Plant Physiology and Biochemistry 136 (2019) 58–66

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Local boron concentrations in tuberous roots of Japanese radish (*Raphanus sativus L.*) negatively correlate with distribution of brown heart

Naoyuki Sotta[a], Bian Bian[a], Danhan Peng[a], Phattharawan Hongkham[a], Takehiro Kamiya[a], Satoshi Niikura[b], Toru Fujiwara[a,*]

[a] Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo, Tokyo, 113-8657, Japan
[b] Tohoku Seed Company, 1625 Nishihara, Himuro, Utsunomiya, Tochigi, 321-3232, Japan

ARTICLE INFO

Keywords:
Internal browning
brown heart symptom
Japanese radish (*Raphanus sativus L.* var. *longipinnatus* L.H. bailey)
Chemical element analysis
Boron

ABSTRACT

Internal browning (or brown heart) in radish is a physiological disorder, manifested as a reddish pigmentation in the central part of the tuberous root. Boron deficiency has been known to induce brown heart, but the relationship between B tissue concentration and the development of brown heart has not been tested. Here, we examined the relationship between these variables. Dissected root tissues of two inbred lines (i.e., cultivars) of East Asian big long radish exhibiting different severity of brown heart were submitted to inductively coupled plasma mass spectrometry (ICP-MS) analysis to reveal the spatial distribution of 19 chemical elements. Statistical analysis revealed that only B correlated negatively with the severity of brown heart. There was no significant difference in the average B concentration between the two cultivars, suggesting that differences in the efficient use of local B may be responsible for the variation in brown heart resistance between the two cultivars.

1. Introduction

Boron (B) is an essential microelement for plants, and its deficiency causes a reduction in crop yield and quality (Brown et al., 2006). B deficiency causes a pleiotropic plant growth disorder, evidenced as an inhibition in root elongation (Bohnsack and Albert. 1977; Josten and Kutschera. 1999), flower formation and seed production (Mozafar. 1993; Misra and Patil. 1987). Internal browning, also known as brown heart, is one of the physiological disorders linked to B deficiency and can be found in radish roots (*Raphanus sativus L.*), turnip (*Brassica rapa L.*), and rutabaga (*Brassica napus L.*) (Brown et al., 2006; Hurst and Macleod. 1936). Brown heart in radish plants is characterized by a brown coloration in the inner part of the root, which significantly reduces the commercial value of radish produce because of its unsightly appearance and bitter taste. The symptom develops under low B soil conditions, and therefore B application acts to decrease the incidence and severity of brown heart (Fadhel et al., 2015). Also, foliar application of B can alleviate brown heart (Shelp et al., 1987). High temperature acts to accelerate internal browning in the roots, which frequently occurs during the summer (Fukuoka and Enomoto. 2001). As radish is one of the most consumed vegetables throughout the year in East Asia, including Japan, it is critical to cultivate radish even during high temperature seasons to satisfy demand. Given this predicament, brown heart is a serious agricultural problem in Asian countries.

In rutabagas, Beauchamp et al. reported that the prevalence of brown heart was correlated with hot water soluble B (HWS-B) concentration in the soil and was more prevalent when the HWS-B concentration was less than 1.3 ppm (Beauchamp and Hussain. 1974). The same study showed that the application of the fertilizer borax, which released 4.5 kg B/ha, did not eliminate brown heart in a majority of their experiments, and further B application (9 kg B/ha) neither affected the yield nor reduced brown heart incidence (Beauchamp and Hussain. 1974). These results suggest that B concentration in the soil is one of the critical factors controlling brown heart but is not the only factor. It was reported that the European small radish (*Raphanus sativus* cv. Cherry Belle) manifested brown heart when the B concentration of the root (dry matter) was lower than 28 $\mu g\ g^{-1}$, and that brown heart severity was dependent on root B concentration (Shelp et al., 1987).

The resistance to brown heart varies among cultivars. In rutabaga, clear significant differences in the incidence of brown heart were reported among cultivars (Fadhel et al., 2015). Resistant rutabaga cultivars have a lower threshold of root B concentration at which they develop brown heart compared to sensitive cultivars (Fadhel et al., 2015). It has been suggested that a higher capacity to translocate B from leaves to roots makes these cultivars less sensitive to contracting brown heart (Shelp and Shattuck. 1987). These results suggest that resistance to

* Corresponding author.
E-mail address: atorufu@mail.ecc.u-tokyo.ac.jp (T. Fujiwara).

https://doi.org/10.1016/j.plaphy.2018.12.027
Received 24 September 2018; Received in revised form 26 December 2018; Accepted 28 December 2018
Available online 02 January 2019
0981-9428/

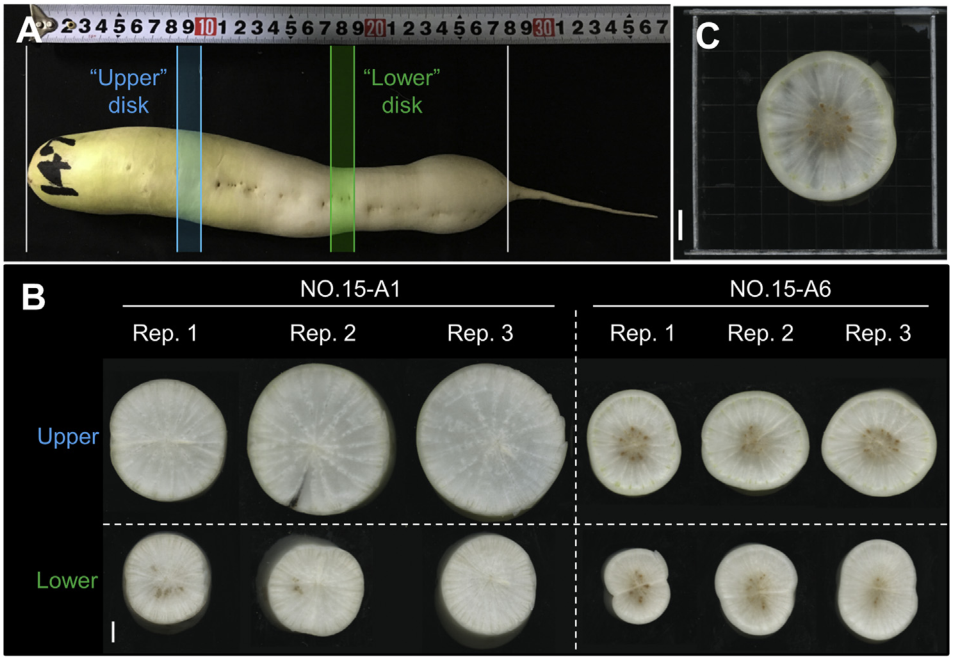

Fig. 1. Sample preparation of radish roots of two cultivars for use with ICP-MS. (A) The approximate position from which cross sections were sampled for ICP-MS. For each individual root, disks (15–20 mm thick) were collected from two positions, corresponding with the "upper disk" and the "lower disk", the former being cut approximately one third from the top of the root, and the latter two thirds from the top. (B) The prepared root disks before dicing (2016). Bars, 10 mm. (C) An example of a squared root disk. Cutting a single root disk with a lattice cutter made at most 36 squares (6 × 6 lattice).

