Distribution of Growth-inhibitory Activity, Mineral Contents, and Functional Components in Different Tissue Parts of Asparagus (*Asparagus officinalis* L.) and Availability of Unusable Parts

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Abstract. Asparagus is a popular vegetable rich in healthy functional components. However, the process of its production leaves ferns from aboveground parts and roots from underground parts as unusable parts, and this is an issue to be resolved. In our previous studies, large amounts of rutin were noted in the cladophylls and storage roots (brown and epidermis), and the protodioscin content was high in buds, in the soil-covered section of spears, and in rhizomes. This study was conducted to examine the distribution of growth-inhibitory activity and mineral contents in different parts of asparagus. Correlations, including representative functional components (rutin and protodioscin), were examined. The results suggest there are differences in growth-inhibitory activity of different parts of asparagus. The growth-inhibitory activity was strong in the buds, rhizome, and absorptive and storage roots, and weak in the cladophylls and lateral branches. The percent N content of the aboveground part of asparagus was high compared with that in the aboveground part of other crops. Although the percent K content was similar to the mean of the aboveground part of other crops, it was higher than that in general green manure, suggesting the residual stems and leaves of the aboveground part of asparagus are effective green manure. In the aboveground part of asparagus, the rutin content and percent N and K content were higher, whereas growth-inhibitory activity tended to be low, suggesting that when no disease developed in the aboveground part, it can be used as an organic substance.

Asparagus (*Asparagus officinalis* L.) is a perennial plant belonging to the Asparagaceae family and a popular vegetable cultivated and

consumed in many different areas of the world (Benson, 2012). Asparagus is rich in healthy functional components and can be divided easily into edible and inedible parts. Asparagus spears are known to contain a large amount of rutin, which has been found to have anti-inflammatory, antitumor, and antibacterial/antiviral properties; and protodioscin, which is an antitumor substance, is present in the bottom parts (8 cm from the cut end) (Brueckner et al., 2010; Chin et al., 2002; Lee et al., 2010; Maeda et al., 2005, 2012; Schwarzbach et al., 2006; Wang et al., 2003). However, its production process leaves ferns from the aboveground parts and roots from the underground parts as

large amounts of unusable parts, and this is an issue to be resolved. In our previous study, large amounts of rutin were noted in the cladophylls and storage roots (brown and epidermis), whereas the protodioscin content was high in buds, in the soil-covered section of spears, and in the rhizome (Motoki et al., 2019).

Few reports are available on the active use of the cladophylls and storage roots as useful functional components or resources. In our previous studies, the functional components of asparagus in different cultivation conditions showed that rutin was found only in green asparagus, and protodioscin was found only in white asparagus cultivated using the blanching-with-soil method (Motoki et al., 2012b). However, the cladophylls of asparagus cultivated in hydroponic culture in a closed cultivation system contained both rutin and protodioscin (Motoki et al. 2012b). Fresh asparagus spears are commonly discarded if their length is out of specification. However, spears that have not been harvested at the optimum time also contain a large amount of rutin and may be useful components or resources of rutin (Motoki et al., 2012a). In addition, although a significant amount of rutin was detected in the aboveground parts, which is consistent with the results of previous studies (Motoki et al., 2012b), it was also found in the storage roots (Motoki et al., 2019). The largest amount of protodioscin was found in the buds as well as in young fruit and seeds among the aboveground parts (Motoki et al., 2019). However, the influences of the cultivation method and environmental conditions on the levels of rutin and protodioscin have not been clarified.

Unlike cladophylls, strong growth-inhibitory activity was observed in the storage roots (Motoki et al., 2006c). In our previous study, we developed a rhizosphere soil bioassay method, and reported that it helped efficiently assess growth-inhibitory activity of asparagus at the laboratory level (Motoki et al., 2006a). Motoki et al. (2002) examined the effect of the flowable agent in activated C (herein referred to as “activated C F”) to mitigate allelopathy in asparagus. The results of tests on lettuce as an assay plant and 1-year-old asparagus showed that activated C F acts to mitigate allelopathy and promote growth in asparagus. This material facilitates the efficient use of activated C materials and the provision of advice on appropriate cultivation methods, and it is a material that reduces inhibitory activity (Motoki et al., 2006a, 2006b).

