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# Molecular Genetics of Glucosinolate Biosynthesis in *Brassicas*: Genetic Manipulation and Application Aspects

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## 1. Introduction

Glucosinolates are sulphur containing secondary metabolites biosynthesized by many plant species in the order *Brassicales*. Physical tissue or cell injury leads to the breakdown of glucosinolates through the hydrolytic action of the enzyme myrosinase, resulting in the production of compounds including isothiocyanates, thiocyanates and nitriles. Derivative compounds of glucosinolates have a wide range of biological functions including anti-carcinogenic properties in humans, anti-nutritional effects of seed meal in animals, insect pest repellent and fungal disease suppression (Mithen et al., 2000; Brader et al., 2006). Glucosinolates play important role in the nutritional qualities of *Brassica* products. *Brassica* products are consumed as oil, meal and as vegetables. Rapeseed (*B. napus*, *B. juncea* and *B. rapa*) is a source of oil and has a protein-rich seed meal. High glucosinolates in the seed meal pose health risks to livestock (Fenwick et al., 1983; Griffiths et al., 1998). Consequently, plant breeders have nearly eliminated erucic acid from the seed oil and have dramatically reduced the level of seed glucosinolates (>100  $\mu\text{mole/g}$  seed to <30  $\mu\text{mole/g}$  seed) via conventional breeding, allowing the nutritious seed meal to be used as an animal feed supplement. There is, however, a significant residual content of glucosinolates in rapeseed/canola seed meal (over 10  $\mu\text{mole/g}$  seed) and further reduction of the total glucosinolate content would be nutritionally beneficial (McVetty et al., 2009). Therefore, to produce healthy seed meal from rapeseed, it is important to genetically manipulate glucosinolate content. *Brassica* vegetables (*B. rapa* and *B. oleracea*) are highly regarded for their nutritional qualities, they are a good source of vitamin A and C, dietary soluble fibres, folic acid, essential micro nutrients and low in calories, fat and health beneficial glucosinolates such as glucoraphanin and sulforaphane. Breeding objectives for these *Brassica* crops include the enhancement of beneficial glucosinolates and reduction of others. It is, therefore, important to understand the genetic, biosynthetic, transportation and accumulation mechanisms for glucosinolates in *Brassica* species.

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## 2. Historical background of *Brassica* species

The crops belonging to the genus *Brassica* have been of great importance to humanity. Since ancient times, *Brassica* crops have been used for many purposes, including vegetables, oilseeds, feed, condiments, fodder, green manure and even medical treatments. Early history suggests that rapeseed has been cultivated for several thousand years with its origin in the Mediterranean region although exact time of domestication and the place of origin are still unknown. Sanskrit writings in 2000-1500 BC characterized species identified as *B. rapa* and *B. napus* as oleiferous forms and mustards, respectively. *Brassica juncea* and *B. rapa* are believed to have been crop plants in India long before the Christian era. The Greek, Roman and Chinese literature of 500-200 BC referred *B. rapa* as rapiferous forms and were also described for various medicinal properties (Downey & Röbellen, 1989). In early times, rapeseed oil was used as a lamp oil, which in later centuries led gradually to its use as a valuable cooking oil.

*Brassica* species are diverse in terms of morphology, agronomy and quality traits. Domestication of rapeseed in Europe seems to have begun in the early Middle Ages. In 1620, *B. rapa* was first recorded in Europe by the Swiss botanist Casper Bahin (Gupta & Pratap, 2007). As a result, *Brassica* crops were adapted and cultivated in many parts of the world (Mehra, 1966). Rapeseed was introduced in Canada before the Second World War (McVetty et al., 2009). Commercial cultivation in Canada began during the Second World War to supply lubricating oil for steamships. Canada's first *B. rapa* rapeseed cultivar, Arlo, with high erucic acid (40 to 45%) and high glucosinolate content (>150  $\mu\text{mole/g}$  seed) was developed in 1958 using selection from open pollinated populations (McVetty et al., 2009). Initially, *B. rapa* was the dominant cultivated species of *Brassica* in western Canada. In late 1980s, a large acreage of *B. rapa* and *B. napus* was grown in the Prairie Provinces. Subsequently, the production area of *B. rapa* declined to about 15 – 20% of its former area in 1990s. The reduction in acreage of *B. rapa* resulted from the introduction of herbicide tolerance canola, which provided the early planting and high yield advantages of *B. napus* cultivars. Currently, *B. rapa* is still grown in small areas in Canada because of its early maturity. Research efforts are underway to develop disease resistant hybrid varieties to increase yield potential of *B. rapa*. *Brassica rapa* are grown as a winter sarson crop in Asian countries such as India, Pakistan, China and Bangladesh. Vegetable forms of *B. rapa* (Chinese cabbage, turnip, pak choi, komatsuna, mizuna green and rapini) are widely cultivated in many parts of the world (Prakash & Hinata, 1980; Takuno et al., 2007).

## 3. Economic importance of *Brassica* species

The family *Brassicaceae* (syn. *Cruciferae*) is one of the crucial plant families for humans and animals and supplies several products from various plant parts. The little cruciferous weed *A. thaliana* has become an important model organism for the study of plant molecular biology, including the related crop species. The mustard family (*Brassicaceae*) is the fifth largest monophyletic angiosperm family, comprising 338 genera and about 3700 species in 25 tribes (Beilstein et al., 2006). The genus *Brassica* is one of the 51 genera of the tribe *Brassiceae* and includes the economically valuable crop species. *B. napus*, *B. rapa*, *B. juncea*, *B. carinata* and *B. nigra* are grown for edible and industrial oil as well as nutritionally valued seed meal.

Globally, rapeseed and canola oil is being utilized for human consumption, industrial applications and as a feedstock for biodiesel production. Canola oil is considered a healthy edible oil due to its high level of monounsaturated fatty acid (61%), lower level of saturated fatty acid (7%) and moderate amount of polyunsaturated fatty acid (22%) in its overall fatty acid profile (McVetty & Scarth, 2002). Rapeseed that has erucic acid levels greater than 45% also has many industrial applications such as plasticizers, slip agents for fibreglass and oil for the lubrication industry. Additionally, the seed meal is a marketable source of protein rich animal feed supplement.

Rapeseed is the world's third leading oil producing crop after palm and soybean, and it contributes about 15% to the global total vegetable oil production. Canada was the top rapeseed producing country in the world with 12.6 million MT productions in 2008 (FAO 2008). Canola/rapeseed contributes about \$14 billion annually to the Canadian economy along with the generation of about 200,000 jobs throughout Canada in the areas of production, transportation, exporting, crushing and refining (Canola Council of Canada, 2010b [http://www.canolacouncil.org/canadian\\_canola\\_industry.aspx](http://www.canolacouncil.org/canadian_canola_industry.aspx)). Canola/rapeseed meal is the second most popular protein feed ingredient in the world after soybean meal. Protein content of canola/rapeseed meal ranges from 36 to 39%, with a good amino acid profile for animal feeding (Newkirk et al., 2003). The major producers and consumers of canola/rapeseed meal are Australia, Canada, China, European Union and India. Along with oil production, *Brassica* species also produce different forms of vegetables and are the most widely cultivated vegetable crops in the world. Most of the production is consumed locally with a small amount of international trade. *B. napus* and *B. juncea* are used as vegetables in Asian countries like China, Japan and India. *B. rapa* is differentiated into seven groups viz., var. *compestris*, *pekinensis*, *chinensis*, *parachinensis*, *narinosa*, *japonica* and *rapa*. *Brassica rapa* is cultivated for leafy and root vegetables in the form of Chinese cabbage, pak choi and turnip; *B. oleracea* is cultivated for leafy and floret vegetables in various morph types such as cabbage, cauliflower, kale, collard, kohlrabi, brussels and broccoli.

#### 4. Genomic relationships in *Brassica* species

Genomic relationships between the three diploid and three amphidiploid *Brassica* species were initially established in the 1930s based on various taxonomical and cytogenetic studies (Fig. 1) (Morinaga 1934; U 1935). Three allotetraploid *Brassica* species namely, *B. napus* (AACC,  $2n=38$ ), *B. juncea* (AABB,  $2n=36$ ) and *B. carinata* (BBCC,  $2n=34$ ) have been derived from three diploid elementary species, *B. rapa* (AA,  $2n=20$ ), *B. nigra* (BB,  $2n=16$ ) and *B. oleracea* (CC,  $2n=18$ ).

The genomic relationships of *B. napus* with *B. rapa* and *B. oleracea* have been confirmed by the resynthesis of *B. napus* from *B. rapa* x *B. oleracea* crosses (U 1935; Downey et al., 1975; Olsson & Ellerstrom, 1980). The close relationship between the six *Brassica* species made it feasible to incorporate a trait from one species into others to make the crops more suitable to agricultural systems. Thus, complex traits like glucosinolates can also be manipulated as required through interspecific hybridization. It has been relatively easier to make interspecific crosses among some of these six species (e.g. *B. napus* x *B. rapa*) compared to others (e.g. *B. rapa* x *B. oleracea*). Wide hybridizations are normally performed by the application of embryo rescue techniques. The most recent advances in genome sequencing technology, bioinformatics and

data mining have opened an avenue for comparative analysis of ESTs, BACs, genes (families), whole chromosomes and even entire genomes to determine evolutionary relationship between these species and their ancestors (Gao et al., 2004; Gao et al., 2006; Punjabi et al., 2008; Mun et al., 2009; Qiu et al., 2009; Nagoaka et al., 2010).