brown heart is not simply explained by the ability of the roots to absorb B from soil, but also related to the efficiency of B usage (e.g., via translocation) after absorption. For instance, the distribution of **B** to specific root tissues may be critical for resistance to brown heart. Previous studies have not examined B distribution within tuberous roots. As brown heart develops unevenly within the root, it is worth examining the relationship between the spatial distribution of chemical elements, including B, and the development of brown heart. To our knowledge, chemical element distribution in radish roots has not been examined except Nitrogen (Seaman et al., 2014) and Cerium (Zhang et al., 2015).

In the present study, we analyzed the spatial distribution of brown heart damage and chemical elements, including B, in the roots of two inbred lines of East Asian big long radish (daikon in Japanese) (*Raphanus sativus* L. var. *longipinnatus* L.H. Bailey). Relationships between radish brown heart damage and elements concentrations were also examined using simple correlations and a multiple regression model.

2. Materials and methods

2.1. Plant material and growth conditions

Two Japanese radish inbred lines with different sensitivities to brown heart, NO.15-A1 (resistant) and NO.15-A6 (sensitive), were grown in a greenhouse in Utsunomiya, Tochigi, Japan (36°34′23.4″N 139°55′12.9″E). NO.15-A1 is derived from crossing between the inbred lines bred from the 'Miyashige' and 'Tokinashi' varietal groups in Japan and has been phenotypically screened over 18 generations by inbreeding or selfing. NO.15-A6 is derived from 'Miyashige' varietal group and has been screened over 16 generations by inbreeding or selfing. Radish was sown in the greenhouse under natural light on August 5, 2016, and July 24, 2017. As base fertilizer, N (110 kg/ha), phosphorous pentoxide (P_2O_5; 171 kg/ha), and K_2O (131 kg/ha) were applied to soil. Element concentrations in the soil and the irrigation water are shown in Tables S1 and S2. Rainfall was not a factor in the greenhouse. Plants were irrigated weekly for the first month, and after that plants were grown without irrigation. Samples were harvested on Oct. 13, 2016, and Oct. 10, 2017 for experimentation.

2.2. Tuberous root sample processing

After harvest, tuberous roots of Japanese radish were washed with tap water to remove soil and rinsed with deionized water. Using a ceramic kitchen knife, the roots were then cut perpendicular to the longitudinal axis to make disks (cross sections) with a 15–20 mm thickness.

2.3. Quantification of radish root brown heart via calculation of a BHS with image analysis

To quantify the brown heart severity, image analysis was employed, in which the cross sections were scanned at 300 dpi (GT-970, EPSON, Japan) and saved as 24-bit color TIFF. Cross sections were subsequently cut into squares with a 6 × 6 latticed Jumbo Potato Cutter (Progressive International, USA). Two metal blades with different size squares were used depending on the root slice diameter (13 × 13 mm or 8.8 × 8.8 mm). The ICP-MS data processing and heat map visualization were performed with custom R scripts. To generate heat maps of the chemical element distribution, element concentrations measured with the ICP-MS were mapped to a 6 × 6 lattice (the same arrangement as the lattice blades) and visualized as a heat map using the R image function. Using the "EBImage" library (Pau et al., 2010), the lattice heat maps were then overlaid with the shape of the scanned images of the root cross sections using the "color threshold" function of Fiji (Schindelin et al., 2012).

Root cross section images were first RGB scanned (8 bit/channel) and then separated into three color channels. As an indicator of brown pigmentation, a red–blue intensity range value was calculated for each pixel using the image calculator function. To quantify the red pigmentation (indicator of brown heart), the area without brown heart was masked using the color threshold function in the hue–saturation–-brightness (HSB) mode with pass ranges as follows: hue = 0–38; saturation = 57–255; and, brightness = 0–255. To exclude pigmentation derived from the vascular bundle, which exists in the peripheral root sections, pigmentation within 0.76 mm (0.3 inch) of the root surface was omitted. After masking and omitting the surface pigmentation, rectangular regions of interest (ROI) were set based on the grid of the lattice blades, and the mean intensities were measured for each ROI. The resultant intensities for each square were designated as the brown