The unusable parts of asparagus with weak growth-inhibitory activity can be used for their mineral contents or reused as fertilizer components the following year. Furthermore, if the cladophylls and storage roots or unusable parts of asparagus can be used as useful component resources, this may not only reduce the amount of waste or plant residue generated during production, but also may help to mitigate the replant problem

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(Motoki et al., 2006c), which will greatly impact the production setting.

Most previous studies on the mineral contents of asparagus have analyzed spears (Amaro-Lopez et al., 1996; Casas and Nunez, 2001; Shalaby et al., 2002; Takahashi et al., 2019). Because root, main stem, fern, fruit, and so forth have not been analyzed separately, the mineral contents in each part of asparagus remain unclear. These contents should be investigated to facilitate the use of asparagus as a fertilizer. In Japan, there have been some cases when production areas that did not implement the correct measures were destroyed. If all storage roots are removed from the cultivated field at the time of replanting, it is possible to avoid continuous crop damage, but there is no established method for detoxifying storage roots or their use. It is necessary to solve the issue of large amounts of unusable parts that are generated during the time of summer growth and post-harvest residues. Although only a small portion of the cladophylls of asparagus harvested in the autumn are used as cladophylls powder, most of the rest is put back into the soil or disposed of. The amount is estimated to be $\approx 130,000$ t annually in Japan (Motoki et al., 2019). If the unusable parts of asparagus can be retained as useful resources for soil improvement and fertilization, the cultivation cost of asparagus can be reduced.

Despite several studies (Hartung et al., 1990; Lake et al., 1993; Young and Chou, 1985), no growth-inhibitory substances involved in the replant problem of asparagus have been identified. Rutin, a functional component of buckwheat, was identified as a growth-inhibitory substance (Golisz et al., 2007). Rutin is also abundant in asparagus, suggesting that it may be a growth-inhibitory substance of asparagus (Motoki et al., 2019). However, the relationship between rutin and protodioscin as representative functional components of asparagus, and growth-inhibitory activity, are unknown.

Our current study aimed to examine whether the unusable parts of asparagus to be discarded can be retained as useful resources. Therefore, we analyzed comprehensively growth-inhibitory activity and mineral contents of different parts of field-grown asparagus to reveal their correlations with functional components. Based on our results, we examined the possibility of using the unusable parts of asparagus.

Materials and Methods

Cultivation method

The cultivation method was conducted in accordance with our previous study (Motoki et al., 2019). The tested material was 6-year-old 'UC157 F₁' cultivated in a long-term harvest production system in Iijima-machi, Kami-ina-gun, Nagano Prefecture. 'UC157 F₁'—a cultivar of green asparagus—is the most common cultivar in Japan, with a short dormant period and high yield. The cultivation conditions were as follows: lat. 36°N, long. 138°E; elevation, 720 m above sea

level; alluvial clay loam; pH 6.0; electrical conductivity, 0.1 dS·m⁻¹; and humus content, 3.3%. The planting pattern was a single row, with a row width of 150 cm, a bed width of 80 cm, and a 30-cm in-row distance (22,222 plants/ha). Chemical fertilizer (200N–200P–200K) was applied preplanting, before the permanent planting of 1-year-old plants, and before the sprouting of 2- to 6-year-old plants. Supplementary application (50N–50K) was provided every 2 weeks from June to August every year. Plants were watered only by rainfall. To prevent lodging, the plants were supported by ridging, the stems were supported with stakes, and the lower branches were cut back to 50 cm from the ground. All the other cultural practices followed the standard procedure of Nagano Prefecture (Motoki, 2003; Motoki et al., 2008).

Samples

The samples required for each experiment were taken from 26 different plant parts of 6-year-old asparagus (Table 1) in accordance with a previous study (Motoki et al., 2019), and were frozen immediately at -40°C , except for samples used for water content investigation.

