アブラナ科作物の種子に含まれるOxazolidinethione含量の種･品種間差異

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著者	生井, 兵治 梶, 敏勝 細田, 友雄
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INTERSPECIFIC AND INTERVARIETAL VARIATIONS IN CONTENT OF OXAZOLIDINETHIONE IN SEED MEALS OF CRUCIFEROUS CROPS[1)]

HYOJI NAMAI, TOSHIKATSU KAJI[2)] AND TOMOWO HOSODA

Faculty of Agriculture, Tokyo Kyoiku University
Tokyo 153

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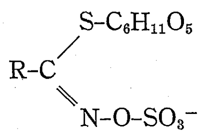
It is known that *Brassica* fodder crops give high protein yields of a reasonably good quality (Schuphan 1958) and solvent-extracted seed meals of rape and turnip rape contain large amounts of protein (40 to 45%) having nutritionally well balanced favorable amino acid composition (Miller *et al.* 1962). However, Cruciferous plants contain mustard oil glucosides which are found especially in the seeds but also in the other parts of the plant. These glucosides are hydrolyzed by an enzyme, called myrosinase, normally present in crushed or ground moist seed (Kjaer 1960). When coming in contact with the emzyme the glucosides yield glucose, sulphate, and an isothiocyanate, or some other products that are formed by reactions of an isothiocyanate. The general structural formula of the thioglucosides was established by Ettlinger and Lundeen (1956) as follow;



To date twelve different thioglucosides are known to occur in *Brassica* and various kinds of hydrolyzate are liberated from their parent thioglucosides. Some of these products are reported to give toxic effects. The known toxic products from the thioglucosides are isothiocyanates, oxazolidinethiones, or thiocyanate ion, which are the goitrogenic substances and are supposed to interfere with proper function of the thyroid (Astwood *et al.* 1949; Virtanen 1962). However, Wetter and Craig (1959) did not recognize the anti-thyroid effect of the isothiocyanates.

Rape seed meals have not been used as feed of cattle and poultry from old time but also it has been known that too heavy feeding with rape, kale or cabbage causes disturbances in the health of cattle. For this reason it is of interest to examine the variation in content of the goitrogenic substances in Cruciferous crops and to examine the possibility of reducing the thioglucoside content by genetic and breeding methods.

There are some observations on the variation in content of thioglucosides in some Cruciferous crops, however, few papers concerning the variation in its content in

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2) Present address: Shukutoku Gakuen Junior College.

Japanese crops. Hence, the authors observed many cultivars of Japanese Cruciferous crops. This paper reports oxazolidinethione measurements after hydrolysis of seed meals from various cultivars in Japanese Cruciferous crops.

MATERIALS AND METHODS

The materials used in this study are composed of six species, *Brassica napus*, *B. campestris*, *B. oleracea*, *B. nigra*, *B. juncea* and *Raphanus sativus* (Table 1). Most of the seed materials except *B. napus* were obtained from Takii Seed Co., Ltd. or Yamato Seed Co., Ltd. and *B. napus* seeds were sampled from our strains which had been maintained at Soshigaya Experimental Farm, Faculty of Agriculture, Tokyo Kyoiku University.

Each seed sample (25 g) was dehydrated with 200 ml of cold acetone in a refrigator at 4°C for 1 hr. After changing the acetone the seed sample was similarly re-dehydrated two times and then the seeds were crushed in a mortar. The crushed seeds were evaporated for 2 to 3 hr at dry room condition. The dehydrated seeds were defatted with Soxhlet apparatus for 10 hr, and the defatted seed materials were evaporated *in vacuo* and continuously in drying chamber at 40°C for 1 to 2 hr. Then the dehydrated and defatted seeds were ground to fine powder in a mortar.

Oxazolidinethiones were analyzed according to Astwood *et al.* (1949) modified by Miguchi *et al.* (1968) and the authors. Two grams of ground seed meals were suspended in 10 ml of distilled water mixed with 10 ml of pH 5.5 phosphate buffer and the mixture was allowed to stand in a incubator at 30°C for 3 hr. The suspension was then filtered with Toyo-Roshi No. 2 filter paper and the residue was refiltered three times with 20 ml of distilled water. The filtrate was measured up to 100 ml with distilled water by measuring flask. Then the liberated oxazolidinethiones from 5 ml of the filtrate were extracted with 15 ml of ethyl ether in a centrifuging tube having a stopper, shaking eighty times, and the ether phase of the mixture was transferred into test tube by a pipette. The residue was reextracted three times with 15 ml of ethyl ether by the same procedure as mentioned above. The extract was evaporated completely in a water bath at 40°C. The test tube was filled up with definite volume of distilled water and the extracted oxazolidinethiones were measured spectrophotometrically at 220 mμ, 240 mμ and 260 mμ and were calculated as *l*-5-vinyl-2-oxazolidinethione according to Astwood *et al.* (1949).

