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REVIEW

Genotypic Differences in Cadmium Concentration and Distribution of Soybean and Rice

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Abstract

In order to investigate the genotypic differences in seed cadmium (Cd) concentration in soybean and rice, 17 soybean and 49 rice varieties were cultivated in Cd-polluted soils or water culture containing Cd. Significant differences in seed Cd concentration were found among soybean and rice varieties. A high level of inheritance of the seed Cd concentration was revealed for soybean. The physiological mechanism underlying the Cd translocation to shoots and seeds in soybean was involved in Cd retention in the roots. The commercial rice varieties (e.g., Koshihikari) were categorized into the low grain Cd group. On the other hand, several indica or indica-japonica rice varieties accumulated considerably high Cd concentrations in grains as well as straws, when they were cultivated under upland conditions, suggesting that these varieties would be most responsive to phytoremediation of Cd-polluted paddy fields. There was no correlation of the Cd concentration between younger shoots and mature seeds in the rice cultivars, so it may be impossible to use rice for evaluating the genotypic variation in seed Cd concentration using relatively younger shoots. On the other hand, a positive correlation between them was found in the soybean cultivars, so it may be possible to evaluate the genotypic variation in soybean seed Cd concentration using relatively younger soybean shoots. Interactions between Cd and other metals (Cu, Fe, Mn, and Zn) in terms of their uptake and translocation to shoots were found among the rice and soybean cultivars.

Discipline: Soils, fertilizers and plant nutrition
Additional key words: Cd-polluted soils, heavy metals, roots, solution culture

Introduction

Cadmium (Cd) is one of the hazardous heavy metals to human health. The Codex Committee on Food Additives and Contaminants has been examining a maximum level for Cd in various foods including staple crops and has recently proposed a draft maximum level for polished rice[3] (0.4 mg kg^{-1} Cd). Rice and soybean are the principle sources of dietary intake of Cd in the Japanese population. Therefore, minimizing the intake of Cd from these staple crops is an important health issue.

Agronomic practices applied to satisfy this criterion, such as soil dressing, soil amendment, and flooding of paddy fields, are effective for reducing grain Cd levels[15]. However, many of these agronomic measures are expensive, and in some cases impractical in Cd-polluted soils. Selecting genotypes that translocate lower levels of Cd to the grain when grown in these Cd-polluted soils is one of the practical options to allow the use of these soils for rice production. From the viewpoint of public health, minimizing the concentrations of Cd and other hazardous heavy metals in grain is an urgent matter. Thus, if the proposed criterion is approved by the Codex Committee, much more research will be needed to identify low Cd genotypes of rice grain and soybean seed across different soil series and environments.

The uptake of Cd by plants varies not only among plant species but also among cultivars. Li et al.[8,9] reported significant variation in the grain Cd level of sunflowers, durum wheat and flax. Morishita et al.[12] found that Cd levels in brown rice grains ranged from 2.1 to 27.0 ppb among 28 japonica varieties and from 4.1 to 55.5 ppb among 23 indica varieties under normal soil conditions. Reddy and Dunn[13] reported significant differences in seed grain Cd levels between two soybean varieties grown on sewage sludge amended soil.

Most of the studies reported on the screening of low-

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Cd cultivars were carried out using a water culture system, by the addition of Cd to non-polluted soils, or by using one soil series. The selection and evaluation of low-Cd cultivars in one soil or water culture for breeding purposes may not be practical, because the uptake of Cd in plants is considerably affected by various soil factors, e.g., pH, CEC, clay content, total carbon content, concentrations of heavy metals including Cd, etc.[8]. In addition, Cd in soils occurs in complicated forms because of its association with a number of physicochemical forms that in turn influence its availability.

It has been suggested that the uptake of Cd by roots and its subsequent translocation to shoots could be considerably affected by other metals coexisting in Cd-polluted soils, such as Zn, Mn, Cu, etc. The synergistic or antagonistic interactions between Cd and other metals were reported in rice[10], wheat[17], maize[5], lettuce[4], etc. The ratio of Cd to other heavy metals in soils depends on the source of Cd pollution, and the uptake and translocation of Cd may also vary depending on the metal ratio in different soils with complex contaminants.

