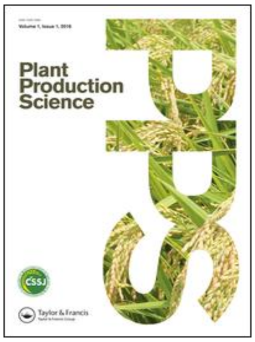

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Cadmium Concentration in Grains of Japanese Wheat Cultivars : Genotypic Difference and Relationship with Agronomic Characteristics

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Abstract : The contamination of cadmium (Cd) into the food chain can be harmful because Cd causes chronic health problems. To evaluate the breeding potential reducing the Cd concentration in wheat grain, we compared Cd concentrations in 237 wheat genotypes including Japanese landraces, Japanese cultivars and introduced alien cultivars for breeding using grain samples collected from upland fields in 2004–5 and 2005–6 growing seasons. The Cd concentration in wheat grain significantly varied with the growing seasons and with the experimental fields. Cultivars bred in northern Japan, including the recent Japanese leading cultivar ‘Hokushin’, tended to have a low Cd concentration in grain compared with that bred in central and southern Japan. Simple correlation analysis between Cd concentration in grain and agronomic characteristics revealed that the Cd concentration in grain showed significant negative correlations with stem number, culm length and spikelet number per spike, and showed significant positive correlation with SPAD value (chlorophyll content) of flag leaf. Stepwise multiple-regression analysis showed that the genotypic variation of Cd concentration in grain was associated with the culm length and spiklet number per spike. This study clarified the geographical pattern of genotypes with different Cd concentrations in grain in Japanese wheat cultivars. Cultivars originating from northern Japan may be useful genetic resources to develop cultivars with a low Cd concentration in grain to be grown in the areas where Cd accumulation in wheat grain is a problem.

Key words : Cadmium, Genotypic variation, Grain, Heavy metal, Soil contamination, *Triticum aestivum*, Wheat.

Cadmium (Cd) is a heavy metal emerging as one of the most important environmental contaminants (McLaughlin et al., 1999). Cd can spread in agricultural soils through irrigation with contaminated water and diffusion of smoke produced by various human activities, including Cd production from ore and use in industrial processing. In addition, Cd may be added to agricultural fields through the use of sewage sludge amendments (Chaudri et al., 2001) and phosphate fertilizer (McBride et al., 1981). In 2005, the Ministry of the Environment of Japan reported that 96 areas (total 6786 hectare) had high Cd concentration in agricultural soil and required countermeasures, such as replacement with non-contaminated soil.

Cd is easily taken up by plant roots and translocated to above ground tissues, where it becomes available for human consumption. Cd is accumulated over many years in the human body via foods, and exerts negative effects on humans (Wagner, 1993). The Codex Alimentarius Commission (2005) proposed a maximum level of 0.2 mg kg^{-1} of Cd for wheat grain as the international criterion. A survey of Cd concentration in wheat grain by the Ministry of Agriculture, Forestry and Fisheries in Japan revealed that 3.1% of wheat cultivated in Japan exceeded 0.2 mg kg^{-1}. The reduction of Cd concentration in wheat grain is therefore an urgent issue.

The development of wheat cultivars with poor Cd accumulation in grains is a promising countermeasure to suppress Cd in humans through the food chain. Genotypic variation in Cd concentration in grain has been reported in durum and bread wheat (Oliver et al., 1995; Li et al., 1997; Greger and Lofstedt, 2004); however, there are few data on the Cd concentration in grain in Japanese wheat cultivars. Evaluation of Cd concentration in grain in broad genetic resources is necessary to understand the mechanisms of grain Cd accumulation and to construct a breeding program to decrease the Cd concentration in grain in wheat. In this study, we compared the Cd concentration in grain among Japanese wheat cultivars, and evaluated the relationship between agronomic characteristics and Cd concentration in grain.

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Table 1. Genotypes used in this study.