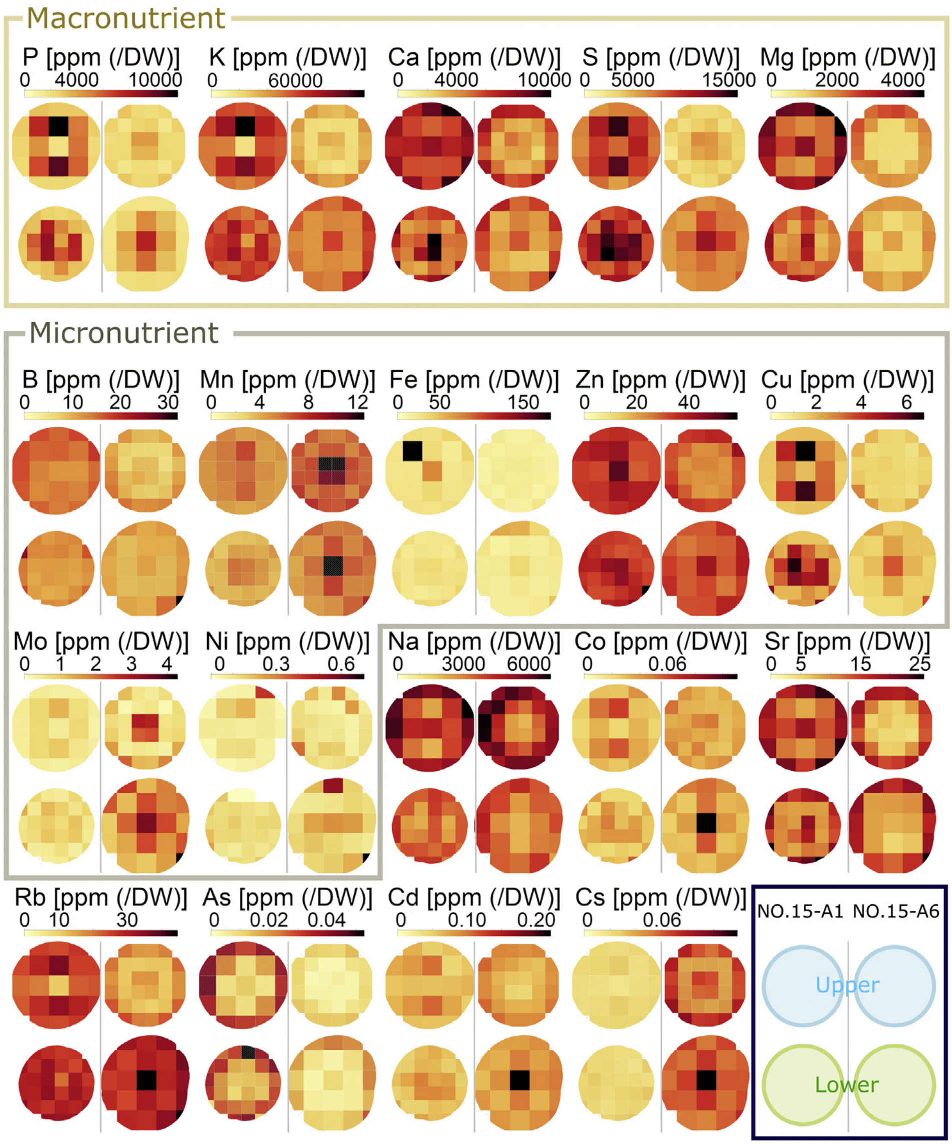

Fig. 2. Spatial distribution of chemical elements in Japanese radish tuberous roots. Chemical element concentrations (ppm dry weight) in the upper and the lower parts of the NO.15-A1 and NO.15-A6 cultivars' tuberous roots are shown with the heat maps. The positional information for each root disk is shown at the bottom right. Crosswise disks of a radish root were dissected into squares and element concentrations were determined by ICP-MS. Fractions whose values are lower than the lowest ICP-MS standard are indicated by white stripes. For each cultivar, one of six individual roots analyzed is shown here.

heart score (BHS).

2.4. Calculation of radish root chemical element concentrations

The square samples were placed in 24 well dish, dried in an oven at 70 °C for more than 24 h, and the dry weight was measured and recorded. The dried samples were then put in heat-resistant Teflon tubes. 2 mL HNO_3 (density 1.38, for B determination; Wako, Osaka, Japan) was added to each sample, and the tubes were heated with heat blocks for 1 h at 80 °C, then for 1 h at 100 °C, and finally overnight at 120 °C. After the HNO_3 in the tubes had completely evaporated, 2 mL of HNO_3 was added and heated at 120 °C until the samples solidified; the same step was then repeated with 1 mL of HNO_3. Finally, 0.5 mL of hydrogen peroxide (H_2O_2) was added to the samples and heated at 120 °C to obtain white pellets. The resultant pellets were dissolved in 5 mL of 0.08 N HNO_3, containing 2 ppb Indium (In) as an internal control. The solution was diluted 10–20 times with 0.08 N HNO_3 containing 2 ppb In and subjected to inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7800 ICP-MS). The measurements were normalized by the signals of In, and concentrations for each element (ppm per dry weight) were calculated.

The average element concentrations for two different positions of the radish root disks (peripheral and center) were calculated. Any part of the surface of the roots was categorized as peripheral, and the remainder of the root was categorized as center.

2.5. Element analysis of soil and irrigation water

Soil was sampled at 10 cm depth from the surface. After dried at 70 °C for more than 72 h, soil were sieved with a 2 mm sieve, To extract water-soluble elements, 1 g (DW) of soil was shaken in 10 mL deionized water for 6 h at 200 rpm. After centrifuge at 3000 rpm for 20 min, supernatant was filtered with Econofilter PES 25 mm diameter 0.45 μm pore (Agilent, USA). The resultant solution or irrigation water was subjected to ICP-MS.

