Differences in Cadmium Accumulation and Root Morphology in Seedlings of Japanese Wheat Varieties with Distinctive Grain Cadmium Concentration

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Abstract: A low cadmium (Cd) concentration in wheat grain is desirable because of Cd toxicity to humans. Grain Cd concentrations in Japanese wheat differed among the varieties in previous study. In this study, we hypothesized that the varieties with a low concentration of Cd in grain have (1) low Cd uptake from the soil through the roots during early growth and/or (2) low Cd translocation from the roots to shoots, and also, that (3) Cd uptake from soil is affected by root morphology. These hypotheses were verified by investigating the concentration and quantity of Cd in root, shoot and leaf tissues, and examining the root morphology of young seedlings of wheat varieties with high and low grain Cd concentrations. Seedlings of ‘Kitahonami’ and ‘Nanbukomugi’ which had low grain Cd concentration (low Cd/G varieties) had a lower Cd quantity in whole plant tissues than ‘Nishikazekomugi’ and ‘Kitakamikomugi’ which had high grain Cd concentration (high Cd/G varieties) during early growth. Low Cd/G varieties also showed lower root to shoot (aerial parts) translocation of Cd than high Cd/G varieties. Seedlings of low Cd/G varieties showed less root branching than high Cd/G varieties. Root frequency showed a significant positive correlation with Cd quantity in whole plant tissues. These results suggest that low Cd/G varieties used in this study have low Cd uptake and translocation from the roots to shoots during early growth, and furthermore, that low Cd uptake at the seedling stage may relate to slow and/or limited development of branching roots.

Key words: Cadmium, Root, Seedling, Translocation, *Triticum aestivum*, Uptake, Wheat.
among varieties with different grain Cd concentration may have occurred since early growth stage. Detection of the differences can contribute to the efficient analysis of morpho-physiological mechanisms of Cd accumulation and the breeding of low Cd accumulation in wheat. In this study, we hypothesized that the varietal difference in Cd concentration in grain is affected by (1) the amount of Cd absorbed from the soil through the roots during early growth and (2) translocation of Cd from the roots to shoots, and moreover, that (3) Cd uptake from soil is affected by root morphology. These hypotheses were verified through field, pot and root box experiments aimed at investigating the concentration and amount of Cd in root, shoot and leaf tissues, and the root distribution in young seedlings of wheat varieties with high and low grain Cd concentrations.

Materials and Methods

1. Plant materials

Four Japanese wheat (*Triticum aestivum* L.) varieties, ‘Nisikazekomugi’ (grain Cd concentration: high), ‘Kitakamikomugi’ (high), ‘Kitahonami’ (low) and ‘Nanbukomugi’ (low) were used for the field, pot and/or root box experiments. Our previous study reveals a varietal difference in grain Cd concentration in Japanese common wheat using diverse materials (Kubo et al., 2008a). Grain Cd concentration in Nishikazekomugi, Kitakamikomugi and Nanbukomugi was 54.5, 43.9 and 29.2 ng g$^{-1}$, respectively. Grain Cd concentration in Kitahonami was evaluated as approximately half compared with that of Nishikazekomugi in subsequent experiments. We defined Nishikazekomugi and Kitakamikomugi as ‘High Cd/G varieties’, and Kitahonami and Nanbukomugi as ‘Low Cd/G varieties’.

2. Field experiment

The four wheat varieties were grown in a drained lowland field with alluvial soil at the National Agricultural Research Center (NARC; Ibaraki, Japan, 36.03°N, 140.10°E). The sowing date was November 7, 2006. The plots were single rows 1.0 m long and 70 cm apart. Sowing density was 10.1 g m$^{-2}$. Fertilizer was applied just before sowing at 40, 60 and 40 kg ha$^{-1}$ of N, P$_2$O$_5$ and K$_2$O, respectively. The experimental design was a randomized block design with two replications. After measurement of plant height and stem number, leaf, stem and root tissues were sampled from 30 cm length row which show normal growth in each plot at 62 days after sowing (DAS) for measurement of dry weight (DW), Cd concentration and quantity. The leaf sheath was included as stem tissue. Root samples were collected from a soil depth of 30 cm and 10 cm away from the seedlings. Roots were separated from the soil in a water tank by rinsing moderately with distilled water.

