— **Regular Article** —

Comparison of Selenium Metabolism in Three Brassicaceae Plants

Ayako Yawata,[a] Yasuko Oishi,[a] Yasumi Anan,[a] and Yasumitsu Ogra*[, a, b]

[a]*Laboratory of Chemical Toxicology and Environmental Health and* [b]*High Technology Research Center, Showa Pharmaceutical University, 3–3165 Higashi-Tamagawagakuen, Machida 194–8543, Japan*

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Although selenium (Se) is not an essential element in plants, Se metabolism in plants is remarkable, mainly for the purpose of phytoremediation and nutritional supplementation of Se. *Brassica juncea* (Indian mustard) is known as an Se accumulator. In addition to Indian mustard, unique Brassicaceae plants are consumed in Japan. *Brassica rapa* var. *hakabura*, commonly known as nozawana, and *Brassica rapa* var. *peruviridis*, commonly known as komatsuna, are typical Brassicaceae plants. In this study, we evaluated the Se-accumulating ability of these unique Brassicaceae plants. There were no significant differences in the Se concentrations in the roots and the leaves of the three Brassicaceae plants. However, nozawana most efficiently accumulated Se among the three Brassicaceae plants because it showed the most rapid growth, resulting in the highest biomass. Speciation revealed that the Se species accumulated in the three plants were identical. In addition, nozawana contained easily extractable essential minerals, such as iron, copper, and zinc. Potentialities of nozawana as a source of minerals and the phytoremediation of soil and water contaminated with Se were discussed.

Key words —— selenium, phytoremediation, speciation, Brassicaceae, inductively coupled plasma-mass spectrometry

INTRODUCTION

Selenium (Se) is an essential micronutrient for humans and animals, and is required to promote the activities of some selenoenzymes, such as glutathione peroxidases, iodothyronine 5′-deiodinase, and thioredoxin reductase.[1–3] It has been reported that organic forms of Se, such as selenoamino acid derivatives, decrease the incidence of cancers and cardiovascular diseases.[4–6] On the other hand, Se is also recognized as an environmental contaminant because it is widely used in the semiconductor and electronic industries. Se is known as a non-essential element in plants. However, Se metabolism in plants is remarkable due to the following reasons. One is the nutritional supplementation of Se. Selenized yeasts and Allium plants belonging to Family Liliaceae, such as garlic, onion, wild leek, and shallot, have been developed for this purpose.[7,8] Se is a metalloid and thus, even if inorganic Se in the form of selenite [Se(IV)] or selenate [Se(VI)] is absorbed by plants, it is metabolized to form selenocompounds having an Se-C covalent bond.[9] It is known that the pharmacological, nutritional, and toxicological effects of Se strongly depend on the chemical species. Inorganic Se salts are more toxic to animals than organic Se compounds and thus, the conversion of inorganic into organic Se in plants is effective to produce Se supplements. The other reason is phytoremediation to remove contaminating Se. Phytoremediation is a low-cost and environmentally friendly technique to remove contaminants. The Indian mustard, *Brassica juncea* (*B. juncea*), has been extensively studied for Se phytoremediation[10–12] because of its high Se-accumulating ability, fast growth, and high biomass.

Unique Brassicaceae plants are consumed in Japan. *Brassica rapa* (*B. rapa*) var. *peruviridis*, commonly known as komatsuna, is one of the most popular vegetables nationwide. It shows fast growth and high biomass. *B. rapa* var. *hakabura*, com-

*To whom correspondence should be addressed: Laboratory of Chemical Toxicology and Environmental Health, Showa Pharmaceutical University, 3–3165 Higashi-Tamagawagakuen, Machida 194–8543, Japan. Tel. & Fax: +81-42-721-1563; E-mail: ogra@ac.shoyaku.ac jp

monly known as nozawana, is mainly consumed in the Shinshu area in Japan. It is pickled according to traditional methods. Nozawana shows faster growth and higher biomass than Indian mustard and komatsuna. However, the Se metabolism in these unique Brassicaceae plants, *i.e.*, komatsuna and nozawana, has not been clarified.

