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## Research Article

# Distribution of Minerals in Young and Mature Leaves of Different Leaf Vegetable Crops Cultivated in a Field

### Abstract

At least 17 elements are known to be essential for plants; however, plants also absorb and accumulate various nonessential elements. Plants re-translocate different elements, including essential and nonessential elements, with differing efficiencies from mature to young developing organs via the phloem transport, resulting in different distributions of minerals in these organs. Therefore, in this study, we analyzed the concentrations of 23 elements in young and mature leaves of different leaf vegetable crops to comprehensively understand the mineral dynamics in plants. The allocation profiles of minerals in leaves of varying ages differed among species. For example, a higher molybdenum allocation profile was observed in young crown daisy (*Glebionis coronaria*) leaves, which might be related to the efficient nitrate assimilation in young leaves of this species. Thus, this study provides a new insight into the mineral uptake and transport mechanisms in plants.

## Introduction

To date, 17 elements have been recognized as essential for plants [1]. However, soil contains various nonessential and essential elements, and plants growing in fields absorb and accumulate both the elements. The accumulation of minerals in plants is largely affected by genetic variation and growth environment [2,3]. In particular, compared with normal plants, specific plant species, known as hyperaccumulators, accumulate extremely high concentrations of nonessential or trace elements, including aluminum (Al), sodium (Na), silicon, and heavy metals, in their above-ground organs [4-6].

Minerals are transported via the xylem and phloem vascular systems in plants. Essential elements are re-translocated from old or mature organs to young developing organs via the phloem transport to increase their efficiency of use [1]. However, the mobilities of essential elements in the phloem differ among elements. For example, the mobilities of nitrogen, potassium (K), phosphorus (P), sulfur (S), and magnesium (Mg) are high, whereas those of calcium (Ca) and manganese (Mn) are low [1]. Moreover, some nonessential elements were found in high concentrations in the phloem sap [7,8].

Ionomics is the study of the accumulation of all metals, metalloids, and nonmetals (ionome) in living organisms [9]. Ionomics aims to systematically profile all elements found in a certain organism and considers the genetic links among the elements, thereby providing insight into the functional status of a complex biological organism. Moreover, ionomics can be applied to the study of the phylogenetic influence on mineral accumulation. Our previous broad survey of various plant species investigated higher-level phylogenetic effects that influenced the leaf composition of a wide range of elements [10]. However, the ionome profile of leaves may not be constant and may change with leaf senescence as described above. Therefore, in

this study, we analyzed the concentrations of 23 elements in young and mature leaves of different leaf vegetable crops and attempted to examine whether there was a phylogenetic influence on the elemental distribution profile and elemental concentration profile in leaves. In this study, we selected the leaf vegetable crops because we could obtain plants of uniform size during field cultivation and their plant type was suitable for collecting leaves of varying ages.

