Original research article

Catechin production in roots of *Thujopsis dolabrata* var. *hondai* in soils on Mt. Hayachine with high Ni concentrations

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Abstract: Mt. Hayachine in Iwate prefecture is characterized by serpentine site, which is known to have high concentrations of Ni. In general, few plants that can tolerate high concentrations of Ni can grow in serpentine soil. *Thujopsis dolabrata* var. *hondai* occurs naturally on Mt. Hayachine. In this study, we hypothesized that *T. dolabrata* var. *hondai* shows Ni tolerance due to detoxification by phenolics. We collected *T. dolabrata* var. *hondai* seedlings and root-zone soil from the Kadoma National Forest on Mt. Hayachine to analyze the concentrations of Ni, other heavy metals, and macronutrients. The seedling roots had high concentrations of Ni. Further, we conducted a pot experiment by using 1-month-old sterile seedlings grown in three types of sterilized soils—Kadoma soil (obtained from the Kadoma National Forest), Tsugaru forest soil, and nursery soil—and compared the concentrations of Ni, nutrients, and catechin, as well as the growth of seedlings in the different soils. The pot experiment indicated that the roots of seedlings grown in Kadoma forest soil contained high concentrations of Ni and catechin, which could act as an antioxidant and a possible Ni-chelating compound that detoxified Ni in the plant cells. We concluded that *T. dolabrata* var. *hondai* seedlings growing in the serpentine site of Mt. Hayachine accumulated Ni and could detoxify it by producing high concentrations of catechin.

Keywords: catechin, hinoki-asunaro, nickel, serpentine site, *Thujopsis dolabrata* var. *hondai*, tolerance

Abbreviations: HPLC, high-performance liquid chromatography; ICP-AES, inductively coupled plasma atomic emission spectroscopy

Introduction

Mt. Hayachine, Iwate prefecture, Japan, is located at a serpentine site, and only specific plant species are known to grow at this site (Matsuda 1989, Tanaka et al. 2008). Serpentine soil is chemically characterized by the following three factors: (1) high levels of heavy metals such as Ni, Mn, Cr, and Co; (2) high concentration of Mg; and (3) low contents of essential plant nutrients such as Ca, P, and K (Walker 1954, Kayama et al. 2002, 2005, 2006, 2009). Among these factors, high levels of heavy metals, mainly Ni, are considered a crucial stress factor for plants growing in serpentine soils (Kruckeberg 1954). Because Ni is an essential micronutrient for higher plants (Brown et al. 1987) and is present at the active site of urease (Polacco 1977, Todd and Hausinger 1989), it has an important physiological role (Seregin and Kozhevnikova 2006). However, non-tolerant plants exposed to higher concentrations of Ni show toxic symptoms such as reduced mitotic activity and root growth, damaged plasma membranes (Alexander et al. 2007), and root branching inhibition (Seregin et al. 2003) because of the production of reactive oxygen species (Seregin and Kozhevnikova 2006). *Thujopsis dolabrata* Sieb. et Zucc. var. *hondai* Makino, also known as Hinoki-asunaro, hiba or ate, is an evergreen, needle-leaved tree species that is distributed from the Oshima Peninsula (42°10′) on Hokkaido Island to Tochigi prefecture (36°47′) on Honshu Island in Japan (Kobayashi and Asakawa 1981). This species is extensively found in the Tsugaru Peninsula in

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Aomori prefecture and on Mt. Hayachine (Aomori Forestry Research Institute 2004). Although *T. dolabrata* var. *hondai* grows at a serpentine site, the mechanism of Ni tolerance in this plant has not yet been investigated.

In general, plants tolerate high concentrations of heavy metals in soils by either restricting heavy metal uptake and translocation from roots to shoots (exclusion) or accumulating the heavy metals in a non-toxic form (accumulation; Baker 1981, Kayama et al. 2005, 2006). Larcher (2003) suggested five tolerance mechanisms in plants induced in response to heavy metals: (1) immobilization of heavy metals in cell walls, thus avoiding their contact with protoplasts and further transport through apoplasts; (2) inhibition of permeation beyond the cell membrane; (3) chelating heavy metals with sulfur-containing polypeptides (glutathione and glutamylcysteine derivatives), SH-containing proteins, and induced stress proteins, which provides protection from metal toxicity in the cytoplasm; (4) compartmentalization and formation of complexes with organic and inorganic acids, phenol derivatives, and glycosides in vacuoles; and (5) active exclusion of heavy metals from cells. Many studies have investigated mechanism (4) for heavy metal detoxification. For example, most Ni in *Sebertia acuminata* is bound to citric acid (Sagner et al. 1998). Further, many *Alyssum* species contain Ni and histidine complexes (Krämer et al. 1996), and phenolics act as metal detoxicants in *Pinus sylvestris* L. (Roitto et al. 2005) and *Vaccinium myrtillus* L. (Białońska et al. 2007).