The main objectives of the present study were to determine varietal differences in uptake, translocation and distribution of Cd among soybean and rice genotypes and to investigate the interactions between Cd and other heavy metals in terms of shoot concentrations in rice and soybean grown in soils with various levels of Cd contamination. We also considered the possibility of evaluating the genotypic variation in seed Cd concentration using relatively younger shoots.

Materials and methods

Seventeen soybean (*Glycine max* (L.) Merr.) varieties were grown in pots filled with 3 types of soils (soil A, soil B, soil C, Table 1) or under field conditions in unpolluted soil (peat soils) in Ibaraki, Japan[1]. The varieties selected were primarily popular Japanese varieties; supernodulating (En-b0-1, Sakukei 4 and En-b2-110) and nonnodulating (En-N0-2 and En1282) soybean lines from Enrei were also selected.

Forty-nine rice (*Oryza sativa* L.) varieties were grown in a fiberglass container filled with 2 types of soils (soil A, soil B) measuring 4.65 × 0.85 × 0.22 m (length × width × depth) under upland conditions in 1999 and/or in 2000[2]. Five rice varieties were grown in pots under upland or paddy conditions[2].

Based on the results of the above experiments, 11 rice cultivars with different genotypes (japonica, indica and japonica-indica crosses) and 10 soybean cultivars were selected and grown in pots to investigate the genotypic differences in shoot Cd concentration and the interactions between Cd and other heavy metals in terms of shoot concentrations in the rice and soybean in 4 types of soils (soil A, soil B, soil D, soil E, Table 1) with various levels of Cd contamination[7].

Table 1. Cd concentration of soils in experiments

Soil	Soil types	0.1 M HCl extractable Cd (mg kg^{-1} soil)
A	Fluvisol	0.3– 0.7
B	Andosol	3.8–10.4
C	Andosol	0.2
D	Fluvisol	1.7
E	Andosol	2.9

Characteristics of Cd-polluted soils and growth conditions of each experiment were described previously[1,2,7].

Four or five varieties of soybean were grown in nutrient solution containing 100 μg L^{-1} Cd. Growth conditions were described previously[1].

Results and discussion

1. Soybean seed Cd

Significant genotypic differences in seed Cd levels were found. The seed Cd concentration was lowest for the Sakukei 4(En-b0-1-2), a cross between En-b0-1 and Tamahomare (Fig. 1)[16], and highest for Harosoy, the grandparent plant of Suzuyutaka (Fig. 2), in both field and pot experiments (Table 2). The seed Cd levels of Hatayutaka (Tohoku 128), a cross between Enrei and Suzuyutaka (Fig. 2), were intermediate between those of the parents. For 4 soil types, containing from 0.2 to 6.5 mg kg^{-1} 0.1 mol L^{-1} HCl extractable Cd, the ranking of soybean genotypes based on seed Cd level was similar, indicating that there is a genetic factor involved in the varietal differences in Cd concentration.

The mean seed Cd concentration of Sakukei 4 was lower than that of Enrei (Table 2). However, the seed Cd concentration of En-b0-1 and En-b2-110 were almost identical to that of Enrei (Table 2). Low Cd genotypes of soybean were produced by mutation, although the supernodulation trait itself may not have contributed to the differences in Cd concentration found in soybean seeds. The growth and yield of Sakukei 4 were similar to those of Enrei[14], suggesting that Sakukei 4 could serve as a mother plant for future breeding of low Cd genotypes of soybean.

