
Review

Glucosinolate metabolism, functionality and breeding for the improvement of Brassicaceae vegetables

Masahiko Ishida*¹⁾, Masakazu Hara²⁾, Nobuko Fukino³⁾, Tomohiro Kakizaki³⁾ and Yasujiro Morimitsu⁴⁾

¹⁾ NARO Institute of Vegetable and Tea Science, Tsukuba Vegetable Research Station, 3-1-1 Kannondai, Tsukuba, Ibaraki 305-8666, Japan

²⁾ Research Institute of Green Science and Technology, Shizuoka University, 836 Ohya, Shizuoka 422-8529, Japan

³⁾ NARO Institute of Vegetable and Tea Science, 360 Kusawa, Ano, Tsu, Mie 514-2392, Japan

⁴⁾ The Department of Food and Nutritional Sciences, The Graduate School of Humanities and Sciences, Ochanomizu University, 2-1-1 Otsuka, Bunkyo, Tokyo 112-8610, Japan

Unique secondary metabolites, glucosinolates (*S*-glucopyranosyl thiohydroximates), are naturally occurring S-linked glucosides found mainly in Brassicaceae plants. They are enzymatically hydrolyzed to produce sulfate ions, D-glucose, and characteristic degradation products such as isothiocyanates. The functions of glucosinolates in the plants remain unclear, but isothiocyanates possessing a pungent or irritating taste and odor might be associated with plant defense from microbes. Isothiocyanates have been studied extensively in experimental *in vitro* and *in vivo* carcinogenesis models for their cancer chemopreventive properties. The beneficial isothiocyanates, glucosinolates that are functional for supporting human health, have received attention from many scientists studying plant breeding, plant physiology, plant genetics, and food functionality. This review presents a summary of recent topics related with glucosinolates in the *Brassica* family, along with a summary of the chemicals, metabolism, and genes of glucosinolates in Brassicaceae. The bioavailabilities of isothiocyanates from certain functional glucosinolates and the importance of breeding will be described with emphasis on glucosinolates.

Key Words: glucosinolate, isothiocyanate, biosynthesis, gene, health, *Brassica*, plant breeding.

Introduction

The Brassicaceae family, also called Cruciferae, includes around 375 genera and about 3,200 species (LeCoz and Ducombs 2006), including many economically important plants such as leaf and root vegetables, oilseed and condiment crops, and the model plant *Arabidopsis thaliana*. Brassicaceae vegetables are widely cultivated, with many genera, species, and cultivars including *Brassica rapa* (Chinese cabbage, Chinese mustard, bok choy and turnip), *B. oleracea* (cabbage, broccoli, cauliflower, kale, Brussels sprouts and kohlrabi), *B. napus* (rapeseed and rutabaga), *B. juncea* (mustard green), and *Raphanus sativus* (radish).

Glucosinolates, secondary metabolites found in Brassicaceae and related families, have three moieties: a β -thioglucose moiety, a sulfonated oxime moiety, and a variable aglycone side chain derived from a α -amino acid. Glucosinolates, of which nearly 200 types having different substituents have been identified, are classifiable into three

classes based on the structure of different amino acid precursors: aliphatic glucosinolates, indole glucosinolates, and aromatic glucosinolates (Fig. 1). Table 1 shows the glucosinolates found in Brassicaceae vegetables. Glucosinolates of each group are synthesized through a metabolic pathway that is independent and which shares a common set of enzymes involved in the core structure formation of glucosinolates under genetic control (Fahey *et al.* 2001, Halkier and Gershenzon 2006, Hirani *et al.* 2012, Kim *et al.* 2002, Mithen *et al.* 2000). The composition and contents of glucosinolates are influenced by the genotype, climate and cultivation conditions including fertilization, harvest time and plant position (Rangkadilok *et al.* 2002, Sang *et al.* 1984, Tripathi and Mishra 2007, Verkerk *et al.* 2009). They differ completely among plant genera and among different organs (Rosa *et al.* 1997).

When plant tissue damage occurs because of disruption, glucosinolates are hydrolyzed quickly with inherent myrosinase (β -thioglucoside glucohydrolase, thioglucosidase, EC3.2.1.147), resulting in production of isothiocyanates, thiocyanates, nitriles, goitrin and epithionitriles, depending upon pH and other conditions (Fig. 2) (Bones and Rossiter 1996, 2006, Fenwick *et al.* 1983). The system in which

Communicated by T. Nishio

Received September 20, 2013. Accepted February 24, 2014.

*Corresponding author (e-mail: ishimame@affrc.go.jp)

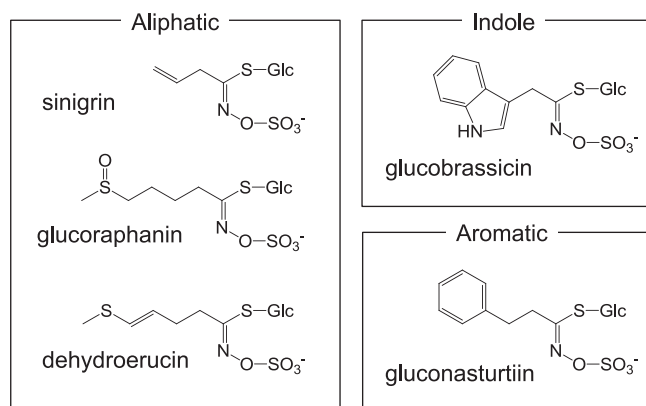


Fig. 1. Examples of glucosinolates found in Brassicaceae vegetables.