The inductively coupled plasma-mass spectrometer (ICP-MS) is a superior instrument for elemental speciation owing to its high sensitivity and specificity when used in combination with such separation instruments as a high-performance liquid chromatograph (HPLC). HPLC-ICP-MS is the technique of choice for Se speciation in samples. In addition, HPLC-ICP-MS can allow multi-elemental speciation in a single run on HPLC. The multi-elemental speciation provides some information regarding not only the distribution of each element but also element-element interaction.[13] Hence, HPLC-ICP-MS was applied to reveal the metabolism of Se and other elements in unique Brassicaceae plants.

The main aim of this study was to compare Se metabolism among the three Brassicaceae plants. In this study, the three Brassicaceae plants were cultivated and fortified with Se in the form of sodium selenate. Se-accumulating ability was evaluated by the determination of Se in the plants. The metabolic pathways of Se were speculated based on the results of HPLC-ICP-MS, and the distributions of other elements were also determined by HPLC-ICP-MS. Finally, the potential use of the three Brassicaceae plants as a nutritional source and for the phytoremediation of Se was discussed.

MATERIALS AND METHODS

Chemicals —— Sodium selenate, nitric acid, and ammonium acetate were purchased from Wako (Osaka, Japan). *Se*-methylselenocysteine (MeSeCys) and selenomethionine (SeMet) were purchased from Acros Organics (Geel, Belgium). Selenohomolanthionine (SeHLan) was synthesized in our laboratory by a previously reported method.[14] γ-Glutamylmethylselenocysteine (GluMeSeCys) and Hoagland-Arnon salt mixture (Hoagland's No. 2) were purchased from Pharmase (Austin, TX, U.S.A.) and Sigma (St. Louis, MO, U.S.A.), respectively. All reagents were of the highest or analytical grade. Deionized water (18.3 MΩ·cm) was used throughout.

Plant Growth and Sample Preparation —— *B. rapa* var. *hakabura* (nozawana), *B. rapa* var. *peruviridis* (komatsuna), and *B. juncea* (Indian mustard) seeds were germinated and grown on a block wool with 1/2 strength solution of Hoagland-Arnon salt mixture in a growth chamber (LH-55-RDS; Nihonikakikai, Osaka) with a photoperiod of 14 hr light (8000 lux)/10 hr dark at 25.0°C. One week after seeding, the plants were thinned out and transferred to a 15-l planter filled with conventional soil. The plants were exposed to a 500 ml portion of sodium selenate at a concentration of 1 μg Se/ml every 6 days for 3 weeks. The plants were harvested and divided into roots and leaves. The plant samples were lyophilized and milled to obtain the homogenized powder.

Determination of Se Concentration —— A 0.1 g portion of the samples was wet-ashed with 1.5 ml of concentrated nitric acid. The ashed samples were diluted with deionized water and Se concentration in the samples was determined by ICP-MS (Agilent 7500ce, Agilent Technologies, Hachioji, Japan). Mass calibration and optimization of the parameters for ICP-MS were performed daily prior to use in accordance with the manufacturer's instructions.

Speciation of Se, Sulfur, and Metals by HPLC-ICP-MS —— The lyophilized samples were incubated with deionized water at room temperature for 1 hr to extract Se, sulfur (S), and metal-containing species, and then ultracentrifuged at 105000 *g* for 60 min to obtain the water extract. A 20 μl aliquot of the water extract and each 0.5 μg Se/ml of standard solution of selenate, SeMet, SeHLan, GluMeSeCys, and MeSeCys was applied to an HPLC coupled with an ICP-MS to analyze the distribution of Se, S, iron (Fe), copper (Cu), and zinc (Zn). The HPLC system consisted of an on-line degasser, an HPLC pump, a Rheodyne six-port injector, and a multi-mode size exclusion column (Shodex Asahipak GS-320HQ, 7.5 internal diameter × 300 mm with a guard column). The column was eluted with 50 mmol/l ammonium acetate, pH 6.5, at a flow rate of 0.6 ml/min. The eluate was introduced into the nebulizer of the ICP-MS to detect Se, S, Fe, Cu, and Zn at m/z 77, 34, 57, 65, and 66, respectively.