Among the 4 soybean varieties tested in one experiment in the present study, the Cd concentrations in leaves, stems and pods as well as the total Cd uptake were lowest for Sakukei 4 (Table 3). The Cd concentrations in the leaves, stem and petiole were lower at both 7

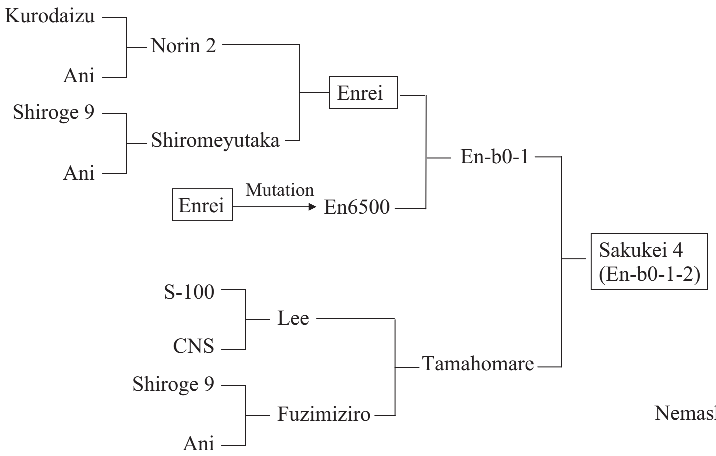

Fig. 1. Estimated dendrogram of Sakukei 4[16]

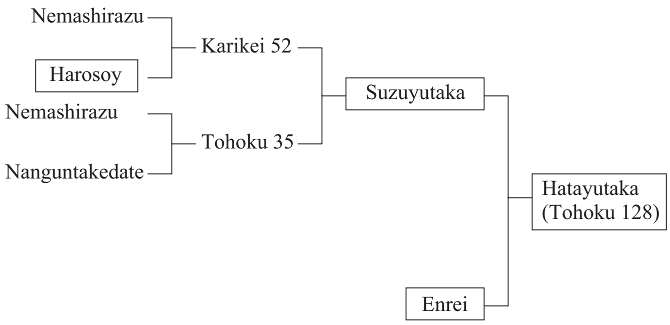

Fig. 2. Dendrogram of Hatayutaka

Table 2. Seed Cd concentration of 17 soybean cultivars grown on soil A, soil B, soil C and low-Cd field

Cultivar name	Pot						Field	
	Soil A (mg kg^{-1}dw)		Soil B (mg kg^{-1}dw)		Soil C (mg kg^{-1}dw)		Low-Cd field (mg kg^{-1}dw)	
Sakukei 4	0.46	a	1.43	a	0.12	a	0.08	a
Tamahomare	0.70	abc	2.52	abc	0.13	ab	0.10	ab
En-b0-1	0.82	bcd	1.96	abc	0.23	cd	0.10	ab
Goyoukuromame	1.16	ef	1.99	abc	0.20	bc	0.10	ab
Hayagin	0.91	cdef	2.22	abc	0.23	cd	0.11	ab
Enrei	0.89	cde	2.09	abc	0.19	abc	0.11	ab
En-b2-110	0.91	cdef	2.06	abc	0.19	abc	0.11	ab
Dewamusume	1.05	def	5.24	d	0.24	cd	0.12	b
Tachiyutaka	1.47	g	3.29	c	0.28	de	0.12	b
En-N0-2	1.91	h	4.94	d	0.18	abc	0.13	b
Tachinagaha	1.17	f	2.88	bc	0.23	cd	0.13	bc
Nattousyouryuu	0.59	ab	2.90	bc	0.18	abc	0.13	bc
Getenshirazu1	0.78	bcd	1.72	ab	0.20	bc	0.13	bc
En1282	2.22	i	5.33	d	0.24	cd	0.16	c
Hatayutaka	0.97	cdef	2.83	bc	0.23	cd	0.22	d
Suzuyutaka	1.50	g	7.46	e	0.33	e	0.31	e
Harosoy	2.68	j	12.68	f	0.34	e	0.40	f
L.S.D. (5%)	0.28		1.35		0.08		0.03	
Average	1.14		3.61		0.21		0.15	

Means followed by the same letter are not significantly different at the 5% level.