Growth-inhibitory activity

The experiment was conducted in accordance with the rhizosphere soil bioassay method (Motoki et al., 2006a) in which 0.02 g powdered sample was placed in six-hole tissue culture multidishes (Nunc Multidish 6; Thermo Fisher Scientific, Tokyo, Japan), and 5 mL 0.75% (w/v) autoclaved (121°C , 20 min; SD–321, Tomy Seiko, Tokyo, Japan) cold-gel agar (gelation temperature, 30 to 31°C ; Nacalaitesque, Kyoto, Japan) and 5 mL agar were added to each of the powdered samples to solidify them. An area consisting solely of 10 mL (5 mL + 5 mL) of agar was also prepared as a control. In addition, the seeds of 'Red Fire', a species of lettuce commonly used to examine growth-inhibitory activity, was sown on multidishes. The dishes were covered with lids, sealed using parafilm, and stored in an incubator (25°C , dark, NK System Biotron LPH200; Nippon Medical & Chemical Instruments, Osaka, Japan) for 72 h before measuring the lengths of the radicles and hypocotyls of the lettuce. Five seeds were sown, and this was repeated four times in each plot. The growth-inhibitory activity was calculated using the following formulas:

$$\text{Growth} - \text{inhibitory activity (\%)} =$$

$$(1 - X_t/X_c) \times 100, \quad [1]$$

where X_t is the length of lettuce radicles or hypocotyls with asparagus tissue and X_c is the length of lettuce radicles or hypocotyls in controls.

Mineral content

Mineral content analysis was conducted in accordance with the crop analysis method for nutrition diagnosis (Crop Analysis Method Editorial Committee, 1975).

Analytical samples were subjected to wet ashing digestion using the nitric acid-perchloric acid method; P was analyzed using the vanadomolybdate method; K, Ca, and Mg were analyzed using atomic absorption analysis; and N was analyzed using a CN coder (JM1000CN; J–Science, Kyoto, Japan).

Wet ashing digestion with nitric acid-perchloric acid. Wet ashing digestion was conducted in accordance with the crop analysis method for nutrition diagnosis (Crop Analysis Method Editorial Committee, 1975; Miller, 1998; Zasoski and Bureau, 1977). A 1000-mg powdered sample was weighed in a 200-mL conical beaker, combined with 30 mL nitric acid-perchloric acid mixed solution using a dispensing pipette, covered with a watch glass, and placed on an electric griddle. Heating started with a low flame (100°C). At the beginning of decomposition, the sample reacted with the mixed solution and generated yellow gas. Organic substances were decomposed while foaming vigorously. After foaming ended, the temperature was elevated gradually to 200°C to promote decomposition. With continuation of decomposition, the solution gradually became colorless and transparent, and generated white smoke over time. Decomposition was continued in this state and stopped immediately before the sample dried and hardened after removing the watch glass. After cooling, the watch glass that covered the beaker was washed by spraying with hot water. The wash solution was added to the beaker. Adhering components were dissolved by rubbing the inner wall of the beaker with a glass rod attached to a rubber tube at the tip while washing off with hot water, then filtered into a 100-mL volumetric flask. In addition, the residue on the filter paper was washed sufficiently with hot water to collect minerals content completely.

Atomic absorption analysis. Analysis of K was conducted in accordance with the crop analysis method for nutrition diagnosis (Crop Analysis Method Editorial Committee, 1975; David, 1960). Potassium chloride was dried at 105°C for several hours, 1.9067 g was dissolved with water, and the volume was adjusted to 1 L to prepare a standard solution containing 1000 ppm K. Analysis of Mg was conducted according to the crop analysis method for nutrition diagnosis (Crop Analysis Method Editorial Committee, 1975; David, 1958). Special-grade Mg metal, 0.1000 g, was measured precisely and dissolved in dilute hydrochloric acid, and the volume was adjusted with water to 1 L (100 ppm Mg). Analysis of Ca was conducted in accordance with the crop analysis method for nutrition diagnosis (Crop Analysis Method Editorial Committee, 1975; Giang et al., 2004). For the Ca standard solution, special-grade calcium carbonate was dried at 110°C , 0.2497 g was measured precisely and dissolved with a small amount of hydrochloric acid, and the volume was adjusted to 1 L with water to 1 L (100 ppm Ca). K, Mg, and Ca standard solutions for atomic absorption analysis were diluted with 1% hydrochloric acid solution, and 2.0- to 10.0-, 0- to 1.0-,

Table 1. Differences in growth-inhibitory activity, mineral contents, functional components, and water content among different asparagus plant parts.

