye H., Chadchawan S., Winge P., Thangstad Ole P., Bones A.M. 2002. Guard Cell- and Phloem Idioblast-Specific Expression of Thioglucoside Glucohydrolase 1 (Myrosinase) in *Arabidopsis. Plant Physiology.* 128; 1180–1188.

Jones A.M.E., Winge P., Bones A.M., Cole R., Rossiter J.T. 2002. Characterization and evolution of a myrosinase from the cabbage aphid *Brevicoryne brassicae*. *Insect Biochem Mol Biol* 32; 275-284.

Josefsson (1970) Glucosinolates content and amino acids composition of rapeseed (*Brassica napus*) meal as affected by sulphur and nitrogen nutrition. *Journals of the science of Food and Agriculture.* 21; 98-103.

Kazana E., Pope T.W., Tibbles L., Bridges M., Pickett J.A. 2007. The cabbage aphid: a walking mustard oil bomb. *Proc. R. Soc. London Sci. Ser. B.* 274; 2271–77.

Keum Y.S., Jeong W.S., Kong A.N.T. 2004. Chemoprevention by isothiocyanates and their underlying molecular signaling mechanisms. *Mutat. Res. Fundam. Mol. Mech. Mutagen.* 555; 191–202.

Kim J.H, Jander G. 2007. *Myzus persicae* (green peach aphid) feeding on *Arabidopsis* induces the formation of a deterrent indole glucosinolate. *Plant J.* 49; 1008–19.

Kliebenstein D.J., Kroymann J., Mitchell-Olds T. 2005. The glucosinolate-myrosinase system in an ecological and evolutionary context. *Current opinion in plant biology* 8; 264-71.

Kliebenstein D.J., Lambrix V.M., Reichelt M., Gershenzon J., Mitchell-Olds T. 2001. Gene duplication in the diversification of secondary metabolism: Tandem 2-oxoglutarate-dependent dioxygenases control glucosinolate biosynthesis in *Arabidopsis*. *Plant Cell* 13; 681-693.

Kogan M., Ortman E.F. 1978. Antixenosis: a new term proposed to define Painter’s nonpreference modality of resistance. *Bull. Entomol. Soc. Am.* 24; 175–76.

Kroymann J., Donnerhacke S., Schnabelrauch D., Mitchell-Olds T. 2003. Evolutionary dynamics of an *Arabidopsis* insect resistance quantitative trait locus. *Proc. Natl. Acad. Sci.* 100; 14587–92.

Kroymann J., Textor S., Tokuhisa J.G., Falk K.L., Bartram S. 2001. A gene controlling variation in Arabidopsis glucosinolate composition is part of the methionine chain elongation pathway. *Plant Physiol.* 127; 1077–88.

Kushad M.M., Brown A.F., Kurilich A.C., Jurik J.A., Klein B.P. Wallig M.A. 1999. Variation in glucosinolates in vegetable crops of *Brassica oleraceae. Journal of Agricultural and food chemistry* 47; 1541-1548.

Lambdon P.W., Hassall M. 2005. How should toxic secondary metabolites be distributed between the leaves of a fast-growing plant to minimize the impact of herbivory? *Funct. Ecol.* 19; 299-305.

Lambrix V., Reichelt M., Mitchell-Olds T., Kliebenstein D.J., Gershenzon J. 2001. The *Arabidopsis* epithiospecifier protein promotes the hydrolysis of glucosinolates to nitriles and influences *Trichoplusia ni* herbivory. *Plant Cell* 13; 2793–807.

Lazzeri L., Curto G., Leoni O., Dallavalle E. 2004. Effects of glucosinolates and their enzymatic hydrolysis products via myrosinase on the root-knot nematode *Meloidogyne incognita*. *J. Agric. Food Chem.* 52; 6703–7.

Lenman M., Rodin J., Josesfsson L.G., Rask L. 1990. Protein measurement with Folin phenol reagent. *J Biol Chem.* 194; 747-753.

Magrath R., Bano F., Parkin I., Sharpe A., Lister C. 1994. Genetics of aliphatic glucosinolates. I. Side chain elongation in *Brassica napus* and *Arabidopsis thaliana*. *Heredity* 72; 290–299.

Marazzi C., Patrian B., Stadler E. 2004. Secondary metabolites of the leaf surface affected by sulphur fertilisation and perceived by the cabbage root fly. *Chemoecology* 14; 87–94.

Miles C.I., del Campo M., Renwick J.A.A. 2005. Behavioral and chemosensory responses to a host recognition cue by larvae of *Pieris rapae*. *J. Comp. Phys. A* 191; 147–55.

Muller C., Agerbirk N., Olsen C.E., Boeve J.L., Schaffner U., Brakefield P.M. 2001. Sequestration of host plant glucosinolates in the defensive hemolymph of the sawfly *Athalia rosae*. *J Chem Ecol* 27; 2505–2516.

Muller C., Boeve J.L., Brakefield P.M. 2002. Host plant derived feeding deterrence towards ants in the turnip sawfly *Athalia rosae*. *Entomol Exp. Appl.* 104; 153–157.

Nielsen J.K., Larsen L.M., Sorensen H.J. 1979. Host plant-selection of the horseradish flea beetle *Phyllotreta armoraciae* (Coleoptera, Chrysomelidae): identification of two flavonol glycosides stimulating feeding in combination with glucosinolates. *Entomol. Exp. Appl.* 26; 40-48.