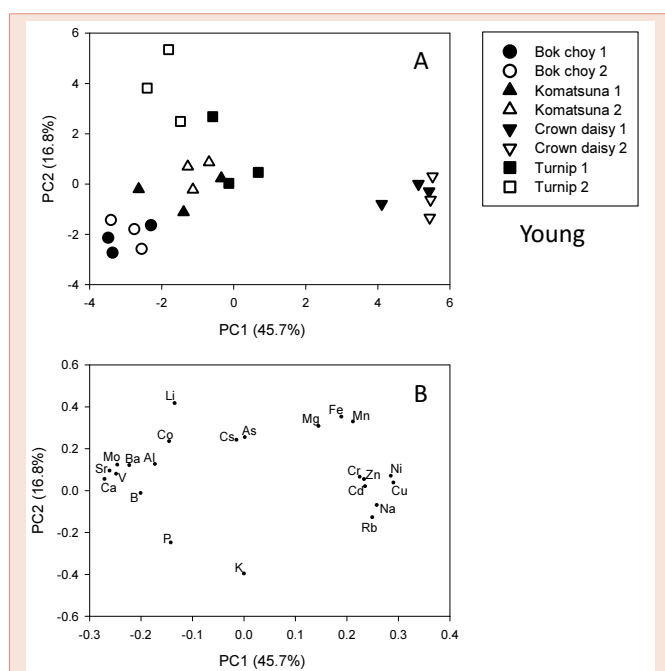
## Materials and Methods

### Cultivation

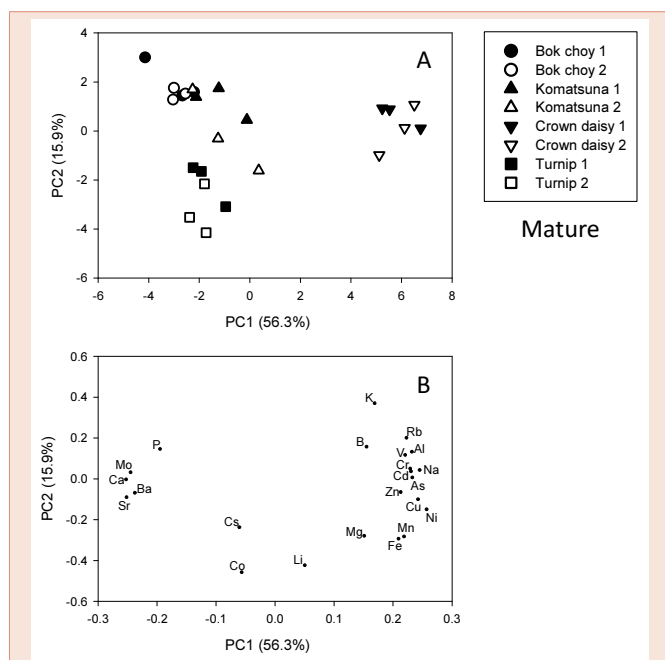
In 2012, four vegetable crop species/varieties were cultivated in an experimental field of the Hokkaido University, Japan. For all cultivations, N, P, and K fertilizers were uniformly applied as ammonium sulfate (140 kg N ha<sup>-1</sup>), superphosphate (130 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>), and potassium sulfate (100 kg K<sub>2</sub>O ha<sup>-1</sup>), respectively. For each species/variety, two different commercial cultivars were used. Seeds of bok choy (*Brassica rapa* var. *chinensis* cv. Fuyushomi and cv. Shikizanmai), komatsuna (*B. rapa* var. *perviridis* cv. Shoten and cv. Hitomikomatsuna), turnip (*B. rapa* var. *rapa* cv. Taibyohikari and cv. Fukukomachi), and crown daisy (*Glebionis coronaria* cv. Kiwamechuba and cv. Chubashingiku) were sown on June 14 in the field. General properties of the field soil are detailed in a previous study [3]. All species used in this study have similar growth rate, and are usually cultivated in this prefecture during spring to autumn. Young and mature leaves were sampled on July 23, with three replicates from at least three plants each. The row and hill spacing used was 13 × 13 cm. Leaf samples were dried in an oven at 70°C for 72 h and ground with a vibrating sample mill (TI-100; CMT, Saitama, Japan) for mineral analysis.

### Mineral analysis

Plant samples were digested in 2 mL of 61% (w/v) HNO<sub>3</sub> (EL grade; Kanto Chemical, Tokyo, Japan) at 110 °C in a DigiPREP



**Figure 1:** Principal component analysis (PCA) of elements in the young leaves of plants analyzed in this study. Scores on PC1 and PC2 with all elements included (A) and the corresponding loading plot (B). Bok choy 1: cv. Fuyushomi, Bok choy 2: cv. Shikizanmai, Komatsuna 1: cv. Shoten, Komatsuna 2: cv. Hitomikomatsuna, Turnip 1: cv. Taibiyohikari, Turnip 2: cv. Fukukomachi, Crown daisy 1: cv. Kiwamechuba, and Crown daisy 2: cv. Chubashingiku.