3. Pot experiment

Nishikazekomugi and Kitahonami were used for the pot experiments in a glasshouse under natural light conditions at NARC. Wagner pots (15000a$^{-1}$) were filled with alluvial soil at a soil density of 0.79 g cm$^{-3}$ and fertilized with 0.15, 0.23 and 0.15 g of N, P$_2$O$_5$ and K$_2$O per pot, respectively. On November 28, 2006, three seeds were sown in each pot with 1 cm depth. The experimental design was a randomized block design with four replications. Plant height and stem number were measured, and aerial parts (shoot: leaf + stem) and root tissues were collected at 37 DAS.

4. Root box experiment

The root box experiment was conducted in a glasshouse under natural light conditions at the National Agricultural Research Center for Kyushu Okinawa Region (KONARC: Chikugo, Fukuoka, Japan, 33.21°N, 130.49°E) from December 8, 2007 to March 14, 2008, using all four varieties. The structure of the root box was similar to that used by Kubo et al. (2008b). Briefly, a filmed rubber tube

Fig. 1. Root box experiment employed in this study. (a), Arrows show the positions of sowing. (b), Boxes were placed in the pool and supported by a frame.

Fig. 2. Root distribution of Kitahonami (a) and Nishikazekomugi (b) at 73 DAS in the root box experiment. Kitahonami had a long seminal root with few branching roots, while Nishikazekomugi had a short seminal root with abundant branching roots. The differences were particularly apparent at 97 DAS.
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(15 mm wide, 15 mm thick; Product No. 10239-0030-12-5, Fuso Rubber Co., Ltd., Hiroshima, Japan) was fastened between two transparent acrylic boards (50 cm wide, 50 cm long and 3 mm thick) using clips at three points on each side (Fig. 1a). The root box was filled with approximately 3.0 L of soil (alluvial soil: sand = 3 : 2) mixed with 0.70 g of N, 0.90 g of P₂O₅ and 0.70 g of K₂O at a soil density of 0.90 g cm⁻³. Two seeds of each variety germinated on wet filter paper were sown at a depth of 1 cm. The boxes were placed in the pool and supported by a frame (Fig. 1b). Irrigation was conducted from holes on the bottom of the boxes using water stored in the pool. The water level was maintained at a depth of 3−5 cm. The experimental design was a randomized block design with three replications. The length of the deepest seminal root and total number of root tips were measured through the acrylic boards at 39 DAS. At 73 DAS, the length of the deepest seminal root and density of the lateral roots on the seminal root were measured. At 73 and 97 DAS, root distribution was evaluated using a method similar to the profile wall method of Böhm (1976). After dividing the acrylic boards into 1.0 × 1.0 cm grids, the number of visible roots in each grid was counted and the root frequency (root number cm⁻² soil surface) was calculated. Shoot and root samples were collected after evaluation of root frequency. At 73 DAS, the density of lateral roots on the