T. dolabrata var. *hondai* is a commercially important tree species that produces many kinds of phenolics and terpenoids that show high defense against insects and pathogens. The seeds (Hasegawa and Hirose 1980), leaves (Takahashi et al. 1981, Nagahama et al. 1996), trunk, and branches of this tree contain characteristic secondary metabolites. Our preliminary study showed high concentrations of phenolic compounds catechin, epicatechin, and totarol in 1- and 2-year-old *T. dolabrata* var. *hondai* seedlings (data not shown). Catechin is reported to chelate Al, an element toxic to plants; Catechin is reported to chelate the toxic element Al in for example *Camellia sinensis* (Nagata et al. 1992). Therefore, we consider that *T. dolabrata* var. *hondai* might be able to detoxify Ni because of the high production of secondary metabolites.

In this study, we hypothesized that *T. dolabrata* var. *hondai* growing on Mt. Hayachine contain high concentrations of Ni, which is detoxified by secondary metabolites, including catechin. We collected *T. dolabrata* var. *hondai* seedlings and root-zone soils from a natural forest of *T. dolabrata* var. *hondai* (Kadoma National Forest) on Mt. Hayachine and analyzed the concentrations of Ni, other heavy metals, and macronutrients in the seedlings and soil. Further, we grew *T. dolabrata* var. *hondai* seedlings in three different soils, namely, nursery soil (Fluvisol), Tsugaru forest soil collected from Tsugaru Peninsula in Aomori prefecture (Cambisol), and Kadoma soil collected from our study site (Cambisol) and compared Ni, nutrient, and phenolic concentrations, as well as seedling growth in the different soils. Finally, we discussed a possible Ni-tolerance mechanism in *T. dolabrata* var. *hondai* seedlings that allow them to survive under adverse conditions, such as at a serpentine site.

Materials and Methods

Field research

Study site

Our study site, Kadoma National Forest (39°35′N, 141°27′E), was located in Mt. Hayachine. In the area close to our study site (39°35′N, 141°25′E), *T. dolabrata* var. *hondai* was the main tree species; *Pinus parviflora* var. *pentaphylla*, *Magnolia obovata*, *Fraxinus lanuginosa* f. *serrata*, *Ilex macropoda*, and *Sorbus commixta* were also found in this area, but these species growing there were small (Ota et al. 2004). There were 523 *T. dolabrata* var. *hondai* trees per hectare (diameter at breast height, >5 cm), and the average peak tree height was 20–24 m (Ota et al. 2004). In June 2013, when we collected samples, the mean temperature at Kuzakai, Miyako (39°39.0′N, 141°21.2′E), the area nearest to our study site, was 15.7°C, and the total precipitation was 49.5 mm (Japan Meteorological Agency, http://www.jma.go.jp/jma/index.html).

Sample collection, growth measurement, and chemical analyses of seedlings and root-zone soil samples

In June 2013, five *T. dolabrata* var. *hondai* seedlings and root-zone soil samples were collected. The root-zone soil (10 × 10 × 10 cm) was sampled together with the seedlings. Seedling samples were carefully rinsed with tap water and Millipore water to remove adhering soil. The samples were then used to measure growth; the number of leaves was counted, and seedling height was measured. Each seedling was separated into leaves, stems, and roots, and their fresh masses were measured. The samples were dried separately for 48 h at 80°C, and then ground using an agate mortar. The ground samples were then pyrolyzed in concentrated HNO_3, and the concentrations of heavy metals (Fe, Mn, Ni, and Pb) and nutrients (K, P, Ca, and Mg) in the samples were quantified using

inductively coupled plasma atomic emission spectroscopy (ICP-AES; Perkin Elmer Optima 7300 DV; Perkin Elmer Inc., MA, USA). Root-zone soil samples were air-dried and passed through a 2-mm sieve mesh. The concentration of heavy metals (Fe, Mn, Ni, and Pb) in the soil samples was quantified using ICP-AES after digestion with concentrated HNO_3-$HClO_3$.