**Figure 2:** Principal component analysis (PCA) of elements in the mature leaves of plants analyzed in this study. Scores on PC1 and PC2 with all elements included (A) and the corresponding loading plot (B). Bok choy 1: cv. Fuyushomi, Bok choy 2: cv. Shikizanmai, Komatsuna 1: cv. Shoten, Komatsuna 2: cv. Hitomikomatsuna, Turnip 1: cv. Taibiyohikari, Turnip 2: cv. Fukukomachi, Crown daisy 1: cv. Kiwamechuba, and Crown daisy 2: cv. Chubashingiku.

apparatus (SCP Science, QC, Canada) for approximately 2 h until the solution had almost disappeared. After the samples had cooled, 0.5 mL  $H_2O_2$  (semiconductor grade; Santoku Chemical, Tokyo, Japan) was added, and the samples were heated at 110 °C for a further 20 min. Once digestion was complete, the tubes were cooled and made up to a volume of 10 mL by adding 2% (w/v)  $HNO_3$  in Milli-Q water. The concentrations of the following elements were measured using inductively coupled plasma-mass spectrometry (ELAN DRC-e; Perkin Elmer, Waltham, MA, USA): lithium (Li), boron (B), Na, K, chromium (Cr), cobalt (Co), nickel (Ni), arsenic (As), molybdenum (Mo), cadmium (Cd), Mg, Al, P, Ca, Mn, iron (Fe), copper (Cu), zinc (Zn), strontium (Sr), barium (Ba), and cesium (Cs).