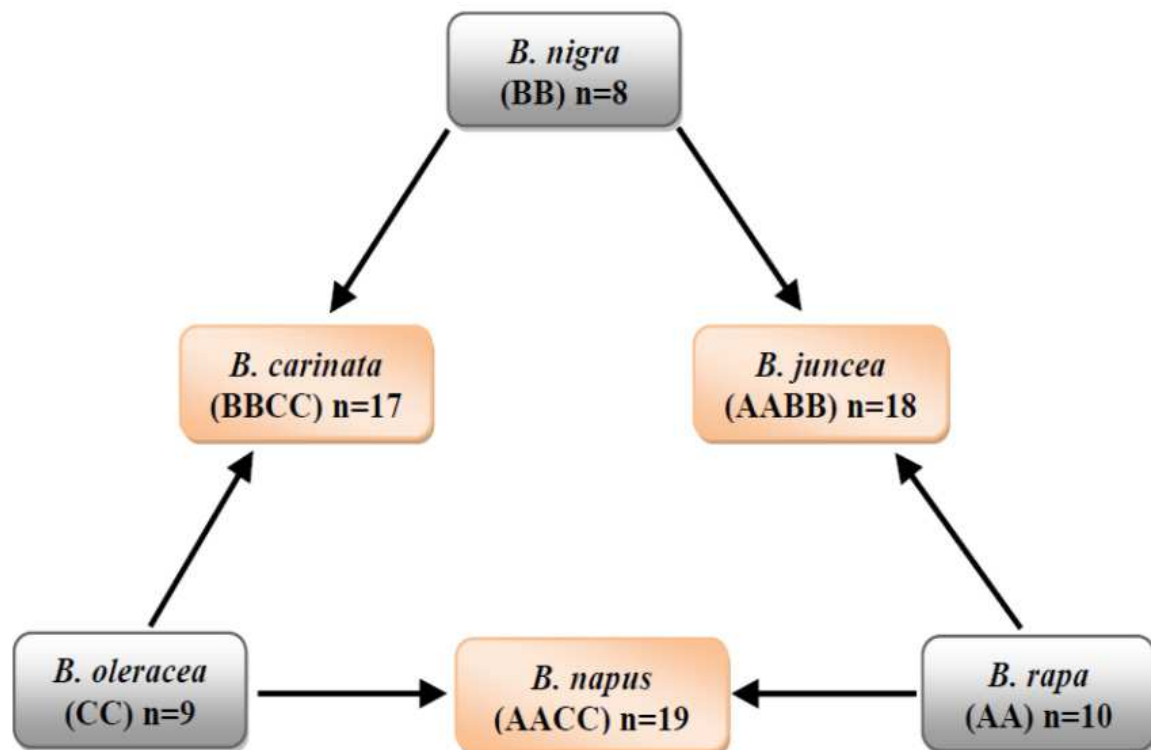


Fig. 1. U-triangle of genomic relationship between diploid and amphidiploid *Brassica* species (U 1935).

#### 4.1 Homoeology between the A, B and C genomes of *Brassica* species

Genome homoeology has been characterized in *Brassica* species by comparative analyses of the genetic and physical maps of *Arabidopsis* with genetic maps of *Brassica* species (Osborn et al., 1997; Lan et al., 2000; Parkin et al., 2002; Lukens et al., 2003; Parkin et al., 2005). These studies indicate that each genomic region has had multiple events of polyploidization and chromosome rearrangements in the *Brassicaceae* lineage after the evolutionary divergence from *Arabidopsis* approximately 14.5 to 20.4 million years ago (MYA) (Yang et al., 1999; Parkin et al., 2002). In the *Brassicaceae*, the *B. nigra* (B) genome separated from the *B. rapa*/*B. oleracea* (AC) genome lineages about 7.9 MYA (Yang et al., 1999; Lysak et al., 2005).

There are high levels of homoeology among the A- and C-genomes of *B. rapa*, *B. oleracea* and *B. napus* (Parkin et al., 2005; Punjabi et al., 2008). Parkin et al., (2003) reported stretches of collinearity on the linkage groups N1 with N11, N2 with N12 and N3 with N13 of the A- and C-genomes, respectively. Similarly, Osborn et al., (2003) reported reciprocal interstitial translocations of homoeologous regions of linkage groups N7 and N16, and their effects on genome rearrangements and seed yield in *B. napus*. This suggests that inter-genomic translocations and rearrangements have taken place during the evolutionary divergence of *B. oleracea* and *B. rapa* from a polyploid ancestor (Sharpe et al., 1995). As a result of genomic



synteny, there have been several reports of homoeologous recombination between the A- and C-genome of *Brassica* species (Udall et al., 2004; Leflon et al., 2006). Cytogenetic and molecular data revealed that small and large collinear genomic regions between the A- and C-genomes of *Brassica* species allow homoeologous recombination-based trait introgression to enhance genetic variability.

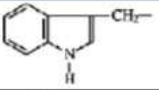
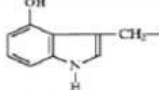
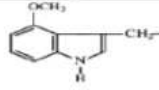
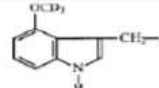
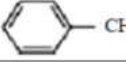
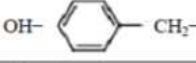
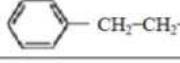
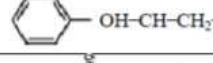
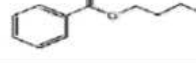
## 5. Plant secondary metabolites and their functions

The sessile nature of plants requires them to produce a large numbers of defence compounds including primary and secondary metabolites. It is believed that the currently discovered plant metabolic compounds account for only about 10% of the actual compounds present naturally within the plant kingdom (Schwab, 2003; Wink, 2003). Plant secondary metabolites are organic biochemical compounds produced in plants during normal growth and development. While they are not directly involved in plant growth, development or reproduction, these secondary metabolites play vital roles in plant defence mechanisms, acting for example, phytoalexins and phytoanticipins. Phytoalexins are antimicrobial defence metabolites synthesized *de novo* in response to biotic and abiotic stresses. Phytoalexins are involved in induced plant defence mechanisms including lytic enzymes, oxidizing agents, cell wall lignifications and pathogenesis-related proteins and transcript stimulation (Pedras et al., 2008). Phytoanticipins are low molecular weight antimicrobial compounds which are constitutively active for defence. Their production may be increased under high biotic or abiotic stresses (Pedras et al., 2007). Certain classes of phytoanticipins require enzymatic modification and derivation in order to become active within the defence systems of the plant. Plant secondary metabolites are broadly categorized into three groups based on their biosynthetic origin

- i. Flavonoids and allied phenolic and polyphenolic compounds
- ii. Terpenoid compounds
- iii. Nitrogen and sulphur containing alkaloid compounds

### 5.1 Glucosinolates as secondary metabolites

Glucosinolates are sulphur rich, nitrogen containing anionic natural products, derived from specific amino acids and their precursors (Fenwick et al., 1983). Glucosinolates are reported almost exclusively from the order *Brassicales*, which possesses about 15 families such as *Brassicaceae*, *Capparaceae* and *Caricaceae*. Glucosinolates are also reported in a few members of the family *Euphorbiaceae*, a very distinct family to other glucosinolate containing families (Rodman et al., 1996). Glucosinolates coexist with endogenous thioglucosidases called myrosinases in cruciferous plant species and activate plant defence mechanism against biotic and abiotic stresses. Tissue disruption causes systemic interactions between glucosinolates and myrosinases in the presence of moisture. The interaction produces numerous compounds with diverse biological activities (Bones & Rossiter, 1996; Halkier, 1999). Glucosinolates are some of the most extensively studied plant secondary metabolites; various enzymes and transcription factors involved in biosynthesis have been studied in the model plant *Arabidopsis* and to some extent in *Brassica* crops species. The broad functionality, physiochemical and genetic studies of glucosinolates have led to a model status for research on secondary metabolites (Sønderby et al., 2010).