RESULTS

The data obtained in the spectrophotometric work were shown in Tables 1 and 2. The contents of oxazolidinethione in the *B. napus* seed meals were generally higher than the other species analyzed and varied from 14.6 (cultivar "Iwashiro natane") to 3.7 mg per gram of dry meal (cultivar "Russian No. 6"). Mean value of oxazolidinethione content was 10.53 ± 3.23 mg per gram of dry meal in 18 cultivars of *B. napus* ssp. *oleifera* (rape) and that of 4 cultivars of ssp. *rapifera* (rutabaga) was 8.08 ± 1.94, however there was no significant differences between these subspecies. Some cultivars

Table 1. Oxazolidinethiones from enzyme-hydrolyzed Cruciferae seed meals

Species (Somatic chromosome number and genome) (English name)	Cultivar	Oxazolidinethione mg/g dry meal Calcd. as vinyl oxazolidinethione
Brassica napus L. ($2n=38$, aacc)		
ssp. *oleifera* (rape)	Kyushu No. 8	13.5
	Kinki No. 28	12.1
	Hokuriku No. 27	13.1
	Abukuma natane	12.8
	Miyuki natane	6.2
	Mikawa natane	12.0
	Asahi natane	10.2
	Kogane natane	12.6
	Tokiwa natane	4.5
	Kongo natane	11.7
	Genkai natane	10.8
	Iwashiro natane	14.6
	French No. 2	11.6
	Russian No. 6	3.7
	Svalöf's orey	8.9
	NABO	8.0
	Zero erucic acid *napus*	14.3
	Artificially synthesized rape CO	9.0
ssp. *rapifera* (rutabaga)	Smooth round	11.0
	White fleshed neckless	7.4
	Green top	7.1
	Wilhelmsburger	6.8
Brassica campestris L. ($2n=20$, aa)		
ssp. *pekinensis* (Chinese cabbage)	*heading variety*	
	Aichi hakusal	0.0
	Nozaki No. 1	0.0
	Nozaki No. 2	0.0
	Chifu hakusai	0.0
	Matsushima new No. 5	0.0
	Kyoto No. 3	0.0
	Hotoren hakusai	0.0
	semi-heading variety	
	Kashin hakusai	0.0
	Chokurei hakusai	0.0
	Ogata santo hakusai	0.0
	Chosen hakusai	0.8
	Honchirimen hakusai	0.0
	Hikoshima haruna	0.0
	non-heading variety	
	Shiroguki santosai	0.0
	Hiroshimana	0.0

Table 1. (continued)

	Mana	0.0
	Osaka shirona	0.0
ssp. *chinensis* (Pak-choi)	Yukijiro taisai	0.0
	Nikanme taisai	0.0
	Shigatsu shirona	0.0
	Sendai yukina	0.0
ssp. *oleifera* (turnip rape)	*vegetable*	
	Shokuyo wase aburana	0.0
ssp. *narinosa* (?)	Kisaragina	0.0
ssp. *japonica* (curled mustard)	Chusei chisuji kyomizuna	0.0
	Chusei maruba mibuna	0.0
	Shizuoka jikyona	0.0
ssp. *rapifera* (turnip)	*vegetable*	
	Fukuyo komatsuna	0.0
	Nozawana	0.0
	Tennoji kabu	0.0
	Yorii kabu	0.0
	Shogoin kabu	0.0
	Kanamachi kokabu	0.0
	Tokyo nagakabu	0.0
	Ono benikabu	0.0
	Hinona kabu	0.0
	Tsuda kabu	0.0
	forage	
	Shimofusa kabu	1.1
	Koiwai kabu	1.3
	Daimaru murasaki kabu	0.8
	Kenshin kabu	0.8
Brassica oleracea L. ($2n=18$, cc)		
var. *capitata* (cabbage)	Fujiwase kanran	6.7
	Toyotawase kanran	9.5
	King kanran	1.3
	Kairyo Nakano kanran	1.9
	Nozakiwase kanran	0.2
	Nozakinakate kanran	0.1
	Watanabe seiko No. 1	3.2
	Yamato Succession kanran	3.8
	Kairyo Nanbu kanran	2.0
	Red cabbage	5.4
	Yoshin kanran	2.0
var. *bullata* (savoy cabbage)	Chirimen kanran	1.0
var. *gemmifera* (brussels sprouts)	Fuji komochikanran	4.1
var. *acephala* (kale)	Green kale	3.6
	Chicken kale	3.4
var. *gongyloides* (kohlrabi)	Early purple vienna	1.5
	Early white vienna	0.6