Name				
Japanese Landraces (26 genotypes)				
Akabozu[4]	Eshimashinriki[6]	Hiroshimashipuree 3[5]	Sanin 1[5]	Shirodaruma[4]
Akadaruma[4]	Fukuokakomugi 18[6]	Igachikugo[6]	Shinchunaga[5]	Shirokomugi[6]
Akakawaaka[2]	Furutsudaruma[3]	Igachikugo-oregon[1,4]	Shinrikikomugi[5]	Shirosanjaku[4]
Akakomugi[4]	Hayakomugi[6]	Saitama 27[4]	Shirochabo[4]	Sojukuakage[6]
Dawson 1[2]	Hirakikomugi[5]	Saitama 29[4]	Shiroboro 21[4]	Yushoki 347[5]
Eshima[5]				
Cultivars (168 genotypes)				
Norin 1[3]	Norin 35[2]	Norin 69[4]	Nichirinkomugi[6]	Taisetsukomugi[2]
Norin 2[3]	Norin 36[6]	Norin 70[4]	Haruhikari[2]	Akitakko[3]
Norin 3[2]	Norin 37[5]	Norin 71[5]	Ushiokomugi[5]	Abukumawase[6]
Norin 4[5]	Norin 38[4]	Norin 72[5]	Omasekomugi[4]	Harunoakebono[2]
Norin 56	Norin 39[3]	Norin 73[5]	Hiyokukomugi[6]	Kinuiroha[6]
Norin 6[3]	Norin 40[3]	Norin 74[5]	Mukakomugi[2]	Chikugoizumi[6]
Norin 7[4]	Norin 41[4]	Norin 75[2]	Zenkojikomugi[4]	Hokushin[2]
Norin 8[2]	Norin 42[4]	Yuyakekomugi[4]	Kobushikomugi[4]	Shunyo[4]
Norin 9[4]	Norin 43[5]	Susonokomugi[3]	Haruminori[2]	Nishihonami[6]
Norin 10[3]	Norin 44[4]	Mutsubenkei[3]	Sakigakekomugi[6]	Tsurupikari[1,4]
Norin 11[5]	Norin 45[6]	Iyokomugi[5]	Hachimankomugi[3]	Iwainodaichi[6]
Norin 12[4]	Norin 46[5]	Hatamasari[6]	Horoshirikomugi[2]	Nishinokaori[6]
Norin 13[4]	Norin 47[5]	Aobakomugi[3]	Takunekomugi[2]	Ayahikari[4]
Norin 143	Norin 48[4]	Nanbukomugi[3]	Hanagasakomugi[3]	Kinuhime[4]
Norin 15[4]	Norin 49[6]	Akatsukikomugi[5]	Shiroganekomugi[6]	Kitamoe[2]
Norin 16[4]	Norin 50[4]	Yukichabo[4]	Gogatsukomugi[6]	Haruhinode[2]
Norin 17[4]	Norin 51[5]	Hikarikomugi[4]	Toyohokomugi[4]	Kinuazuma[4]
Norin 18[3]	Norin 52[4]	Myokokomugi[4]	Setokomugi[6]	Nebarigoshi[3]
Norin 19[5]	Norin 53[4]	Ebisukomugi[6]	Chikushikomugi[6]	Sanukinoyume 2000[5]
Norin 20[6]	Norin 54[4]	Hitsumikomugi[3]	Shirowasekomugi[6]	Kinunonami[4]
Norin 21[5]	Norin 55[3]	Kokeshikomugi[4]	Asakazekomugi[1,6]	Daburu 8[1,4]
Norin 22[3]	Norin 56[5]	Okukomugi[3]	Fukuhokomugi[1,4]	Haruyokoi[1,2]
Norin 23[5]	Norin 57[4]	Sakyukomugi[3]	Minaminokomugi[6]	Haruibuki[3]
Norin 24[4]	Norin 58[3]	Yutakakomugi[6]	Chihokukomugi[2]	Yumeseiki[4]
Norin 25[5]	Norin 59[5]	Danchikomugi[6]	Wakamatsukomugi[3]	Tamaizumi[4]
Norin 26[5]	Norin 60[6]	Furutsumasari[3]	Fukuwasekomugi[5]	Fukusayaka[5]
Norin 27[3]	Norin 61[6]	Shirasagikomugi[5]	Nishikazekomugi[6]	Yukichikara[3]
Norin 28[4]	Norin 62[2]	Junreikomugi[5]	Haruyutaka[2]	Kitanokaori[2]
Norin 29[2]	Norin 63[5]	Kitakamikomugi[3]	Shiranekomugi[4]	Fusetsu[4]
Norin 30[4]	Norin 64[4]	Fujimikomugi[4]	Airakomugi[4]	Minaminokaori[6]
Norin 31[4]	Norin 65[5]	Hayatokomugi[6]	Koyukikomugi[3]	Harunokagayaki[1,4]
Norin 32[5]	Norin 66[4]	Mikunikomugi[4]	Daichinominori[6]	Yumeasahi[4]
Norin 33[3]	Norin 67[4]	Shimofusakomugi[3]	Bandowase[4]	Hanamanten[4]
Norin 34[6]	Norin 68[4]	Miyaginokomugi[3]		
Introduced cultivars (43 genotypes)				
Alsen	Chinese spring[1]	Jagger	Panpa INTA	Suweon 235
Ardito	Combine	Joshuu	Parchall	Tapdongmil
Aroona	Corringin	Kanred	Reeder	Timstein
Arrino	Creopatra	KS 831957	Rieti	Tordo
AUS 1408	D 6899	Martin	Roblin	Turkey Red II
Australia 8	Eradu	Nainari S60	Rosella	U-11(Purcam)
Australia 13	Geurmil	Nodai 16	Shikan	Victria INTA
Cadoux	Hope	Olgrumil	Shisen 1	Wichita
California	IA 7873	Oregon		

