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Glucosinolate in Japanese Radish, Raphanus sativus L.

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Abstract
Daikon (Raphanus sativus L.), which is one of the cruciferous crops, has the largest production and consumption among the vegetables grown in Japan. Daikon quality such as flavor and color depends mainly on the contents of 4-methylthio-3-butenyl glucosinolate (MTB-GSL) and its breakdown products in both fresh and processed foods. The pungent flavor of daikon root is enzymatically converted from MTB-GSL to the corresponding volatile isothiocyanate (MTB-ITC) by myrosinase. MTB-ITC is known to possess antifungal and antibacterial activities against a range of organisms. The degradation product of MTB-ITC in water is likely to be the precursor of yellow pigment in daikon pickles. A quantitative method for determining MTB-GSL was developed by using a gas liquid chromatography after enzymatic hydrolysis of glucosinolates to elucidate the effect of environmental and genetic factors on it. Regarding the distribution of MTB-GSL content in a root, the phloem of tip was the highest with a gradual decline toward the stem and xylem. The change of MTB-GSL content after sowing was low at the early stage and rapidly reached a maximal peak followed by a gradual decline. There were great varietal differences in MTB-GSL contents among the 20 daikon cultivars tested with a coefficient of variation of 39%, and the contents in the 13 cultivars in two years were positively correlated. Application of sulfate to a nutrient solution caused a high accumulation of total glucosinolate in daikon roots.

Discipline: Post harvest
Additional key words: daikon, evaluation of quality, 4-methylthio-3-butenyl glucosinolate (MTB-GSL), myrosinase, pungent principle, sulfate ion

Introduction

Japanese radish, or daikon in Japanese, is one of the most popular and important cruciferous crops in Japan, where its large amount has been produced and consumed as a vegetable. Various types of cooking and pickling have traditionally been developed, including eating it raw, boiled and salted. A fresh 100 g root contains, on an average, 95 g of water, 15 mg of vitamin C, 3 g of glucose, 1 g of dietary fiber and 240 mg of potassium with a β-amylase activity. It is well known that glucosinolates (Fig. 1) and their breakdown products of cruciferous crops contribute to human foods in providing special flavor and acceptability of those foodstuffs[3]. The pungent flavor of Japanese radish root depends on 4-methylthio-3-butenyl isothiocyanate (MTB–ITC)[2,4]. MTB–ITC, being enzymatically converted from its corresponding glucosinolate, plays a great role in inducing flavor and color of cooked or processed daikon, as shown in Fig. 2.

In recent years, high quality of agricultural products, including daikon, has been strongly required in Japanese markets. It is therefore necessary in research work to identify the components relevant to daikon quality for its improvement. Daikon quality as a raw food consists of various components, such as appearance, taste and nutritional

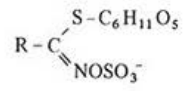

Fig. 1. General structure of glucosinolate

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Table 1. Change of 4-methylthio-3-butenyl glucosinolate in roots of Japanese radish

Cultivar (Genetic group)	Days after seeding*	Root weight (gFW)	MTB-GLS (μmol/ 100 gFW)
Heiantokinashi (Ninengo· tokinashi)	30	2.7±1.3	96
	37	29.8±5.2	257
	44	89.0±17	387
	51	320±29	219
Taibyosofutori (Miyashige)	30	3.0±1.1	196
	37	33.5±13.1	532
	44	86.0±37	449
	51	384±89	416
Tenshun (Minowase)	30	6.1±2.1	179
	37	42.5±3.6	315
	44	229±53	292
	51	670±90	267
Natsuminowase No. 3 (Minowase)	30	3.1±1.2	254
	37	30.2±10.3	549
	44	130±32	491
	51	493±139	189

* Seeded on 28 April 1986.

value. Among the various constituents in daikon root, contents of the MTB-glucosinolate (MTB-GSL), which is the precursor of MTB-ITC, have predominant influence on the quality of both fresh and processed daikon. The present paper reviews results of the study on some factors affecting MTB-GSL contents, such as cultivars, growing stages and fertilizer applications.