Table 3. Cd concentration in leaves, stems and pods of soybean (66 days after sowing, soil B)

	Cultivars	Cd content (mg kg^{-1})	Cd uptake (μg plant^{-1})
Leaves	Sakukei 4	5.5 a	67.6 a
	Hatayutaka	12.2 b	152.8 b
	Suzuyutaka	12.9 b	86.9 a
	L.S.D. (5%)	1.6	27.1
Stems	Sakukei 4	4.3 a	48.0 a
	Hatayutaka	10.3 b	130.1 b
	Suzuyutaka	20.3 c	120.1 b
	L.S.D. (5%)	5.1	51.2
Pods	Sakukei 4	2.1 a	8.4 a
	Hatayutaka	5.5 ab	8.3 a
	Suzuyutaka	13.7 b	8.6 a
	L.S.D. (5%)	9.4	5.9
Total	Sakukei 4		124.0 a
	Hatayutaka		291.3 b
	Suzuyutaka		215.6 b
	L.S.D. (5%)		83.8

Values followed by the same letter are not significant at the 5% level.

and 15 days after the addition of Cd to the nutrient solution for Sakukei 4 and Enrei than for Hatayutaka, Suzuyutaka and Harosoy; however, the Cd concentrations of roots for Sakukei 4 and Enrei were higher than for the other varieties (Table 4). Florijn and Van Beusichem[5] reported that two groups of maize inbred lines were distinguished: a group with low shoot, but high root Cd concentrations ('shoot Cd excluder') and a group with similar shoot and root Cd concentrations ('non-shoot Cd excluder'). In the results obtained in the present study, the percentage of total Cd uptake in the roots of En-bo-1-2 and Enrei was much higher than that in Hatayutaka, Suzuyutaka and Harosoy (Table 4). Our results indicate that Cd retention in roots is an important mechanism in regulating or minimizing its translocation to the shoots and particularly to the seeds.

2. Rice grain Cd

Results of ranking of rice grain Cd concentrations were similar in 1999[2] and 2000 (Table 5). NIPPONBARE, KOSHIHIKARI and HU-LO-TAO belonged to the rice group with the lowest grain Cd contents. KASALATH could be categorized as a variety with a medium grain Cd content and MILYANG23 and PEH-KUH-TSAO-TU could be categorized as varieties with high grain Cd contents. These 5 varieties except for HU-LO-TAO from the above 6 typical varieties were grown in submerged pots (paddy conditions), and the ranking of the grain Cd content was almost the same as that in the upland condition pots (Table 6). It is well known that Cd uptake of rice under upland conditions is remarkably higher than under paddy conditions in association with chemical speciation of Cd in soil[11]. So, these results imply that it may be possible to select paddy rice varieties with a low Cd content even when growth occurred under upland conditions.

Morishita et al.[12] reported that Cd levels in the grains of japonica varieties were lower than in those of indica, javanica, or hybrid varieties in non-polluted fields. In our results, japonica varieties such as NIPPONBARE belonged to the lowest Cd level group in both types of Cd-polluted soils (Table 5) in both years[2]. The grain Cd concentrations in LAC23 (African upland rice variety) and HU-LO-TAO (Chinese variety) were as low as that for NIPPONBARE (Table 5). In order to produce varieties with lower Cd levels than those in the varieties cur-

Table 4. Cd concentration and percentage of total uptake in leaves, petioles, stems and roots of soybean (solution culture)*