[1] Genotype not used in the 2004–5 growing season.

Genotype originating in [2]Hokkaido region, [3]Tohoku region, [4]Kanto-Tokai-Hokuriku region, [5]Kinki-Chugoku-Shikoku region and [6]Kyushu-Okinawa region.

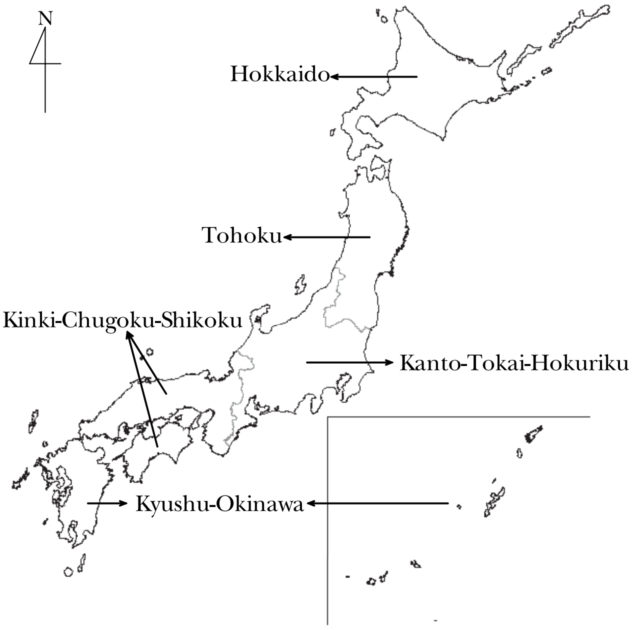

Fig. 1. Geographical location of Japanese regions in this study.

Materials and Methods

1. Evaluation of agronomic characteristics and grain sample collection in fields

A total of 237 wheat genotypes (Table 1), including Japanese landraces, commercial cultivars released in Japan and alien cultivars introduced for breeding, were grown in upland fields of the National Agricultural Research Center (NARC: Ibaraki, Japan, 36°N, 140 °E) located in the Kanto region (Fig. 1) in 2004–5 and 2005–6 wheat growing seasons. The sowing dates were November 4 in the 2004–5 growing season and November 10 in the 2005–6 growing season. In the 2004–5 growing season, eight genotypes were not grown due to poor germination. The plots were single rows of 1.0 m long for the fields in both growing seasons. Planting distance was 70 cm between rows and 8.5 cm between plants. Fertilizer was applied just before seeding: 40, 60 and 40 kg ha^{-1} of N, P_2O_5 and K_2O, respectively. Insects, diseases and weed were controlled by NARC standard practice. Wheat grain was harvested and threshed at maturity. In the 2005–6 growing season, the days of panicle emergence and flowering of each genotype were determined. At the panicle emergence stage of each genotype, stem number and the chlorophyll content of flag leaf were estimated. The chlorophyll content was measured with a SPAD502 meter (Konica Minolta Holdings, Inc.). At maturity in each genotype, culm length and the number of fertile spikelets were measured. The 1000-grains weight was measured after harvest and threshing. To confirm the genotypic difference in the Cd concentration in grain under different environmental conditions, we cultivated three genotypes (Chihokukomugi, Hokushin and Kitamoe) with a low Cd concentration bred in northern Japan (Hokkaido region) and three genotypes (Norin 61, Shiroganekomugi and Nishikazekomugi) with a relatively high Cd concentration bred in southern Japan (Kyushu-Okinawa region) in NARC fields, in upland fields in Hokkaido where the growing condition of wheat differed from the Kanto region. The sowing date was September 21, 2004 and April 19, 2005 in Hokkaido cultivars and Kyushu-Okinawa cultivars, respectively. Planting distance was 60 cm between rows. Fertilizer was applied just before seeding: 50, 90 and 60 kg ha^{-1} of N, P_2O_5 and K_2O, respectively.