Pot experiment

Sterile seedling preparation

Ripe seeds were directly collected from two mature *T. dolabrata* var. *hondai* trees (40°58′N, 140°28′E, Aomori prefecture) in October 2013. They were soaked in water and peeled using a scalpel and tweezers to remove epiphytes from seed coats. The seeds were then placed in 70% ethanol for 1 min, transferred to 15% hydrogen peroxide for 15 min, and again placed in 70% ethanol for 1 min. They were rinsed with sterile Millipore water and incubated in 3.5-times diluted Hoagland solution containing 1.5% agar at 25°C for a 14-h light period and at 20°C for a 10-h dark period (Biotron LH-220S; NK System, Osaka, Japan). The photosynthetic photon flux density of 105.5 ± 3.7 μmol photons·m^{-2}·s^{-1} was measured using an LI-1400 data logger equipped with a quantum sensor (Licor; Lincoln, NE, USA). Seed germination was observed after about 1 month of incubation, and seedlings with open cotyledons were used for the pot experiment.

Soil sterilization

Kadoma soil, Tsugaru forest soil, and nursery soil were used for the pot experiment. Root-zone soil, randomly collected from 10 locations at our study site corresponding to the root zone of *T. dolabrata* var. *hondai* seedlings (depth, 10 cm) and then mixed, was referred to as Kadoma soil. Tsugaru forest soil was randomly collected from 15 sites around the forest from where the *T. dolabrata* var. *hondai* seeds were collected (as described above). Nursery soil was randomly collected from 15 sites in a nursery at Yamagata University (38°43′N, 139°49′E). On the basis of the FAO-UNESCO system (FAO 1988), Kadoma soil, Tsugaru forest soil, and nursery soil are classified as Cambisol, Cambisol, and Fluvisol, respectively. Each soil sample was air-dried at room temperature and passed through a sieve (<2 mm), and the chemical properties were analyzed. Total Ni and exchangeable Ni^{2+} were determined using 1 M ammonium acetate solution (pH 7.0; Mizuno and Hayashi 1967); exchangeable K^+, Ca^{2+}, and Mg^{2+} were measured using 0.05 M ammonium acetate and 0.0114 M strontium chloride solution (Committee of Soil Environment Analysis 1997); available P_2O_5 was determined according to Truog's method (Committee of Soil Environment Analysis 1997); and pH (H_2O) of the three soils was also recorded (Table 1). Each soil sample was sealed in a plastic bag and sterilized by irradiation (30 kGy of gamma radiation). The pH (H_2O), available P_2O_5, and exchangeable Ni and cation were not significantly different between sterilized and non-sterile soils ($p > 0.05$, Student *t*-test, data not shown). Subsequently, sterilized soil (77 g) was transferred to sterilized pots (Agri-pot; Kirin, Tokyo, Japan). Sterilized water was added to the sterilized soil in each pot until the moisture content equilibrated and reached field capacity.

Incubation, harvest, growth measurement, and chemical analyses of seedlings in pots

Two sterile seedlings were aseptically transplanted to each pot containing sterilized soil. Four replicate pots were prepared for each soil type. Seedlings were incubated for about 1 month (light: 25°C, 14 h/dark: 20°C, 10 h; photosynthetic photon flux density, 105.5 ± 3.7 μmol photons·m^{-2}·s^{-1}; Biotron LH-220S; NK System, Osaka, Japan). After incubation, seedlings were harvested to measure growth and subjected to chemical analyses. The numbers of leaves were counted, and the seedling height, root length, fresh

Table 1. Total Ni, exchangeable ions, available P_2O_5, and pH (H_2O) in the soils used for the pot experiment. Results of three replicate analyses; values are mean ± standard error. n.d.: not detected. Different letters indicate significant difference of each element concentration in different tissues at the 5% level.