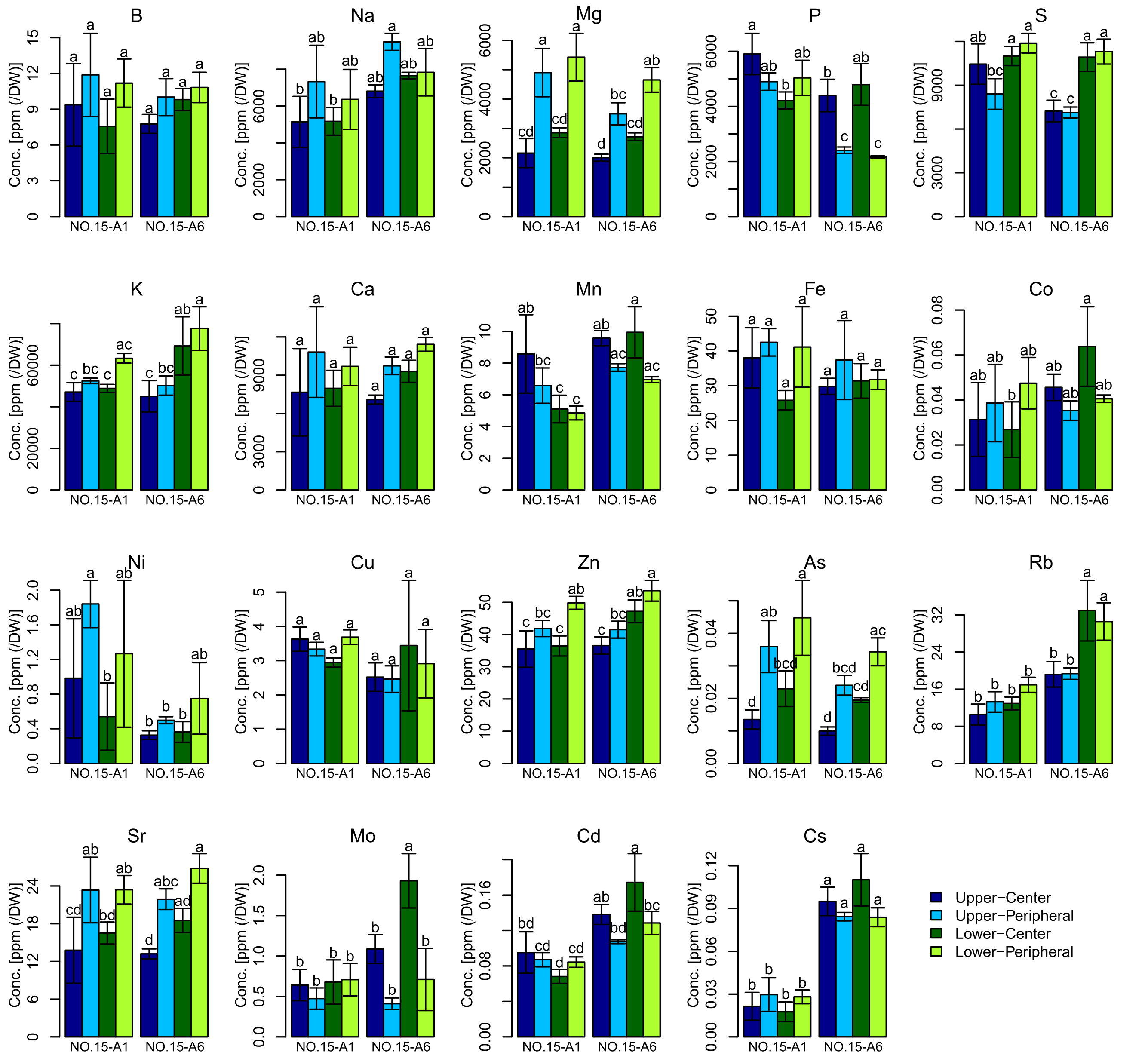

Fig. 3. Quantitative comparison of chemical elements of roots at two positions for cultivars NO.15-A1 and NO.15-A6 harvested in 2016. Average element concentrations in the root central and peripheral regions as determined using root squares from root disks were calculated with an ICP-MS. The average element concentrations (ppm dry weight) were calculated for each root slice. The mean and standard deviation of measurements from three individual roots are shown for four root positions (upper center, upper peripheral, lower center, and lower peripheral). Groups sharing the same letter were not significantly different from each other at $p < 0.05$ by Tukey's multiple test.

2.6. Statistical analysis

Correlation analysis among element concentrations was performed with R PerformanceAnalytics package (Peterson et al., 2015).

For multiple regression analysis between the root BHS and chemical element concentrations, the Tobit model was applied using the VGAM package (Yee. 2010) in R. To build the model, 330 squares derived from 12 root disks (three replicates from two cultivars and two positions) sampled in 2016 were used. The element concentrations in dry weight (ppb) of the 19 measured elements were used as the predictor variables, and the BHS was used as the dependent variable.

3. Results and discussion

3.1. Severity of brown heart between cultivars and years

For analysis of the spatial distribution of elements in radish roots, the two radish lines of NO.15-A1 (resistant) and NO.15-A6 (sensitive) were grown in a greenhouse, and their tuberous roots were harvested, and subsequently sampled, at 69 days (2016) and 78 days (2017) after sowing, corresponding with their harvest time for use in commercial products. Samples were processed for experiments in both years (2016 and 2017), in which three individuals were used for root brown heart damage and chemical element analysis.

From the series of root cross sections, we selected two disks per root, one corresponding with a distance of 1/3 the length of the root from the root top and the other 2/3 the length of the roots from the root top

Table 1

Statistical comparison of chemical element concentrations between the peripheral and central regions of tuberous roots of radish cultivars NO.15-A1 and NO.15-A6. Differences in the element chemical concentrations between the central and peripheral regions of radish tuberous roots were tested for the upper and lower root parts of NO.15-A1 and NO.15-A6 cultivars, respectively. The one-tailed Welch's *t*-test was applied with a *p*-value threshold of 0.05 to test the following two alternative hypotheses in relation to each element: element concentration of the central root part is significantly lower than that of the peripheral root part (c < p); element concentration of the central root part is significantly higher than that of the peripheral root part (c > p). Significant differences which are consistent between the two years are highlighted; n.s. = not significant.

Cultivar	Tissue	Year	B	Na	Mg	P	S	K	Ca	Mn	Fe	Co	Ni	Cu	Zn	As	Rb	Sr	Mo	Cd	Cs
NO.15-A1	Upper	2016	c<p	c<p	c<p	c>p	c>p	c<p	c<p	n.s.	n.s.	c<p	n.s.	n.s.	c<p	c<p	c<p	c<p	c>p	n.s.	c<p
		2017	c<p	c<p	c<p	c>p	c>p	n.s.	c<p	n.s.	n.s.	n.s.	n.s.	c>p	n.s.	c<p	n.s.	c<p	n.s.	n.s.	n.s.
	Lower	2016	c<p	n.s.	c<p	n.s.	c<p	c<p	c<p	n.s.	c<p	c<p	n.s.	c<p	c<p	c<p	c<p	c<p	n.s.	n.s.	c<p
		2017	c<p	c<p	c<p	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	c<p	n.s.	c<p	n.s.	n.s.	c<p
NO.15-A6	Upper	2016	c<p	c<p	c<p	c>p	n.s.	n.s.	c<p	c>p	n.s.	n.s.	c<p	n.s.	c<p	c<p	n.s.	c<p	c>p	c>p	n.s.
		2017	c<p	c<p	c<p	n.s.	n.s.	c<p	c<p	c>p	c>p	n.s.	n.s.	n.s.	c<p	c<p	c<p	c<p	n.s.	c<p	n.s.
	Lower	2016	n.s.	n.s.	c<p	c>p	n.s.	c<p	c<p	c>p	n.s.	n.s.	n.s.	n.s.	c<p	c<p	n.s.	c<p	c>p	c>p	c>p
		2017	c<p	c<p	c<p	c>p	c>p	c<p	c<p	c>p	n.s.	c>p	n.s.	c>p	n.s.	n.s.	n.s.	c<p	n.s.	n.s.	c>p

Table 2

Statistical comparison of radish root chemical element concentrations between the NO.15-A1 and NO.15-A6. Differences in the element concentrations between NO.15-A1 and NO.15-A6 cultivars were tested for the peripheral and center root parts of the upper and lower parts of tuberous roots, respectively. The one-tailed Welch's *t*-test was applied with a *p*-value threshold of 0.05 to test the following two alternative hypotheses in relation to each element: the concentration of NO.15-A1 is significantly lower than that of NO.15-A6 (1 < 6); the concentration of NO.15-A1 is significantly higher than that of NO.15-A6 (1 > 6). Significant differences which are consistent between the two years are highlighted. n.s. = not significant.