Table 1. Common and chemical name of major glucosinolates present in Brassicaceae vegetables

Common name	Chemical name (side chain R)
<i>Aliphatic glucosinolate</i>	
3 carbon chain length	
Glucoberverin	3-Methylthiopropyl
Glucobrassin	3-Methylsulfinylpropyl
Sinigrin	2-Propenyl
4 carbon chain length	
Glucorucin	4-Methylthiobutyl
Dehydroerucin	4-Methylthio-3-butenyl
Glucoraphanin	4-Methylsulfinylbutyl
Glucoraphenin	4-Methylsulfinyl-3-butenyl
Gluconapin	3-Butenyl
Progoitrin	2-Hydroxy-3-butenyl
5 carbon chain length	
Glucoberteroin	5-Methylthiopentyl
Glucosylsin	5-Methylsulfinylpentyl
Glucobrassicinapin	4-Pentenyl
Gluconapoleiferin	2-Hydroxy-4-pentenyl
<i>Aromatic glucosinolate</i>	
Gluconasturtiin	2-Phenylethyl
<i>Indole glucosinolate</i>	
Glucobrassicin	3-Indolyl methyl
4-Hydroxyglucobrassicin	4-Hydroxy-3-indolymethyl
4-Methoxyglucobrassicin	4-Methoxy-3-indolymethyl
Neoglucobrassicin	N-methoxy-3-indolymethyl

glucosinolate and myrosinase come into contact in cases of tissue destruction is called “the glucosinolate-myrosinase system” (Bones and Rossiter, 1996, 2006, Rask *et al.* 2000). Isothiocyanates are important pungent compounds that affect the taste and odor of Brassicaceae plants (Fahey *et al.* 2001, Mithen *et al.* 2000, Williams and Pun 2011). However, the consumption of Brassicaceae vegetables containing glucosinolates might reduce the risk of carcinogenesis and particular disease in humans (Traka and Mithen 2009). Therefore, glucosinolate, an isothiocyanate precursor, has also been in the spotlight.

Along with the acute progression of aging and the increase of lifestyle diseases in economically developed countries, maintaining and enhancing good health through dietary habits has become a crucially important social issue. For this purpose, phytochemicals in dietary vegetables are

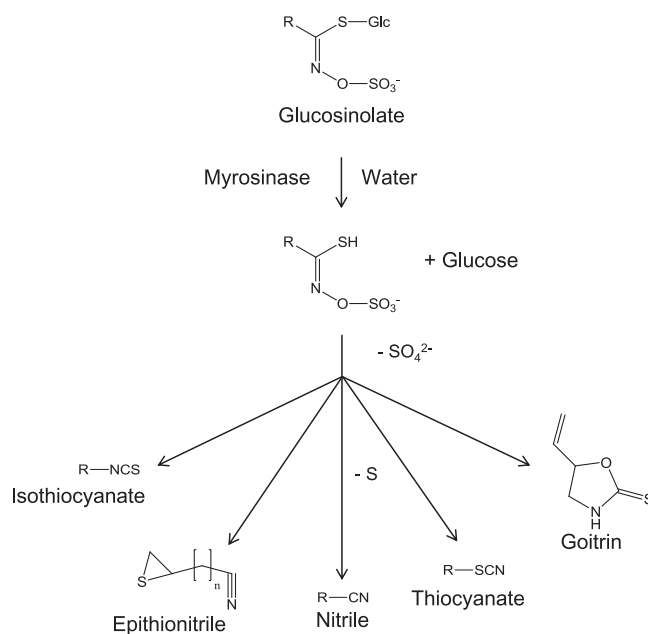


Fig. 2. Isothiocyanate, epithionitrile, nitrile, thiocyanate, and goitrin generated from glucosinolate.

noteworthy. Among the many varieties of vegetables, Brassicaceae vegetables have received the most attention because their unique constituents, glucosinolates, are abundant in edible parts and are regarded as most likely to maintain human health through continuous consumption. For the future, the breeding of Brassicaceae vegetables by particularly addressing beneficial glucosinolates is expected to grow in importance. The purpose of this review was to collect research findings related to recent developments in glucosinolates and their breakdown products for the breeding of Brassicaceae vegetables.

1. Structure, metabolism, and biosynthetic genes of glucosinolates in Brassicaceae vegetables

Glucosinolate biosynthesis

The biosynthetic pathway of glucosinolates has been almost entirely elucidated mainly by *Arabidopsis*. Many reviews have presented summaries of the results (such as Grubb and Abel 2006, Halkier and Gershenzon 2006, Sønderby *et al.* 2010b). Glucosinolates are biosynthesized from amino acids. The three glucosinolate subtypes have their corresponding precursors. Aliphatic glucosinolates are derived from alanine, leucine, isoleucine, valine, and methionine. Indole glucosinolates and aromatic glucosinolates are derived respectively from tryptophan and phenylalanine or tyrosine. The glucosinolate biosynthetic pathways comprise three independent steps: the chain elongation stage, formation of a core glucosinolate structure, and secondary modification (Fig. 3).

Aliphatic glucosinolates have various side chains with different lengths determined by the chain elongation steps.