GSL Name	Trivial name	RF	R Side chain	Chemical Structure	Mol. Wt. #
Aliphatic 3C	Sinigrin	1.00	2-Propenyl	$\text{CH}_2=\text{CH}-\text{CH}_2-$	279
	Glucobervirin	0.80	3-Methylthiopropyl	$\text{CH}_3\text{S}-\text{CH}_2-\text{CH}_2-\text{CH}_2-$	327
	Gluciberin	1.07	3-Methylsulfinylpropyl	$\text{CH}_3\text{SO}-\text{CH}_2-\text{CH}_2-\text{CH}_2-$	343
	Glucoscheirolin	1.26	3-Methylsulfonylpropyl	$\text{CH}_3\text{SO}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-$	179.26
	Glucoputranjivin	1.00	1-Methylethyl	$\text{CH}_3-\text{CH}-\text{CH}_3$	281
	Glucosismbrin	1.32	2-hydroxy-1-methylethyl	$\text{OH}-\text{CH}_2-\text{CH}-\text{CH}_3$	298
Aliphatic 4C	Gluconapin	1.11	3-Butenyl	$\text{CH}_2=\text{CH}-\text{CH}_2-\text{CH}_2-$	293
	Progoitrin	1.09	(2R)-2-Hydroxy-3-butenyl	$\text{CH}_2=\text{CH}-\text{CH}(\text{OH})-\text{CH}_2-$	309
	Epi-progoitrin	1.09	(2S)-2-Hydroxy-3-butenyl	$\text{CH}_2=\text{CH}-\text{CH}(\text{OH})-\text{CH}_2-$	309
	Glucorucin	1.04	4-Methylthiobutyl	$\text{CH}_3\text{S}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-$	341
	Glucoraphasatin	0.40	4-Methylthio-3-butenyl	$\text{CH}_3\text{S}-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}_2-$	340
	Glucoraphanin	1.07	4-Methylsulfinylbutyl	$\text{CH}_3\text{SO}-\text{CH}_2-(\text{CH}_2)_2-\text{CH}_2-$	357
	Glucoraphenin	0.90	4-Methylsulfinyl-3-butenyl	$\text{CH}_3\text{SO}-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}_2-$	355
	Glucosconringiin	1.00	2-Hydroxy-2-methylpropyl	$\text{CH}_3=\text{CH}(\text{OH})-\text{CH}_2$	312
	Glucosallysin	1.07	5-Methylsulfinylpentyl	$\text{CH}_3\text{SO}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-$	371
Aliphatic 5C	Glucobrassicinapin	1.15	Pent-4-enyl	$\text{CH}_2=\text{CH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-$	307
	Glucoberteroiin	1.05	5-Methylthiopentyl	$\text{CH}_3\text{S}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-$	354
	Gluconapoleiferin	1.00	2-Hydroxy-pent-4-enyl	$\text{CH}_2=\text{CH}-\text{CH}_2-\text{CH}-\text{OH}-\text{CH}_2-$	323
	Glucocleomin	1.07	2-Hydroxy-2-methylbutyl	$\text{CH}_3-\text{CH}_2-\text{CH}(\text{OH})-\text{CH}_2-\text{CH}_2-$	326
	Glucosquerelein	1.00	6-Methylthiohexyl	$\text{CH}_3\text{S}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-(\text{CH}_2)_2-$	370
Aliphatic 6C	Glucosesperin	1.00	6-Methylsulfinylhexyl	$\text{CH}_3\text{SO}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-(\text{CH}_2)_2-$	385
	Glucosarabishirsutain	1.00	7-Methylthioheptyl	$\text{CH}_3\text{S}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-(\text{CH}_2)_3-$	384
Aliphatic 7C	Glucobarbarin	1.00	7-Methylsulfinylheptyl	$\text{CH}_3\text{SO}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-(\text{CH}_2)_3-$	399
	Glucosarabishirsuin	1.10	8-Methylthiooctyl	$\text{CH}_3\text{S}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-(\text{CH}_2)_4-$	398
Aliphatic 8C	Glucosarabishirsutin	1.10	8-Methylsulfinyloctyl	$\text{CH}_3\text{SO}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-(\text{CH}_2)_4-$	414
GSL Name	Trivial name	RF	R Side chain	Chemical Structure	Mol. Wt. #
Indole	Glucobrassicin	0.29	3-Indolylmethyl		368
	4-Hydroxyglucobrassicin	0.28	4-Hydroxy-3-indolylmethyl		384
	4-Methoxyglucobrassicin	0.25	4-Methoxy-3-indolylmethyl		398
	Neoglucobrassicin	0.20	N-Methoxy-3-indolylmethyl		398
Aromatic	Glucotropaeolin	0.95	Benzyl		329
	Glucosinalbin	0.50	p-Hydroxybenzyl		345
	Gluconasturtiin	0.95	2-Phenethyl		343
	Glucobarbarin	1.09	(2S)-2-Hydroxy-2-phenethyl		360
	Glucomalcomiin	0.40	3-Benzoyloxypropyl		402

Trivial name and chemical formula of R side-chains of glucosinolates identified in *Brassica* species, Mol. Wt.#: molecular weight of desulfoglucosinolates, RF: response factor (Haughn et al., 1991; Griffiths et al., 2000; Brown et al., 2003).

Table 1. Chemical structures of glucosinolates in Brassica species.

### 5.1.1 Glucosinolates and their biological functions in agriculture and nature

Glucosinolates are a uniform group of thioglucosides with an identical core structure called  $\beta$ -D-glucopyranose bound to a (Z)-N-hydroximiniosulfate ester by a sulphur atom with a variable R group. Approximately 120 glucosinolates differing in their R group side chains have been identified (Halkier & Gershenzon, 2006). These glucosinolates are categorized into three classes based on their precursor amino acids and side chain modifications (Table 1). Kliebenstein et al., (2001a) suggested that these three classes of glucosinolates are independently biosynthesized and regulated by different sets of gene families from separate amino acids. Each class is briefly discussed below.

#### 5.1.1.1 Aliphatic glucosinolates

Aliphatic glucosinolates are the major group of glucosinolates in *Brassica* species, contributing about 90% of the total glucosinolate content of the plant. Glucosinolates are constitutively biosynthesized *de novo* in cruciferous plants, although their degradation is highly regulated by spatial and temporal separation of glucosinolates and myrosinases within the plant based on environmental and biotic stresses (Drozdowska et al., 1992). Hydrolysis of glucosinolates produces a large number of biologically active compounds that have a variety of functions. The most common hydrolysis products of aliphatic glucosinolates in many cruciferous species are isothiocyanates that are formed by the rearrangement of aglycone with carbon oxime adjacent to the nitrogen at neutral pH while at acidic pH, nitriles are the predominant products (Fahey et al., 2001). These unstable compounds are cyclised to a class of substances responsible for goiter in animals (Griffiths et al., 1998).

By contrast, sulforaphane is one of the derivatives of glucoraphanin, an aliphatic glucosinolate that has several beneficial properties for humans and animals. It is known as an inducer of phase II enzymes such as glutathione transferases and quinone reductases of the xenobiotic pathway in human prostate cells (Zhang et al., 1992; Faulkner et al., 1998). The phase II enzymes are involved in the detoxification of electrophilic carcinogens that can lead to mutations in DNA and cause different types of cancers (Mithen et al., 2000). Enhanced consumption of cruciferous vegetables appears to reduce the risk of cancers (Nestle, 1997; Talalay 2000; Brooks et al., 2001). The sulforaphane content of these vegetables could be a leading factor in the reduction. Another less documented health benefit of sulforaphane is the inhibition of *Helicobacter pylori*, a pathogen of peptic ulcers and gastric cancer (Fahey et al., 2002). Sulforaphane also protects human retinal cells against severe oxidative stresses (Gao et al., 2001).

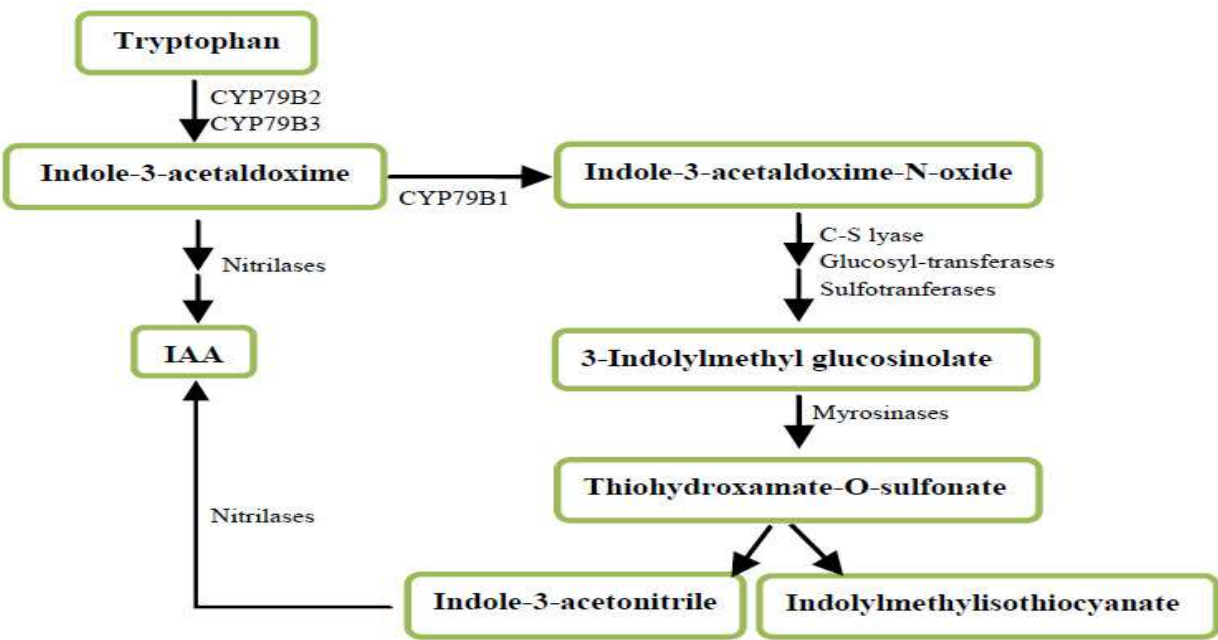
Isothiocyanates and other breakdown products of glucosinolates play important roles as repellents of certain insects and pests (Rask et al., 2000; Agrawal & Kurashige, 2003; Barth & Jander, 2006; Benderoth et al., 2006). Leaves of the mutant *myb28myb29* in *Arabidopsis* with low aliphatic glucosinolate content, when fed to the lepidopteran insect *Mamestra brassicae*, enhanced larval weight by 2.6 fold (Beekwilder et al., 2008). Glucosinolates may have specific repellent or anti-nutritional effects on specific classes of insects and microorganisms. Some *in vitro* studies demonstrated that glucosinolate degradation products, isothiocyanates and nitriles, inhibited fungal and bacterial pathogen growth (Brader et al., 2001; Tierens et al., 2001). In *Arabidopsis*, over expression of CYP79D2 from cassava increased accumulation of isopropyl and methylpropyl aliphatic glucosinolates and transformed plants showed