Materials and method

1) A quantitative determination of MTB-GSL in daikon roots by a gas chromatography (GC)

The method employed consisted of extraction of glucosinolate with ethanol, purification with anion exchange resin, and hydrolysis with crude myrosinase prepared from daikon roots. The MBT-ITC produced was dissolved into methylene chloride and measured by GC. The coefficients of variation of 5 samples from each daikon root was approximately 4% throughout the measurements taken by this method.

(1) Extraction of GSL from daikon roots and preparation of ITC solution

Fresh roots of daikon were cut into ca. 1 cm^3 pieces, fully blended and then an amount of 50 g was quickly put into 100 m*l* of hot 80% ethanol

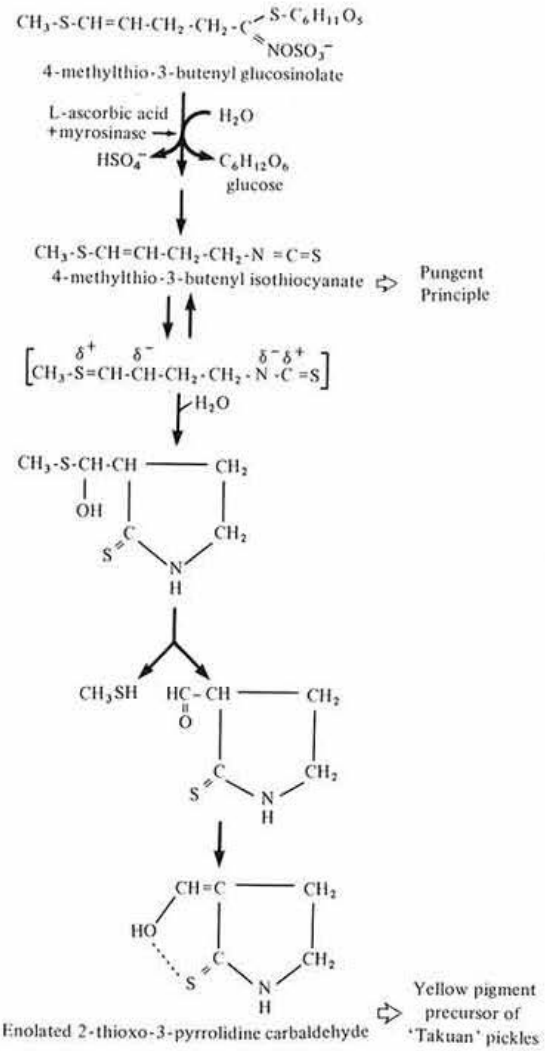

Fig. 2. Proposed pathway for the formation of flavor and color from MTB-GSL in daikon root[1,2,11,12]

in a flask. The contents of the flask were boiled for 15 min with a cooling circulator, cooled and homogenized with a blender. The suspension was filtrated through a glass filter. The same procedure for extraction and filtration was repeated again for the residue. The filtrates were combined and concentrated to ca. 25 m*l* *in vacuo* under 40°C. After centrifugation, the supernatant was filled up to 50 m*l* with distilled water.

An aliquot 20 m*l* of the extracted solution was passed through anion exchange column (5 m*l* of Dowex 1-X2, Cl^- form). The ion exchange column

was washed with ca. 100 m*l* of distilled water until no glucose reaction was detected. The ion exchange resin with glucosinolates was transferred into the enzyme reaction mixture, which contained 5 m*l* of 0.1 M sodium phosphate buffer (pH 7.0), 1 m*l* of 10 mM L-ascorbic acid, 50 mg of crude myrosinase and 5 m*l* of methylene chloride in a 50 m*l* Erlenmeyer flask with a silicone stopper. The enzymatic hydrolysis of glucosinolates took place while the flask was shaked for 18 hr by a reciproshaker. After centrifugation, the methylene chloride layer was transferred into a 5 m*l* glass tube, and then dehydrated with anhydrous sodium sulfate.