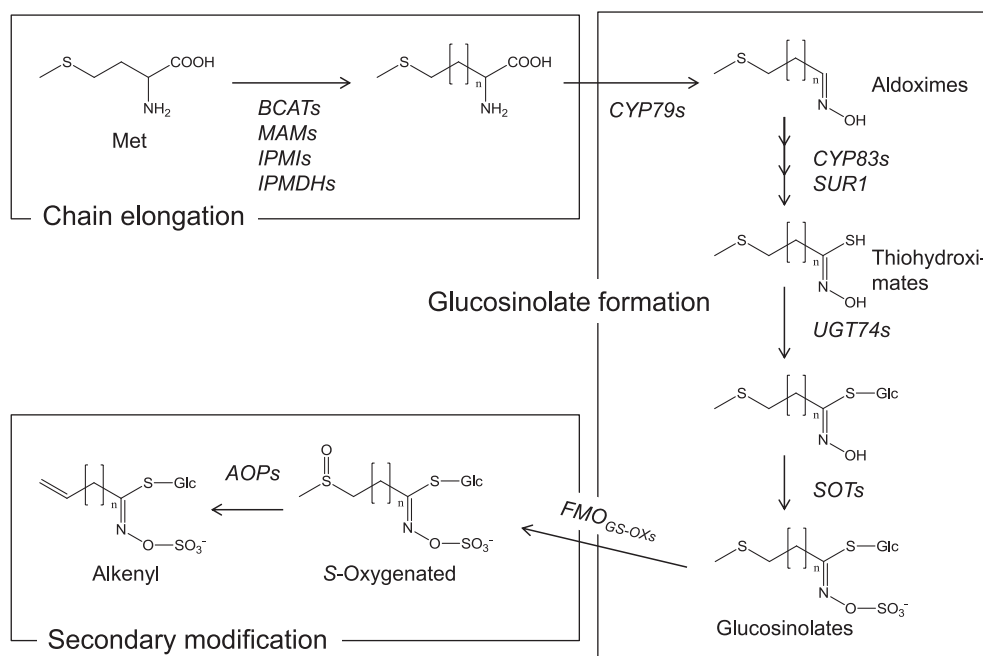


Fig. 3. Schematic pathway of aliphatic glucosinolate biosynthesis. The pathway consists of chain elongation, glucosinolate formation, and secondary modification. Details can be found in the review articles presented in the text.

The first process of chain elongation is the deamination of amino acids such as methionine to the corresponding 2-oxo acids by a branched-chain amino acid aminotransferase (BCAT). The 2-oxo acids are precursors of the elongation reaction by a methylene group. The elongation proceeds by methylthioalkylmalate synthase (MAM), isopropylmalate isomerase (IPMI), and isopropylmalate dehydrogenase (IPMDH). Finally, the elongated 2-oxo acids are transformed to the corresponding amino acids by BCAT. This chain elongation also takes place in the biosynthesis of aromatic glucosinolates, but does not occur in the formation of indole glucosinolates.

Amino acids, including elongated ones, then undergo following step: the formation of core glucosinolate structure. Cytochromes P450 (CYP79s) convert the amino acids to aldoximes, which are then oxidized to the activated forms by CYP83s. The activated forms are transformed to thiohydroximates via glutathione conjugation and the C-S lyase (SUR1) reaction. The thiohydroximates are finally converted to the glucosinolate structure by the S-glucosyltransferases of the UGT74 family and the sulfotransferases SOTs. After the glucosinolate structure formation, the side chains are modified by oxygenation, hydroxylation, alkenylation, benzoylation, and methoxylation. The S-oxygenation of aliphatic glucosinolates is a common modification conducted by flavin monooxygenases *FMO_{GS-OXs}*. The S-oxygenated aliphatic glucosinolates, such as glucoraphanin, are found in many Brassicaceae vegetables. Alkenyl glucosinolates such as sinigrin are produced by 2-oxoglutarate-dependent dioxygenases *AOPs* from S-oxygenated glucosinolates. Glucobrassicin, which is a common indole glucosinolate, is hydroxylated by CYP81F2 in *Arabidopsis*. Methoxylations

might occur by unidentified O-methyltransferases. The level and profile of glucosinolates are determined respectively in *Arabidopsis* by transcription factors such as MYB28, MYB29, and MYB76 for aliphatic glucosinolates and by MYB34, MYB51, and MYB122 for indole glucosinolates (Sønderby *et al.* 2010a).

Biosynthetic genes and transcription factors related to glucosinolates have been identified using *Arabidopsis*. The backbone of biosynthesis found in *Arabidopsis* is expected to be the same as those in Brassicaceae vegetables. The association between the glucosinolate biosynthetic genes of *Arabidopsis* and those found in Brassicaceae vegetables is described below.

Glucosinolate-myrosinase system

Glucosinolates are hydrolyzed by thioglucosidases called myrosinases to isothiocyanates, thiocyanates, nitriles, epithionitriles etc., which are bioactive compounds (Fig. 2) (Kissen *et al.* 2009). Presumably, glucosinolates are hydrolyzed only slightly under intact conditions, in which myrosinases are separated from the location of glucosinolates (Husebye *et al.* 2002, Kelly *et al.* 1998, Koroleva *et al.* 2000). Once tissues are mechanically damaged, however, glucosinolates are hydrolyzed intensively by myrosinases. This hydrolyzing reaction is called the glucosinolate-myrosinase system (Bones and Rossiter 1996, 2006, Rask *et al.* 2000). Although isothiocyanates are main products from the myrosinase reaction, nitriles/epithionitriles and thiocyanates can be generated respectively by the associations of an epithiospecifier protein and a thiocyanate-forming protein (Bones and Rossiter 2006). The epithiospecifier protein determines the composition of hydrolysis products in each

Brassicaceae crop. Unlike *Brassica* species, no epithiospecifier protein is detected in *Sinapis alba* or *R. sativus*, which produce few nitriles/epithionitriles (Foo *et al.* 2000). A glucosinolate, progoitrin, is hydrolyzed by myrosinases to form a cyclic thiocarbamate goitrin which is an antinutritional compound that reduces the production of thyroid hormones (Stoewsand 1995). Hydrolysis products of glucosinolates are related to pigment formation. Carboline compounds derived from 4-methylthio-3-butenyl isothiocyanate, which is a hydrolyzation compound of dehydroerucin, are necessary factors for the formation of yellow pigments in salted radish roots (Ozawa *et al.* 1990). Moreover, this glucosinolate-myrosinase system has been described as related to plant-insect and plant-pathogen interactions. Several studies have implicated glucosinolate degradation products in plant defense against insects, pathogens, and herbivores (Agrawal and Kurashige 2003, Hopkins *et al.* 2009, Manici *et al.* 1997, Rask *et al.* 2000, Tierens *et al.* 2001).