enhanced resistance against a bacterial soft-rot disease (Brader et al., 2006). Birch et al., (1992) reported that biotic stresses such as pest damage in *Brassica* species alters glucosinolate profiles in roots, stems, leaves and flowers. This suggests that a phytoanticipin property of glucosinolates is involved in the plant defence mechanisms of *Brassica*. Glucosinolates and their breakdown products have many biological functions, with a few compounds acting as biopesticides, biofungicides and soil fumigants, while others play roles in attraction of pollinators and provide oviposition cues to certain insects. The attraction of specialized insects could be due to the glucosinolate-sequestering phenomenon of some insects including harlequin bugs, sawflies, and some homoptera including aphids (Bridges et al., 2002; Mewis et al., 2002).

5.1.1.2 Indole glucosinolates

Indole (heterocyclic) glucosinolates in cruciferous plants (including *Arabidopsis*) are derived from tryptophan and possess variable R group side chains. The relatively high content of indole glucosinolates in the model plant *Arabidopsis* has enhanced our knowledge of the biosynthesis, transportation and functional properties of this class of glucosinolates (Petersen et al., 2002; Brown et al., 2003). Side chain modification in indole glucosinolates occurs through hydroxylations and methoxylations catalysed by several enzymes. Indole glucosinolate types and contents in different organs of the plant are strongly affected by environmental conditions. Four main indole glucosinolates have been identified in most cultivated *Brassica* species: glucobrassicin, neoglucobrassicin, 4-methoxyglucobrassicin and 4-hydroxyglucobrassicin. Similar to aliphatic glucosinolates, breakdown products of indole glucosinolates have multiple biological functions. Indole-3-carbinol derived from glucobrassicin has potent anticarcinogenic activity (Hrncirik et al., 2001). The indole



In this pathway, IAA produces from precursors and derivatives of 3-indolylmethyl glucosinolate by various nitrilases.

Fig. 2. Biosynthetic pathway and breakdown products of indole glucosinolates (De Vos et al., 2008).

glucosinolate derived compound 4-methoxyglucobrassicin has strong insect deterrent activity (Kim & Jander, 2007; De Vos et al., 2008). Osbourn, (1996) reported antimicrobial activities of indole glucosinolates and their breakdown products in *Brassica* species. Several studies suggest that there is a metabolic association between indole glucosinolates and the plant hormone indole-3-acetic acid (IAA). In the consecutive reactions, indole glucosinolates are degraded into indole acetonitrile (IAN), which is then hydrolyzed by nitrilases into IAA (Fig. 2). In clubroot infected *Brassica* roots, indole glucosinolate-based induction of IAA was observed to be responsible for gall formation. The IAA production from indole glucosinolates during gall formation is associated with a signalling cascade of IAA and cytokinin complex (Ugajin et al., 2003). Structural similarity data indicates that the indole alkaloid, brassinin, and possibly other cruciferous phytoalexins are derived from glucobrassicin. Studies in rapeseed, mustard and *Arabidopsis* have suggested that methyl jasmonate and wounding induce the biosynthesis of particular indole glucosinolates (Bodnaryk 1992; Brader et al., 2001).

### 5.1.1.3 Aromatic glucosinolates

The third class of glucosinolates in cruciferous species is aromatic or benzylic glucosinolates, derived from the aromatic parental amino acids phenylalanine and tyrosine. Very limited information is available regarding aromatic glucosinolates at qualitative or quantitative levels. Aromatic glucosinolates are biosynthesized independently from other glucosinolates, which is apparently due to involvement of different amino acid precursors in the biosynthesis of the different classes of glucosinolates (Kliebenstein et al., 2001a). Cloning and functional characterization of the *CYP79A* gene of *Arabidopsis* suggests that cytochrome P450-dependent monooxygenase catalyzes the reaction from phenylalanine to phenylacetaldoxime in aromatic glucosinolate biosynthesis (Wittstock & Halkier, 2000). Five aromatic glucosinolates have been identified in *Brassicaceae*: glucotropaeolin, glucosinalbin, gluconasturtiin, glucobarbarin and glucomalcomiin. The distinctive aroma and spiciness of condiment *Brassica* plant parts, such as the leaves and seeds of white (*Sinapis alba*) and black (*B. nigra*) mustards, is due to the presence of these aromatic glucosinolates (Fenwich et al., 1983).

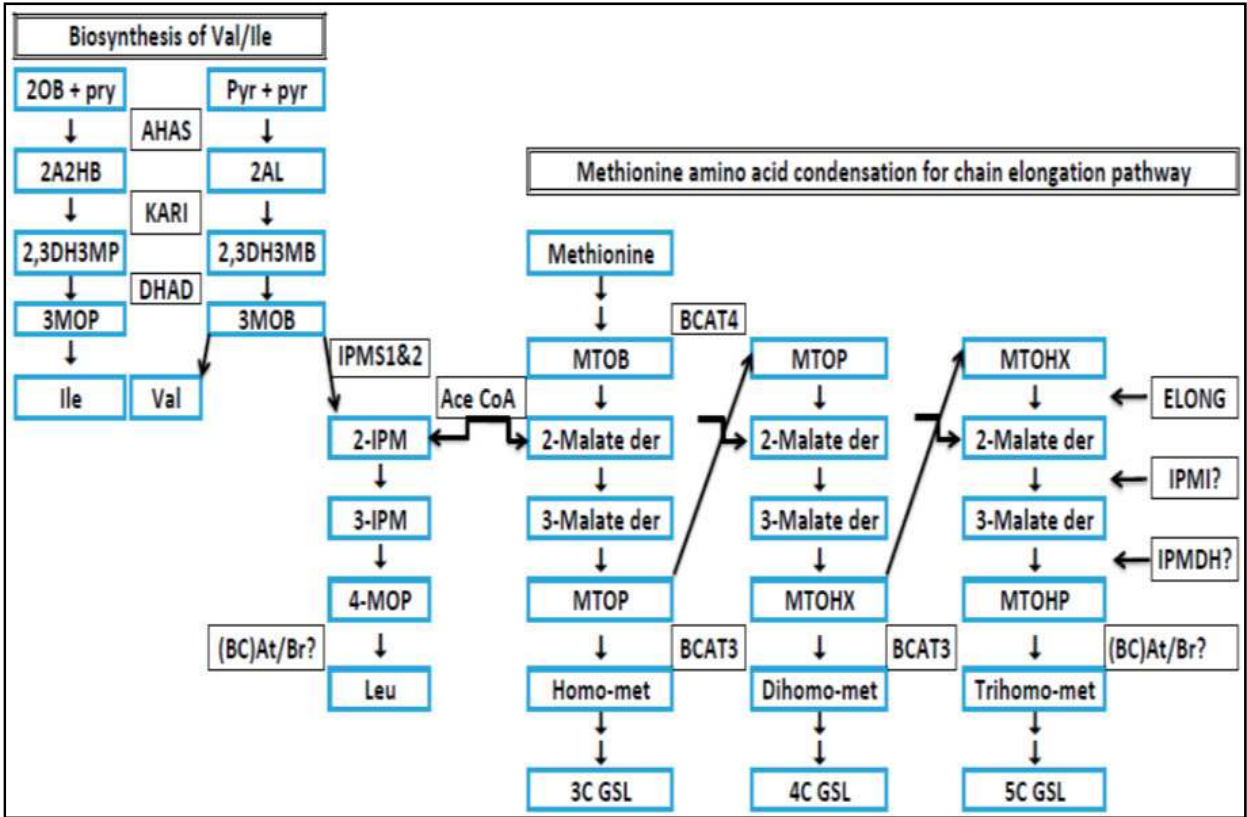
### 5.1.2 Biosynthesis of aliphatic glucosinolates