About 5 μl was taken for analysis of MTB-ITC by GC. The water layer contained glucose which was released from glucosinolates. Total glucosinolate content was calculated from the glucose concentration in the water layer after enzymatic hydrolysis of glucosinolates.

(2) Preparation of crude myrosinase

About 1 kg of a fresh and intact root (chilled under ca. 5°C) of daikon was homogenized, and squeezed with two layers of cotton gauze. Cold acetone was then added to the filtrate (1.5 : 1, v/v). The combined filtrates were centrifuged, and the precipitate was lyophilized *in vacuo*. All operations were conducted under a low temperature of below 5°C. The dried powder was stored before use in a sealed vial under below −20°C.

(3) Preparation of authentic MTB-ITC

Two kg of daikon roots were used for extraction of MTB-ITC by the same method as above. After enzymatic conversion of glucosinolate to the corresponding isothiocyanate, the layers of methylene chloride solution containing volatile isothiocyanate were collected and combined. Methylene chloride was evaporated *in vacuo*, and the remaining liquid was analyzed by GC-MS, IR and ^{1}H-NMR. The spectra obtained showed a good agreement with those of MTB-ITC reported by Friis and Kjaer[4]. The purity of MTB-ITC was of over 99%.

2) Distribution of contents of MTB-GSL in a root and their change with growth stage after sowing[8]

(1) Distribution of MTB-GSL in a root

A daikon root (ca. 850 g) was divided into four portions with an equal length each, between the tip (diameter 1 cm) and the stem. Each of them was then separated into xylem and phloem parts.

After removal of cortex and phloem, the xylem of hypocotyl in a root was divided into inner and outer zones with a cork borer (diameter 13 mm) in parallel with vessel. The samples taken from those two zones were subjected to measurements of MTB-GSL by GC.

(2) Change of MTB-GSL in roots after sowing

Five or ten roots of four cultivars each were sampled intermittently four times each during the growing stage in an open field of NIVOT at the end of April in 1988. Each root was washed and divided vertically into 2, 4 or 8 equal-sized sections according to their growing stage. The materials gathered from the five or ten roots, or sections, were used as a sample for chemical analysis in each stage.

3) Varietal difference of MTB-GSL contents in roots[9]

In order to identify a varietal difference in contents of total glucosinolate and MTB-GSL, 20 cultivars seeded in late summer in an open field were subjected to analyses. Two roots with a medium size each sampled from 20 to 40 roots in each cultivar were chosen. Each root was divided vertically into 8 or 16 equal-sized sections and the materials gathered from the two roots, or sections, were used