Myrosinases are localized in myrosin cells, which are protein-rich idioblasts found mainly in the tissues of imbibed seeds. However, such myrosin cells have been observed in Brassicaceae crop vegetative organs. Myrosinase was localized in the epidermis and the vascular cambium of the radish and turnip taproots and the Japanese horseradish (wasabi) rhizome (Hara *et al.* 2000, 2001). This distribution of myrosinases, called a “double castle wall structure” is likely to be common among Brassicaceae vegetables. In the flower stalk of *Arabidopsis*, myrosinases are expressed both in the phloem cells and the guard cells, but glucosinolates accumulate in the S-cells which are adjacent to the phloem cells. In fact, spatial separation of myrosinases from glucosinolates is the basis of the glucosinolate-myrosinase system.

Nutritional and environmental factors regulating glucosinolate production

The accumulation of aliphatic glucosinolates in *B. rapa* is enhanced by low nitrogen and high sulfur supplies (Chen *et al.* 2006). Glucose promoted aliphatic glucosinolate biosynthesis in *Arabidopsis* (Miao *et al.* 2013). The glucosinolate levels in *B. napus* were elevated by wounding, methyl jasmonate, and fungal infection (Brader *et al.* 2001). Some intriguing results have been obtained for the relation between high-temperature stress and glucosinolate synthesis. Exposure of Brassicaceae vegetables to high temperatures is known to enhance the higher aliphatic glucosinolate contents (Charron and Sams 2004, Pereira *et al.* 2002). A glucosinolate-deficient mutant of *Arabidopsis* showed thermosensitivity and less heat shock protein (HSP) 90 expression after high-temperature stress (Ludwig-Müller *et al.* 2000). Moreover, exogenous application of isothiocyanates to *Arabidopsis* enhanced its thermotolerance and induced the expression of HSP genes (Hara *et al.* 2013). These results suggest that isothiocyanate is a signaling molecule that promotes thermotolerance in plants.

2. Bioavailability and functionality of isothiocyanates derived from glucosinolates of Brassicaceae vegetables

Isothiocyanates against human pathogens

Brassicaceae vegetables have been used by human beings not only as food materials but also for their medicinal properties. The well-known bioactivities of Brassicaceae vegetables have been investigated for antibacterial and antifungal activities of isothiocyanates (Kojima and Ogawa 1971, Uda *et al.* 1993) from ancient times in human history. Glucosinolates and their breakdown products, isothiocyanates, have also been used for their fungicidal, bactericidal and nematocidal properties, which are readily linked to plant defenses. Not only the plant, but also the antimicrobial activities of isothiocyanates such as allyl isothiocyanate in mustard or Japanese horseradish (wasabi) against various human pathogens have been worth using for their medicinal effects. Even in recent research, sulforaphane (4-methylsulfinylbutyl isothiocyanate), a distinguished isothiocyanate in broccoli and broccoli sprouts, has shown an inhibitory effect for urease from *Helicobacter pylori* (Fahey *et al.* 2013).

Promise of cancer chemopreventive agents by inducing phase 2 enzymes

The development of natural anticancer constituents from edible plants is extremely important for the inclusion of potential cancer chemopreventive agents in dairy foods. Epidemiological reports have described that Brassicaceae vegetables lower the risk of many cancers (Herr and Büchler 2010). Glutathione *S*-transferase (GST) is a family of detoxification enzymes (phase 2 enzymes) consisting of class α , μ , π , and θ isoforms. Induction of phase 2 enzymes such as GST and NADP(H) quinone oxidoreductase 1 (NQO1) have been studied in Brassicaceae vegetables such as broccoli sprouts (Kostova *et al.* 2007, Munday *et al.* 2008, Zhang *et al.* 2006), cabbage (Whitty and Bjeldanes 1987), and Japanese horseradish (Morimitsu *et al.* 2000, 2002). Results demonstrated that many isothiocyanates derived from Brassicaceae vegetables and certain fruits (papaya and so on) show phase 2 induction in cultured cells and rodents. Sulforaphane was identified initially as a potential inducer of NQO1 in Hepa lcl7 cell culture (Zhang *et al.* 1992). To date, sulforaphane has been proved in other mechanisms of chemopreventive activities such as cell cycle arrest and apoptosis induction (Gamet-Payraastre *et al.* 2000, Parnaud *et al.* 2004).

Phase 2 induction mechanisms of isothiocyanates sensing by Keap1

Although isothiocyanates are widely known as effective phase 2 inducers, phase 2 enzymes are also induced by phenols (Piero *et al.* 2006), organosulfurs (Wu *et al.* 2002), and indoles (van Lieshout *et al.* 1998). Many detoxification enzymes including GST are regulated through the antioxidant response element (ARE), a gene control region (Fig. 4). Actually, ARE is regulated by two proteins: nuclear respiratory