	Kadoma soil		Tsugaru forest soil		Nursery soil	
Total Ni (mg kg^{-1} DM)	425.3 ± 24.8	a	10.8 ± 0.2	b	8.5 ± 0.8	b
Exchangeable ion (mg kg^{-1} DM)						
Ni^{2+}	1.0 ± 0.04		n.d.		n.d.	
K^+	97.4 ± 1.6	c	514.6 ± 15.0	a	295.6 ± 22.6	b
Ca^{2+}	342.9 ± 34.2	c	652.0 ± 12.9	a	1210.4 ± 55.5	b
Mg^{2+}	32.4 ± 0.1	c	36.6 ± 0.4	a	37.1 ± 0.6	b
Available P_2O_5 (mg kg^{-1} DM)	6.7 ± 1.4	b	2.4 ± 0.3	b	31.0 ± 2.7	a
pH (H_2O)	4.1 ± 0.1	b	4.1 ± 0.1	b	5.0 ± 0.1	a

and dry mass of each part (leaves, stems, and roots), and branching nodes were measured for all seedlings (8 seedlings for each soil type). One seedling was randomly selected from each pot and used for analysis of Ni and other inorganic elements. The four samples were dried separately for 48 h at 80°C, and then ground. The ground materials were pyrolyzed in concentrated HNO_3, and the inorganic elements (Ni, K, P, Ca, and Mg) in the samples were analyzed using ICP-AES. One seedling root from each pot was used for catechin analysis (Yamaji and Ichihara 2012, Yamaji et al. 2003). Fresh roots were cut into pieces with scissors in 100% methanol and extracted overnight in the dark. The extract was evaporated in vacuo and dissolved in 50% methanol (600 μL). Next, 10 μL of the concentrated sample was analyzed using high-performance liquid chromatography (HPLC; LC-20A series; Shimadzu, Kyoto, Japan), according to spectral characteristics by using a diode array detector (SPD-M20A; Shimadzu) at 220, 280, and 320 nm. The HPLC conditions were as follows: column, Mightysil RP-18 GP (75-mm length × 4.6-mm diameter; Kanto, Tokyo, Japan); eluent, aq. 1.5% tetrahydrofuran + 0.25% *O*-phosphoric acid (solvent A) and methanol (solvent B); flow rate, 1.0 mL min^{-1}; and temperature, 30°C. The following gradient was used for the eluent system: 0–5 min, 85% A and 15% B; 5–10 min, 70% A and 30% B; 10–20 min, 50% A and 50% B; 20–40 min, 50% A and 50% B; 40–50 min, 25% A and 75% B, and 50–60 min, 100% B. The dried sample was dissolved in 600 μL of 50% methanol, and 10-μL sample was used for HPLC analysis. The following phenolics in the seedling roots were analyzed: (+)-catechin (standard purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan) and (-)-epicatechin and totarol (standards purchased from Sigma-Aldrich, Tokyo, Japan). Each phenolic compound was quantified using a standard curve. Four replicate analysis results were averaged, and the amounts were expressed as $\mu g \cdot mg^{-1}$ FM (± standard error).

Statistical analyses

IBM SPSS Statistics software (Version 22 for Windows; IBM, Armonk, NY, USA) was used for statistical analyses. The heavy metal and nutrient concentrations among plant tissues of *T. dolabrata* var. *hondai* seedlings collected from our study site, as well as the differences in seedling growth; Ni, K, P, Ca, and Mg concentrations; and catechin content among Kadoma soil, Tsugaru forest soil, and nursery soil were evaluated using one-factor analysis of variance (ANOVA) by using Tukey test. Differences were considered significant at $P < 0.05$.

Results

Seedling growth and heavy metal and nutrient concentrations at the serpentine site

Seedling growth data (mean ± standard errors) were as follows: number of leaves, 10 ± 1.1; seedling height (mm), 105 ± 9.2; fresh mass of leaves (mg), 1720.1 ± 547.7; fresh mass of stems (mg), 730.5 ± 390.1, and fresh mass of roots (mg), 379.4 ± 93.9. Heavy metal and nutrient concentrations of the seedlings at our study site are shown in Table 2. All heavy metals except Pb (Fe, Mn, and Ni) showed the highest concentrations in roots of; there were no significant differences in the heavy metal concentrations among the any of the tissues (Table 2; all the values are marked by "a"). Lead was detected only in the roots (Table 2). Ni was higher in the roots (43.4 ± 27.3 mg kg^{-1} DM), compared with leaves and stems, of seedlings grown at our study site. Potassium was significantly higher in the leaves than in the stems and roots (Table 2). Phosphorus and calcium levels were the highest in the leaves, and their levels were significantly different from those in the stems and roots, respectively (Table 2). Magnesium concentration was the highest in the roots, and the concentration was significantly higher than that in the stems (Table 2).