Aliphatic glucosinolates are the most abundant class in *Brassica* species, therefore, the genetic of biosynthesis is described in more detail. Aliphatic glucosinolates are biosynthesized from five amino acids (methionine, alanine, leucine, isoleucine and valine) (Halkier & Gershenzon, 2006). Biosynthesis of aliphatic glucosinolates occurs in three stages at two different locations. The first chain elongation step is catalyzed by *BCAT4* in the cytosol (Schuster et al., 2006), whereas development of core structures and secondary side chain modification reactions take place in the chloroplasts (Textor et al., 2007; Sawada et al., 2009). Chain elongation steps produce propyls (3C), butyls (4C), pentyls (5C), hexyls (6C), heptyls (7C) and octyls (8C) aliphatic glucosinolates in cruciferous species including *Arabidopsis*. Glucosinolate side chain modification reactions involve oxygenation, hydroxylation, alkenylation and benzylation, which are controlled by several gene families. The pattern of glucosinolate biosynthesis varies from organ to organ within the plant; young leaves, buds, flowers and silique walls all have higher rates of glucosinolate biosynthesis than roots, old leaves and presumably seeds (Brown et al., 2003). Various studies also suggest that transportation of glucosinolates and their breakdown products from organ to

organ via phloem occurs upon requirement to protect the plant. Seeds, however, are the most important store of total glucosinolates produced by the plants (Brudnell et al., 1999). Seeds contain much higher glucosinolates concentrations than other plant parts and it is thought that leaf glucosinolates are the basis for accumulations of total glucosinolates in seeds (Klienbestein et al., 2001a). This suggests that long distance transportation of glucosinolates from source to sink occurs. A few reports discuss an independent pathway of glucosinolate biosynthesis in seeds, resulting in the high concentration of glucosinolate in seeds (Du & Halkier, 1998; Osbourn, 1996). Experimental evidence, however, is not strong enough to support a separate pathway at this time.

5.1.2.1 Parental amino acid biosynthesis and condensation

Methionine is the main precursor of aliphatic glucosinolates in *Brassica* species. The enzyme BCAT4 catalyzes the initial chain elongation reaction to produce 2-oxo acid from methionine, an analogous process to the formation of the branched chain amino acid valine



All the reactions are catalyzed by *BCATs*, *ELONGs*, *IPMIs* and *IPM-DHs* gene families for 3C, 4C and 5C glucosinolates. Genes shown in gray boxes and derivative products shown in blue boxes. BCAT- branched chain amino transferase, MTOB- 4-methylthio-2-oxobutanoate, MTOP- 6 methylthio-2-oxopentanoate, MTOHX- 4-methylthio-2-oxohexanoate, IPMI- isopropylmalate isomerases, IPMDH- isopropylmalate dehydrogenases, MOB- methyl-2-oxobutanoate, MOP- methyl-2-oxopentanoate, AHAS- acetohydroxyacid synthase, KARI- ketolacid reductoisomerase, DHAD- dihydroxyacid dehydratase, 2AL- 2-acetolactate, 2A2HB- 2-aceto-2-hydroxybutyrate, 2OB- 2-oxobutyrate, 2,3DH3MB- 2,3-dihydroxy-3-methylbutyrate, 2,3DH3MP- 2,3-dihydroxy-3-methylpentanoate.

Fig. 3. Methionine amino acid condensation pathway regulated by several gene families (Kroymann et al., 2001; Sawada et al., 2009).

to its chain-elongated homolog leucine (Fig. 3). In *Arabidopsis*, a *bcat4* mutant showed about a 50% reduction in total aliphatic glucosinolates and at the same time increased the level of free methionine and S-methyl-methionine (Schuster et al., 2006). This suggests that the *BCAT4* gene produces an enzyme which is involved in the first deamination reaction. Subsequently, three consecutive reactions of transformations occur. The first is a transamination and condensation reaction with acetyl-CoA catalyzed by *GSL-ELONG* in *Brassica* species (Li & Quiros, 2002). This is homologous to *MAM1* in *Arabidopsis* (Campos de Quirose et al., 2000; Benderoth et al., 2006; Textor et al., 2007). The same reaction occurs for 3C aliphatic glucosinolates which is controlled by isopropylmalate synthase (*IPMS1*, *IPMS2*). Isopropylmalate synthase is homologous to *MAM1* in *Arabidopsis* (Kliebenstein et al., 2001b; Field et al., 2004) and to *GSL-PRO* in *Brassica* species (Li et al., 2003; Gao et al., 2006). The second isomerisation reaction is controlled by isopropylmalate isomerises (*IPMI*) and third reaction is oxidative decarboxylation controlled by isopropylmalate dehydrogenases (*IPM-DH*) (Fig. 3) (Wentzell et al., 2007; Sawada et al., 2009).

These three consecutive reactions produce elongated 2-oxo acids with one or more methylene groups. These compounds are either transaminated by the *BCAT* enzyme to yield homo-methionine, which can enter into the core glucosinolate skeleton structure formation, or proceed through another round of chain elongation (Fig. 3). Overall, the methionine amino acid condensation pathway produces a range of methionine derivatives such as homo-methionine, di-homo-methionine, and tri-homo-methionine, which proceed to the next biosynthesis step called glucosinolate core skeleton formation (Fig. 3).

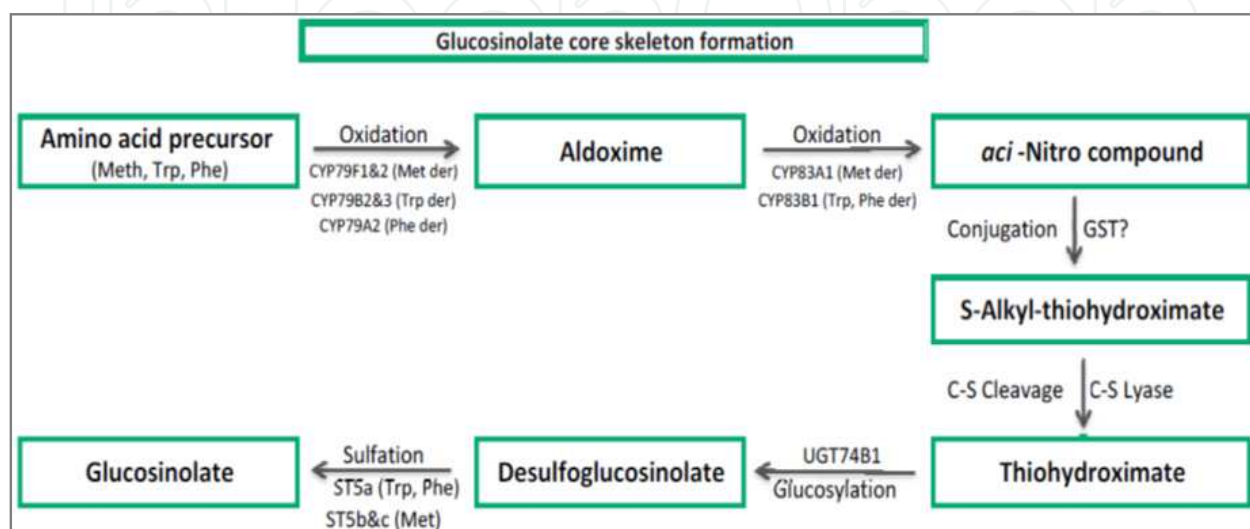
#### 5.1.2.2 Glucosinolate core skeleton formation

Glucosinolate core skeleton structure formation has been well characterized in *Arabidopsis*, with at least 13 enzymes and five different biochemical reactions, i.e., oxidation, oxidation with conjugation, C-S cleavage, glucosylation and sulfation (Grubb & Abel, 2006; Halkier & Gershenzon, 2006) involved in the formation. The precursors are catalyzed into aldoxime by cytochromes belonging to the *CYP79* gene family (Fig. 4). At least seven *CYP79s* were identified and functionally characterized in *Arabidopsis*. The *CYP79F1* gene converts all short chain methionine derivatives, whereas *CYP79F2* gene is involved in conversions of the long chain methionine derivatives. Similarly, *CYP79B2* and *CYP79B3* catalyze tryptophan derivatives, and *CYP79A2* catalyzes phenylalanine substrates (Fig. 4) (Zang et al., 2008). Subsequently, aldoximes are oxidized into either nitrile oxides or aci-nitro compounds by *CYP83A1* for methionine derivatives and *CYP83B1* for tryptophan as well as phenylalanine derivatives. This proceeds to a non-enzymatic conjugation to produce S-alkyl-thiohydroximates. In this sulphur rich chemical pathway, the next step is C-S cleavage by C-S lyase from S-alkyl-thiohydroximate to thiohydroxamic acid; C-S lyase forms an enzymatic complex with an S-donating enzyme. The *c-s lyase* mutant of *Arabidopsis* showed complete lack of aliphatic and aromatic glucosinolates in *Arabidopsis*, suggesting that this single gene family has a crucial role in skeleton processing (Mikkelsen et al., 2004).