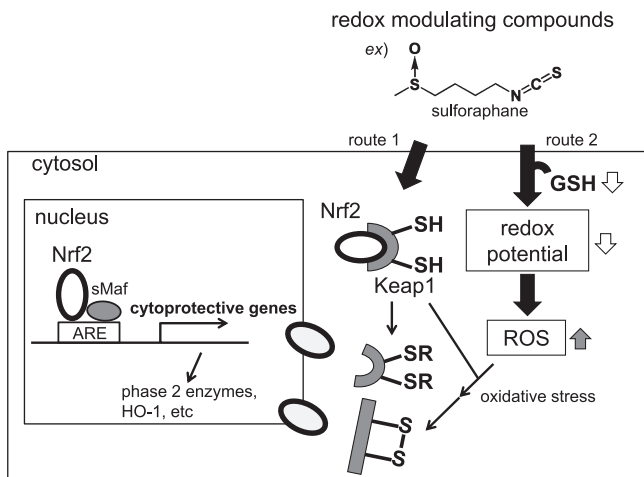


Fig. 4. Reliable scheme for isothiocyanates (redox inducers) activates the Keap1/Nrf2/ARE pathway. When oxidoreductive compounds such as isothiocyanates in Brassicaceae vegetables are absorbed into the cell, the highly reactive cysteine residues of sensor protein Keap1 are modified. Conformational changes of Keap1 repress Nrf2, which subsequently undergoes nuclear translocation. In heterodimeric combination with small Maf transcription factors, it binds to ARE and recruits the basal transcriptional machinery to activate transcription of cytoprotective genes.

factor 2 (Nrf2) and Kelch-like ECH-associated protein 1 (Keap1). Keap1 is an inhibitor of Nrf2, which is a transcriptional activator of ARE. If inducers such as sulforaphane disrupt the Keap1-Nrf2 complex by reacting with thiol residues in Keap1 (route 1 in Fig. 4), or by reacting with glutathione (GSH), which cause the formation of reactive oxygen species (ROS) in the cytosol of cells (route 2 in Fig. 4), then Nrf2 migrates to the nucleus where it forms heterodimers with other transcription factors such as small Maf binding to ARE, and accelerates the transcriptional activity. Many studies have clarified phase 2 induction mechanisms by redox-modulating compounds such as isothiocyanates (Dinkova *et al.* 2002, Watai *et al.* 2007).

Necessary structures of isothiocyanates for potent phase 2 inducers and other functions

The chemical moiety of isothiocyanate ($-N=C=S$) is a reactive function with electrophiles such as glutathione and other sulfhydryl-containing molecules (RSH) in the cells. Therefore, the isothiocyanate moiety has been proved to be necessary for the induction of phase 2 enzymes (Morimitsu *et al.* 2002). Additionally, a potent antimicrobial effect must exist for the isothiocyanate moiety among related compounds. Regarding the kinds of alk(en)yl side-chains of isothiocyanates, ω -methylthioalk(en)yl or ω -methylsulfinylalk(en)yl moieties were preferred rather than normal alk(en)yl ones. The length of the alk(en)yl side chain is affected by the hydrophobicities of isothiocyanates. Fewer than 10 carbons (10C) would be better (Morimitsu *et al.* 2002). Considering sulforaphane in broccoli, it has the ω -methylsulfinylbutyl moiety and four-carbon (4C) chain

length between both ends. Based on these results, the chemical structures of isothiocyanates are extremely important for their functionality. Therefore, the accumulation of beneficial glucosinolates such as glucoraphanin (the precursor glucosinolate of sulforaphane) in edible parts is expected to be a good strategy for improving Brassicaceae vegetables using breeding techniques as described hereinafter.

3. Breeding emphasizing glucosinolates

Glucosinolate analysis

To date, numerous methods have been developed for the optimized extraction, and analysis of individual and total glucosinolates according to the need for quantitative or qualitative information, analytical speed, analytical accuracy, or combinations of these factors. Depending on the purpose, determining a suitable pretreatment method and analytical method is important. Detailed information for these analyses was presented in earlier reviews (Clarke 2010, Fahey *et al.* 2001, Griffiths *et al.* 1998, Kiddle *et al.* 2001, Mithen *et al.* 2000).

Prevention of myrosinase activity is necessary during the glucosinolate extraction procedure in vegetative tissue. For this reason, several extraction methods have been used specifically to prevent activation of myrosinase. Generally, extractions are conducted at temperatures of 65–100°C, close to the water solvent or 70% aqueous methanol boiling point. The most widely used extraction method is that of The International Organization for Standardization (1992), which uses a sample that has been powdered after freeze-drying. Myrosinase can also be deactivated in wet tissues by a microwaving treatment. In radish, a method has also been used by which a sample is cut into 1–3 cm fragments from leaf, stem, root, etc., chilled below 5°C and extracted with 70–80% hot methanol (Carlson *et al.* 1985, Ishii *et al.* 1989). However, these processes were restricted to extraction of a few samples because of time-consuming sample preparation. Moreover, hot methanol vapor is poisonous and flammable, presenting dangers of high-temperature conditions. Ishida *et al.* (2011) previously reported a simple method for the extraction of glucosinolates from lyophilized radish roots using methanol at room temperature without hot methanol or hot water. This method provides rapid extraction of glucosinolates from radish roots. It is expected that this is applied to other Brassicaceae vegetables.

A well-known analytical method used for glucosinolates is desulfonation of glucosinolates with sulfatase with subsequent analysis using a reversed phase high-performance liquid chromatography (HPLC) gradient system (Bjerg and Sørensen 1987, Bjorkqvist and Hase 1988). An ion-pair method has also been developed, by which extracted glucosinolates are analyzed directly using HPLC with a reverse-phase column using an ion-pair reagent without desulfonation (Mellon *et al.* 2002, Rangkadilok *et al.* 2002). These methods provide reliable quantitative data and information related to glucosinolate variation, but they require much time

and labor for analyses and for special equipment. Therefore, a simple method for quantitative analysis of glucosinolates is necessary for the fields of plant breeding and food processing. For the Japanese common or pungent radish, extremely high percentages of dehydroerucin were confirmed in the roots. For this reason, Ishida *et al.* (2012a), by modification of the method of Thies (1982), developed a palladium colorimetric method that can be used simply and rapidly for the estimation of total glucosinolate contents. The colorimetric method does not provide quantitative or qualitative information related to individual glucosinolates, but it is extremely useful for analyzing the total glucosinolate contents of Japanese radish cultivars and breeding lines developed from a cross between Japanese radish cultivars.