| Field experiment | Concentration (ng g⁻¹) | Quantity (ng) |
|------------------|------------------------|--------------|
|                   | Whole plant | Leaf | Stem | Root | Shoot/Root | Whole plant | Leaf | Stem | Root | Shoot/Root |
| High Cd/G varieties |           |       |      |      |             |           |       |      |      |             |
| Nishikazekomugi  | 152 b)     | 62 b  | 181 ab | 545  | 0.196       | 39.5 ab   | 9.5 ab  | 15.1  | 14.9  | 1.651        |
| Kitakamikomugi   | 186 a      | 76 a  | 229 a  | 662  | 0.197       | 48.3 a    | 10.7 a  | 19.3  | 18.3  | 1.639        |
| Low Cd/G varieties |           |       |      |      |             |           |       |      |      |             |
| Kitahonami       | 120 c      | 42 c  | 91 c  | 482  | 0.132       | 24.1 b    | 4.2 b   | 6.6   | 13.3  | 0.812        |
| Ananbukomugi     | 134 bc     | 47 c  | 155 b  | 490  | 0.175       | 34.7 ab   | 6.5 b   | 13.2  | 15.1  | 1.305        |
| ANOVA           | **         | **    | *     |     | ns          | *         | *      | ns    | ns    |              |
| Pot experiment   |           |       |      |      |             |           |       |      |      |             |
| Nishikazekomugi  | 908        | 401 b | 2049  | 0.196 | 145.2       | 47.5      | 97.7   | 0.486 |
| Kitahonami       | 736        | 215   | 1908  | 0.113 | 103.0       | 23.3      | 79.7   | 0.203 |
| ANOVA           | ns         | *     | ns    |     |             | *         | *      | ns    | ns    |              |
| Root Box experiment |         |       |      |      |             |           |       |      |      |             |
| 73DAS            |           |       |      |      |             |           |       |      |      |             |
| High Cd/G varieties |           |       |      |      |             |           |       |      |      |             |
| Nishikazekomugi  | 1317 b     | 297 b | 1322 a | 0.224 | 3622 a      | 514 a     | 1348 a | 0.381 |
| Kitakamikomugi   | 2044 a     | 432 a | 1816 a | 0.238 | 3822 a      | 514 a     | 1235 a | 0.416 |
| Low Cd/G varieties |           |       |      |      |             |           |       |      |      |             |
| Kitahonami       | 1331 b     | 213 b | 1687 a | 0.126 | 2396 ab     | 262 ab    | 962 a  | 0.272 |
| Ananbukomugi     | 1291 b     | 226 b | 1496 a | 0.151 | 1704 b      | 190 b     | 718 a  | 0.265 |
| ANOVA           | **         | **    | *     |     | ns          | *         | *      | ns    | ns    |              |
| 97DAS            |           |       |      |      |             |           |       |      |      |             |
| High Cd/G varieties |           |       |      |      |             |           |       |      |      |             |
| Nishikazekomugi  | 504 b      | 186 b | 1312 a | 0.142 | 6270        | 833 a     | 2283 a | 0.365 |
| Kitakamikomugi   | 750 a      | 262 a | 1461 a | 0.179 | 7202        | 749 a     | 2835 a | 0.264 |
| Low Cd/G varieties |           |       |      |      |             |           |       |      |      |             |
| Kitahonami       | 785 a      | 172 b | 1889 a | 0.092 | 5357        | 377 b     | 2905 a | 0.164 |
| Ananbukomugi     | 768 a      | 172 b | 1902 a | 0.091 | 5239        | 380 b     | 2922 a | 0.166 |
| ANOVA           | *          | **    | *     |     | ns          | *         | *      | ns    | ns    |              |

1) Means followed by common letters under each trait were not significantly different according to the multiple test of Ryan-Einot-Gabriel-Welsch (P < 0.05).
2) ** and * show significance at P < 0.01 and 0.01 ≤ P < 0.05, respectively, and ns is not significant according to ANOVA.
3) Values of aerial parts (shoot: leaf + stem).
1. Semiconductor root was also measured. Plant height and stem number were obtained at 39, 73 and 97 DAS. Shoot and root tissues were collected at 73 and 97 DAS. The plants at 39, 73 and 97 DAS were around seedling stage (Zadoks growth stage (ZGS) 15–16) (Zadoks et al., 1974), tillering stage (ZGS 29) and tillering stage (ZGS 29), respectively, in Kitakamikomugi, Kitahonami and Nanbukomugi. In Nishikazekomugi, plants were at seedling stage (ZGS 16), stem elongation stage (ZGS 30) and stem elongation stage (ZGS 31–32) at 39, 73 and 97 DAS, respectively.