2. Analysis of Cd in grain

Grains (10 g) were ground using a laboratory mill with stainless steel blades, and 0.1 g was digested in 20 mL HNO_3 (0.1 M) for 1 hr at room temperature. The samples were then strained with 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 by this method corresponds with that digested in an acid mixture in a microwave oven (analysis by Nittech Research, Co., Hyogo, Japan).

3. Data analysis

Meteorological data during the wheat growing period were obtained from the Japan Meteorological Agency (2006). To evaluate the relationship between Cd concentration in grain and morpho-physiological characteristics, we performed simple correlation analysis and stepwise multiple regression analysis by forward inclusion of the best variables using computer software (SPSS Ver. 13.0 J for Windows, SPSS Japan Inc.).

Results and Discussion

1. Weather conditions and soil properties in the fields

The monthly average temperature in the early growth stage of wheat in the 2005–6 growing season was lower than that in the 2004–5 growing season, although sunshine hours were longer in the 2005–6 than 2004–5 growing season (Fig. 2). In the reproductive growth stage of wheat, sunshine hours were drastically shorter in the 2005–6 growing season than in the 2004–5 growing season. These weather conditions in the 2005–6 growing season delayed the maturity (about 10 days) compared with the 2004–5 growing season. The soil type of fields in NARC and Hokkaido was volcanic ash soil. The pH values of the soil solution were 6.25 and 5.62 in NARC and Hokkaido, respectively. Cd concentration in soil of

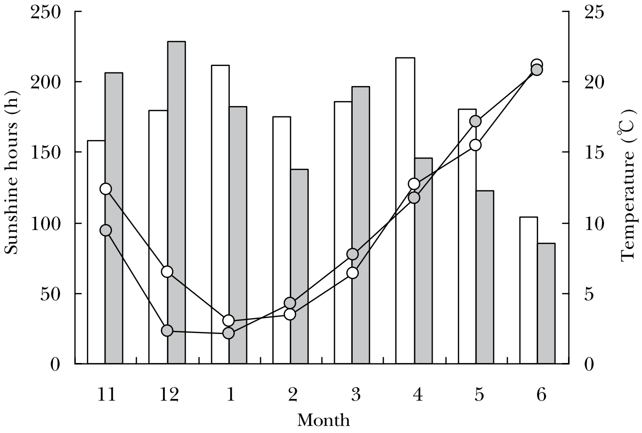

Fig. 2. Monthly sunshine hours (square symbol) and mean temperature (circle symbol) during 2004–5 and 2005–6 growing season of wheat in NARC.
□, ○; 2004–5 growing season. ■, ●; 2005–6 growing season.

Table 2. Mean and coefficient of variation of Cd concentration in wheat grain.

	Mean (ppb)	CV (%)	n
2004–5	42.2	26.0	230
2005–6	34.7	24.8	238

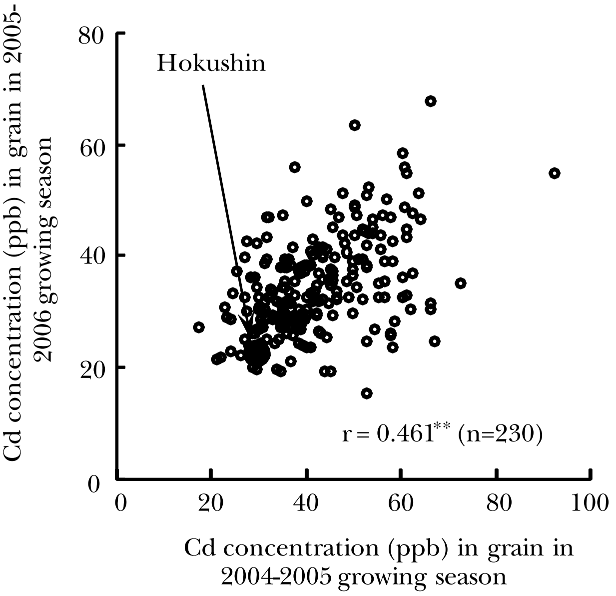

Fig. 3. Relationship between Cd concentration in grain in 2004–5 and 2005–6 growing seasons.
** shows significance at P<0.01 according to simple correlation analysis.