Tissue		Year	B	Na	Mg	P	S	K	Ca	Mn	Fe	Co	Ni	Cu	Zn	As	Rb	Sr	Mo	Cd	Cs
Upper	Peripheral	2016	n.s.	n.s.	1>6	1>6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	1>6	1>6	n.s.	n.s.	1<6	n.s.	n.s.	1<6	1<6
		2017	n.s.	n.s.	1>6	1>6	1>6	n.s.	n.s.	1<6	1>6	n.s.	n.s.	1>6	n.s.	1>6	n.s.	n.s.	1<6	1<6	1<6
	Center	2016	n.s.	n.s.	n.s.	1>6	1>6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	1>6	n.s.	n.s.	1<6	n.s.	1<6	1<6	1<6
		2017	n.s.	n.s.	n.s.	1>6	1>6	n.s.	n.s.	1<6	n.s.	n.s.	1>6	1>6	1>6	1>6	n.s.	n.s.	1<6	n.s.	1<6
Lower	Peripheral	2016	n.s.	n.s.	n.s.	1>6	n.s.	n.s.	n.s.	1<6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	1<6	n.s.	n.s.	1<6	1<6
		2017	n.s.	n.s.	1>6	1>6	1>6	n.s.	n.s.	1<6	n.s.	n.s.	n.s.	1>6	n.s.	1>6	n.s.	n.s.	n.s.	1<6	1<6
	Center	2016	n.s.	1<6	n.s.	n.s.	n.s.	n.s.	n.s.	1<6	n.s.	1<6	n.s.	n.s.	1<6	n.s.	1<6	n.s.	1<6	1<6	1<6
		2017	n.s.	n.s.	1>6	n.s.	1>6	n.s.	n.s.	1<6	n.s.	1<6	n.s.	1>6	n.s.	n.s.	n.s.	n.s.	1<6	1<6	1<6

(Fig. 1 A). Hereafter, these disks are referred to as "upper disk" and "lower disk", respectively. Brown heart was evident in the cross sections in NO.15-A6, but not evident or minimally evident in NO.15-A1 (Fig. 1 B). The difference in sensitivity to brown heart between the cultivars was observed in both years, although the difference was generally greater in 2016 than in 2017 (Fig. S1). The difference in the severity between the two years may be attributable to differences in the position in the greenhouse where the radish was grown: in 2017, radish was planted relatively close to the ventilator, where it is cooler in relation to other areas of the greenhouse. Because low temperature suppresses brown heart (Fukuoka and Enomoto. 2001), we speculate that the difference in the local environment resulted in the difference in brown heart development between the two years.

3.2. Variation in chemical element distribution between the two cultivars

Some elements, including P, S, Cu and Mo, were observed to accumulate more in the radish roots' central part compared to the peripheral part. The elements Mg, B, and As exhibited the opposite pattern, with a higher accumulation in the peripheral part (Figs. 2 and 3, S2).

B concentrations were between 8 and 12 ppm across our samples and across years, whereas Shelp et al. reported brown heart in roots of radish (*Raphanus sativus* cv. Cherry Belle) when B concentration was less than 28 ppm (dry matter) at harvest, and concluded that its severity was dependent on B root concentration (Shelp et al., 1987). In our experiments, the average B concentration in the squares was lower than the 28 ppm threshold of the aforementioned study, irrespective of upper or lower part of the root. This difference may be due to the radish growth conditions and the cultivars subjected to the experiment and suggests that B concentration is not the only determinant of brown heart development.

Differences in element concentration were tested statistically between 1) peripheral and central radish tissue, 2) NO.15-A1 and NO.15-A6 radish cultivars. The results of the statistical tests are summarized in Tables 1 and 2, respectively. Because we tested the data from each year separately, we were able to determine whether the tendency in these variables for each year which was consistent.

The radial profile of the elements was variable, with some elements exhibiting an uneven distribution within the cross sections. In both cultivars, B, Na, Mg, K, Ca, Zn, As, and Sr exhibited a tendency to accumulate in the root's peripheral part, compared to the central part, which was consistent in both years (Table 2). In contrast, P was higher in the central part, compared to the peripheral part, in both cultivars. Mn was highly accumulated in the central part in NO.15-A6, which was not the case in NO.15-A1. Cs exhibited a different pattern between NO.15-A1 and NO.15-A6, whereby in the former Cs accumulated more in the peripheral part, and in the latter it accumulated more in the central part (Tables 1 and 2).

3.3. Correlations among element concentrations and severity of brown heart

To evaluate the similarity in element concentration between cultivars, we calculated the correlation coefficients among elements by pooling the data of all the square samples for each root for each cultivar (Fig. 4, S3). Significant correlations with Spearman's coefficient greater than 0.8 ($p < 0.05$) were observed between Ca and Sr consistently in both 2016 and 2017. Ca and Sr are congeners and are known to show similar behavior in plants (Mengel and Kirkby. 1978). Our analysis