Statistics —— The results are presented as means ± standard deviation (S.D.) of 5–10 samples. Statistical analysis was performed with the Student's t-test.

Table 1. Se Concentrations in Brassicaceae Plants

	B. rapa var. *hakabura* (Nozawana)	*B. juncea* (Indian mustard)	*B. rapa* var. *peruviridis* (Komatsuna)
Leaves	0.37 ± 0.08	0.36 ± 0.09	0.34 ± 0.17
Roots	1.07 ± 0.18	1.36 ± 0.98	1.64 ± 0.79

Data are expressed as means ± S.D. in μg Se/g wet weight. n = 5–10.

RESULTS AND DISCUSSION

Concentration and Accumulation of Se in Three Brassicaceae Plants

There were no significant differences in the Se concentrations in the roots and the leaves of the three Brassicaceae plants (Table 1). Although Se concentration in the roots of komatsuna tended to be higher than that of nozawana, the difference was not significant. The results indicate that the three Brassicaceae plants have comparable ability to accumulate Se in the leaves and roots. Se concentration in the roots was apparently higher than that in the leaves in all the three plants, suggesting that Se was absorbed and preferably accumulated in the roots. Tellurium (Te), an element that belongs to the same group as Se, is, like Se, a non-essential element for plants. It was reported that Te was more preferably accumulated in the roots than in the shoots of *B. juncea*.[15] Thus, the three Brassicaceae plants may have a common mechanism for the accumulation of non-essential elements, *i.e.*, Se and Te, in the roots to prevent the distribution of Se and Te to the leaves. Figure 1 shows the total amounts of Se accumulated in the leaves and the roots of the three plants. The growth of Indian mustard and komatsuna for 3 weeks almost equaled each other, whereas the leaves and the roots of nozawana grew most rapidly to result in the highest biomass. The leaf biomasses of nozawana, komatsuna and Indian mustard were 55.1 ± 27.9, 41.7 ± 30.4 and 22.0 ± 13.8 g wet weight, respectively. Whereas, the root biomasses of nozawana, komatsuna and Indian mustard were 1.33 ± 0.98, 0.34 ± 0.14 and 0.24 ± 0.31 g wet weight, respectively. As shown in Table 1, the Se-accumulating abilities of these plants were comparable. Hence, it can be concluded that nozawana has the greatest potential for the accumulation of Se. As mentioned above, the three plants preferably accumulated Se in the roots to prevent Se distribution to the leaves. However, the biomass of the leaves was considerably higher than those of the roots. Thus, the leaves practically act as an Se reservoir in Brassicaceae plants. Indeed, the leaves of nozawana accumulated much larger amounts of Se (20.8 ± 12.7 μg) than those of Indian mustard (7.71 ± 4.02 μg) and komatsuna (11.6 ± 7.8 μg, Fig. 1 a). In contrast, the amounts of Se in the roots of nozawana, Indian mustard, and komatsuna were 1.48 ± 1.15, 0.57 ± 0.59, and 0.52 ± 0.27 μg, respectively (Fig. 1 b).