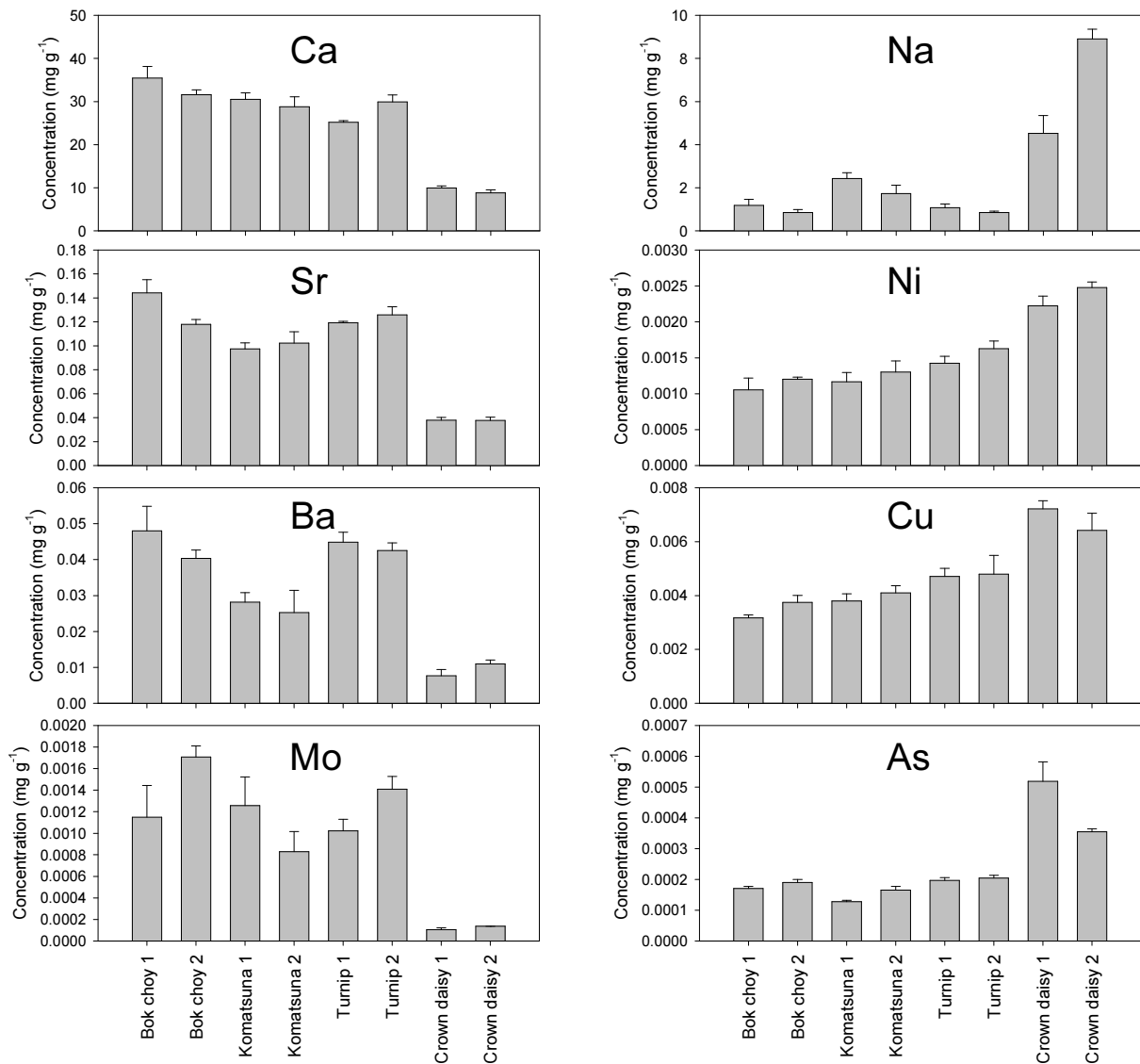
## Statistical analyses

All statistical analyses were performed using the Sigmaplot 11.0 (Systat Software, Inc., San Jose, CA, USA) and Minitab 14 (Minitab Inc., State College, PA, USA) softwares.

## Results and Discussion

First, we comprehensively compared the characteristics of element accumulation in leaves by comparing the ionomes of young and mature leaves among species, varieties, and cultivars using the principal component analysis (PCA). PCA provides a dimension reduction method for ionome data and is widely used in ionomics [11]. The PCA scores were then presented according to the combinations of PC1 and PC2. In the score plots of both young and mature leaves, Brassicaceae (bok choy, komatsuna, and turnip) and Asteraceae (crown daisy) were separately plotted along PC1 (Figures 1,2, both significant at  $P < 0.01$ , Student's  $t$ -test). Moreover, different varieties of *B. rapa* were separately plotted along PC2, and a significant difference ( $P < 0.05$ , Tukey's multiple comparison test) between turnip and the other *B. rapa* varieties was observed. These differences in PC2 might be because of their phylogenetic distance [12]. Differences in the ionome profile between two different commercial cultivars were small for all species/varieties (Figures 1,2, closed and opened symbols). The loading plot shows which variables (element concentrations) had the greater discriminating power to each PC1 and PC2. Results of loading plots for both young and mature leaves indicated that the concentrations of Ca, Sr, Ba, and Mo negatively contributed, whereas those of many other elements positively contributed to the PC1 score (Figures 1,2). The concentrations of elements with higher negative (Ca, Sr, Ba, and Mo) or positive (Na, Ni, Cu, and As) contributions to PC1 are shown in Figure 3. As expected, Ca, Sr, Ba, and Mo concentrations were higher in Brassicaceae, whereas Na, Ni, Cu, and As concentrations were higher in crown daisy. Because Ca, Sr, and Ba are homologous elements, the same absorption and transport mechanisms may be involved in the accumulation of these elements.