Glucosinolate profiles in Brassicaceae vegetables

Glucosinolate contents of Brassicaceae are influenced by environmental factors such as soil, climate and cultivation conditions including fertilization, harvest time, and plant position. However, wide genetic variations in the contents and composition of glucosinolates have been reported from previous studies (Carlson *et al.* 1987, Ishida *et al.* 2012a, Padilla *et al.* 2007, Rosa *et al.* 1997, Verkerk *et al.* 2009, Yang and Quiros 2010). In general, there is greater diversity exists in both the amount and profile of glucosinolate in *B. oleracea* as opposed to *B. rapa*. In *R. sativus*, the genetic diversity of the glucosinolate profile is extremely narrow. Similarly, each vegetable species contains many major and minor glucosinolates (Fahey *et al.* 2001). The Brassicaceae

vegetable tissues include one or more major aliphatic glucosinolates. Table 2 shows aliphatic glucosinolate profiles of main Brassicaceae vegetables. Generally, Brassicaceae vegetables include an alkyl side chain with 3–5 carbons. Glucoiberin is contained in cabbage, broccoli, and cauliflower in *B. oleracea*. Sinigrin is produced in *B. oleracea* vegetables, mustard green (*B. juncea*). Glucoerucin is included mainly in garden rocket (*Eruca sativa*). Glucoraphanin, a functional component, is found in *B. oleracea* such as broccoli, cauliflower, and kohlrabi. Gluconapin and progoitrin are included in many *Brassica* vegetables such as *B. rapa* (Chinese cabbage, mustard spinach, mizuna, and turnip), *B. oleracea* (cabbage, broccoli and cauliflower), *B. juncea* (mustard green), and *B. napus* (rapeseed vegetable). Dehydroerucin, which is specific to radish (*R. sativus*), is the dominant aliphatic glucosinolate, accounting for over 80% of the all glucosinolates. Glucobrassicinapin is the main glucosinolate constituent of *B. rapa* vegetables.

In *Brassica* species, diversity based on glucosinolates composition is related to A, B and C genomes. With regard to the three ancestral *Brassica* species with diploid genome chromosomes: *B. nigra* (BB, $2n = 16$) contains glucosinolates with three carbon (C) side chains, derived from a single elongation reaction: *B. oleracea* (CC, $2n = 18$) contains glucosinolates with either 3C or 4C side chains; and *B. rapa* (AA, $2n = 20$) contains glucosinolates with either 4C or 5C side chains. Three amphidiploid *Brassica* species, *B. juncea* (AABB, $2n = 36$), *B. napus* (AACC, $2n = 38$), and *B. carinata* (BBCC, $2n = 34$), possess glucosinolate

Table 2. Distribution of aliphatic glucosinolates among main Brassicaceae vegetables (Cartea and Velasco 2008)

Botanical classification	3 carbon side chains			4 carbon side chains						5 carbon side chains		
	Glucoiberin	Glucoiberin	Sinigrin	Glucoerucin	Dehydroerucin	Gluco-raphanin	Gluco-raphenin	Gluconapin	Progoitrin	Gluco-alyssin	Glucobrassicinapin	Gluconapoleiferin
<i>Brassica rapa</i>												
Chinese cabbage		+						*	+		*	+
bok choy ^a								*	*		+	+
turnip		+		+				*	*		*	+
turnip greens	+	+		+		+		*	+	+	*	+
<i>Brassica nigra</i> ^b												
<i>Brassica oleracea</i>												
white cabbage	+	*	*	+		+		+	+	+	+	
red cabbage	+	*	*			+		+	+			
broccoli		+	+	+		*		*	*	+	+	+
cauliflower	+	*	*			+			*			
kale	+	*	*			+		+	+			
Brussels sprouts	+	+	*			+		+	*			
kohlrabi	+	+	*			+		+	+	+		
<i>Brassica juncea</i> ^b												
<i>Brassica napus</i>												
rapeseed						+			+		+	+
swede	+							+	+	+	*	+
<i>Brassica carinata</i> ^b												
<i>Raphanus sativus</i>												
radish ^c					*		+					
<i>Eruca sativa</i>												
garden rocket ^d				*		*						

Major aliphatic glucosinolates found in each crop are shown in * symbol.

Data sources: ^a Wiesner *et al.* (2013), ^b Kirkegaard and Sarwar (1998), ^c Ishida *et al.* (2012a), ^d Kim and Ishii (2007).

composition that consists of the profiles of two elementary species. As with elongation, modifications of side chains in *Brassica* are also more limited than those found throughout the entire family.

Genetic system controlling glucosinolate biosynthesis in Brassicaceae vegetables

Glucosinolate contents have quantitative inheritance, which is regulated by complex genetic factors and which is affected by environmental factors (Hirani *et al.* 2012). As described in the “Glucosinolate biosynthesis” section above, most genes involved in the glucosinolate biosynthetic pathway have been identified in *Arabidopsis*. In Brassicaceae vegetables, mainly using syntenic information with the model plant *Arabidopsis*, attempts to study genetic system controlling glucosinolate biosynthesis have been made.