In the glucosylation step, desulfoglucosinolate is formed by a member of the *UGT74* family. The final reaction of core skeleton formation is accomplished with sulfation of desulfoglucosinolates to produce intact glucosinolates by sulfotransferases *AtST5a*, *AtST5b* and *AtST5C* in *Arabidopsis*. Biochemical characterization of sulfotransferases in *Arabidopsis* revealed that *AtST5a* favour to sulfate phenylalanine and tryptophan derived



desulfoglucosinolates, whereas *AtST5b* and *AtST5c* favour to sulfate long chain aliphatic glucosinolates (Piotrowski et al., 2004). In a comparative analysis study between *Arabidopsis* and *B. rapa*, at least 12 paralogs of sulfotransferases were known to be responsible for this reaction (Zang et al., 2008). In glucosinolate skeleton formation reactions, the first four biosynthesis reactions take place in the chloroplast and the last reaction of sulfation occurs in the cytosol. This suggests that shuttle transporters play important roles in the entire biosynthesis process (Klein et al., 2006).



ST- sulfotransferase, UGT- glucuronosyltransferases, GST- glutathione S-transferase.

Fig. 4. Glucosinolate core skeleton structure formation by cytochromes. Methionine amino acid precursors produce aliphatic, tryptophan produces indole and phenylalanine produces aromatic glucosinolate core structures (Grubb & Abel, 2006; Halkier & Gershenzon, 2006).

### 5.1.2.3 Side chain modification in aliphatic glucosinolates

After glucosinolate core skeleton structure formation, the core skeletons are subjected to a set of reactions known as side chain modification or secondary transformation. Side chain modifications of glucosinolates are the last crucial enzymatic reactions on intact glucosinolates before their transport to sinks or biological degradation by myrosinases occur. Hydrolysis products of individual glucosinolates are recognized based on side chain variation in R groups. A hydrolysis product of glucoraphanin has anticancer properties. The R group modifications of glucoraphanin change their chemical properties, therefore, hydrolysis products have anticarcinogenic functions. Hydrolysis products of progoitrin, however, have anti-nutritional effect in animals, which reduce the palatability of rapeseed meal.

Side chain modification begins with the oxidation of sulphur in the methylthio precursor to produce methylsulfinyl and then methylsulfonyl moieties (Fig. 5). In *Arabidopsis*, this reaction is catalyzed by the flavin monooxygenases, *GSL-FMO<sub>OX1-5</sub>* located within the *GSL-OX1* locus on chromosome I. Phylogenetic analysis revealed a main group of *GSL-FMOs* for cruciferous species, which is further categorized according to subspecies, indicating that functional diversity of S-oxygenation of glucosinolates exists (Hansen et al., 2007; Li et al., 2008). Knockout mutant and over expression studies suggested that *GSL-FMO<sub>OX1-4</sub>* catalyzes

the 4-methylthiobutyl to 4-methylsulfinyl reaction and *GSL-FMO<sub>OX5</sub>* is involved in the S-oxygenation of long chain glucosinolates in *Arabidopsis* (Li et al., 2008). In *Brassica* vegetables, products of *GSL-FMO*s catalyses are the sources of anticancer compounds from aliphatic glucosinolates. It will be beneficial to identify these genes/loci in *Brassica* species so that they might be further used to manipulate aliphatic glucosinolates towards favourable forms.

A second round of binary side chain modification changes methylsulfinyl to alkenyl- and to hydroxyl- aliphatic glucosinolates (Fig. 5). In *Arabidopsis* these reactions are controlled by a *GSL-ALK/GSL-OHP* locus that has three tandem repeats (*GSL-AOP1*, *GSL-AOP2* and *GSL-AOP3*), which encode 2-oxoglutarate-dependent dioxygenases located on chromosome IV. Functional characterization indicates that *GSL-AOP2* catalyzes the reaction to alkenyl, whereas *GSL-AOP3* controls the reaction toward hydroxyalkenyl. The function of *GSL-AOP1*, however, is not clear in *Arabidopsis*, it might be involved in both reactions (Fig. 5) (Hall et al., 2001; Kliebenstein et al., 2001c; Mithen et al., 1995). The *GSL-ALK* and *GSL-OHP* are either closely linked on the same genomic region or allelic variants of a single genetic locus though they may show variable functions. In *Arabidopsis*, *GSL-OHP* catalyzes the reaction only for 3C aliphatic glucosinolate branches, whereas *GSL-ALK* is involved in 3C, 4C and 5C aliphatic glucosinolate branches. There is no clear functional information available for long chain (6C and so on) aliphatic glucosinolate branches and presumably

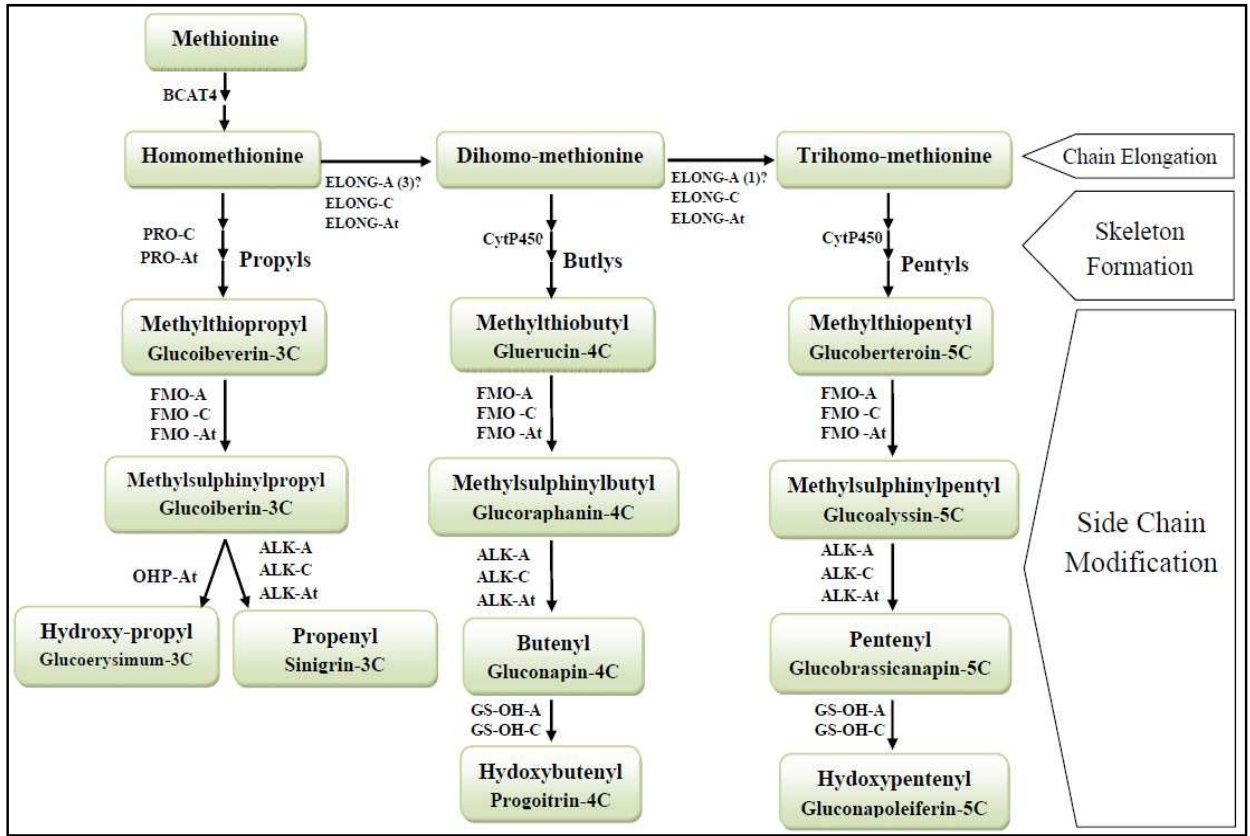


Fig. 5. Glucosinolate core structure and side chain modification pathway for 3C, 4C and 5C aliphatic glucosinolates. In the biosynthesis steps, gene symbols ending with A indicate A genome, C for C genome and At for *A. thaliana* (Magrath et al., 1994; Mithen et al., 1995; Li & Quiros, 2003; Mahmood et al., 2003).

*GSL-ALK* accomplishes these reactions in *Arabidopsis* (Kliebenstein et al., 2001c; Parkin et al., 1994). In *B. oleracea*, *GSL-ALK* was inferred by positional cloning and biochemical analysis. The functional allele in collard and the non-functional allele (with 2 bp deletion creating a frame-shift mutation) in broccoli were confirmed. A locus or loci of *GSL-ALK* is also believed to have a role in the catalysis of methylsulfinyl to alkenyl glucosinolates (Li & Quiros, 2003). Hydroxylation changes alkenyl to hydroxy aliphatic glucosinolate (in butyls, pentyls, hexyls and so on) biosynthesis branches in *Arabidopsis* and *Brassica* species; these sets of reactions are controlled by *GSL-OH* dependant on the presence of both *GSL-AOP2* and *GSL-ELONG*. In *Brassica*, the final product of this reaction in 4C glucosinolate biosynthesis is progoitrin and its hydrolytic derivative, oxazolidine-2-thione which causes goiter in animals. These compounds are major obstacles to the use of *Brassica* crops as animal feed (Fenwick et al., 1983).