	Cultivars	7 days after adding Cd		15 days after adding Cd	
		Cd concentration (mg kg^{-1})	Percentage of total uptake (%)	Cd concentration (mg kg^{-1})	Percentage of total uptake (%)
Leaves	Sakukei 4	6.2 a	9.0 a	12.3 a	13.1 a
	Enrei	5.0 a	8.3 a	11.4 a	13.7 a
	Hatayutaka	12.1 b	27.2 b	21.6 b	30.8 b
	Suzuyutaka	13.5 bc	26.0 b	25.5 b	26.8 b
	Harosoy	15.2 c	24.5 b	26.4 b	37.2 c
	L.S.D. (5%)	2.1	4.1	6.1	6.0
Petioles	Sakukei 4			24.6 a	4.1 a
	Enrei			25.4 a	4.1 a
	Hatayutaka			43.7 b	8.9 b
	Suzuyutaka			82.0 d	11.3 c
	Harosoy			55.1 c	8.7 b
	L.S.D. (5%)			9.1	1.3
Stems	Sakukei 4	9.0 ** a	6.0** a	14.0 a	3.8 a
	Enrei	6.9 ** a	6.0** a	11.0 a	4.6 a
	Hatayutaka	17.6 ** b	20.4** b	20.0 b	13.1 b
	Suzuyutaka	21.4 ** b	19.3** b	39.6 d	15.1 b
	Harosoy	27.1 ** c	23.6** b	27.4 c	17.2 b
	L.S.D. (5%)	5.3	7.2	5.4	4.1
Roots	Sakukei 4	198 a	85.1 a	291 a	79.0 a
	Enrei	153 b	85.8 a	210 b	77.6 a
	Hatayutaka	59 c	52.4 b	107 cd	47.2 b
	Suzuyutaka	70 c	54.6 b	125 c	46.8 b
	Harosoy	62 c	51.9 b	76 d	36.9 c
	L.S.D. (5%)	30	8.4	47	7.0

*: Cd was added 7 days after transfer of the plants. **: Stems + petioles.
Means followed by the same letter are not significantly different at the 5% level.

Table 5. Mean grain Cd concentration of rice cultivars grown in soil A and soil B (container experiments, 2000)

Cultivar name	Country of origin	Soil A ($mg\ kg^{-1}dw$)		Soil B ($mg\ kg^{-1}dw$)	
LAC 23	SLE	0.19	a	1.06	a
HU-LO-TAO	CHN	0.39	a b	0.79	a
AKITAKOMACHI	JPN	0.66	a b c	1.28	a b
NIPPONBARE	JPN	0.71	a b c	1.71	a b c
SASANISHIKI	JPN	0.80	a b c d	1.16	a b
KOSHIHIKARI	JPN	0.80	a b c d	1.83	a b c
BATATAIS	BRZ	0.87	a b c d e	1.46	a b c
KETAN	IDN	0.88	a b c d e	2.36	a b c
AKIHIKARI	JPN	0.96	a b c d e	1.05	a
CAIAPO	BRZ	1.02	a b c d e f	1.88	a b c
PEROLA	BRZ	1.03	a b c d e f	1.58	a b c
GURANI	unknown	1.06	a b c d e f g	1.69	a b c
DHILI BORO	BGD	1.22	a b c d e f g h	3.28	a b c d e
DENNYUU1GOU	JPN	1.24	a b c d e f g h	2.78	a b c d
KHAO DAM	LAO	1.36	a b c d e f g h i	2.73	a b c
MIZUHATAMOCHI	JPN	1.48	a b c d e f g h i	3.94	a b c d e f
BARAN BORO	BGD	1.54	a b c d e f g h i	2.29	a b c
GHARIB	IRN	1.56	a b c d e f g h i	2.58	a b c
IRAT212	CIV	1.62	b c d e f g h i j	2.71	a b c
SHORT GRAIN	THA	1.67	b c d e f g h i j	7.24	f g
BG1	JPN	1.78	c d e f g h i j	2.50	a b c
TSUKUBAHATAMOCHI	JPN	1.87	c d e f g h i j	2.14	a b c
NOURIN24GOU	JPN	1.89	c d e f g h i j	2.76	a b c d
GERDEH	IRN	1.90	c d e f g h i j k	2.23	a b c
KUROMOKU	JPN	2.15	d e f g h i j k	2.80	a b c d
KASALATH	IND	2.22	e f g h i j k l	2.81	a b c d
HATAKINUMOCHI	JPN	2.34	f g h i j k l	2.27	a b c
CHIYOMINORI	JPN	2.36	f g h i j k l	4.85	c d e f g
CHAHORA 144	PAK	2.42	g h i j k l	4.51	b c d e f g
RD7	THA	2.58	h i j k l	6.16	d e f g
LMN111	THA	2.66	i j k l	7.05	f g
PEH-KUH-TSAO-TU	TWN	2.98	j k l m	6.66	e f g
IR-8	PHL	3.27	k l m	7.65	g
HABATAKI	JPN	3.56	l m	6.94	f g
MILYANG23	KOR	4.31	m	7.18	f g
Average		1.70		3.25	

Means followed by the same letter are not significantly different at the 5% level according to the Tukey HSD test.
BGD: Bangladesh, BRZ: Brazil, CHN: China, CIV: Cote d'Ivoire, IDN: Indonesia, IND: India, IRN: Iran, JPN: Japan, KOR: Korea, LAO: Laos, PAK: Pakistan, PHL: Philippines, SLE: Sierra Leone, THA: Thailand, TWN: Taiwan.