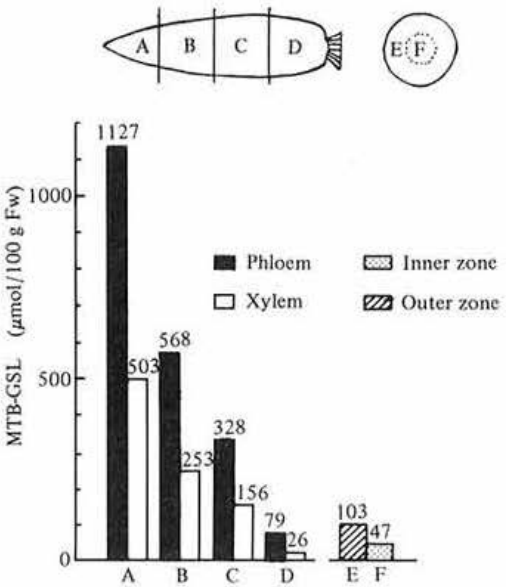

Fig. 3. Distribution of MTB-GSL content in a root and a hypocotyl

Table 2. Contents of glucosinolate in 20 cultivars of daikon roots

Cultivar (Genetic group)	Days after sowing	Root weight (gFW)	Total GSL[a] (μmol/100 gFW)	MTB-GSL[b] (μmol/100 gFW)
Wakakoma	83	1167±65	334	321
(Ninengo·tokinashi)	85	1116±86	369	369
Tenman	64	447±34	320	316
(Shiroagari)	65	591±21	345	340
Kurodaikon A	80	608±13	311	294
(European)	75	619±27	339	303
Shougoindaimaru	68	1004±12	311	286
(Shougoin)				
Houshunsangatsu	90	1212±115	265	240
(Ninengo·tokinashi)	93	1008±113	366	321
Wakayama	64	656±5	275	251
(Shiroagari)	65	750±86	287	286
Nerimashirimaru	76	929±2	247	217
(Nerima)	85	1014±84	303	295
Miura	90	1611±224	263	237
(Nerima)	93	1736±287	324	251
Houryou	65	672±11	259	244
(Houryou)				
Harumakiminowase	65	898±36	259	230
(Minowase)				
Heiantokinashi	83	795±191	234	166
(Ninengo·tokinashi)	75	878±133	309	259
Shigatsuwase	67	800±25	228	209
(Minowase)	65	1155±16	208	177
Eberesuto	59	799±54	227	166
(South China)	59	752±26	210	169
Taibyosofutori	66	1086±3	232	165
(Miyashige)				
Tenshun	67	1048±33	199	156
(Minowase)	59	1060±74	211	159
Natsuminowase No. 3	67	934±2	151	136
(Minowase)				
Futomiya	68	1154±55	192	123
(Miyashige)				
Koutoaonaga	59	382±55	264	121
(North China)	59	394±9	296	113
Sakurajimadaimaru	94	2052±20	196	98
(Didaikon)				
Ten-ankoushin	73	701±45	222	41
(North China)	71	703±69	190	42
Mean ± SD of each mean for 2 years of 20 cultivars			259±55	210±82

a): Total glucosinolate content was calculated by measuring glucose released after enzymatic hydrolysis of glucosinolates.

b): Content of 4-methylthio-3-butenyl glucosinolate was determined as that of its corresponding isothiocyanate.

for the chemical analyses. The test was repeated in two years.

4) Effects of sulfate and nitrogen applications on GSL accumulation in roots[7]

Relationships between fertilizer applications and glucosinolate contents were investigated with a nutrient-solution culture method in a greenhouse. Two different concentrations of sulfate and nitrogen solution each was applied intermittently to daikon planted in a 1/2,000 a pot packed with vermiculite. At the harvest time, two daikon roots under each

Table 3. Effects of fertilized sulfate on accumulation of total glucosinolate contents in roots of Japanese radish

Cultivar	Sulfate conc. (mM)	Root weight (gFW)	Leaf weight (gFW)	Total GSL (μmol/100 gFW)
Shougoindaimaru	0.5	702±93	224±8	173±6
	2.0	663±13	225±13	353±21
Natsuminowase No. 3	0.5	900±56	192±2	158±4
	2.0	737±70	198±26	409±44

The same amount of nutrient solution was applied except for sulfate.

Table 4. Effects of fertilized nitrogen on accumulation of total glucosinolate contents in roots of Japanese radish

Nitrogen conc. (mM)	Root weight (gFW)	Leaf weight (gFW)	Total GSL (μmol/100 gFW)
5	435±4	160±19	461±13
10	507±81	223±27	293±41

Cultivar: Natsuminowase No. 3
The same amount of nutrient solution was applied except for nitrogen.

treatment were subjected to measurement of total glucosinolate contents after the same preparation as described above.

Results

1) Distribution of MTB-GSL contents in a root

More MTB-GSL contents were observed in the tip (A) than in the upper part (B, C, D), and in the phloem than in the xylem as shown in Fig. 3. The greatest amount of MTB-GSL, i.e. 1,127 μmol/100 g of fresh weight, was recognized in the phloem of the tip. In the xylem of hypocotyl, MTB-GSL contents were two times as much in the outer zone (E) near cambium than in the inner zone (F).

2) Change of MTB-GSL in roots after seeding

Four cultivars (Heiantokinashi, Taibyosofutori, Tenshun and Natsuminowase No. 3) were subjected to analyses once a week between 30 and 51 days after seeding. MTB-GSL contents were relatively low in 30 days after sowing: their maximum came out on the 37th day, followed by a gradual decline as shown in Table 1. Their maximum levels reached 300 to 500 μmol/100 g of fresh weight.