5. **Analysis of Cd concentration and quantity**

The DW of each plant tissue was measured after drying at 80°C for seven days. They were then ground using a laboratory mill with stainless steel blades, and 0.1 g was digested in 20 mL of HNO₃ (0.1 M) for 1 hr at room temperature. The samples were then strained through filter paper (Grade 2, Toyo Roshi Kaisha, Ltd.). A reagent blank was processed with each set of 40 samples. Cd concentration was determined with an ELAN6100DRC (Perkin Elmer, Inc.) inductively coupled plasma mass spectrometer. Watanabe et al. (2006) showed that Cd concentration in wheat grain extracted according to this method corresponds with that digested in an acid mixture in a microwave oven (analysis by Nittech Research, Co., Hyogo, Japan). Cd quantity was calculated as the product of Cd concentration multiplied by the DW of each plant tissue for each replication.

6. **Data analysis**

Analysis of variance was conducted using a linear regression model with computer software SPSS (Ver. 13.0 J for Windows, SPSS Japan Inc.).

### Results

Cd concentration in whole plant tissues showed significant differences among varieties in field and root box experiments (73 and 97 DAS) (Table 1). In the field experiment, low Cd/G varieties showed lower values than high Cd/G varieties. Cd quantity in whole plant tissues showed significant varietal differences in field, pot and root box experiments (73 DAS). High Cd/G varieties had 1.5 times (field experiment), 1.4 times (pot experiment) and 1.8 times (root box experiment) higher values, respectively than low Cd/G varieties on average.

The Cd concentration and quantity in the leaf, stem and root tissues, and the shoot / root ratio are shown in Table 1. Cd concentration and quantity in the root tissues did not show significant differences among varieties in any experiments. The shoot / root ratio of Cd concentration and quantity were lower in low Cd/G varieties than high Cd/G varieties on average.

At 39 DAS, the number of root tips significantly differed among varieties, ranging from 81 (Nanbukomugi) to 161 (Nishikazekomugi) (Table 2). The number of root tips was about two times greater in Nishikazekomugi than in Kitahonami and Nanbukomugi (low Cd/G varieties). At 73 DAS, a significant difference in the length of the longest primary root was found among varieties, and the primary root was longer in Nishikazekomugi and Kitahonami than

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**Table 1.** Length of the longest primary root, number of root tips and the density of secondary roots on the primary root in the root box experiment.

| Variety            | 39DAS Length of the longest primary root (cm) | 39DAS Number of root tips | 73DAS Length of the longest primary root (cm) | 73DAS Density of secondary root on primary root (cm⁻¹) |
|--------------------|-----------------------------------------------|---------------------------|-----------------------------------------------|-----------------------------------------------------|
| High Cd/G varieties |                                               |                           |                                               |                                                     |
| Nishikazekomugi    | 14.8 a                                        | 161 a                     | 47.2 a                                        | 3.05 a                                              |
| Kitakamikomugi     | 13.3 a                                        | 110 b                     | 38.5 b                                        | 3.12 a                                              |
| Low Cd/G varieties |                                               |                           |                                               |                                                     |
| Kitahonami         | 15.2 a                                        | 85 bc                     | 53.1 a                                        | 2.37 a                                              |
| Nanbukomugi        | 11.0 a                                        | 81 c                      | 37.5 b                                        | 3.05 a                                              |
| ANOVA              | ns                                            | **                        | **                                            | ns                                                  |

1) Means followed by common letters under each trait were not significantly different according to the multiple test of Ryan-Einot-Gabriel-Welsch (P < 0.05).