Table 1. (continued)

var. *italica* (Italian broccoli)	Italian broccoli	0.0
	Waltham 29	0.0
	Docico	1.2
var. *botrytis* (cauliflower)	Nozakiwase hanayasai	0.0
	Shirotama chogokuwase hanayasai	0.0
Brassica nigra Koch (2*n*=16, bb) (black mustard)	?	0.0
Brassica juncea Hemsel (2*n*=36, aabb) (mustard)	Miike oba chirimen takana	0.0
	Katsuona	0.0
	Kigarashina	0.0
	Hagarashina	0.0
Brassica carinata (2*n*=34, bbcc) (Abyssinian cress)	?	0.0[1)]
Raphanus sativus L. (2*n*=18, RR) (radish)	Daimaru hatsukadaikon	0.0
	Heian sushirazu shogoin daikon	0.0

1) This data was quoted from Daxenbichler *et al.* (1964).

Table 2. Mean content of oxazolidinethiones in each species

Species	Number of cultivar	Oxazolidinethione mg/g dry meal and standard deviation
Brassica napus L.	22	10.09±2.34
ssp. *oleifera*	18	10.53±3.23
ssp. *rapifera*	4	8.08±1.94
Brassica campestris L.	40	0.12±0.33
ssp. *pekinensis*	17	0.05±0.19
ssp. *chinensis*	4	0.00±0.00
ssp. *oleifera*	1	0.00 —
ssp. *narinosa*	1	0.00 —
ssp. *japonica*	3	0.00±0.00
ssp. *rapifera*	14	0.28±0.48
Brassica oleracea L.	22	2.37±2.44
var. *capitata*	11	3.28±2.90
var. *bullata*	1	1.00 —
var. *gemmifera*	1	4.10 —
var. *acephala*	2	3.50±0.16
var. *gongyloides*	2	1.05±0.64
var. *italica*	3	0.67±0.50
var. *botrytis*	2	0.00±0.00
Brassica nigra Koch	1	0.00 —
Brassica juncea Hemsel	4	0.00±0.00
Raphanus sativus L.	2	0.00±0.00

such as Russian No. 6 (3.7 mg) and Tokiwa natane (4.5 mg), Miyuki natane (6.2 mg) were lower in the content of oxazolidinethione significantly at the 5 per cent level and a few cultivars such as Iwashiro natane (14.6 mg) and Zero erucic acid *napus* (14.3 mg), were significantly higher at the same level, as compared with other *napus* cultivars.

Almost all of 40 *campestris* cultivars did not form oxazolidinethiones. Only trace amounts (1.3 mg per gram of dry meal or less) were found from all of 4 forage turnips and 1 Chinese cabbage cultivar named Chosen hakusai, which were significant statistically at the 1 per cent level.

In the *B. oleracea* cultivars the oxazolidinethione content ruggedly varied from 9.5 to 0.0 mg. The mean value of 22 cultivars was 2.37±2.44 mg and it seemed to vary with varieties, however there was no significant difference among them. Nevertheless, some of the cultivars such as Toyotawase kanran (9.5 mg), Fujiwase kanran (6.7 mg), were significantly higher than the other cultivars at the 5 per cent level, and all of cauliflowers (0.0 mg), 2 cabbage cultivars, Nozakinakate kanran (0.1 mg) and Nozakiwase kanran (0.2 mg), and 2 cultivars of Italian bloccoli, Italian broccoli (0.0 mg) and Waltham 29 (0.0 mg), were significantly lower at the same level. No oxazolidinethione was found in the seed materials from *B. nigra*, *B. juncea* and *Raphanus sativus*.

DISCUSSION

All seed meals from *B. napus* cultivars formed oxazolidinethiones, the yields of which were generally higher than that of other species. Mean content of oxazolidinethiones in 22 cultivars of *B. napus* was 10.09±2.34 mg, with a range from 14.6 to 3.7 mg per gram of dry meal. The 12 cultivars, Kyushu No. 8 to Iwashiro natane in Table 1, have been bred in Japan. Upper 4 rape cultivars, Kyushu No. 8 to Abukuma natane, were bred from pure line selection or *B. napus*×*B. napus* crosses and the others originated from interspecific crosses between *B. napus* and *B. campestris* ssp. *oleifera* (turnip rape). Mean content of oxazolidinethiones was 12.88±0.42 in the cultivars derived from the former breeding methods and 10.33±3.35 in the latter. Though there was no significant difference between them, the standard deviation was clearly different.