	Sampling time	Inhibition rate (%)		Mineral contents (%DW)					Rutin content ^z (mg·g ⁻¹ DW)	Protodioscin content ^z (mg·g ⁻¹ DW)	Water content ^z (%)
		Radicle	Hypocotyl	N	P	K	Ca	Mg			
Aboveground parts											
Cladophylls	10 June 2012	28.3 f ^y	19.6 c–e	3.4	0.28	2.2	0.71	0.19	20.456 a	ND	71.9 j ^z
Primary lateral branch	10 June 2012	51.1 d–f	2.6 e	1.9	0.19	2.5	0.21	0.08	2.928 de	0.026 f	77.1 i
Second lateral branch	10 June 2012	44.7 ef	17.6 c–e	2.2	0.18	2.2	0.35	0.10	5.655 c	0.027 f	70.9 j
Main stem (whole)	10 June 2012	52.8 d–f	–4.2 e	1.7	0.23	3.3	0.07	0.05	2.431 ef	0.027 f	83.5 f–h
Main stem (cortex)	10 June 2012	64.3 b–f	5.7 e	1.6	0.26	3.5	0.09	0.06	ND	0.033 f	86.7 c–f
Main stem (epidermis)	10 June 2012	27.5 f	–1.7 e	1.1	0.09	1.8	0.09	0.06	9.718 b	0.046 f	63.4 k
Male flower	27 May 2012	60.3 c–f	11.6 de	4.5	0.63	3.9	0.40	0.25	4.095 cd	0.088 f	85.2 d–g
Female flower	27 May 2012	NT	NT	NT	NT	NT	NT	NT	NT	NT	84.9 e–g
Young fruit	25 June 2012	82.1 a–d	66.3 a–d	3.2	0.48	2.6	0.25	0.18	1.889 e–g	3.792 c	88.3 c–e
Ripe fruit	5 Sept. 2012	42.1 ef	22.4 b–e	2.6	0.61	2.6	0.05	0.13	NT	NT	54.2 l
Seed ^x	21 May 2012	40.5 ef	26.9 b–e	3.6	0.44	0.6	0.03	0.18	ND	5.487 b	6.5 m
Spear											
Tip of spear (harvest in spring)	2 May 2012	88.1 a–c	82.1 a	6.3	1.02	3.7	0.27	0.17	3.160 de	ND	89.6 bc
Tip of spear (harvest in summer–autumn)	25 June 2012	89.3 ab	83.5 a	6.1	1.00	4.1	0.12	0.17	NT	NT	91.9 ab
Base of spear (harvest in spring)	2 May 2012	79.7 a–d	64.6 a–d	4.0	0.63	3.2	0.25	0.15	0.766 gh	0.061 f	93.2 a
Base of spear (harvest in summer–autumn)	25 June 2012	82.6 a–d	67.3 a–c	2.8	0.49	3.0	0.05	0.09	NT	NT	93.5 a
Scale of spear (harvest in spring)	11 May 2012	NT	NT	NT	NT	NT	NT	NT	NT	NT	85.6 d–g
Epidermis of spear (harvest in spring)	9 May 2012	NT	NT	NT	NT	NT	NT	NT	NT	NT	89.5 bc
Cortex of spear (harvest in spring)	9 May 2012	NT	NT	NT	NT	NT	NT	NT	NT	NT	93.3 a
Spears covered with soil	10 June 2012	72.3 a–e	51.0 a–e	1.3	0.28	3.4	0.10	0.05	ND	3.870 c	82.5 gh
Underground parts											
Buds	10 June 2012	92.3 a	88.8 a	2.7	0.44	3.4	0.33	0.14	0.631 gh	7.514 a	84.7 fg
Rhizome	10 June 2012	88.4 a–c	83.9 a	1.6	0.62	5.9	0.69	0.11	ND	3.022 d	79.8 hi
Storage root (white)	10 June 2012	80.9 a–d	65.7 a–d	4.0	0.57	3.7	0.40	0.11	0.243 h	1.141 e	89.9 bc
Storage root (yellow)	10 June 2012	85.2 a–c	73.8 ab	3.1	0.47	2.9	0.44	0.15	1.116 f–h	1.509 e	89.7 bc
Storage root (brown)	10 June 2012	84.8 a–c	70.6 a–c	2.7	0.29	2.2	0.58	0.17	3.008 de	0.942 e	88.4 cd
Storage root (epidermis)	10 June 2012	64.3 b–f	23.1 b–e	1.5	0.31	0.7	0.48	0.18	3.242 de	0.028 f	63.7 k
Absorptive root	10 June 2012	89.8 ab	82.6 a	1.6	0.62	5.9	0.69	0.11	NT	NT	NT

^zAccording to a previous study by the authors (Motoki et al., 2019).^yAccording to the Tukey-Kramer test, any two means within a column not followed by same letter are significant at $P < 0.05$ ($n = 3-6$). Statistical analysis was conducted after arcsine conversion of water content.^xSeeds produced in 2011; freeze-dried on 21 May 2012.