2) ** shows significance at P < 0.01 and ns is not significant according to ANOVA.
in Kitakamikomugi and Nanbukomugi. Root frequency at a soil depth of 0−25 cm significantly differed among varieties at 73 DAS and 97 DAS, ranging from 0.79 cm
\(^{-2}\) (Nanbukomugi) to 2.12 cm
\(^{-2}\) (Nishikazekomugi) and from 1.70 cm
\(^{-2}\) (Kitahonami) to 2.97 cm
\(^{-2}\) (Nishikazekomugi), respectively (Table 3). Low Cd/G varieties showed lower values than high Cd/G varieties at 73 DAS and 97 DAS. At a soil depth of 25−50 cm, the varietal difference was significant at 97 DAS, with values ranging from 0.78 cm
\(^{-2}\) (Nanbukomugi) to 1.81 cm
\(^{-2}\) (Kitakamikomugi). Root frequency in the total depth (0−50 cm) was lower in low Cd/G varieties than in high Cd/G varieties. The root profiles of Kitahonami and Nishikazekomugi are shown in Fig. 2. Higher root frequency significantly related to accumulate a larger quantity of Cd (Fig. 3).

### Table 3. Root frequency (cm
\(^{-2}\)) in the root box experiment.

| Soil depth (cm) | 0−25 | 25−50 | 0−50 |
|----------------|------|-------|------|
| **73DAS**      |      |       |      |
| High Cd/G varieties |      |       |      |
| Nishikazekomugi | 2.12 \(^{a1}\) | 0.36 \(^{a}\) | 0.61 \(^{a}\) |
| Kitakamikomugi  | 1.72 \(^{a}\) | 0.21 \(^{a}\) | 0.48 \(^{ab}\) |
| Low Cd/G varieties |      |       |      |
| Kitahonami      | 0.84 \(^{b}\) | 0.16 \(^{a}\) | 0.25 \(^{bc}\) |
| Nanbukomugi     | 0.79 \(^{a}\) | 0.07 \(^{a}\) | 0.21 \(^{c}\) |
| ANOVA \(^{2}\)  | **\*\*** | ns | *    |
| **97DAS**       |      |       |      |
| High Cd/G varieties |      |       |      |
| Nishikazekomugi | 2.97 \(^{a}\) | 1.09 \(^{bc}\) | 1.02 \(^{ab}\) |
| Kitakamikomugi  | 2.91 \(^{a}\) | 1.81 \(^{a}\) | 1.18 \(^{a}\) |
| Low Cd/G varieties |      |       |      |
| Kitahonami      | 1.70 \(^{b}\) | 1.36 \(^{ab}\) | 0.77 \(^{bc}\) |
| Nanbukomugi     | 1.78 \(^{b}\) | 0.78 \(^{c}\) | 0.64 \(^{c}\) |
| ANOVA \(^{2}\)  | **\*\*** | ns | *    |

1) Means followed by common letters under each trait were not significantly different according to the multiple test of Ryan-Einot-Gabriel-Welsch (P < 0.05).
2) ** and * show significance at P < 0.01 and 0.01 \(\leq P < 0.05\), respectively, and ns is not significant according to ANOVA.

Fig. 3. Relationships between root frequency and Cd quantity in whole plant tissues in the root box experiment.

Kitahonami, Kitakamikomugi and Nanbukomugi showed intermediate values compared to Nishikazekomugi and Kitahonami. Shoot DW was larger in Nishikazekomugi than in other varieties. Kitahonami and Nanbukomugi (low Cd/G varieties) had a smaller root DW than Nishikazekomugi and Kitakamikomugi (high Cd/G varieties).

### Discussion

We examined the concentration and quantity of Cd in seedlings of four Japanese wheat varieties with a high or low grain Cd concentration. These varieties were selected from 237 varieties and used in field, pot and/or root box experiments. The varietal differences in tested traits had a similar tendency among the three experiments. Stolt et al. (2006) reported that genetic variation in shoot and grain Cd concentration remains consistent, regardless of soil type or growing season. The results obtained in this study therefore appear reliable. Cd concentration of soil used in the experiments ranged from 0.170 to 0.210 ug g
\(^{-1}\) (n=16).

Asami et al. (1988) reported that Cd concentration of natural non-contaminated soil in Japan was 0.295 ug g
\(^{-1}\) (0.056−0.801 ug g
\(^{-1}\)). Soil used in this study is considered to be non-contaminated soil.