Heavy metal concentrations of the root-zone soil

Total heavy metal concentrations of the root-zone soil (mean ± standard errors, mg kg^{-1} DM) were as follows: Fe, 17472.1 ± 5830.6; Mn, 141.3 ± 39.7; Ni, 111.0 ± 52.9; Pb, 90.4 ± 23.0. The concentrations of Ni (111.0 ± 52.9 mg kg^{-1} DM) and Pb (90.4 ± 23.0 mg kg^{-1} DM) were higher in the root-zone soil than in the agricultural soils of Japan (Ni, 26 mg kg^{-1} DM; Pb, 24 mg kg^{-1} DM; Takeda et al. 2004).

Seedling growth and concentrations of nutrients, Ni, and phenolics in the pot experiment

Seedling growth data for the pot experiment are shown in Table 3. There were no significant differences in seedling growth among the three soil types. Nutrient concentrations of the seedlings grown in the pot experiment are shown in Figure 1. They were not significantly different across the three soil types. Ni concentration of seedlings grown on Kadoma soil was higher than that of seedlings grown on Tsugaru forest or nursery soil, but the differences were not significant (Fig. 2). The amounts of K, P, Mg, Ca, and Ni in each plant part were calculated as mg element per plant. There were no significant differences between plants grown on the different soils for

Table 2. Heavy metal and macronutrient concentrations of seedlings growing at the serpentine site (mg kg^{-1} DM, n = 5). Results of five replicate analyses; results are shown as mean ± standard error. n.d.: not detected. Different letters indicate significant difference of each element concentration in different tissues at the 5% level.

	Leaves				Stems				Roots			
Fe	53.8	±	11.3	a	162.2	±	73.0	a	497.8	±	308.4	a
Mn	16.3	±	4.9	a	0.4	±	0.4	a	65.0	±	48.0	a
Ni	7.1	±	2.7	a	3.1	±	2.0	a	43.4	±	27.3	a
Pb	n.d.				n.d.				2.3	±	1.4	
K	5075.7	±	362.7	a	1959.7	±	154.1	b	2166.2	±	341.6	b
P	1390.9	±	69.0	a	657.6	±	90.5	b	1007.7	±	192.3	ab
Ca	10967.7	±	1221.6	a	7797.2	±	809.1	ab	4570.0	±	645.0	b
Mg	1029.2	±	125.9	ab	396.2	±	66.6	b	1122.5	±	296.1	a

Table 3. Seedling growth in the pot experiment. Results of eight replicate analyses for height, root length, and fresh mass are shown as mean ± standard error. Results of four replicate analyses for dry mass are shown. Different letters indicate significant difference of each element concentration in different tissues at the 5% level. There were no significant differences in these parameters among the three soil types ($p > 0.05$).

	Soil type											
	Kadoma soil				Tsugaru Forest soil				Nursery soil			
Height (mm)	13.5	±	2.3	a	19.0	±	2.0	a	18.5	±	2.5	a
Root length (mm)	75.3	±	21.3	a	40.4	±	7.2	a	52.5	±	15.4	a
Fresh mass (mg)												
Leaves	12.5	±	1.7	a	16.6	±	2.4	a	15.6	±	3.2	a
Stems	4.1	±	0.3	a	3.9	±	0.3	a	4.6	±	0.5	a
Roots	8.7	±	2.3	a	6.8	±	1.3	a	9.0	±	3.0	a
Dry mass (mg)												
Leaves	3.4	±	0.8	a	3.5	±	0.7	a	4.2	±	1.4	a
Stems	0.9	±	0.2	a	0.8	±	0.1	a	1.0	±	0.2	a
Roots	1.7	±	0.6	a	1.3	±	0.3	a	1.6	±	0.7	a
Branching node (per plant)	2.3	±	0.8	a	1.3	±	0.6	a	1.9	±	0.6	a

any element or any plant part (data not shown). Because Ni concentration tended to be the highest in the roots, phenolic analyses of the roots were conducted to determine the possible detoxification mechanism of Ni. Phenolic analysis showed that in the roots of seedlings grown on Kadoma soil, the (+)-catechin concentration was significantly higher than in the roots of seedlings grown on the two other soil types (Fig. 3). In contrast, totarol and (-)-epicatechin were not detected in any seedlings; this could be because their values were lower than the detection limit of HPLC analysis.