NARC fields extracted with 0.1 M HCl was 87.8 ppb and 121.9 ppb in 2004–5 and 2005–6 growing seasons, respectively.

2. Geographical pattern of genotypic difference in Cd concentration in grain

Well-fertilized grains of all the genotypes were collected in the 2004–5 and 2005–6 growing seasons. Table 2 shows the mean and coefficient of variation of Cd concentration in grain in cultivars used in this study. In the grain sample collected in the 2004–5 growing season, the Cd concentration of each cultivar ranged from 17.4 to 92.5 ppb. In the 2005–6 growing season, it ranged from 15.4 to 67.6 ppb. No cultivars exceeded 200 ppb in Cd concentration in grain, which was the draft maximum level advanced by the Codex Alimentarius Commission (2005). A significant correlation was observed in Cd concentration in grain in each cultivar between the 2004–5 and 2005–6 growing seasons (Fig. 3). These results suggest that there is a genotypic difference in Cd concentration in grain in Japanese wheat cultivars, and it is difficult to affect the rank of cultivars in Cd concentration in grain by environmental conditions. The Cd concentration in grain of a recent leading cultivar in Japan, 'Hokushin', was relatively low in this collection. After the cultivars were classified by region of origin, the frequency distribution of Cd concentration in grain was shown for each region (Fig. 4). Although the Cd concentration in grain differed among cultivars in each region, it tended to be low in cultivars bred in Hokkaido and high in cultivars bred in southern Japan (Kinki-Chugoku-Shikoku and Kyushu-Okinawa regions). The cultivars developed in Hokkaido are genetically different from those developed in southern Japan because the difference in environmental conditions between Hokkaido and southern Japan require different ecotypes for wheat genotypes. The low Cd concentration in grain in Hokkaido cultivars may be related to the use of breeding materials with a low Cd concentration in grain and the inheritance of characteristics. The large difference in Cd concentration in grain in the Japanese wheat collection in this study indicates the potential of breeding to reduce the Cd concentration in grain.

3. Relationship between Cd concentration in grain and grain filling period

The grain filling period (duration from flowering to maturity) was about 10 days shorter in Hokkaido cultivars than in Kyushu-Okinawa cultivars when cultivated in NARC fields located in the Kanto region (Table 3) suggesting that the low Cd concentration in Hokkaido cultivars is related to the shorter duration of grain filling. To evaluate the relationship between the grain filling period and Cd concentration in grain, we compared the Cd concentration of Hokkaido cultivars (Chihokukomugi, Hokushin and Kitamoe) with that of Kyushu-Okinawa cultivars (Norin 61, Shiroganekomugi and Nishikazekomugi) using the grains collected from upland fields in Hokkaido. Although the grain filling period in the Hokkaido cultivars was similar to that in