3) Varietal difference of GSL contents in daikon roots

Twenty cultivars seeded in late summer in an open field were analyzed at harvest. The test was repeated in two years. Table 2 shows the results obtained in the order of high contents of MBT-GSL on the basis of means for two years. The range and mean of MTB-GSL contents was 42 to 345 μmol/100 g (an average of the two-year measurements) and 210 μmol/100 g of fresh weight, respectively. A coefficient of variation of the estimated values was 39%. Among the cultivars examined, the highest cultivar in total glucosinolate contents contained 2.3 times higher as much than the lowest. The mean content of MTB-GSL corresponded to about 80% of that of total glucosinolate. The MTB-GSL contents of 13 cultivars were positively correlated between the two years ($r=0.9$).

4) Effects of sulfate and nitrogen applications on GSL accumulation in roots

Cultivars of Shougoindaimaru and Natsuminowase No. 3 received two levels of sulfate application to each, i.e. 0.5 mM and 2.0 mM per pot, as a fertilizer. Their roots supplied with more sulfate contained greater glucosinolate contents than those with less

sulfate (Table 3). Natsuminowase No. 3 fertilized with 5 mM nitrogen per pot were nearly two times more in total glucosinolate contents but less in weight of root and top, as compared with the same cultivar supplied with 10 mM nitrogen per pot (Table 4).

Discussion

In order to improve the quality of Japanese radish, it is required in a research program to establish a methodology for determining chemical components of a root and to identify critical factors affecting their contents. This procedure could contribute to evaluating daikon quality. In cruciferous crops, isothiocyanates are usual products of the myrosinase-induced breakdown of glucosinolates and they are key compounds for grading a cooked radish[1]. This characteristic odor and taste of radish is given by the formation of MTB-ITC derived from a glucosinolate, comprising more than 80% of the total glucosinolate, which appears to be confined to *Raphanus* species. A remaining portion of the total glucosinolate is mainly 3-indolyl methyl-glucosinolate. MTB-ITC of the grated daikon roots is chemically unstable in water[2,10].

Some results of the analyses of glucosinolates by HPLC, GC-MS, FAB-MS and FD-MS[6] are available as a reference. However, detailed conditions for the measurements are not specified in their paper. In the present study, a method for quantitative measurements of MTB glucosinolate in daikon roots has been formulated. It was recognized that this method could be applicable in identifying the fluctuations of MTB-GSL contents of roots in various growing stages and different parts. The results obtained suggest that higher MTB-GSL contents be seen in the physiologically active stage and active part as well. For this reason, it seems to be concluded that the following biosynthesis pathway of glucosinolate operates: α-amino acids → N-hydroxyamino acid → aldoxime → [*aci* tautomer of primary nitro compound + cysteine?] → thiohydroximic acid → desulfoglucosinolate → glucosinolate[3], though further details are to be identified yet. Since amino acid metabolism is supposed to proceed more actively at younger stage or in the phloem and tip of a root, more glucosinolates would be biosynthesized or accumulated there.

In addition, sulfate ion is incorporated into the basic structure of glucosinolate via the initial and final steps of biosynthesis pathway. Total glucosinolate and isothiocyanate contents were increased with the application of a greater amount of sulfate. Also lack of sulfur in the nutrient solution caused a symptom of sulfur defeciency in leaves as well as a considerable decrease in plant weight[5]. These results obtained suggest that the sulfur nutrient of plants give a primary influence on the contents of glucosinolate, and the nitrogen nutrient follow.

The differences among 13 cultivars in their contents of MTB-GSL in two years were highly correlated. This suggests that a relative rank of varieties in their contents of MTB-GSL do not greatly vary from year to year. However, those contents in the cultivar Heiantokinashi fluctuated by 1.6 times between the two years. Such discrepancy might have been caused by the varietal difference in response to sulfate ion. Further studies are required to identify the physiological roles of glucosinolate in plant growth of daikon and processing after harvest, including storage and cooking.

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