Discussion

Sensitive plant species show toxicity at less than 10 mg kg^{-1} Ni in plant tissues (Broadley et al. 2012). For example, Ni content in the leaves of *Cryptomeria japonica*, which belongs to the same family as *T. dolabrata* var. *hondai*, was 3.0 mg kg^{-1} DM (Memon et al. 1980). One of the abundant species in Japan, pine trees (*Pinus* spp.) contain Ni at concentrations of 1.1 mg kg^{-1} in the roots of 1 mm diameter (Kabata-Pendias 2011). In general, the growth of needles is decreased by the accumulation of Ni. Kayama et al. (2006) performed an experiment by using serpentine soil and showed that the dry mass of *Picea jezoensis* needles was suppressed due to Ni accumulation (14.67 mg kg^{-1} DM in needles), and that the photosynthetic rate of the needles was suppressed at Ni concentration of 17.6 mg kg^{-1} in needles. Moderately tolerant species are known to tolerate 50 mg kg^{-1} Ni (Broadley et al. 2012). In contrast, Ni hyper-accumulation of 3,180 mg kg^{-1} Ni in the shoots and 1,610 mg kg^{-1} Ni in the roots was observed in a tolerant species, *Thlaspi goesingense*, which natively grows on serpentine soil (Wenzel et al. 2003).

At our study site, *T. dolabrata* var. *hondai* seedlings, especially roots, tended to accumulate high concentrations of Ni (Table 2), which was comparable to the bearable limit of moderately tolerant species described above. Thus, *T. dolabrata* var. *hondai* can be considered a moderately Ni-tolerant species. Moreover, the pot experiment yielded the same results, because *T. dolabrata* var. *hondai* seedlings accumulated higher concentrations of Ni when grown in Kadoma soil than when grown in Tsugaru forest soil or nursery soil, without showing inhibited seedling growth (Table 3) and nutrient uptake (Fig. 1). In addition, the calcium concentration was tended to be