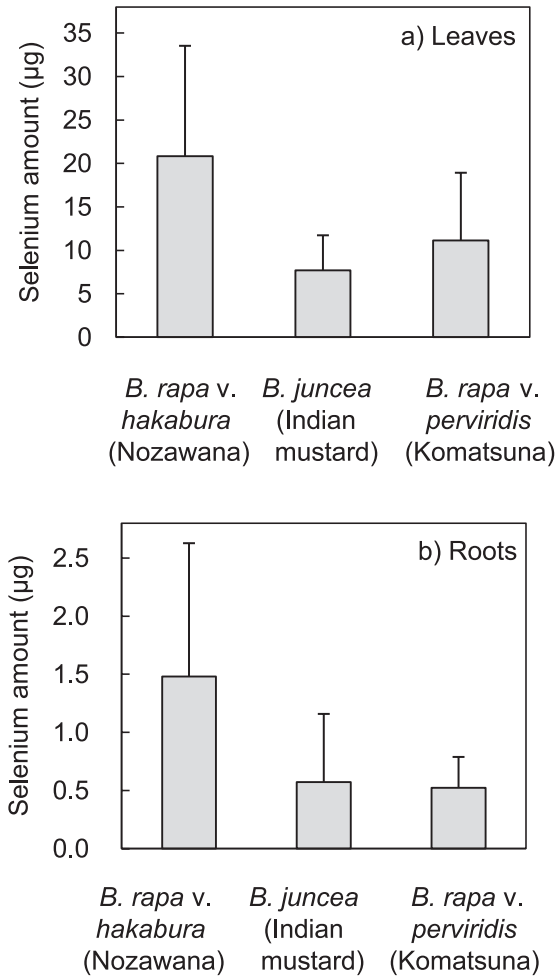

Fig. 1. Amounts of Se Accumulated in the Leaves (a) and Roots (b) of Three Brassicaceae Plants

The amounts of Se in the plants are the products of the Se concentration multiplied by the biomass. The columns and bars are means ± S.D. for 5–10 samples.

Speciation of Se, S, and Metals in Extracts of Three Brassicaceae Plants

For the purpose of the nutritional supplementation of Se, the chemical species of Se accumulated in the plant is crucial. Therefore, the speciation of Se, S, and other metals was determined. The major Se compound extractable with water in all the three plants was selenate because the retention time of the largest peak on the Se profile of the samples matched that of authentic selenate (Figs. 2 and 3). The extraction efficiencies of nozawana, Indian mustard, and komatsuna were 77.3, 77.4, and 78.2%, respectively. Thus, almost all Se existing in the three plants was recovered as selenate. A minor Se compound was detected at the retention time of 15.7 min in the three plants although it was not assigned yet. It was reported that SeHLan and GluMeSeCys were detected in another Brassicaceae plant, radish (*Raphanus sativus*).[16] However, no apparent SeHLan and GluMeSeCys were detected in the extracts of the three plants. Trace amounts of SeMet and MeSeCys were detected at the retention times of 20.2 and 19.2 min, respectively, in the extracts of the three plants. These two selenoamino acids are typical Se species detected in Se-accumulating plants.[17,18] Consequently, nozawana and komatsuna showed the same Se metabolism as Indian mustard, which is already known as an Se accumulator.

As the retention time of the largest S peak matched that of sulfate (data not shown), the peak was assigned to sulfate. The chromatographic behavior of sulfate was similar to that of selenate, hence suggesting that the major S extracted from the plants is sulfate. Other minor S compounds varied among species and have yet to be assigned.

Two Fe peaks were commonly detected in all the extracts of the three plants. The intensity of the peak that had a retention time of 13.9 min dif-