Table 6. Mean grain Cd concentration of 5 rice cultivars (pot experiment)

	Cultivar name	Paddy conditions	Upland conditions
		Grain Cd (mg kg^{-1}dw)	Grain Cd (mg kg^{-1}dw)
Soil A	NIPPONBARE	0.013 a	0.30 a
	KOSHIHIKARI	0.050 ab	0.58 ab
	KASALATH	0.089 b	0.67 ab
	PEH-KUH-TSAO-TU	0.098 bc	1.06 b
	MILYANG 23	0.157 c	1.85 c
Soil B	NIPPONBARE	0.023 a	1.42 a
	KOSHIHIKARI	0.092 ab	1.43 a
	KASALATH	0.141 bc	1.60 a
	PEH-KUH-TSAO-TU	0.194 c	3.11 b
	MILYANG 23	0.150 bc	4.95 c

Means followed by the same letter are not significantly different at the 5% level according to the Tukey HSD test.

Table 7. Shoot Cd concentration and percentage of grain Cd uptake (pot experiment, 136 days after transplanting, soil B)

Cultivar name	Cd concentration		Dry weight		Cd uptake		Grain Cd uptake / (shoot + grain) Cd uptake	Grain dry weight / (shoot + grain) dry weight	
	Shoot (mg kg^{-1}dw)	Grain (mg kg^{-1}dw)	Shoot (g/pot)	Grain (g/pot)	Shoot (μg/pot)	Grain (μg/pot)	a (%)	b (%)	a/b
NIPPONBARE	5.5 a	1.0 a	34.7	2.7	191	2.8	1.5	7.3	20.0
KOSHIHIKARI	8.0 ab	1.2 a	27.3	2.5	218	3.1	1.4	8.5	16.3
KASALATH	12.2 abc	2.0 a	27.0	0.8	329	1.6	0.5	2.9	16.8
PEH-KUH-TSAO-TU	21.0 c	3.2 b	19.6	2.0	412	6.2	1.5	9.1	16.4
MILYANG 23	15.9 bc	3.9 b	19.7	2.2	312	8.4	2.6	9.9	26.5

Means followed by the same letter are not significantly different at the 5% level according to the Tukey HSD test.

rently under cultivation, it would be useful to elucidate and compare the properties of these low Cd varieties.

Although heavy metal hyper-accumulating plants such as *Thalaspi carulescens* are reported to be promising for phytoremediation of Cd polluted soils[6], it is unclear whether these plants could exert Cd accumulation activity in paddy fields. The shoot Cd concentrations in MILYANG23 and PEH-KUH-TSAO-TU in the pot experiments in soil B under upland condition were very high (Table 7), which suggests that these varieties would be most applicable to phytoremediation of Cd-polluted paddy fields. Field trails of rice–based phytoremediation including post-harvest technologies for handling of rice plants are in progress.

Cd distribution for grain was evaluated (Table 7). KASALATH absorbed a relatively high amount of Cd, although Cd distribution to grain in KASALATH was the lowest among the 5 varieties. On the other hand, in MILYANG23, a much higher amount of Cd was translocated to the grain than in the other 4 varieties, suggesting the existence of genetic variability in Cd translocation from shoots to grains.