DW = dry weight; ND = not detectable; NT = not tested.

and 0- to 20.0-ppm standard solutions for the construction of calibration curves were prepared. The absorbance of the sample solution was measured using an atomic absorption spectrophotometer (AA-7000; Shimadzu, Kyoto, Japan), and its concentration was determined from the calibration curve prepared beforehand. The measurement wavelength of K was set at 766.5 nm. A 1% hydrochloric acid sample solution was used as a sample solution for measurement without dilution. The measurement wavelength of Mg was set at 285.2 nm. Strontium chloride solution added to the 1% hydrochloric acid sample solution with a strontium concentration adjusted to 0.5% was used as a sample solution for measurement. The measurement wavelength of Ca was set at 422.7 nm. To a 50-mL volumetric flask, 1 mL sample solution was added, then strontium solution was added, followed by a constant volume with 1% hydrochloric acid solution, which was used as a sample solution for measurement. The sample solution was aspirated and sprayed using a nebulizer, and was introduced into an acetylene-air flame using an atomic absorption spectrophotometer. The K, Mg, and Ca contents in the sample solution were calculated using the following formula:

$$\text{K, Mg, and Ca contents (mg} \cdot 100 \text{ g}^{-1}) =$$

$$(A \times V \times d) / (W \times 1000) \times f \times 100 \quad [2]$$

where A is the K, Mg, or Ca concentration in the sample solution for measurement was determined from the calibration curve (measured in micrograms per milliliter), V is the amount of sample solution (measured in milliliters), d is the dilution factor, f is the factor of standard solution, W is the amount of collected sample (measured in grams).

Colorimetry using the vanadomolybdate method. Analysis of P was conducted in accordance with the crop analysis method for nutrition diagnosis (Crop Analysis Method Editorial Committee, 1975; Quinlan and DeSesa, 1955; Sunaga et al., 2012). Into a color comparison tube with a 50-mL volume, 1 mL sample solution of P was measured within a range of 35 mL and combined with water, adjusting the volume to about 35 mL, to which 5 mL nitric acid solution and then 5 mL ammonium metavanadate solution was added and stirred well. Last, 5 mL ammonium molybdate solution was added, and the solution was stirred to be colored, followed by a constant volume with 50 mL of water. After being left for 5 min or longer, colorimetric determination at 430 to 460 nm was performed using a spectrophotometer (V630; JASCO Corporation, Tokyo, Japan).

Analysis of N. Analysis of N was conducted in accordance with the crop analysis method for nutrition diagnosis (Crop Analysis Method Editorial Committee, 1975; Jimenez and Ladha, 1993). The total N concentration was measured using a CN coder. A dried-powder sample was measured precisely using an electronic balance and transferred carefully into a nickel boat. The calibration curve was prepared with 40 to 80 mg hippuric acid (N, 7.82%) for analysis of the standard substance of N using a four-point calibration curve method within a range of high linearity.

Statistical analysis

Tukey-Kramer tests were performed using Statcel software (version 2; OMS Publishing, Saitama, Japan) to identify significant differences ($P < 0.05$) in growth-inhibitory activity and mineral contents of different plant parts.

Results and Discussion

Distribution of growth-inhibitory activity

Tang and Motoki (2018) stored powdered samples of the same asparagus parts as this study prepared in 2015 at 25°C for 2 years, growth-inhibitory activity was reevaluated in 2017, and this was used in the activated C

Fig. 1. Relationship of growth-inhibitory activity of analytical samples between 2015 and 2017. (A) Radicle. (B) Hypocotyl inhibition rate. ******Significant at $P < 0.01$.

addition test. Regarding the correlation of growth-inhibitory activity of the analytical samples between 2015 and 2017, the radicle inhibition rate was $r = 0.89$ (Fig. 1A), and the hypocotyl inhibition rate was $r = 0.89$ (Fig. 1B), showing a significant positive correlation between 2015 and 2017. Therefore, the discussion in this study was based on the results of the analytical sample created in 2015 because the samples were prepared at a time point close to the sampling time point.