In the following step, the young/mature ratio of the leaf concentration of each element was calculated. These ratios were then used as variables for PCA. The score plot of these ratios revealed a clear significant separation between Brassicaceae and crown daisy (Figure 4,  $P < 0.01$ , Student's  $t$ -test), suggesting that the allocation profiles of minerals in leaves of varying ages are also phylogenetically influenced. Minerals are transported from mature leaves (source) to young leaves (sink, newly developing tissue) via the phloem along

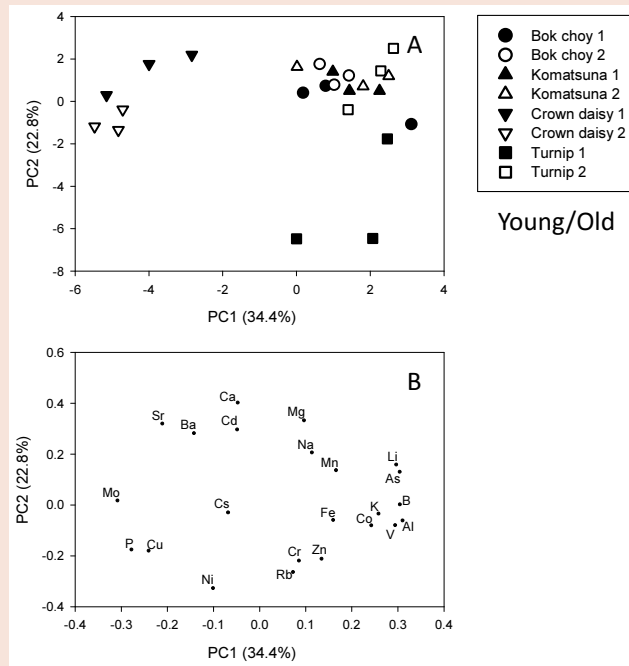


**Figure 3:** Concentration of Ca, Sr, Ba, Mo, Na, Ni, Cu, and As in mature leaves of each plant. Bars represent  $\pm$  SEs ( $n=3$ ). Bok choy 1: cv. Fuyushomi, Bok choy 2: cv. Shikizanmai, Komatsuna 1: cv. Shoten, Komatsuna 2: cv. Hitomikomatsuna, Turnip 1: cv. Taibyo hikari, Turnip 2: cv. Fukukomachi, Crown daisy 1: cv. Kiwamechuba, and Crown daisy 2: cv. Chubashingiku.

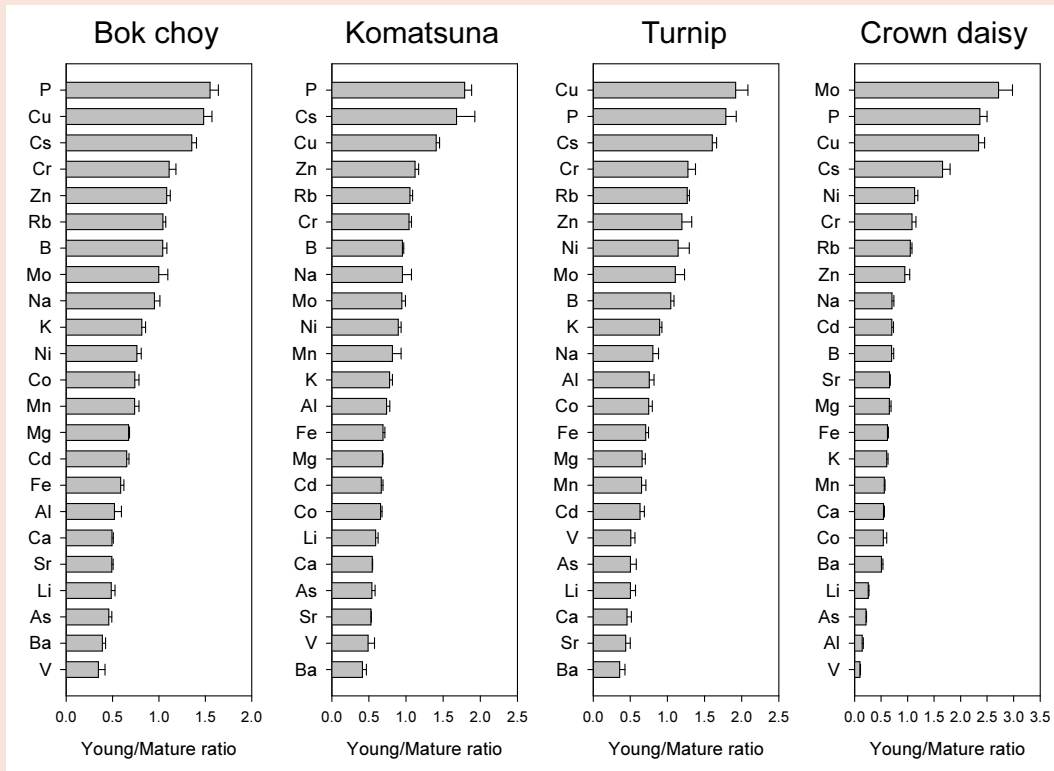
with the translocation of photosynthates [1]. However, as described in the Introduction, the mobilities of elements in the phloem sap differ, which can be because of their different availability in source cells and/or different efficiencies of phloem loading. These differences are possibly related to the different allocation profiles of minerals in leaves of varying ages as observed in this study. Transporters and channels responsible for phloem loading of minerals have been reported for some elements, such as  $K^+$  [13], phosphate [14], and  $Na^+$  [15], but not for other elements, particularly nonessential elements.