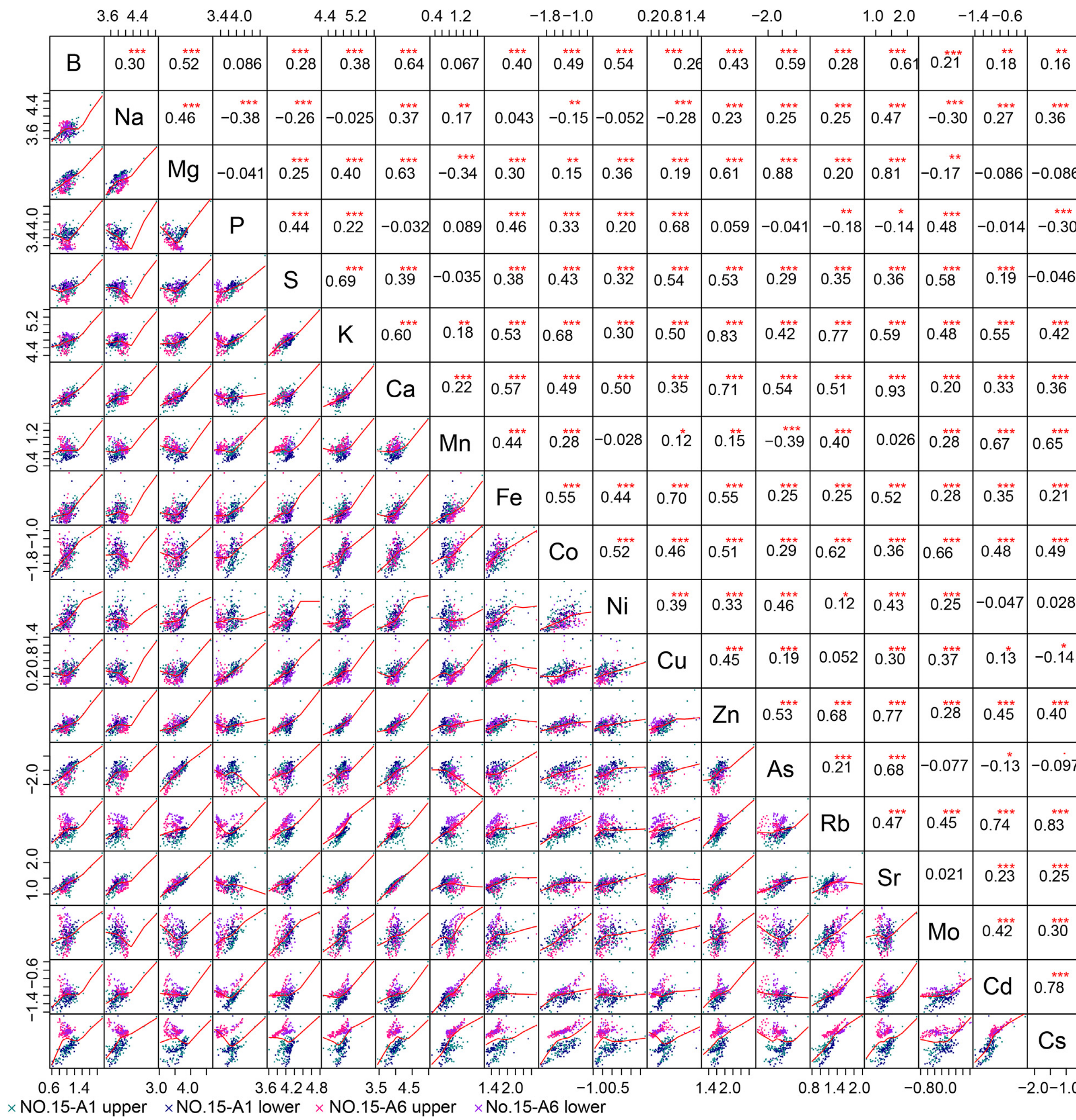

Fig. 4. Correlations of radish root chemical elements based on location within the root in 2016 Correlations of chemical element concentrations based on their distribution in radish tuberous roots. Each data point represents a measurement from a single root square, which was obtained from root disks and corresponded with one of two root positions (upper disk and lower disk); there were three disk replicates. Each single data point corresponding with a square is shown in the heat map in Fig. 2. Both axes represent $\log_{10}$ values of element concentrations (ppm dry weight). Numbers in the upper right half are Spearman's correlation coefficients; asterisks represent statistical significance.

revealed that the two elements share a similar spatial distribution in Japanese radish roots.

Next, to evaluate the correlations between element concentrations and brown heart severity, we first quantified the extent of brown pigmented area in each square by image analysis. The calculated scores reflected the degree of brown heart severity observed by eye (Fig. 5, S1).

We examined correlations between the BHS and the element concentrations using the data of the square samples (Fig. 6). Positive correlations of the BHS with element concentration were observed for P, K, Co, Mo, Cd, and Cs. Alternatively, B exhibited a weak negative correlation with the BHS. The correlation analysis between the BHS and B concentration revealed that some radish tissues without brown heart had lower B concentrations than some tissues with brown heart (Fig. 6, S4). This suggests that low B concentration in the tissue at this stage is not the sole determinant in the development of brown heart in these radish lines.

Next, we performed a multiple regression analysis to explain the BHS in terms of the predictor variables of element concentrations using a Tobit model, a linear regression that uses cutoff thresholds for the latent variables. We set 0 as the cutoff threshold because the BHS were nonnegative values. To generate the model, we used the BHS and the concentrations of 19 elements obtained from the 319 square root samples obtained in 2016. The resultant model predicted the BHS for

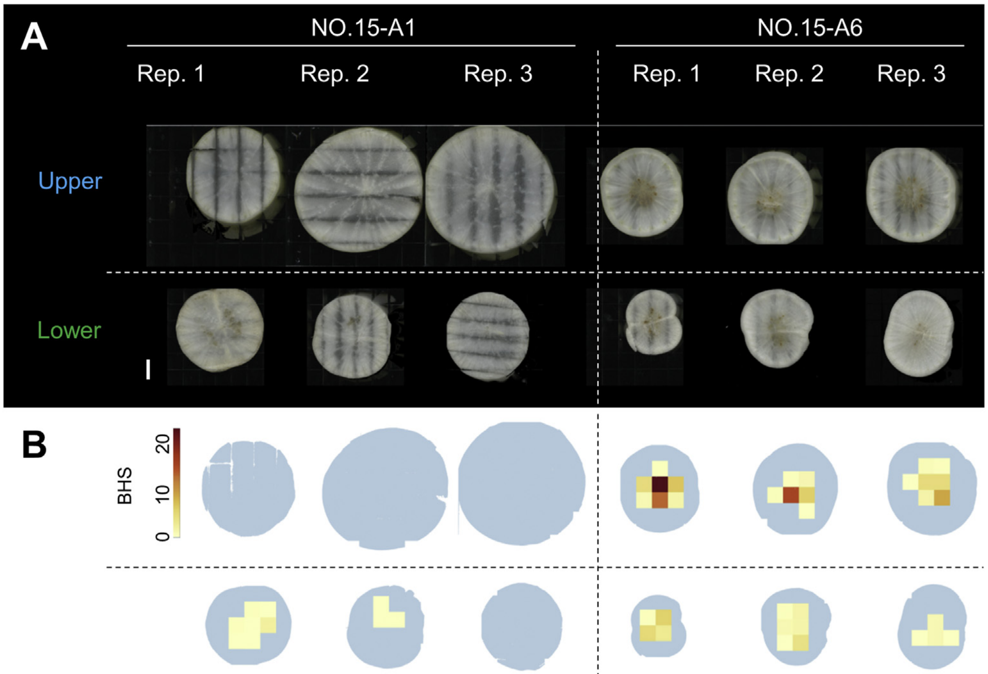

Fig. 5. Quantification of radish root brown heart based on location with the root using image analysis in 2016. (A) Root disk images from which brown heart damage was quantified. The process involved cutting 15–20 mm disks with a kitchen knife, then cutting them into 6 × 6 squares, after which an image of each square was obtained with a scanner. (B) Brown heart damage in the square samples were quantified as scores (BHS), which are shown on the heat map. Bar, 10 mm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

each element, which correlated well with the observed 2016 BHS (Fig. 7 A).

To validate the model, we predicted the BHS from element concentrations using the 2017 dataset, and the model developed for the 2016 dataset (Fig. 7 B). As a result, most of the observed positive BHS had positive predicted BHS, though there was a tendency for the predicted scores to be higher than the observed scores. This suggests that the model qualitatively captures the relationship between element concentrations and brown heart symptom emergence, although it failed to capture quantitative fluctuations between the two years.

In the multiple regression model, B was the only element with a negative coefficient that significantly explained part of the variance in the BHS. This suggests that B concentration is negatively correlated with the extent and frequency of brown heart. On the other hand, the model indicated a significantly positive coefficient of Co with brown heart (Table 3). Co is known to be beneficial for radish growth but is also toxic at higher concentrations (Jayakumar et al., 2007). As excessive amount of Co induces an oxidative stress response (Jayakumar et al., 2007), and therefore it is possible that Co is involved in the development of brown heart. However, this possibility should be further analyzed, because our analysis does not test the cause and effect relationship between Co accumulation and the development of brown heart.