Table 1 shows differences in growth-inhibitory activity among different parts of asparagus. The growth inhibition rates for different parts of lettuce were calculated to examine growth-inhibitory activity of asparagus, and the rates for the radicles and hypocotyls of lettuce were 27.5% [main stem (epidermis) of aboveground parts] to 92.3% (buds of underground parts) and -4.2% [main stem (whole) of aboveground parts] to 88.8% (buds of underground parts). The rate for the radicles was greater than that for the hypocotyls. There were differences in growth-inhibitory activity of different parts of asparagus. The growth-inhibitory activity was strong in the buds, rhizome, absorptive and storage roots, and spears, and weak in the cladophylls, lateral

branches, and main stem. These results are consistent with those of previous studies (Motoki et al., 2006c; Tang and Motoki, 2018), which suggested that growth-inhibitory activity is strong in the underground parts and weak in the aboveground parts. The underground parts, such as the storage roots and rhizome of asparagus, are considered to contain larger volumes of growth-inhibitory substances because the underground parts have stronger growth-inhibitory activity than the aboveground parts. Regarding the spear aboveground parts, growth-inhibitory rates of the radicles and hypocotyls were high: 79.7% for the base of the spear (harvest in spring) to 89.3% for the tip of spear (harvest in summer-autumn), and 64.6% for the base of spear (harvest in spring) to 83.5% for the tip of spear (harvest in summer-autumn). Regarding the buds, growth-inhibitory rate of the radicles was the highest (92.3%), followed by that of the hypocotyls (88.8%). Because growth-inhibitory activity was also strong in the tips of the spears and the buds, the tissues involved directly in sprouting may contain large volumes of growth inhibitors.

Distribution of mineral contents

Table 1 shows differences in the mineral contents among different parts of asparagus. In the underground parts, percent Ca and Mg content in the storage roots increased in old brown roots, whereas percent N, P, and K content tended to increase in new white roots. Because the sugar content of asparagus increases in old storage roots (Haynes, 1987), the percent N, P, and K content may have tended to decrease relatively, and these may have been transferred quickly as a result of absorption compared with Ca and Mg. In a comparison among main stem, primary lateral branch, secondary lateral branch, and cladophylls, percent P and K content tended to be greater in the main stem, whereas percent N, Ca, and Mg content tended to increase as the region progressed farther from the main stem. In the main stem, percent N, P, and K content in the cortex tended to be greater than that in the epidermis. Percent N, P, K, Ca, and Mg content in spears harvested in the spring and that harvested in the summer-autumn tended to be greater in the tip than in the base. Mineral contents are concentrated more efficiently at the tip of the spear than at the base of the spear (Amaro-Lopez et al., 1996; Moreno-Rojas et al., 1992). Thus, our study supports the findings of previous ones. Regarding the water content of the spears, the content in the base was equivalent to or significantly greater than that in the tip in both those harvested in the spring and the summer-autumn. Percent P content was greater in ripe fruit than in young fruit, but percent N, Ca, and Mg content tended to be less in ripe fruit than in young fruit.

Nitrogen and Mg are termed leaf fertilizers, and these are components required in the period during which the plant is young or stems and leaves are developing (FAO, 1984). Nitrogen and Mg are also components of chlorophyll and increase the green color of leaves, suggesting that N and Mg concentrations increased in the tip of the fern. Phosphorus is termed a top-dressing at the ripening stage. Because it accumulates in fruit from the surrounding ferns with the ripening of fruit (FAO, 1984), the P concentration may have decreased in the tip of the fern on which fruit were growing. Phosphorus is essential to ensure root spread and the growth of leaves during the early growth period. For example, in strawberry, about 1 to 2 lb (0.5–0.9 kg) is absorbed per 1 t of fruit yield (Tagliavini et al., 2004). During fruit growth, the P concentration decreases in the other organs, promoting translocation to the fruit, and, finally, $\approx 40\%$ of P is incorporated into strawberries accumulated in the fruit (Tagliavini et al., 2004). In our study, percent P content was greater in ripe fruit than in young fruit, suggesting that P accumulated in the fruit with ripening. Potassium is termed a root fertilizer, and it promotes mainly root development (FAO, 1984), suggesting that the concentration of K was greater in the main stem close to the root than in the tip of the fern. Percent N content of the aboveground part of