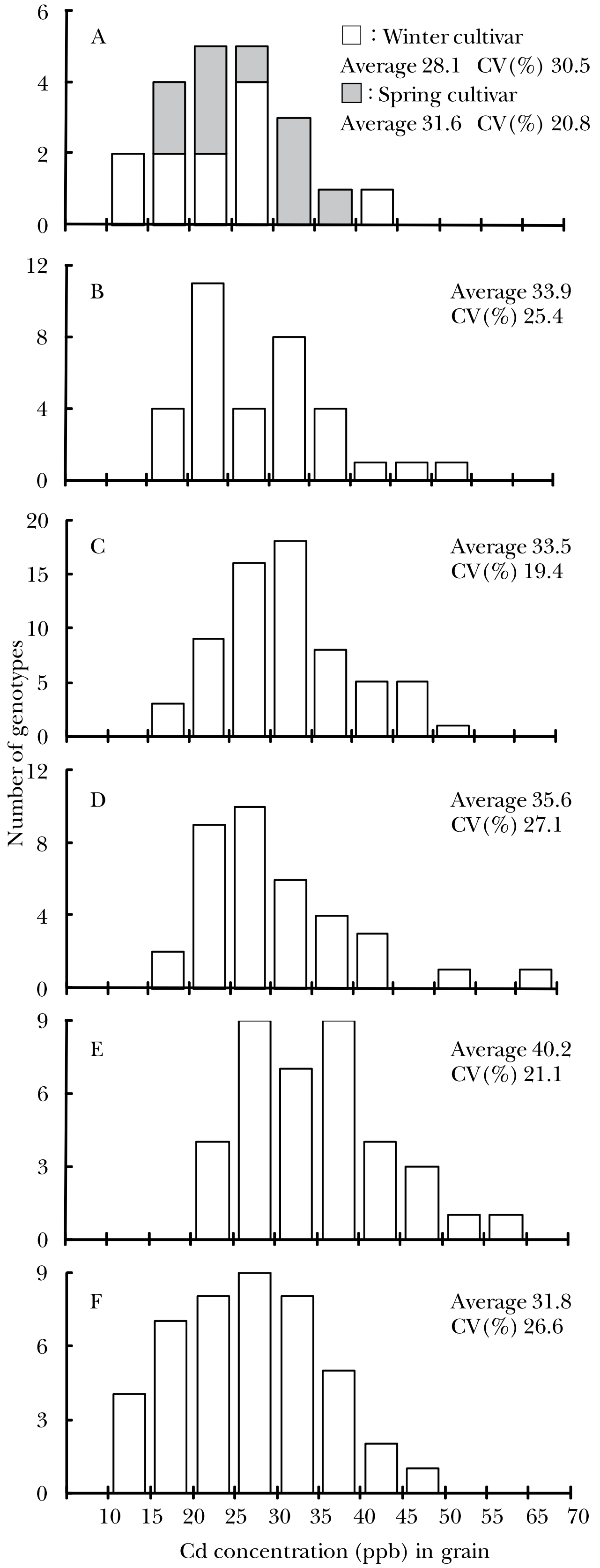

Fig. 4. Frequency distribution of Cd concentration in grain. Genotype group originating in Hokkaido region (A), Tohoku region (B), Kanto-Tokai-Hokuriku region (C), Kinki-Chugoku-Shikoku region (D), Kyushu-Okinawa region (E) and foreign countries (F).

the Kyushu-Okinawa cultivars in the growth conditions of Hokkaido, the Cd concentration in grain was lower in the Hokkaido cultivars than in the Kyushu-Okinawa cultivars. The genotype-region interaction was not significant. These results suggest that the low Cd concentration of Hokkaido cultivars compared with Kyushu-Okinawa cultivars was not due to the difference in the grain filling period.

4. Relationship between the Cd concentration in grain and morpho-physiological characteristics

Table 4 shows the coefficients of correlation between Cd concentration in grain and the morpho-physiological characteristics. Stem number, culm length and spikelets number per panicle showed significant negative correlations with Cd concentration in grain. The genotypes with a longer culm tended to retain more Cd in stems than those with a shorter culm, and the genotypes with a larger number of stems and spikelets tended to contain a smaller amount of Cd per stem and spikelet than those with a smaller number of stems and spikelets. The SPAD value of the flag leaf at panicle emergence stage had a significant positive correlation with Cd concentration in grain. Rharrabti et al. (2001) reported that the chlorophyll content of the flag leaf, measured by a SPAD meter at flowering, is related with the grain protein in durum wheat. The mobilization of Cd to the grain may be related to the process of protein accumulation in the grain. The spikelet number was significantly larger in Hokkaido cultivars than in Kyushu-Okinawa cultivars (Table 5). These results also suggest that the Cd concentration in grain can be reduced with the agricultural management such as by increasing stem number.