3.4. Possible mechanisms driving brown heart in Japanese radish

Our analysis revealed that tissue with brown heart tends to have lower B concentrations compared with other parts. However, at whole tissue level, the average B concentration was not significantly different between the resistant and sensitive cultivars (Table 1). This suggests that B requirements could differ between the cultivars. In plants, B is essential for maintenance of cell wall function via the formation of boric acid esters between pectin chains in cell walls, which is required for cell wall maintenance (Hu and Brown. 1994). Among plant species, requirement of B correlates with cell wall pectin concentrations (Hu et al., 1996). In this sense, a variation in pectin concentration may be a determinant in the resistance to brown heart. It has been suggested that the pigmentation from brown heart is related to polyphenol oxidation and that cell wall integrity is important as a physical barrier against this oxidation (Fukuoka et al., 2010). In this vein, our results suggest that local B depletion may result in reduced cell wall integrity, inducing polyphenol oxidation. However, it is important to note that our measurements were performed on mature roots, at which stage of development the symptoms are already apparent, and that the results of our experiment do not exclude the possibility that some elements may be important to development of brown heart depending on the plant's growth stage.

In conclusion, our study revealed that local B concentration in tuberous roots at harvest stage correlates with the development of brown heart but is not the single determinant of brown heart; it is likely that differences in B requirement in cell wall or in a threshold whereby a B deficiency response is triggered underlie the differences in brown heart resistance between cultivars.

Abbreviations used

BHS, brown heart score; ICP-MS, inductively coupled plasma mass spectrometry.

Funding

This work was supported in part by a grant from the Japan Society for the Promotion of Science (No. 15J11021 to NS), a Grant-in-Aid for Scientific Research, Kiban S (No. 25221202 to TF).

Conflicts of interest

The authors declare no competing financial interest.

CRediT information

Naoyuki Sotta: Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Writing – Original Draft, Visualization, Funding Acquisition.

Bian Bian: Investigation.

Danhan Peng: Investigation.

Phattharawan Hongkham: Investigation.

Takehiro Kamiya: Conceptualization, Methodology, Investigation,

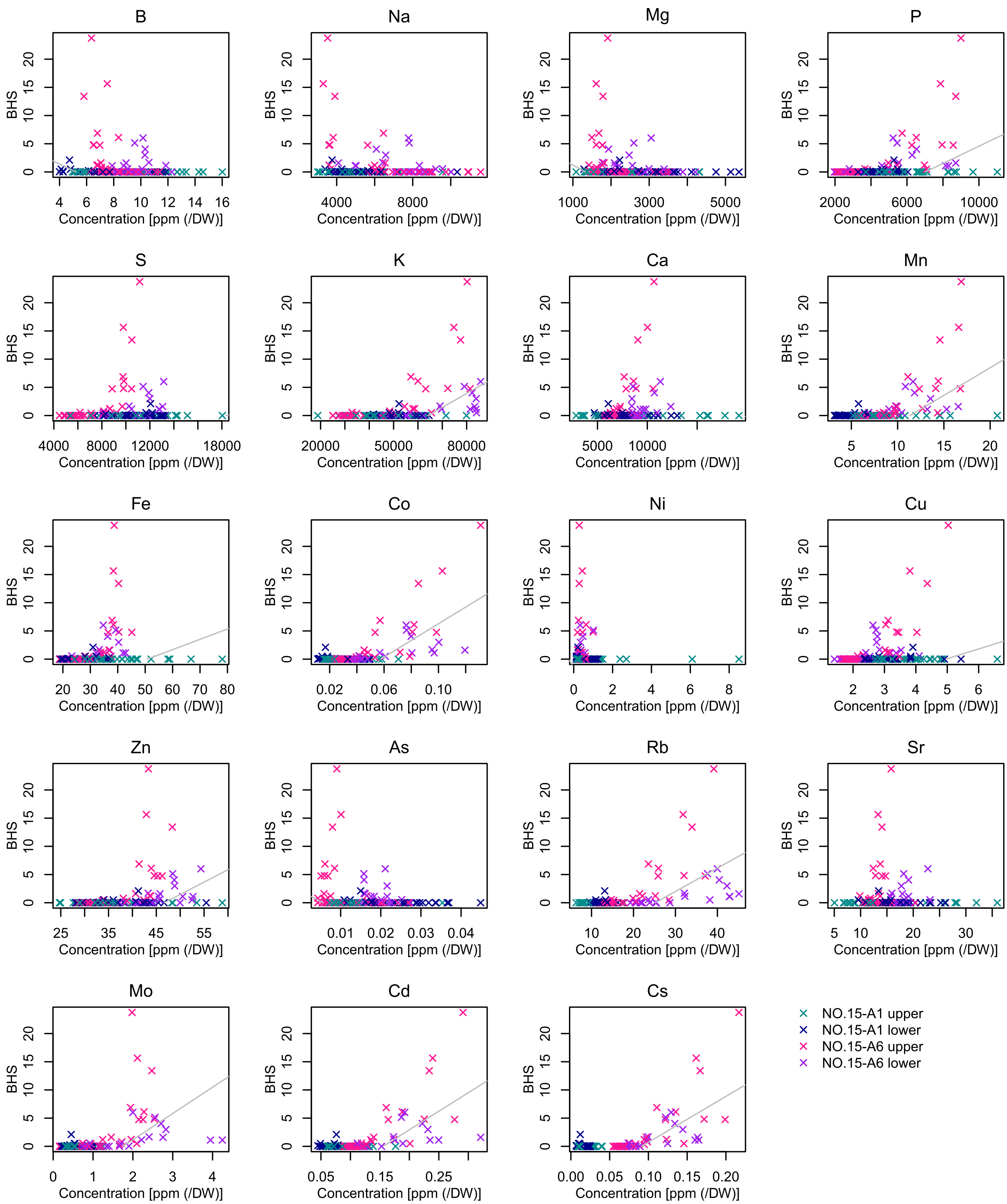

Fig. 6. Correlation between radish root chemical elements and brown heart damage based on location within the root in 2016. Correlations between the element concentrations and the brown heart score (BHS) (indicating brown heart severity) were plotted. Each data point represents a single square subjected to ICP-MS (318 dices from 12 root disks in total). The brown heart score (BHS) was calculated by quantifying the reddish pigments in root squares by image analysis. The gray lines are regression lines based on the Tobit regression model. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Writing – Review & Editing.

Satoshi Niikura: Conceptualization, Methodology, Resources, Investigation, Writing – Review & Editing, Supervision, Project Administration.