Fig. 2. Relationship between the rutin content and growth-inhibitory activity. (A) Radicle. (B) Hypocotyl inhibition rate. DW, dry weight; n.s., not significant; **, significant at $P < 0.01$.

asparagus was high compared with that in the aboveground part of other crops. Although percent K content was similar to the mean of the aboveground parts of other crops, it was greater than that in general green manure, suggesting that the residual ferns of the aboveground parts of asparagus generated every year are effective green manure (Müller and Sundman, 1988).

Correlation between growth-inhibitory activity, mineral contents, and functional components

Table 2 shows the correlations among growth-inhibitory activity, mineral contents, functional components, and water content among different parts of asparagus. The results show that in the evaluation of growth-inhibitory activity, a significant positive correlation was noted between the radicle inhibition rate and hypocotyl inhibition rate ($r = 0.87$). Regarding the relationship between growth-inhibitory activity and mineral contents, significant positive correlations of the radicle inhibition rate with P and K were also noted, with $r = 0.57$ and $r = 0.56$,

respectively. Significant positive correlations of the hypocotyl inhibition rate with N, P, and K were noted, with $r = 0.47$, $r = 0.64$, and $r = 0.46$, respectively. In the relationship between growth-inhibitory activity and functional components, a significant negative correlation was noted between radicle inhibition rate and rutin content ($r = -0.66$) (Fig. 2A), whereas no significant correlation was noted between hypocotyl inhibition rate and rutin content ($r = -0.38$) (Fig. 2B). On the other hand, a significant positive correlation was noted between hypocotyl inhibition rate and protodioscin content ($r = 0.49$), but not between radicle inhibition rate and protodioscin content ($r = 0.34$). Regarding the relationship between growth-inhibitory activity and water content, a significant positive correlation was noted between radicle inhibition rate and water content ($r = 0.64$). In accordance with our previous study, the rutin contents in white, yellow, and brown storage roots were 0.243, 1.116, and 3.008 $\text{mg} \cdot \text{g}^{-1}$ dry weight, respectively, demonstrating that rutin content increases as the asparagus stocks age (Motoki et al., 2019). The radicle inhibition rate was slightly greater in brown and yellow storage roots (84.8% and 85.2%,

respectively) than in white storage roots (80.9%), but the difference was not marked compared with the difference noted in rutin content. No clear relationship was observed between growth-inhibitory activity and contents of the functional components, rutin and protodioscin, suggesting that not only the rutin content but also the other conditions, such as P (involved in the early growth period and root spread), K (mainly promotes root development) (FAO, 1984), and water content involved in the underground parts, are involved in growth-inhibitory activity in a complex way. When replant problems of asparagus are considered, it may be necessary to take biomass quantity into consideration.

In the mineral contents, significant positive correlations of N with P and Mg were noted at a level of 1% ($r = 0.85$ and $r = 0.63$, respectively). As described, N, P, and Mg are mineral contents promoting the growth of leaves (FAO, 1984), and the components may have a mutual relationship with each other. In addition, positive correlations of P with K and Mg, and that of Ca with Mg at a significance level of 5% ($r = 0.50$, $r = 0.49$, and $r = 0.46$, respectively), and that of K with water content ($r = 0.63$) at a significance level of 1% were noted.

There were differences in growth-inhibitory activity and mineral contents, as well as functional components and water content in different parts of asparagus. Growth-inhibitory activity was strong in the underground parts and weak in some of the aboveground parts. In the underground parts of asparagus, the percentage of mineral contents were high, and the percent K, Ca, and Mg contents tended to be greater than those in the aboveground parts. However, it has been reported that many substances with strong growth-inhibitory activity are present in underground parts (Motoki et al., 2006b, 2006c). Because growth-inhibitory activity was also strong in our study, by combining with activated C (Motoki et al., 2002), reuse as an organic resource in the field may be possible. In our previous study, when activated C was added, growth-inhibitory activity of asparagus was reduced markedly (Tang and Motoki, 2018). On the other hand, it has been reported that fewer substances with a strong growth-inhibitory activity (allelopathic agent) are present in the aboveground parts of asparagus (Motoki et al., 2006c), and the results of our study support the previous report. Furthermore, based on our study results, percent N and K content was especially high in the fern among the aboveground part.