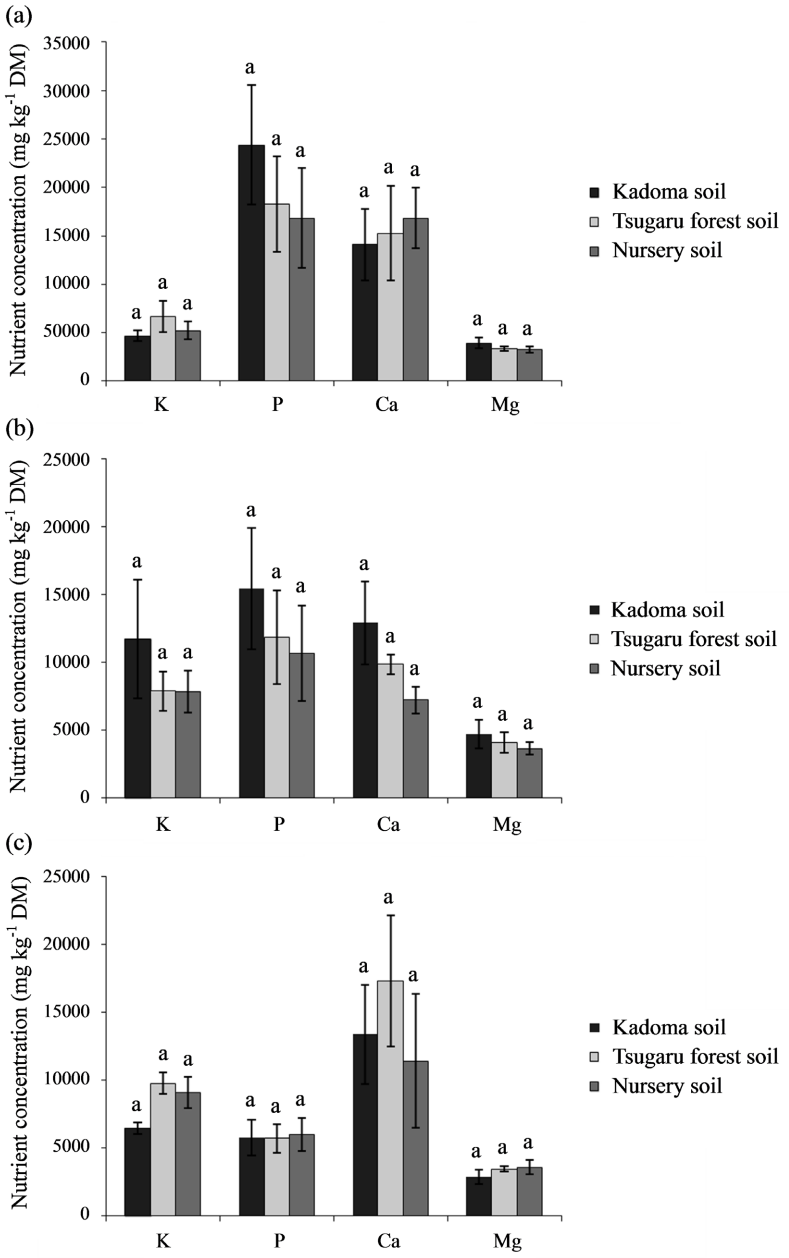

Fig. 1. Nutrient concentrations of seedlings in each soil type. (a) Leaves, (b) stems, and (c) roots. Results of four replicate analyses are shown as mean ± standard error. Different letters indicate significant difference of each element concentration in different tissues at the 5% level. There were no significant differences among the three soil types.

considerably high, especially in the leaves of *T. dolabrata* var. *hondai* seedlings growing at our study site (Table 2). In general, calcium is known to compete with Ni for calcium channels, resulting in decreased Ni absorption and Ni toxicity alleviation in plant roots (Seregin and Kozhevnikova 2006, Ehlken and Kirchner 2002). Endemic plant species growing in serpentine soil, such as *Alyssum bertolonii*, accumulate Ca in the roots in order to decrease Ni absorption (Gabbrielli and Pandolfini 1984). Therefore, the growth of *T. dolabrata* var. *hondai* seedlings might be maintained by the selective translocation of calcium from the roots to shoots. However, further studies are warranted to investigate the involvement of calcium against Ni toxicity in *T. dolabrata* var. *hondai* seedlings.

In general, serpentine soil contains high concentrations of exchangeable Mg (Alexander et al. 2007); however, Kadoma soil did not show a high concentration of Mg (Table 1). The Kadoma soil was collected from the forest edge that contained organic matter at the surface. Therefore, the organic matter in Kadoma soil might have affected the exchangeable Mg concentration (Rajakaruna et al. 2009).

Our pot experiments indicated that catechin and Ni concentrations tended to be the highest in the roots of seedlings grown on Kadoma soil (Figs. 2, 3). Phenolics, including catechin, can scavenge reactive oxygen species (e.g., hydrogen peroxide), which are produced by metal ions, and thereby control heavy metal toxicity (Michalak 2006). In particular, catechin and flavonoids have been shown to have antioxidant

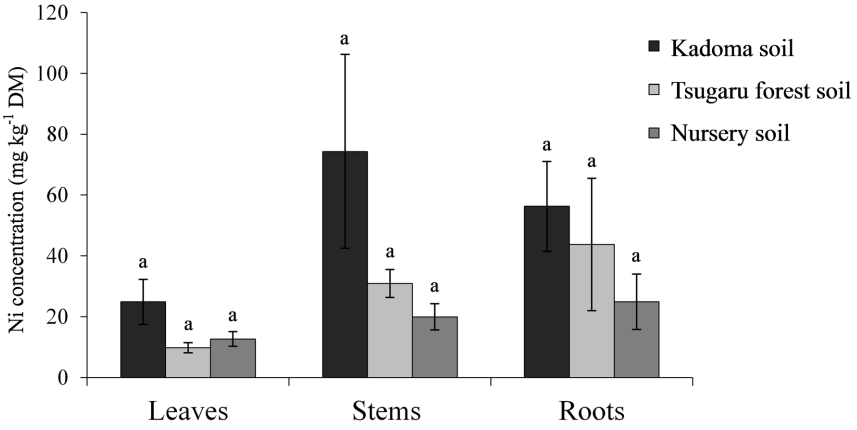

Fig. 2. Ni concentration of seedlings in each soil type. Results of four replicate analyses are shown as mean ± standard error. Different letters indicate significant difference of each element concentration in different tissues at the 5% level. There were no significant differences among the three soil types.