3. Rapid screening of varieties with low seed Cd

If the genotypic variation in seed Cd concentration could be evaluated in relatively younger shoots, the effort to develop cultivars with a low seed Cd concentration would be reduced. Therefore, we examined the varietal differences in the Cd concentration of younger shoots in the rice and soybean in the 4 soils with different levels of Cd contamination and evaluated the genotypic variation in seed Cd concentration using their younger shoots. Significant differences in shoot Cd concentration were found among rice and soybean cultivars in the 4 soils[7]. The ranking of the rice cultivars for the shoot Cd concentration varied considerably among the soils[7]. On the other hand, soybean cultivars were ranked similarly in terms of shoot Cd concentration in the 4 soils[7]. The Cd concentration of younger shoots was highly correlated with the mature seed Cd concentration, irrespective of the soil types (Fig. 3, Soybean). This indicates that it could be possible to evaluate the genotypic variation in seed Cd concentration using relatively younger shoots in the soybean cultivars. Meanwhile, it appears to be difficult to conduct such an evaluation for the rice cultivars, because there was no correlation between them in two soils (Fig. 3, Rice). Other approaches for efficiently selecting rice cultivars with low grain Cd should be investigated.

4. Interaction between Cd and other heavy metals in shoots

Significant and positive correlations were found between the Cd and Zn concentrations and between the Cd and Mn concentrations in the shoots of rice cultivars, when they were grown in the D and E soils with relatively moderate levels of Cd contamination (Fig. 4, Rice). This result suggests that the physiological mechanisms of the uptake of Cd and its translocation to shoots in rice may be partly associated with several metal ions that are chemically related. The shoot Cd concentration in the soybean cultivars was not correlated with the concentrations determined for any of the metals (Zn, Mn, Cu, and Fe) across the 4 soils (Fig. 4, Soybean). It is likely that the concentrations of Cd and other metals in the shoots of the soybean cultivars are controlled by independent mechanisms.

In conclusion, we elucidated the genetic and physiological traits in terms of the Cd uptake and its translocation to shoots and seeds of rice and soybean. Information provided in the present study would be useful for the development of new varieties in rice and soybean with lower seed Cd concentration than the present ones.

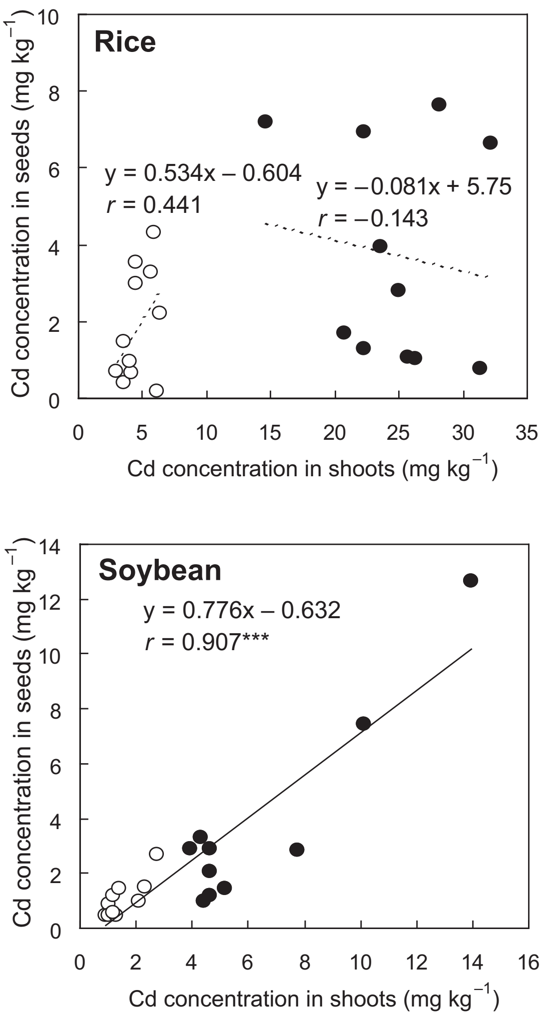

Fig. 3. Relationships between the concentrations of Cd in shoots and seeds in rice and soybean cultivars

○ : A soil, ● : B soil. The straight or dotted regression lines indicate significant or non-significant differences, respectively.