In *B. oleracea*, a gene for *BoGSL-ELONG* and *BoGSL-PRO* has been cloned based on *Arabidopsis* sequence infor-

mation (Fig. 5) (Gao *et al.* 2005, Li and Quiros 2002, Li *et al.* 2003). In addition, *BoGSL-ALK* was cloned using a positional cloning approach in *B. oleracea* (Li and Quiros 2003). *BoGSL-ELONG*, a side chain elongation gene leading to 4C glucosinolates, and *BoGSL-PRO* control propyl glucosinolate biosynthesis of 3C. These two genes segregate independently of each other. *BoGSL-ALK* gene is involved in the desaturation of glucosinolate side chain. Moreover, the presence of genes for *BoGSL-OXID* loci and *BoGSL-OH* loci has been inferred from the inspection of glucosinolate profiles (Giamoustaris and Mithen 1996, Mithen *et al.* 1995). These genes are involved in the oxidation of glucosinolate side chain and hydroxylation. Genes for *BoGSL-ELONG*, *BoGSL-PRO*, and *BoGSL-ALK* were mapped on a high-density *B. oleracea* linkage map together with sequences of *BoGSL-OH*. Moreover, a linkage between *BoGSL-ALK*, and *BoGSL-OH* were reported (Gao *et al.* 2007). Comparative genomic analysis revealed potential

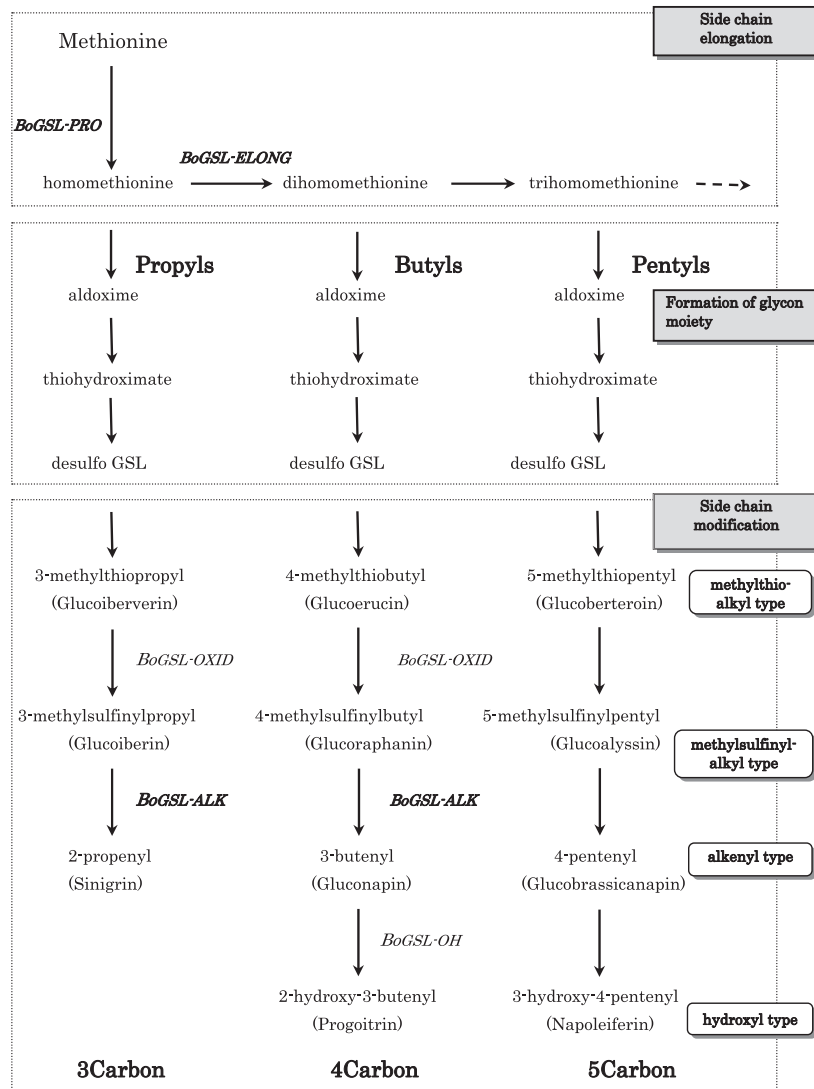


Fig. 5. Glucosinolate biosynthesis pathway for 3C, 4C and 5C aliphatic glucosinolates of *Brassica*. Genes set in bold have been cloned. Others have been inferred from inspection of glucosinolate profiles in *B. oleracea*.

candidate genes within the regions of the quantitative trait locus (QTL) affecting glucosinolate biosynthesis (Issa 2010). Furthermore, in high-glucoraphanin broccoli, which was developed via marker-assisted selection through interspecific cross with a wild species of *B. oleracea*, *B. villosa* (Mithen *et al.* 2003), enhanced glucoraphanin content is regarded as attributable to a *B. villosa* *Myb28* allele. *Myb28* is a transcription factor gene. Its increased expression was confirmed in high-glucoraphanin broccoli (Traka *et al.* 2013).

In *B. rapa*, QTLs for leaf and seed glucosinolates were identified. The genes involved in the glucosinolate biosynthesis pathway that might account for the QTL were inferred from comparative genomic analyses based on synteny with *Arabidopsis* and mapping of candidate orthologous genes in *B. rapa* (Hirani 2011, Lou *et al.* 2008). Chinese cabbage lines with altered glucosinolate profiles were achieved through a cross between *B. rapa* and *B. oleracea* using marker-assisted selection (Hirani 2011). Recently, glucosinolate biosynthetic genes were identified from cDNA/BAC libraries and whole genome sequence of *B. rapa*. High collinearity in the glucosinolate biosynthetic pathway between *A. thaliana* and *B. rapa* has been established using comparative genomic analysis (Wang *et al.* 2011, Zang *et al.* 2009). Based on the DNA sequences from *Arabidopsis* and *B. oleracea* for genes involved in glucosinolate biosynthesis, candidate genes were co-mapped with glucosinolate QTLs reported by Ramchiary *et al.* (2007). The functionality and contribution of candidate genes/QTLs were assessed.