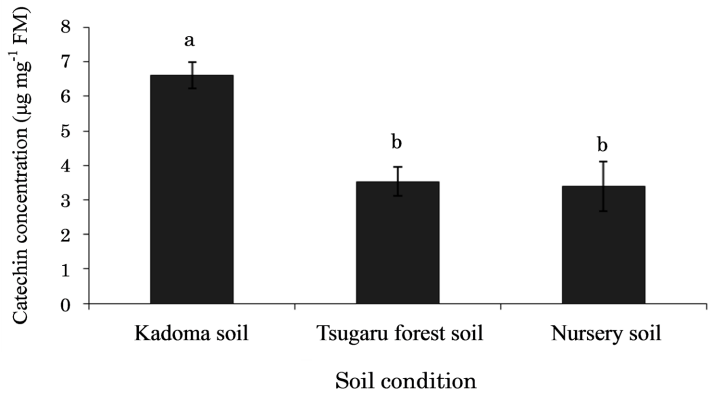

Fig. 3. Catechin concentration of the seedling roots in each soil type. Results of four replicate analyses are shown as mean ± standard error. Different letters show significant difference at the 5% level.

activities against various oxidizable compounds (Larson 1988, Rice-Evans et al. 1996). Catechin produced in *T. dolabrata* var. *hondai* seedling roots might act as an antioxidant and decrease Ni toxicity by scavenging reactive oxygen species. Additionally, phenolics directly reduce heavy metal toxicity by chelating and compartmenting heavy metals in vacuoles (Larcher 2003). Although catechin has not yet been reported to form complexes with Ni^{2+}, according to its partial structural similarity to quercetin, catechin might be able to chelate Ni. Catechin and quercetin have two aromatic rings that are linked by an oxygen-containing heterocycle. Quercetin possesses three possible chelating sites for metal ions, including Ni, Zn, Cu, and Al: 3-hydroxy-carbonyl, 5-hydroxy-carbonyl, and 3′,4′-dihydroxyl (catechol). Further, it is known to chelate Ni at a mole ratio of 2:1 *in vitro* (Ni^{2+}:quercetin; Kakavand et al. 2012). Catechin also possesses a catechol function; thus, it might be able to chelate Ni in a similar manner as quercetin. Catechin production might protect the *T. dolabrata* var. *hondai* seedlings grown on Kadoma soil by reducing Ni toxicity. This is also supported by the fact that there was no significant difference in seedling growth (Table 3) and nutrient concentrations (Fig. 1) among the three soil types, despite the high accumulation of Ni (Fig. 2, Table 2).

Compared with the Ni concentration of *Betula ermanii*, which shows high Ni tolerance on serpentine soil (Kayama and Koike 2015), Ni concentration of *T. dolabrata* var. *hondai* grown on Kadoma soil was considerably low (Fig. 2), but was high enough to produce Ni toxicity in plant cells (Ni toxicity threshold level in sensitive plants, >10 mg kg^{-1} Ni; Broadley et al. 2012). We propose two mechanisms by which *T. dolabrata* var. *hondai* plants growing at our study site tolerate high concentrations of heavy metals: (1) exclusion strategy via catechin release to the root zone, and (2) detoxification strategy via the formation of catechin-Ni complexes in plant cells. In this study, we provided possibility for the second mechanism; however, studies involving the growth of *T. dolabrata* var. *hondai* in hydroponic culture condition to directly detect catechin as root exudates are necessary.

In conclusion, our findings indicate that the roots of *T. dolabrata* var. *hondai* growing in the serpentine site of Mt. Hayachine accumulate Ni and might be able to detoxify Ni by increasing the absorption of Ca and producing catechin. In the future, chemical Ni-detoxification mechanisms need to be elucidated to determine how *T. dolabrata* var. *hondai* seedlings grow naturally in the serpentine site.

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