We firstly hypothesized that low Cd/G varieties had a small amount of Cd absorbed from the soil through the roots during early growth. This was confirmed by the experiments shown in Table 1. The amount of Cd absorbed during the early growth stage may affect to the grain Cd accumulation. Stolt et al. (2006) also showed that it is possible to identify the genotypes that accumulate the most Cd in the grain at an early plant development stage in common wheat and durum wheat. The knowledge should contribute to the research on Cd absorption in wheat seedlings and efficient selection of low Cd/G lines in breeding programs decreasing Cd concentration in wheat grain.
Second, we hypothesized that low Cd/G varieties had a low translocation of Cd from the roots to shoots. While Cd concentration and quantity in root tissues showed no differences among varieties, aerial parts (shoot: leaf + stem) values were lower in low Cd/G varieties than in high Cd/G varieties (Table 1). In fact, the shoot / root ratio of

### Table 4. Plant height, stem number, and DW of leaf, stem and root samples.

| Plant height (cm) | Stem number (plant⁻¹) | Leaf DW (g plant⁻¹) | Stem DW (g plant⁻¹) | Root DW (g plant⁻¹) |
|--------------------|-----------------------|---------------------|---------------------|--------------------|
| **Field experiment** |
| High Cd/G varieties |
| Nishikazekomugi | 19.2 a²) | 4.3 | 0.15 | 0.08 | 0.03 |
| Kitakamikomugi | 16.9 ab | 4.4 | 0.14 | 0.09 | 0.03 |
| Low Cd/G varieties |
| Kitahonami | 12.1 b | 3.5 | 0.10 | 0.07 | 0.03 |
| Nanbukomugi | 13.2 b | 3.5 | 0.14 | 0.09 | 0.03 |
| ANOVA³) | ** | ns | ns | ns | ns |
| **Pot experiment** |
| Nishikazekomugi | 21.1 | 2.3 | 0.11 b | 0.05 |
| Kitahonami | 18.7 | 2.4 | 0.10 | 0.04 |
| ANOVA | ns | ns | ns | ns |
| **Root Box experiment** |
| 39DAS |
| High Cd/G varieties |
| Nishikazekomugi | 21.0 a | 3.8 a | 1.73 a | 1.02 a |
| Kitakamikomugi | 20.0 a | 3.3 b | 1.19 ab | 0.68 ab |
| Low Cd/G varieties |
| Kitahonami | 16.8 b | 3.0 b | 1.23 ab | 0.57 ab |
| Nanbukomugi | 16.4 c | 3.0 b | 0.84 b | 0.48 b |
| ANOVA | ** | ** | * | * |
| 73DAS |
| High Cd/G varieties |
| Nishikazekomugi | 32.3 a | 8.5 a | 4.48 a | 1.74 ab |
| Kitakamikomugi | 19.6 b | 11.5 a | 2.86 b | 1.94 a |
| Low Cd/G varieties |
| Kitahonami | 17.7 b | 15.7 b | 1.23 ab | 0.57 ab |
| Nanbukomugi | 16.4 b | 10.3 a | 0.84 b | 0.48 b |
| ANOVA | ** | ** | * | * |
| 97DAS |
| High Cd/G varieties |
| Nishikazekomugi | 51.3 a | 8.5 c | 4.48 a | 1.74 ab |
| Kitakamikomugi | 26.7 b | 14.8 b | 2.86 b | 1.94 a |
| Low Cd/G varieties |
| Kitahonami | 20.6 c | 29.0 a | 2.19 b | 1.22 b |
| Nanbukomugi | 26.1 b | 11.8 bc | 2.21 b | 1.21 b |
| ANOVA | ** | ** | * | * |

¹) Means followed by common letters under each trait were not significantly different according to the multiple test of Ryan-Einot-Gabriel-Weich (P < 0.05).

²) ** and * show significance at P < 0.01 and 0.01 ≦ P < 0.05, respectively, and ns is not significant according to ANOVA.