Toru Fujiwara: Conceptualization, Investigation, Writing – Review & Editing, Supervision, Project Administration, Funding Acquisition

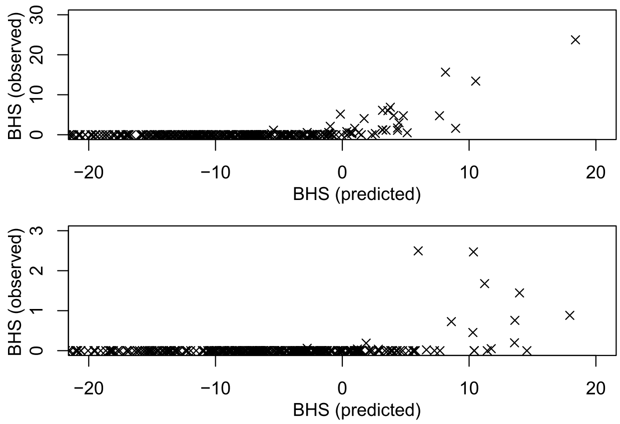

Fig. 7. Multiple regression model between brown heart and chemical element concentrations. A Tobit model multiple regression was applied to analyze the relationship of the brown heart score (BHS) with the element concentrations. The correlations between the observed brown heart score (BHS) values and the model predicted values are plotted. Each data point represents a single root square, and was generated with ICP-MS. (A) The BHS of the 2016 dataset were predicted by the model constructed with the 2016 dataset. (B) The BHS of 2017 were predicted by the model constructed with the 2016 dataset.

Table 3
Coefficients of the Tobit model where brown heart score (BHS) are explained by element concentrations.

	Estimate	Std.	Error	z value
(Intercept):1	−0.690	4.759	−0.145	0.885
(Intercept):2	1.057	0.127	8.307	< 2e-16 ***
B	−1.270	0.412	−3.084	0.002 **
Na	0.001	0.001	1.177	0.239
Mg	−0.002	0.002	−0.945	0.345
P	0.002	0.001	1.783	0.075
S	0.001	0.000	1.248	0.212
K	0.000	0.000	−0.599	0.549
Ca	−0.001	0.001	−1.004	0.315
Mn	0.878	0.634	1.385	0.166
Fe	−0.447	0.190	−2.350	0.019 *
Co	174.800	58.040	3.013	0.003 **
Ni	−3.263	2.177	−1.499	0.134
Cu	0.375	0.189	1.985	0.047 *
Zn	0.077	0.130	0.597	0.551
As	−236.200	163.400	−1.446	0.148
Rb	0.184	0.388	0.474	0.635
Sr	0.972	0.839	1.159	0.246
Mo	−0.906	1.329	−0.682	0.495
Cd	−62.160	50.850	−1.222	0.222
Cs	16.960	38.670	0.439	0.661

Significance: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '' 1.

Acknowledgment

We thank Yuko Kawara, Maki Saiki and Makiha Fukuda for excellent technical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2018.12.027.

References

Beauchamp, E., Hussain, I., 1974. Brown heart in rutabagas grown on southern Ontario soils. Can. J. Soil Sci. 54 (2), 171–178.
Bohnsack, C.W., Albert, L.S., 1977 Jun. Early effects of boron deficiency on indoleacetic Acid oxidase levels of squash root tips. Plant Physiol. 59 (6), 1047–1050.
Brown, P., Barker, A., Pilbeam, D., 2006. Handbook of plant nutrition. Handbook of Plant Nutrition.
Fadhel, F., Jellings, A., Kennedy, S., Fuller, M., 2015. Genotypic resistance to brown heart incidence in swede parent lines and F1 hybrids and the influence of applied boron. J. Agric. Sci. 153 (2), 195–204.
Fukuoka, N., Enomoto, T., 2001. The occurrence of internal browning induced by high soil temperature treatment and its physiological function in Raphanus root. Plant Sci. 161 (1), 117–124.
Fukuoka, N., Ikeshita, Y., Enomoto, T., 2010. Relationship between the occurrence of internal browning and size of xylem parenchymatous cells in roots of Japanese radish. J. Jpn. Soc. Hortic. Sci. 79 (1), 27–33.
Hu, H., Brown, P.H., Labavitch, J.M., 1996. Species variability in boron requirement is correlated with cell wall pectin. J. Exp. Bot. 47 (2), 227–232.
Hu, H., Brown, P.H., 1994 Jun. Localization of boron in cell walls of squash and tobacco and its association with pectin (evidence for a structural role of boron in the cell wall). Plant Physiol. 105 (2), 681–689.
Hurst, R., Macleod, D., 1936. Turnip brown heart. Scientific Agriculture 17 (4), 209–214.
Jayakumar, K., Jaleel, C.A., Vijayarengan, P., 2007. Changes in growth, biochemical constituents, and antioxidant potentials in radish (Raphanus sativus L.) under cobalt stress. Turkish J. Biol. 31 (3), 127–136.
Josten, P., Kutschera, U., 1999. The micronutrient boron causes the development of adventitious roots in sunflower cuttings. Ann. Bot. 84 (3), 337–342.
Mengel, K., Kirkby, E.A., 1978. Principles of plant nutrition. Principles of Plant Nutrition.
Misra, S., Patil, B., 1987. Effect of boron on seed yield in Lucerne (Medicago sativa L.). J. Agron. Crop Sci. 158 (1), 34–37.
Mozafar, A., 1993. Role of boron in seed production. In: Gupta, U.C. (Ed.), Boron and its Role in Crop Production, pp. 186–206.
Pau, G., Fuchs, F., Sklyar, O., Boutros, M., Huber, W., 2010. EBImage—an R package for image processing with applications to cellular phenotypes. Bioinformatics 26 (7), 979–981.
Peterson, B.G., Carl, P., Boudt, K., Bennett, R., Ulrich, J., Zivot, E., et al., 2015. Package 'PerformanceAnalytics'.
Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., et al., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9 (7), 676–682.
Seaman, C., Flinders, B., Eijkel, G., Heeren, R.M., Bricklebank, N., Clench, M.R., 2014. "Afterlife experiment": use of MALDI-MS and SIMS imaging for the study of the nitrogen cycle within plants. Anal. Chem. 86 (20), 10071–10077.
Shelp, B., Shattuck, V., 1987. Boron nutrition and mobility, and its relation to the elemental composition of greenhouse grown root crops I. Rutabaga. Communications in Soil Science & Plant Analysis 18 (2), 187–201.
Shelp, B., Shattuck, V., Proctor, J., 1987. Boron nutrition and mobility, and its relation to the elemental composition of greenhouse grown root crops. II Radish. Commun. Soil Sci. Plant Anal. 18 (2), 203–219.
Yee, T.W., 2010. The VGAM package for categorical data analysis. J. Stat. Software 32 (10), 1–34.
Zhang, W., Ebbs, S.D., Musante, C., White, J.C., Gao, C., Ma, X., 2015. Uptake and accumulation of bulk and nanosized cerium oxide particles and ionic cerium by radish (Raphanus sativus L.). J. Agric. Food Chem. 63 (2), 382–390.