Nishie K., Daxenbichler M.E. 1980. Toxicology of glucosinolates, related compounds (nitriles, R-goitrin, isothiocyanates) and vitamin U found in cruciferae. *Food Cosmet Toxicol* 18; 159-172.

Nishie K., Daxenbichler M.E. 1982. Hepatic effects of R-goitrin in sprague-dawley rats. *Food Chem Toxicol* 20; 279-287.

Painter R.H. 1941. The economic value and biologic significance of insect resistance in plants. *J. Econ. Entomol.* 34; 358–67.

Piotrowski M., Schemenewitz A., Lopukhina A., Muller A., Janowitz T., Weiler E.W., Oecking C. 2004. Desulfoglucosinolate sulfotransferases from *Arabidopsis thaliana* catalyze the final step in the biosynthesis of the glucosinolate core structure. *J Biol Chem* 279; 50717-50725.

Potter M.J., Vanstone V.A., Davies K.A., Rathjen A.J. 2000. Breeding to increase the concentration of 2-phenylethyl glucosinolate in the roots of *Brassica napus*. *J. Chem. Ecol.* 26; 1811–20.

Rask L., Andreasson E., Ekbom B., Eriksson S., Pontoppidan B., Meijer J. 2000. Myrosinase: gene family evolution and herbivore defense in Brassicaceae. *Plant Mol. Biol.* 42; 93–113.

Reed D.W., Pivnick K.A., Underhill E.W. 1989. Identification of chemical oviposition stimulants for the diamondback moth, *Plutella xylostella*, present in three species of Brassicaceae. *Entomol. Exp. Appl.* 53; 277–86.

Renwick J.A.A., Haribal M., Gouinguene S., Stadler E. 2006. Isothiocyanates stimulating oviposition by the diamondback moth, *Plutella xylostella*. *J. Chem. Ecol.* 32; 755–66.

Roessingh P., Stadler E., Baur R., Hurter J., Ramp T. 1997. Tarsal chemoreceptors and oviposition behaviour of the cabbage root fly (*Delia radicum*) sensitive to fractions and new compounds of host-leaf surface extracts. *Physiol. Entomol.* 22**;** 140–48.

Schoonhoven L.M., van Loon J.J.A., Dicke M. 2005. Insect-Plant Biology. *Oxford Univ. Press.*

Smallegange R.C., van Loon J.J.A., Blatt S.E., Harvey J.A., Agerbirk N., Dicke M. 2007. Flower vs leaf feeding by *Pieris brassicae*: Glucosinolate-rich flower tissues are preferred and sustain higher growth rate. *J. Chem. Ecol.* 33**;** 1831-44.

Textor S., Bartram S., Kroymann J., Falk K.L., Hick A. 2004. Biosynthesis of methionine-derived glucosinolates in *Arabidopsis thaliana*: recombinant expression and characterization of methylthioalkylmalate synthase, the condensing enzyme of the chainelongation cycle. *Planta*. 218; 1026–35.

Thies W. 1979. Detection and utilization of a glucosinolate sulfohydrolase in the edible snail, *Helix pomatia*. *Naturwissenschaften* 66; 364–65.

Tokuhisa J.G., Kraker D., Textor S., Gershenzon J. 2004. The biochemical and molecular origins of aliphatic glucosinolate diversity in *Arabidopsis Thaliana*. *Secondary Metabolism in Model Systems* 38; 19-38.

Toroser D., Thormann C.E., Osborn T.C., Mithen R. 1995. RFLP mapping of quantitative trait loci controlling seed aliphatic-glucosinolate content in oilseed rape (*Brassica napus* L.). *Theor. Appl. Genet*. 91; 802–8.

Uzunova M., Ecke W., Weissleder K., Robbelen G. 1995. Mapping the genome of rapeseed (*Brassica napus* L).1. Construction of an RFLP linkage map and localization of QTLS for seed glucosinolate content. *Theor. Appl. Genet.* 90; 194–204.

van Dam N.M., Tytgat T.O.G., Kirkegaard J. 2008. Root and shoot glucosinolates: a comparison of their diversity, function and interactions in natural and managed ecosystems. *Phytochem. Rev.* 8; 171-186.

Wheat C.W., Vogel H., Wittstock U., Braby M.F., Underwood D., Olds T.M. 2007. The genetic basis of a plant–insect coevolutionary key innovation. *Proc Natl Acad Sci*. 104; 20427–20431.

Wittstock U., Kliebenstein D.J., Lambrix V.M., Reichelt M., Gershenzon J. 2003. Glucosinolate hydrolysis and its impact on generalist and specialist insect herbivores. *Integrative Phytochemistry: from Ethnobotany to Molecular Ecology*.

Xue P., Jorgensen M., Pihlgren U., Rask L. 1995. The myrosinase gene family in *Arabidopsis thaliana*: gene organisation, expression and evolution. *Plant Molecular Biology*. 27; 911-922.

Xue P., Lenman M., Falk A., Rask L. 1992. The glucosinolates-degrading enzyme myrosinase in Brassicaceae is encoded by gene family. *Plant Molecular Biology*. 18; 387-898.

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