For amphidiploid crop *B. napus*, several QTL mapping studies of seed glucosinolate content (Quijada *et al.* 2006, Toroser *et al.* 1995, Uzunova *et al.* 1995, Zhao and Meng 2003), and gene-linked SSR markers have been used to ascertain the relation of functionally characterized *Arabidopsis* genes to seed glucosinolate content of *B. napus* (Hasan *et al.* 2008). Recently, a glucosinolate metabolic network was constructed. Functions of genes that underlie QTLs in it were inferred (Feng *et al.* 2012). In another amphidiploid crop *B. juncea*, QTLs (Lionneton *et al.* 2004, Mahmood *et al.* 2003) and SCAR markers (Ripley and Roslinsky 2005) have been reported for seed glucosinolate contents.

For a crop other than *Brassica*, *R. sativus*, QTL analyses using high-density mapping and identification of candidate genes controlling glucosinolate content in roots have been reported (Zou *et al.* 2013). Five QTLs were detected in two F₂ populations, with three of them accounting for more than 50% of the total phenotypic variance being detected repeatedly. By synteny analysis of the QTL regions with *A. thaliana* and *B. rapa* genome sequences, three candidate genes were indicated. Their possible function in dehydroerucin biosynthesis in radish roots was suggested by nucleotide sequences and expression of these genes.

Present and future of glucosinolate breeding

The main breeding objectives for Brassicaceae vegetables are the enhancement of beneficial glucosinolates such

as glucoraphanin for anticarcinogenic compounds in broccoli or dehydroerucin for pungent condiments in radish, and the reduction of precursor glucosinolate of antinutritional compounds, such as progoitrin in *B. oleracea*, which includes cabbage.

In *B. oleracea*, a gene has been cloned for *BoGSL-ELONG*, *BoGSL-PRO* and *BoGSL-ALK*, which control the metabolism of aliphatic glucosinolate. The inheritance of these genes is simple. Li and Quiros (2002) were elucidating the phenotype *BoGSL-ELONG/BoGSL-PRO/BoGSL-ALK* of broccoli, cauliflower and purple cauliflower, and provided a historic scenario for the origin of purple cauliflower. Elucidation of the inheritance of major genes controlling glucosinolate biosynthesis is necessary not only to clarify the genetic relation, but also to develop a new cultivar with specific glucosinolate profiles. Even among them, DNA marker for both Mendelian genes and major QTLs would be useful for glucosinolate breeding programs.

The best known example is, as described above, the development of super broccoli. Much more than standard broccoli cultivars, it contains higher levels of methylsulphinylalkyl glucosinolate (glucoiberin and glucoraphanin), the precursors of the functionality isothiocyanates iberin and sulforaphane. The wild forms of *B. oleracea*, *B. villosa*, which accumulate high levels of glucoiberin in flower buds, were used as a parent in this program. The hybrid lines, which had a high level of methylsulphinylalkyl glucosinolate, were selected using DNA markers linked to *BoGSL-ELONG* gene. These high glucosinolate broccolis had shown higher functionality than that shown by standard cultivars (Gasper *et al.* 2005). These might be suitable for increasing the amount of functional isothiocyanates in human food.

In radish, which is an important vegetable in Japan, a novel mutant that lacked dehydroerucin was identified (Ishida *et al.* 2012b). This mutant plant was discovered from a Japanese local variety through an intensive evaluation of glucosinolate profiles in many genetic resources using HPLC analyses. From the null mutants of dehydroerucin, a new cultivar, 'Daikon Chukanbohon Nou 5' ('Daikon parental line 5'), was bred in 2012. This cultivar is characterized by its abundance of glucoerucin compared with common cultivars. This characteristic is stable throughout the growing period. When used with this cultivar for making Japanese pickled radish (*takuan*), no yellow color and sulfurous odor is generated. Using this cultivar as a breeding material is expected to engender the development of new cultivars providing excellent agronomic performance for the production of novel processed foods.

The most commonly consumed vegetable in eastern Asia is *B. rapa*. Its glucosinolate profiles differ from those of *B. oleracea*. However, the breeding for altering glucosinolate profiles does not progress because its study has been delayed compared with that of *B. oleracea*. In the near future, development of molecular markers using sequenced genome information will promote marker-assisted selection

of glucosinolates breeding to increase beneficial glucosinolates such as glucoraphanin in Brassicaceae vegetables including *B. rapa*.

Certain glucosinolates such as sinigrin and progoitrin, and their respective breakdown products are often bitter or astringent (Drewnowski and Gomez-Carneros 2000). Humans often reject foods that taste excessively bitter. This instinctive rejection is believed to have been important for human safety because it can protect people from consuming some potential toxins. The removal of specific glucosinolates and their breakdown products is thought to reduce bitterness and to increase consumer acceptance. Consumer reports have described that taste, rather than recognized nutrition or health value, is the key to food selection (Schonhof *et al.* 2004). For this reason, expectations of consumer willingness to compromise on the taste of glucosinolate-enriched vegetables for health value are risky. Moreover, those efforts might only appeal to a niche market (Williams and Pun 2011). Improvement of glucosinolate compounds for human health or processing fitness requires not only the pursuit of breeding efficiency by marker-assisted selection or new analytical methods but also careful consideration of the taste of Brassicaceae vegetables.

Acknowledgments

This work was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (Genomics-based Technology for Agricultural Improvement, HOR-1006).

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