***: Significant at the 0.001 probability levels.

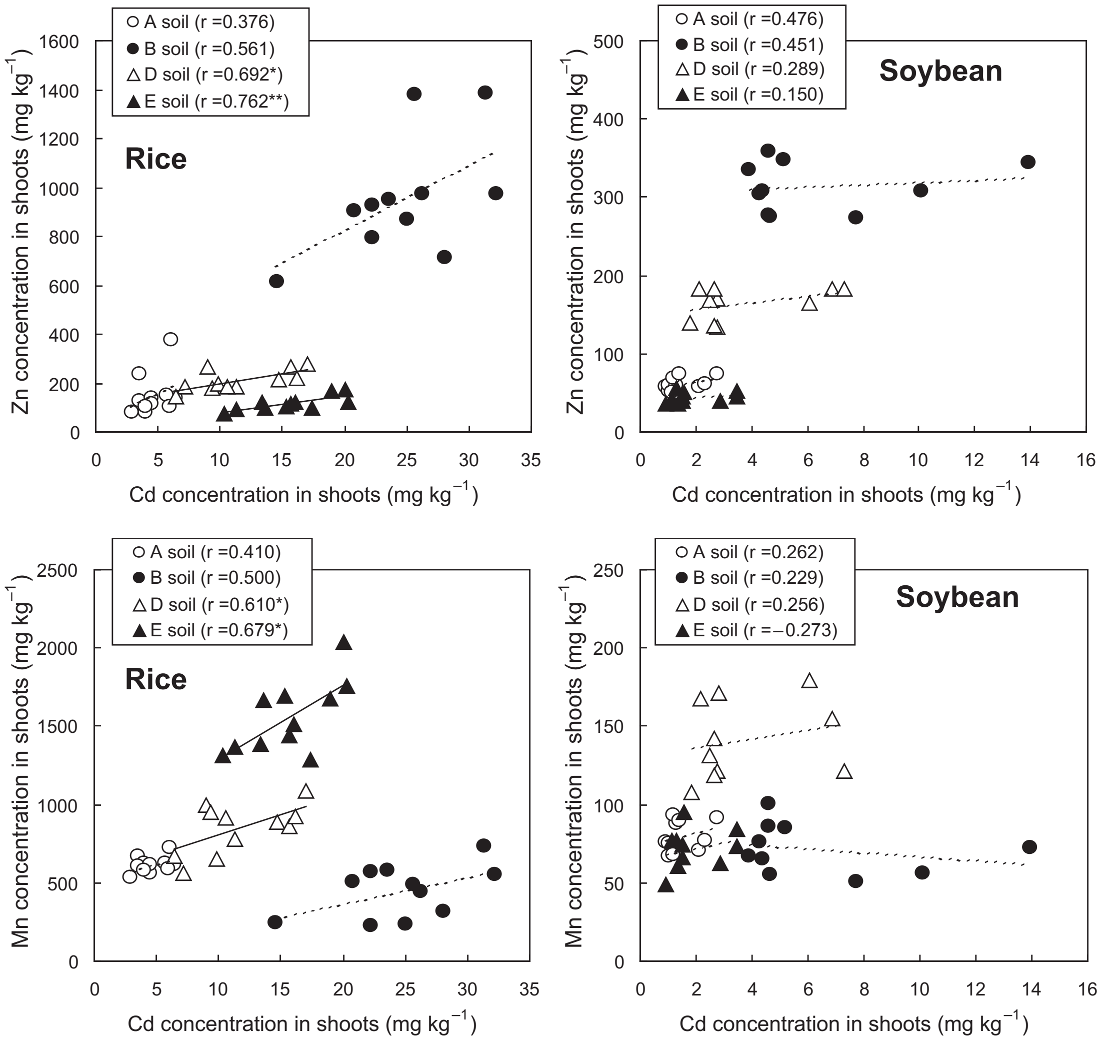

Fig. 4. Correlations between the concentrations of Cd and other metals (Zn and Mn) in the shoots of rice and soybean cultivars

The straight or dotted regression lines indicate significant or non-significant differences, respectively. *&**: Significant at the 0.05 and 0.01 probability levels, respectively.

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