Of all characteristics tested in multiple regression, spikelet number and culm length were significantly correlated with the Cd concentration in grain ($P<0.05$). The combination of spikelet number and culm length resulted in a significant ($P<0.01$) regression model that explained 20% of the variance in the Cd concentration in grain. The model obtained was:

Cd concentration in grain (ppb) $= 76.54 - 1.74$ ($\pm$ 0.31) Spikelet number $- 0.08$ (± 0.03) Culm length; $n = 221$, $R^2_{adj} = 0.20$; $SE = 7.8$

The relatively low variance in Cd concentration in grain estimated from these characteristics may indicate that it is affected by additional characteristics such as the ability of root to absorb Cd and/or translocation pattern of Cd between the root, culm, leaf and grain. Properties controlling Cd accumulation in plants have been studied from various viewpoints in durum wheat. Berkelaar and Hale (1999) reported that Cd accumulation in seedlings was related to the root surface area and the number of root tips. Chan and Hale (2004) showed that root-to-shoot and shoot-to-root xylem translocation of Cd controlled

Table 3. Cd concentration in grain in three Hokkaido genotypes and three southern Japanese genotypes cultivated in Kanto and Hokkaido fields.

Genotype	Region genotype origin	Days for grain filling		Cd concentration (ppb)		
		Kanto 2005–6	Hokkaido 2004–5	Kanto 2004–5	Kanto 2005–6	Hokkaido 2004–5
Chihokukomugi	Hokkaido	37	42	35.00	30.30	45.69
Hokushin	Hokkaido	38	42	29.60	22.42	42.47
Kitamoe	Hokkaido	38	42	42.10	29.33	44.30
Norin 61	Kyushu-Okinawa	48	40-45	60.50	36.16	69.25
Shiroganekomugi	Kyushu-Okinawa	47	40-45	62.70	47.60	63.13
Nishikazekomugi	Kyushu-Okinawa	48	40-45	61.10	54.51	79.60
Region genotype origin (O)					*	
Region and year sample collected (S)					**	
O×S					ns	

** and * show significance at $P<0.01$ and $0.01 \leqq P<0.05$, respectively, and ns is not significant according to two-factor factorial ANOVA.

Table 4. Correlation coefficients of Cd concentration in grain with other morpho-physiological characteristics.

	Cd concentration in grain
Stem number	–0.222**
Culm length	–0.295**
Spikelet number	–0.384**
SPAD value	0.226**
1000-grain weight	0.052

** shows significance at $P<0.01$ according to simple correlation analyses.

Cd accumulation in shoots and grain. Herren and Feller (1997) and Hart et al. (1998) also showed that transport of Cd via the xylem and phloem was related to grain Cd accumulation. Harris and Taylor (2001) concluded that translocation of Cd from the leaves and stem to maturing grain may be responsible for the accumulation of Cd in grain. These reports indicate that the mechanism of Cd accumulation in grain is complex, and such a mechanism may relate to the difference in Cd concentration in grain between Hokkaido cultivars and Kyushu-Okinawa cultivars. Further morpho-physiological analyses are needed to clarify the mechanisms of the Cd accumulation process and to conduct effective breeding using genotypes with a low Cd concentration in grain.

5. Conclusion

This study showed the geographical pattern of genotypic difference in Cd concentration in grain in Japanese wheat. The cultivars bred in Hokkaido, which tended to have a low Cd concentration in grain, have many different breeding materials in their pedigrees from cultivars bred in south Japan, and the characteristic of low Cd concentration in grain may have been introduced from such materials. In Canadian durum wheat, genetic analysis revealed that the Cd concentration in grain is heritable (Clarke et al., 1997). Japanese wheat cultivars with a low Cd concentration in the grain may be bred using Hokkaido genotypes with a low Cd concentration in grain. In addition to breeding of cultivars with a low Cd concentration in grain, the agricultural practice to reduce the Cd accumulation in wheat grain, such as increasing the soil pH (Adams et al., 2004), may be necessary to develop an efficient system to decrease the Cd concentration in wheat.

Table 5. Morpho-physiological characteristics of three Hokkaido cultivars and three Kyusyu-Okinawa cultivars.

	Hokkaido cultivars	Kyushu-Okinawa cultivars	Significance
Stem number (plant^{-1})	20.0±1.5	16.3±1.7	ns
Culm length (cm)	86.3±4.1	92.7±1.5	ns
Spikelet number (spike^{-1})	21.3±0.9	18.0±0.6	*
SPAD value	50.9±1.2	52.4±0.7	ns
1000-grain weight (g)	3.35±0.25	3.25±0.26	ns

* shows significance at $0.01 \leqq P<0.05$, and ns is not significant according to unpaired t-test between Hokkaido cultivars and Kyushu-Okinawa cultivars (mean±standard error, n=3).

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