When comparing the young/mature ratio of the leaf concentrations of each element among different varieties of Brassicaceae, the order and value of the ratio was almost the same among different varieties (Figure 5). The order was partly correlated with the mobility of each

essential element in the phloem [1], indicating that the young/mature ratio of the leaf concentration of the element can reflect its mobility in the phloem. The young/mature ratio of the leaf K concentration was relatively low (Figure 5) and did not correspond with the mobility in the phloem [1]. This might be because of the high K supply from the xylem sap in mature leaves. In contrast, when comparing the young/mature ratio of the leaf concentration of each element between Brassicaceae and crown daisy, several differences were found. The most remarkable difference was that the young/mature ratio of the leaf Mo concentration was much higher in crown daisy than in the three varieties of Brassicaceae (Figure 5). One of the most important roles of Mo is as a component of nitrate reductase. Pate [16], found that the ratio of nitrate-N to total-N was very low (<20%) in radish (Brassicaceae), whereas this ratio was >50% in sunflower (Asteraceae).



**Figure 4:** Principal component analysis (PCA) of the young/mature ratio in leaf concentration of each element analyzed in this study. Scores on PC1 and PC2 with all elements included (A) and the corresponding loading plot (B). Bok choy 1: cv. Fuyushomi, Bok choy 2: cv. Shikizanmai, Komatsuna 1: cv. Shoten, Komatsuna 2: cv. Hitomikomatsuna, Turnip 1: cv. Taibyohikari, Turnip 2: cv. Fukukomachi, Crown daisy 1: cv. Kiwamechuba, and Crown daisy 2: cv. Chubashingiku.



**Figure 5:** Young/mature ratio in leaf concentration of each element in each plant species or variety. Bars represent  $\pm$  SEs (n=6). Bok choy 1: cv. Fuyushomi, Bok choy 2: cv. Shikizanmai, Komatsuna 1: cv. Shoten, Komatsuna 2: cv. Hitomikomatsuna, Turnip 1: cv. Taibyohikari, Turnip 2: cv. Fukukomachi, Crown daisy 1: cv. Kiwamechuba, and Crown daisy 2: cv. Chubashingiku.



This may imply that crown daisy (Asteraceae) efficiently translocates Mo from mature leaves to newly developing leaves via the phloem to enhance its nitrate assimilation.

## Conclusion

Using ionomics, this study first demonstrated that the allocation profiles of minerals in leaves of varying ages differed among species. Moreover, we provided the possibility to estimate the efficiency of mineral re-translocation via the phloem for each element by comparing the ionome between developing and mature tissues. In the future, we can determine the mineral dynamics in plants in greater detail by comparing the ionome profiles between leaves and other organs (e.g., fruit) or between different growth stages. Furthermore, direct ionome analyses of the xylem and phloem saps can comprehensively provide a more exact prediction of mineral transport in plants.

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