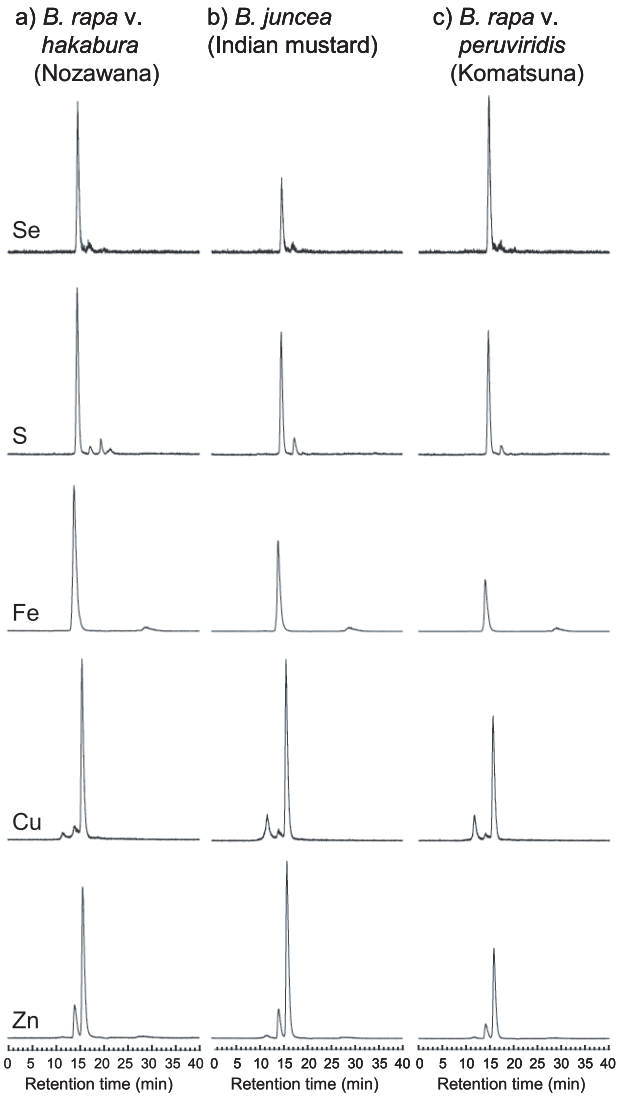

Fig. 2. Elution Profiles of Se, S, Fe, Cu, and Zn in the Extracts of Three Brassicaceae Plants

Soluble compounds in lyophilized *B. rapa* var. *hakabura* (nozawana) (a), *B. juncea* (Indian mustard) (b), and *B. rapa* var. *peruviridis* (komatsuna) (c) were extracted with deionized water. A 20 µl aliquot of the water extract was subjected to HPLC coupled with ICP-MS to measure the distributions of Se, S, Fe, Cu, and Zn. The multi-mode size exclusion column (GS-320HQ) was eluted with 50 mmol/l ammonium acetate, pH 6.5, at a flow rate of 0.6 ml/min.

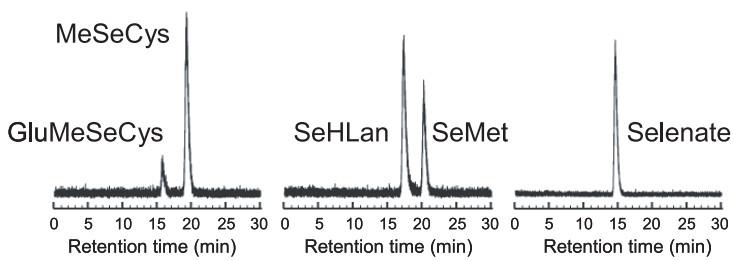

Fig. 3. Elution Profiles of Se Standards

Authentic standards of GluMeSeCys, MeSeCys, SeHLan, SeMet, and selenate were applied to a GS-320HQ column, and the column was eluted under the same conditions as those mentioned in Fig. 2 caption.

fered among the species. Meanwhile, the intensity of the broad Fe peak that had a retention time of 29.2 min was comparable among the species. These two peaks were not assigned yet.

Three apparent Cu peaks were detected at the retention times of 11.7, 14.1, and 15.6 min. The intensities of the two more rapidly eluting peaks (11.7 and 14.1 min) seemed to be identical for Indian mustard and komatsuna extracts. The intensity of the largest peak having a retention time of 15.6 min for komatsuna extract was smaller than those of the other two extracts. Further studies are needed to reveal ligands that bind Cu in the extracts.

Two sharp Zn peaks were detected at the retention times of 14.1 and 15.8 min. The retention time of the large peak was identical to that of the largest Cu peak. Metal cations without any ligands could not be eluted from the column. Thus, Zn and Cu may be bound to an identical ligand. HPLC-ICP-MS cannot identify molecular structures whose authentic standards are not available. To identify the structure of the ligand binding Zn and Cu, other techniques, such as electrospray ionization tandem mass spectrometry (ESI-MS-MS), are required.

The peak intensity of Fe in nozawana extract was the largest among the three species although those of Cu and Zn were comparable between nozawana and Indian mustard. This suggests that Fe is more easily extractable in nozawana than in the other Brassicaceae plants. Hence, nozawana is expected to become a suitable nutritional source of these minerals in addition to Se.

In conclusion, unique Brassicaceae plants consumed in Japan, such as nozawana and komatsuna, showed the same Se-accumulating ability and metabolism as Indian mustard, a known Se accumulator. Nozawana showed the most efficient Se-accumulating ability among the three plants because its biomass was higher than those of the other two plants. In addition, nozawana contained easily extractable essential minerals. Consequently, nozawana may be a potential source of minerals and be applicable to the phytoremediation of soil and water contaminated with Se.

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