³) Total leaf DW and stem DW.

⁴) No measurements obtained.
the concentration and quantity of Cd was also lower in low Cd/G varieties than in high Cd/G varieties (Table 1). Significant relationships between restricted root to shoot translocation of Cd and a low Cd concentration in grain has also been reported in durum wheat cultivars (Berkelaar and Hale, 2000) and in near isogenic lines (Archambault et al., 2001; Harris and Taylor, 2004). Our results indicate that the root to shoot translocation of Cd in the vegetative growth stage may relate to grain Cd concentration in wheat varieties at least used in this study. Mechanisms for restriction of root to shoot translocation of Cd have been studied in the past. In response to Cd, for example, higher plants synthesize sulphur-rich peptides and phytochelatins (PCs), and PC-heavy metal complexes accumulate in the vacuole (Vogeli-Lange and Wagner, 1990; Stolt et al., 2003). Chen and Hale (2004) also reported the translocation of Cd from the shoot to root. Pineros et al. (1998) and Page et al. (2006) further showed an efflux of Cd from the root. These factors may be related to the difference in root to shoot translocation of Cd in the wheat varieties used in this study, and these varieties can be useful materials to investigate Cd translocation in wheat. To clarify detailed mechanisms on absorption and translocation of Cd in plants, serial assessment will be needed over plant growth including stable isotope analyses.

The third hypothesis that the amount of Cd absorption from soil is related to root morphology was investigated with a root box experiment. Root DW was smaller in low Cd/G varieties than in high Cd/G varieties at 73 DAS and 97 DAS (Table 4). The small root DW of low Cd/G varieties was related to the development of lateral roots (Table 2). That is, the low density of lateral roots could have resulted in a low root frequency in these varieties (Table 3), and varieties with a low root frequency had a smaller quantity of Cd in the seedlings (Fig. 3). Kubo et al. (2008b) showed that root frequency had significant positive correlation with root length in wheat. From these results, it is suggested that slow and/or restricted root branching is related to low Cd uptake from soil at the seedling stage in these varieties. Pineros et al. (1998) showed that an influx of Cd\(^{2+}\) into the roots mainly occurs at the root apex. Cieslinski et al. (1998) also reported that low-molecular-weight organic acids, which are mainly released from the root apex to rhizosphere soil, play an important role in the solubilization of particulate-bound Cd in soil solution and its subsequent phytoaccumulation in durum wheat cultivars. Such phenomena may support our suggestion since increased root branching will also result in an increased number of root apaxes. On the other hand, screening and analysis of the difference in Cd absorption by individual roots among wheat varieties may be another groundbreaking research.

Variatel differences were found in shoot morphological traits (Table 4). However, the relationship between shoot growth pattern and Cd accumulation at the seedling stage was not close. At the maturity stage in wheat, Kubo et al. (2008a) reported negative correlations of stem number and plant height with grain Cd concentration. Harris and Taylor (2001) also reported that the size of the shoot as Cd pools may control the remobilization of Cd to the grain. Further investigation may be needed to analyze the correlation of shoot growth and traits with Cd accumulation in grain.

This study revealed the differences in root morphology, Cd uptake and Cd translocation in wheat seedlings with low and high Cd/G varieties. Cd concentration and quantity in shoot parts at the seedling stage appeared to be a useful indicator of grain Cd concentration for selecting lines with a low grain Cd concentration from the progenies of low Cd/G varieties. However, mechanisms of Cd uptake and translocation should also be studied from micro-morphological and physiological aspects based on these varieties. Understanding on Cd mobilization at a later growth stage after stem elongation in these wheat varieties is also needed in the future. Overall, the findings of this study will contribute to further morpho-physiological analyses, genetic improvement and establishment of efficient cropping systems to decrease the Cd concentration in wheat.

Acknowledgements

We are grateful to T. Matsunaga and S. Ito of NARC, and S. Komae of NICS for valuable advice. We would like to thank T. Miike of KONARC for constructing the root boxes and for experimental management.

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