### 5.1.3 Diversity of glucosinolates in *Brassicaceae*

Glucosinolates are united by their unique basic skeleton ( $\beta$ -D-glucopyranose) but glucosinolates are diverse in their origins, side chain modifications, degradations and final biological functions. In addition to structural diversity, a diversity of glucosinolates is seen between families, genera, species, subspecies and different accessions of subspecies. This diversity provides insight into glucosinolate biosynthesis at the genomic, physiological, biochemical and host-pathogen interaction levels. The natural variation of glucosinolate profiles between species or different cultivars of same species permits the investigation of the effects of QTL or genes and gene interactions. This can be utilized for advanced breeding applications like MAS, trait introgression and gene pyramiding for beneficial glucosinolates. In *Arabidopsis*, naturally occurring variations in glucosinolates were identified and quantified for 34 types of glucosinolates in the leaves of 39 ecotypes (Hogge et al., 1988; Reichelt et al., 2002). Similarly, different morphotypes of *B. rapa* possess eight different glucosinolates with gluconapin and glucobrassicinapin as predominant aliphatic glucosinolates (He et al., 2000). Padilla et al., (2007) reported 16 different glucosinolates among 116 accessions of turnip greens.

The wide range of variation in glucosinolate profiles provides the opportunity to study individual glucosinolates for their potent biological activities *in planta*. Within different forms of *B. oleracea*, 12 different glucosinolates have been detected. The beneficial glucosinolate glucoraphanin showed significant variation ranging from 44 to 274  $\mu$ mole/g seed in different genotypes of broccoli (Mithen et al., 2000; Rangkadilok et al., 2002). Furthermore, variation in concentration of individual glucosinolates also exists in cultivars of the same species.

## 6. Low glucosinolate rapeseed and canola

Early forms of domesticated rapeseed and their cultivars possessed a high concentration of glucosinolates (100 to 180  $\mu$ mole/g) in their oil-free seed meal. The presence of glucosinolates in rapeseed had hindered the use of rapeseed meal in livestock industries due to anti-nutritional effects of its hydrolysis products in animals. As a result, in the 1970s, plant breeders searched germplasm collections for low glucosinolate contents. A Polish

spring rape cultivar, Bronowski, with low glucosinolate content was discovered by The Agriculture Canada Research Station in Saskatoon (Kondra & Stefansson, 1970). This sole genetic source of the low glucosinolate trait has been used to develop all the low glucosinolate cultivars in *B. napus* and *B. rapa* worldwide through conventional plant breeding. *B. napus* and *B. rapa* cultivars with low content of erucic acid and glucosinolate were developed, which ushered in a new era for *Brassica* crop production and its consumption. The world's first double low (low erucic acid and low glucosinolate content) *B. napus* and *B. rapa* cultivars, Tower and Candle, respectively, were developed by pedigree selection in the progenies of interspecific crosses in 1970s (Stefansson & Downey, 1995; McVetty et al., 2009). In Canada, this new type of oilseed rape was designated "Canola". The term "Canola" applies to any rapeseed cultivars with erucic acid content of <2% and glucosinolates content of <30  $\mu\text{mol/g}$  in oil-free seed meal. The Canola term is a registered trademark of the Canadian Canola Association. The name is derived from **Canadian Oil Low Acid** (Canola Council of Canada, 2010a, [http://www.canola-council.org/canola\\_the\\_official\\_definition.aspx](http://www.canola-council.org/canola_the_official_definition.aspx)). Currently, most rapeseed (high erucic acid) and canola cultivars have glucosinolate levels <15  $\mu\text{mole/g}$  in oil-free seed meal. The development of low erucic acid and low glucosinolate cultivars has also been undertaken for other *Brassica* rapeseed species (e.g. *B. juncea*) and in other parts of the world for the quality improvement of their oils and seed meals.

## 7. Quantitative trait loci for glucosinolates in major *Brassica* species

Glucosinolate biosynthesis in *Brassica* crops has quantitative inheritance, which is regulated by complex genetic factors and affected by environmental factors. Glucosinolates are functionally diverse and well recognized plant secondary metabolites; so they have been extensively studied in terms of QTL mapping, biosynthesis gene cloning and functional characterization in *Arabidopsis* (Kliebenstein et al., 2001a; Kliebenstein et al., 2001c; Compos de Quiros et al., 2000; Brown et al., 2003; Benderoth et al., 2006; Textor et al., 2007; Li et al., 2008). However, very limited genetic, biochemical and metabolomic information is available on glucosinolate biosynthesis, transport and final product utilization in *Brassica* crops including *B. rapa*. There has been a few QTL mapping studies reported for major *Brassica* crop seed glucosinolates. Uzunova et al., (1995) mapped four QTL for total seed glucosinolate content in a *B. napus* DH population, which accounted 61% total phenotypic variance. Similarly, Toroser et al., (1995), based on a RFLP linkage map, identified two larger and three small effect QTL for total aliphatic glucosinolate content using a DH population in *B. napus*. These QTL explained 70% of the total phenotypic variance. This suggests that several loci with additive or epistatic effect are involved in total seed glucosinolate biosynthesis in different genetic backgrounds. Howell et al., (2003) reported QTL mapping for total seed glucosinolates analyzed by X-ray fluorescence (XRF) and near-infrared reflection spectroscopy (NIRS) in two inter-varietal *B. napus* backcross populations. They identified four QTLs accounting for 76% of the phenotypic variance in the Victor x Tapidor population. These three QTL accounted for 86% of phenotypic variance in this second population. These studies, however, were limited to either total seed glucosinolates or 3C, 4C and 5C aliphatic glucosinolates, and did not infer the genetic loci for individual aliphatic, indole or aromatic glucosinolates. Furthermore, there were no reports of publicly available



molecular markers for marker assisted selection of glucosinolates. Such markers, if developed, could be used in breeding to manipulate glucosinolate profiles and contents in *Brassica* crop species.

In another amphidiploid species, *B. juncea*, several studies were conducted for QTL mapping of seed glucosinolates. Cheung et al., (1998) detected two QTL for 2-propenyl and three QTL for 3-butenyl glucosinolates which explained between 89% and 81% of total phenotypic variance. This QTL mapping study was carried out in a DH population derived from the F<sub>1</sub> of two *B. juncea* parental lines, J90-4317 (low glucosinolates) and J90-2733 (high glucosinolates). Mahmood et al., (2003) reported three QTL for 2-propenyl glucosinolate content which explained 78% of the phenotypic variance, while five QTL for total seed aliphatic glucosinolates explained phenotypic variance between 30% and 45%. In this study a DH population and an RFLP linkage map was used. Similarly, Ramchiary et al., (2007) reported six QTL for seed glucosinolate content in the F<sub>1</sub>DH and advanced backcross DH (BC<sub>4</sub>DH) of *B. juncea*. Some of the large effect QTL in advanced backcross (BC<sub>4</sub>DH) of *B. juncea* were fine mapped using a candidate gene approach and comparative sequence analyses of *Arabidopsis* and *B. oleracea* (Bisht et al., 2009). The results suggested that epistasis and additive effects of glucosinolate genes in different genetic backgrounds in *B. juncea* exist. This study, however, could not explain the homoeologous effects of genes/loci from the A- and B-genomes on the individual or total seed glucosinolate content.

In *B. oleracea*, *BoGSL-ELONG* a side chain elongation gene was cloned based on the *Arabidopsis* sequence information, and functionally characterized using an RNA interference (RNAi) approach. The results suggested that *BoGSL-ELONG* is involved in 4C and 5C aliphatic glucosinolate biosynthesis in *Brassica* species. The RNAi lines displayed an increased level of propyl glucosinolates suggesting that the precursor homo-methionine concentration enhances the activity of 3C aliphatic glucosinolate biosynthesis in *B. napus* (Li & Quiros, 2002, Liu et al., 2010). A natural mutation in *BoGSL-ELONG* resulting in the failure of excision of the third intron and thus producing a long cDNA fragment has been identified in a white cauliflower genotype (*B. oleracea*) lacking 4C and 5C aliphatic glucosinolates (Li & Quiros, 2002). A molecular marker for this mutation would be useful in *Brassica* breeding programs for modification of glucosinolate profiles. Additionally, a gene *BoGSL-PRO* which control propyl glucosinolate biosynthesis in *B. oleracea* was sequenced using comparative analysis of the *MAM* (methylthioalkylmalate synthase) gene family in *Arabidopsis* (Li et al., 2003; Gao et al., 2006).