The unusable parts of asparagus aboveground with the spears removed had weak growth-inhibitory activity. Therefore, the aboveground parts of asparagus can be mixed with the soil as an organic resource rather than being disposed of, if they have no disease or pests when the ferns are clipped or are not overgrown with weeds after the ripening of the fruit. On the other hand, the unusable underground parts of the asparagus had strong growth-inhibitory activity, which was consistent with the results of previous studies

Table 2. Correlation among growth-inhibitory activity, mineral contents, functional components, and water content among different asparagus plant parts.

	Inhibition rate (%)		Mineral contents (%DW)					Functional component		
	Radicle	Hypocotyl	N	P	K	Ca	Mg	Rutin content (mg·g ⁻¹ DW)	Protodioscin content (mg·g ⁻¹ DW)	Water content (%)
Inhibition rate (%)	1.00	0.87**	0.36	0.57**	0.56**	0.18	0.15	-0.66**	0.34	0.64**
Mineral contents (%DW)	Hypocotyl	1.00	0.47*	0.64**	0.46*	0.27	0.27	-0.38	0.49*	0.42
	N		1.00	0.85**	0.23	0.03	0.63**	0.03	-0.05	0.18
	P			1.00	0.50*	0.00	0.49*	-0.30	0.11	0.24
	K				1.00	0.18	-0.12	-0.28	0.04	0.63**
	Ca					1.00	0.46*	0.37	-0.13	0.22
	Mg						1.00	0.19	0.08	-0.10
Rutin content (mg·g ⁻¹ DW)								1.00	-0.38	-0.10
Protodioscin content (mg·g ⁻¹ DW)									1.00	-0.29
Water content (%)										1.00

DW = dry weight. **, *Significant at $P < 0.01$ and $P < 0.05$, respectively.

(Motoki et al., 2006a, 2006b, 2006c). Therefore, the underground parts of asparagus can be reused in the field by adding activated C (Motoki et al., 2002) to them if they are not dug up and discarded during replanting (Tang and Motoki, 2018). As with our previous study, the results of this study suggested that growth-inhibitory activity in the tips of the spears and the buds is also active, and that the tissues involved directly in sprouting may contain large amounts of growth inhibitors (Motoki et al., 2019). In the aboveground parts of asparagus, rutin content and percent N and K content were greater, whereas growth-inhibitory activity tended to be less, suggesting that when no disease or pest was present in aboveground parts, they can be used as an organic substance. When converted from the content percentage of each mineral content, the fern of the aboveground parts at the time of reaping may have contained 17.2 to 34.5 kg N, 1.8 to 2.8 kg P, 22.5 to 32.5 kg K, 0.7 to 7.1 kg Ca, and 0.5 to 1.9 kg Mg·t⁻¹ dry matter. Data on the aboveground parts of asparagus demonstrated no significant correlation between growth-inhibitory activity and mineral contents (S. Motoki, unpublished data). Therefore, the aboveground parts (i.e., fern and main stem) of asparagus can be used effectively as fertilizer without causing any growth inhibition even if they are plowed into fields. Ferns from the aboveground parts are thought to contribute to long-term soil agglomeration and the physical properties of soil improvement. It is thought that there is an economic and labor-saving aspect associated with ferns from the aboveground parts, which has previously been discarded as a residue outside the field, but can be reused as an organic resource. It is not necessary to transport them to a distant vegetable residue dump. In addition, even in fields where crops other than asparagus are cultivated, it is thought that ferns from the aboveground parts can be used as an organic substance to maintain soil in good condition. Therefore, in this study, the result that ferns from the aboveground parts, which have been treated as garbage, may be a useful resource.

The results of this study suggest that the unusable parts of asparagus can be a resource of useful components, and the findings of this study may create value for the unusable parts of asparagus. However, to examine the possibility of using the unusable parts of asparagus, it is necessary to examine differences in plant biomass. In addition, the cultivation conditions may influence the contents of the useful components contained in the unusable parts of asparagus. Based on the findings from this study, further research will examine differences in rutin and protodioscin content among cultivars of asparagus and how cultivation techniques can increase the content of functional components, and will suggest new methods for using the unusable parts of asparagus.

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