In the present experiment oxazolidinethione was not found in turnip rape that was a vegetative cultivar, though it is well known that oil turnip rape cultivars yield much less oxazolidinethiones than the *B. napus* cultivars (Astwood *et al.* 1949; Appelqvist and Josefsson 1967; Nakabayashi *et al.* 1972). The seed meals of all forage turnips yielded some oxazolidinethiones (about 1.0 mg), although, none of vegetative turnips did. It is commonly supposed that the Japanese cultivars of forage turnip such as Shimofusa kabu, Koiwai kabu and Daimaru murasaki kabu had derived from spontaneous interspecific hybrids between rutabaga (*B. napus* ssp. *rapifera*) and turnip (*B. capmestris* ssp. *rapifera*). A cultivar Kenshin kabu was bred by mass selections of the progenies from mutual crossing among some strains and cultivars of forage turnip (Tsuchiya *et al.* 1968).

It seemed to be connected with hereditability of oxazolidinethione content that low content cultivars, Tokiwa natane and Miyuki natane, were bred from the progenies of

B. napus×*B. campestris* and forage turnips yielded some oxazolidinethiones in general.

Large variation was seen in oxazolidinethione content of *B. oleracea*. Josefsson (1967) described the same phenomenon in root and stem of *B. oleracea* varieties. According to these results it is believed that *B. oleracea* var. *botrytis* (cauliflower) does not yield oxazolidinethione, var. *italica* (Italian broccoli) and var. *gongyloides* (kohlrabi) yield a little oxazolidinethiones and var. *capitata* (cabbage) cultivars vary in content of oxazolidinethiones from fairly high to pretty low.

Oxazolidinethione was not found in the seed meals of *B. nigra*, *B. juncea* and *Raphanus sativus*, confirming the results of Astwood *et al.* (1949) and Daxenbichler *et al.* (1964). In the present experiment the seed meals of *B. carinata* were not analyzed. According to Daxenbichler *et al.* (1964) oxazolidinethione was not found in the seed meals of *B. carinata* and Nakabayashi *et al.* (1972) described some yields of oxazolidinethione in them.

As seen in the Table 1 genus *Brassica* comprises 3 basic species, aa-, bb- and cc-genomes, and 3 amphidiploid species, aabb-, aacc- and bbcc-genomes. From the results of the authors and the other workers the content of oxazolidinethione seems to be strongly connected with genome constitution. Oxazolidinethione is not contained in all cultivars having bb- and aabb-genome and a little or less in bbcc-genome species. The cultivars having aacc-genome yield many oxazolidinethiones than the other genome species and the cultivars having cc-genome vary in content of oxazolidinethione from fairly high to zero. The cultivars having aa-genome yield slightly or not at all. Genomic differences of oxazolidinethione content are significant at the 1 per cent level between aacc- and other genomes, and cc- and other genomes.

Although it is difficult to decide genetic background of oxazolidinethione content in genus *Brassica*, the authors suppose that b-genome has not genetic factors producing the oxazolidinethiones and the source of the factors is mainly in c-genome.

As mentioned above *B. napus* plants, including rape and rutabaga, are the amphidiploids of *B. campestris* and *B. oleracea*. This fact was early discovered cytologically by Morinaga (1929) and U (1935) succeeded in the artificial synthesis of napus plants from the cross, *B. campestris*×*B. oleracea*. Since then some workers have carried out studies on the breeding of artificially synthesized rape or rutabaga and some promising cultivars have been bred. Therefore, there should be an assured possibility to breed the synthesized *napus* cultivars containing no oxazolidinethione by means of interspecific crossing between some selected parents of *B. campestris* and *B. oleracea*.

SUMMARY

Oxazolidinethione, one of the main goitrogenic substances in the seed meals of various cultivars of Cruciferous species such as *B. napus*, *B. campestris*, *B. oleracea*, *B. nigra*, *B. juncea* and *Raphanus sativus*, was measured spectrophotometrically and was calculated as *l*-5-vinyl-2-oxazolidinethione. The number of cultivars examined in each species was 22, 40, 22, 1, 4 and 2, respectively.

No oxazolidinethione was found in the seed meals of *B. nigra*, *B. juncea* and *R.*

sativus, confirming the results of previous workers.

Mean value of oxazolidinethione content of seed meals from *napus* cultivars was 10.09±2.3 mg per gram of dry meals, with a range from 14.6 to 3.7 mg. Low content cultivars seemed to belong to those derived from interspecific crosses between *B. napus* and *B. campestris*.

B. campestris cultivars yielded slightly or not at all, however all forage turnips yielded about 1.0 mg, which are commonly supposed to be originated from spontaneous interspecific hybrids between rutabaga (*B. napus*) and turnip (*B. campestris*).

B. oleracea varied in oxazolidinethione content and mean value was 2.37±2.44 mg, with a range from 9.5 to 0.0 mg.

It seemed that b-genome had not genetic factors producing the oxazolidinethiones and the source of the factors was mainly in c-genome.

Though it must not be easy to breed the *napus* cultivars containing no or a small amount of oxazolidinethione, there should be an assured possibility to synthesize such cultivars by interspecific crosses between selected cultivars of *B. campestris* and *B. oleracea*.

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