A glucosinolate side chain modification gene, *BoGSL-ALK*, was cloned using a positional cloning approach based on a closely linked SRAP marker in *B. oleracea* (Li & Quiros, 2003). Functional characterization of *BoGSL-ALK* by overexpression in *Arabidopsis* and RNA interference (RNAi) in *B. napus* suggests that *BoGSL-ALK* is involved in catalyzation of either sulfinylbutyl to butenyl or hydroxybutenyl with high functional redundancy (Li & Quiros, 2003, Liu et al., 2012). Interestingly, a natural frame shift mutation of 2 bp deletions was identified in broccoli, which accumulates sulfinylbutyl glucosinolate by ceasing downstream biosynthesis of other 4C aliphatic glucosinolates.

In *B. rapa*, a single QTL mapping study for leaf glucosinolates has been reported, although it is one of the widely distributed *Brassica* species for oil and vegetable production. Lou et al., (2008) identified six QTL for leaf total aliphatic glucosinolate content, three QTL for total leaf

indole glucosinolate content and three QTL for leaf aromatic glucosinolates in two DH populations of *B. rapa* using an AFLP and SSR based linkage map. There was no information regarding QTL for seed glucosinolates. Glucosinolate content varies greatly between leaves and seeds (Brown et al., 2003). As well, there is variation in the expression patterns of the genetic loci underpinning glucosinolate production in leaves and seeds (Kliebenstein et al., 2001b).

## 8. Glucosinolate identification and quantification approaches

Early analysis of glucosinolates began with detection of glucosinolates and possible hydrolysis products by paper and thin-layer chromatography. The paper chromatography was applied in combination with high voltage electrophoresis, but it had many complications and low yield (Greer, 1962). Danielak & Borkowski, (1969) analyzed glucosinolates from seeds of 150 different cruciferous species using thin-layer chromatography. Since then, numerous techniques have been employed for quantification of total glucosinolate content with various modifications including steam distillation and titration of isothiocyanates, ELISA, sulfate-release assay, UV spectroscopy and gas chromatography of isothiocyanates. Near infrared reflectance spectroscopy (NIRS) is one of the widely used techniques for seed total glucosinolates quantification, which detects N-H, C-H and O-H groups of total glucosinolates. NIRS is a preferred technique because it can simultaneously quantify oil and protein along with total glucosinolates in canola/rapeseed (Velasco & Becker, 1998). Individual intact glucosinolates can be determined using techniques such as reverse phase HPLC-MS, thermospray LC with tandem MS in the two most common interfaces (ESI or APCI), capillary GC-MS and GC-MS-MS.

Desulfoglucosinolates usually are analyzed by reverse phase HPLC or by X-ray fluorescence spectroscopy (XRF). The reverse-phase HPLC analytical approach has been widely used for quantification of individual intact or desulfo- glucosinolates. The technique was developed in 1984 with UV based detection of either intact glucosinolates or an on-column enzymatic desulfation from plant extracts (Spinks et al., 1984). The photodiode array (PDA) with UV detector can distinguish spectra of aliphatic from indole and aromatic glucosinolates; the indole and aromatic glucosinolates spectra end with a shoulder. This widely applicable method for glucosinolate separation is yet subject to difficulties in interpretation of results because of differences in the time and enzymatic activity for the desulfation reaction, pH effects and mobile phase solvents with an appropriate gradient. Desulfoglucosinolates also have been analyzed by the determination of the sulfur content of the seeds using X-ray fluorescence spectroscopy (XRF) (Schnug & Haneklaus, 1990). The hydrolysis products of glucosinolates, isothiocyanates, nitriles, thiocyanates and benzenedithiol, have been analyzed using techniques including GC or GC-MS and HPLC with or without fluorescent labelling (Kiddle et al., 2001).

## 9. Molecular markers and their applications for glucosinolates

Molecular markers are efficient, reliable, time saving and cost effective tools that may enhance the capacity of conventional breeding for improvement in agronomy, quality and yield related traits of crop species without adverse effects. Morphological traits such as petal

color, leaf shape *etc* were used as markers in classical breeding, where significant time and effort was required to refine crosses. There have been many practical difficulties with the use of morphological markers, including:

- i. a paucity of suitable markers and associations with agriculturally important traits (Ranade et al., 2001),
- ii. undesirable pleiotropic effects of many morphological markers on plant phenotypes (Ranade et al., 2001),
- iii. high linkage drag (Ranade et al., 2001), and
- iv. trait of interest easily can be lost in a breeding cycle if there is no strong linkage between marker and traits (Ranade et al., 2001).

Advancements in molecular biology tools and techniques have overcome some of the difficulties of classical breeding. Different types of DNA molecular markers (hybridization based e.g. RFLP; PCR based e.g. SSR, RAPD, SCAR, and SRAP) have been used for gene/QTL mapping, cloning, genetic map construction and marker assisted selection in plant breeding. Most recently, the conversion of various molecular markers (RFLP, RAPD, SRAP, AFLP, SSR, SNP etc.) to simple PCR based SCAR markers for marker assisted selection has overcome the difficulties of other markers. It is feasible and cost effective to use SCAR markers for marker assisted selection of populations.

Marker assisted selection in plant breeding is well supported by the availability of molecular maps developed using various marker systems in different mapping populations. The use of molecular markers has facilitated introgression of important traits through intra or interspecific as well as inter-generic crosses. Similar to agronomic, disease resistance and yield related traits, seed quality traits such as glucosinolates can be genetically manipulated using interspecific hybridization followed by marker assisted selection for introgression or replacement of a native gene with the allied gene. Natural mutations for glucosinolate biosynthesis genes have been identified in accessions of *B. oleracea* (Li & Quiros, 2002; 2003) and molecular markers have been developed. These molecular markers have been employed for the manipulation of glucosinolate profiles in *Brassica* through interspecific hybridization and marker assisted selection. In our QTL mapping study in *B. rapa* RIL mapping population, we identified single major QTL for 5C aliphatic glucosinolates (glucobrassicinapin, glucoalyssin and gluconapoleiferin) on chromosome A3 and gene specific SCAR molecular markers were developed and utilized that markers for marker assisted selection in other *Brassica* interspecific crosses (unpublished). Hasan et al., (2008) reported linkage of SSR markers to candidate genetic loci of glucosinolate biosynthesis genes in *Brassica napus* through structure-based allele-trait association studies, and found potential application of these markers in marker assisted selection for glucosinolates.

On the other hands, Niu (2008) attempted to replace the functional *GSL-ALK* gene of *B. rapa* by the null allele from *B. oleracea* (broccoli) using a gene specific SCAR marker. However, introgression of the *GSL-ALK* null allele or replacement of a single locus with small effect did not change the glucosinolate profile of the *B. rapa* in this study. This suggests that multiple loci with functional redundancy play important roles in glucosinolate biosynthesis in *Brassica* species. This approach has met with very little or no success. This might be due to many reasons, such as:

- i. duplicated or triplicated genomic regions may mask the effect of the single locus being replaced for a quantitative traits like glucosinolate profile and concentration
- ii. lack of similarity of gene and spacer sequences between alien and host chromosomes in monosomic or disomic alien chromosome addition lines
- iii. presence of active homoeologous recombination regulator genes during meiosis
- iv. directional exchange of genetic materials in trivalent formations during meiosis because of distinct chromosome behaviour
- v. host genome chromosome numbers and amount of homology between host and alien chromosomes

Several traits in *Brassica* species have been improved through introgression of functional genes from allied species through interspecific or inter-generic crosses such as *B. rapa* × *B. oleracea* and *B. rapa* × *B. oxyrrhina* (Srinivasan et al., 1998). In near future, development of molecular markers using sequenced genome information of *B. rapa* and *Arabidopsis* will hasten marker assisted selection of glucosinolates to increase beneficial glucosinolates such as glucoraphanin and glucoerucin in *Brassica* vegetables and to reduce total glucosinolates in rapeseed meal.

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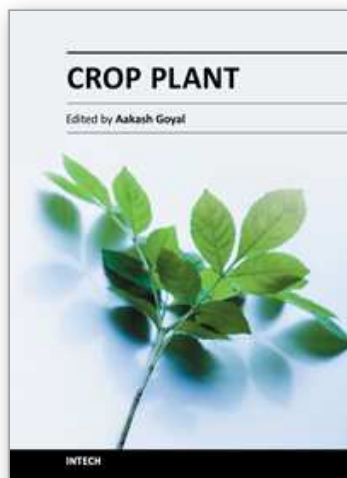
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This book provides us a thorough overview of Crop Plant with current advance in research. Divided into two section based on the chapters contents. Chapter 1 provides information about markers and next generation sequencing technology and its use. Chapter 2 is about how we can use Silicon for Drought tolerance. Chapter 3 is to deal with the major problem of rising CO<sub>2</sub> and O<sub>3</sub> causing environmental pollution. Chapter 4 covers the phenomena of RNAi and its use, application in crop science. Chapter 5 is a review for boron deficiency in soils and how to deal with it for better crops. Chapter 6-10 provide some information regarding recent works going on in crop science.

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