Breeding Science 70: 631–636 (2020)
doi: 10.1270/jsbbs.20070

Note

Breeding and characterization of the high cadmium-accumulating rice line 'Akita 119'

Ryuichi Takahashi*[1)], Masashi Ito[1)], Kazunao Kato[1)], Ikuko Kodama[1)], Satoru Shibata[1)], Kensuke Sato[1)], Riyako Takahashi[1,2)], Shinichi Matsumoto[1)] and Tomohiko Kawamoto[1)]

[1)] *Akita Prefectural Agricultural Experiment Station*, 34-1 Genpachizawa, Yuwa-Aikawa, Akita city, Akita 010-1231, Japan
[2)] *Present address: Akita Prefectural Office*, 4-1-1 Sanno, Akita city, Akita 010-8570, Japan

Cadmium (Cd) is a toxic heavy metal that is mainly accumulated through the consumption of foods produced in Cd-contaminated fields. Phytoremediation is one of the most effective methods to reduce the soil Cd concentration. In this study, we bred a new rice line, 'Akita 119', for Cd phytoremediation. 'Akita 119' was obtained by a soft X-ray mutation of 'Cho-ko-koku', a naturally high-Cd-accumulating rice cultivar. The heading date of 'Akita 119' was about 2 weeks later than that of 'Akitakomachi', which is the leading cultivar in Akita Prefecture, Japan. 'Akita 119' has a short culm length and many panicles. The shattering resistance and lodging resistance of 'Akita 119' were improved compared to 'Cho-ko-koku'. The thousand-grain weight of 'Akita 119' was much smaller than that of 'Akitakomachi', and grains of 'Akita 119' could be easily distinguished from general japonica cultivars. When 'Akita 119' was grown in Cd-contaminated fields, the shoot dry weight and Cd concentration were similar to those of 'Cho-ko-koku'. These results demonstrate that 'Akita 119' has improved agronomic characteristics compared to 'Cho-ko-koku' while retaining the ability to extract Cd. Therefore, it should be considered a promising candidate for Cd phytoremediation in paddy fields in northern parts of Japan.

Key Words: 'Akita 119', cadmium, phytoremediation, rice breeding, rice line.

Introduction

Cadmium (Cd) is a heavy metal that is toxic for humans and causes various health problems (Horiguchi *et al.* 2013, Trzcinka-Ochocka *et al.* 2010). Cadmium accumulates in the human body following the consumption of contaminated foods. A Cd limit in some agricultural products was established by the Codex Alimentarius Commission to provide food safety and human health (CODEX 2006). Some agricultural products that are harvested from Cd-contaminated fields exceed the Cd limit value, which is becoming a serious human health problem. In Japan, Cd limits are exceeded on 2.7 km^2 of farmland, requiring remediation measures (Ministry of the Environment 2017). Techniques to reduce Cd concentrations in soil are therefore urgently needed.

Phytoremediation, which is the removal of soil contaminants using plants, is one of the most effective methods to reduce soil Cd concentrations. Rice has been used effectively for Cd phytoremediation of paddy fields due to its large biomass yield and well-established cultivation and harvest methods (Ishikawa *et al.* 2006). Various Cd levels have been measured in the shoots and grains of different rice cultivars (Arao and Ae 2003, Ishikawa *et al.* 2005, Takahashi *et al.* 2011, Uraguchi *et al.* 2009). 'Cho-ko-koku', 'Jarjan', and 'Anjana Dhan' accumulate significantly higher levels of Cd in their shoots and grains than do other cultivars grown worldwide (Uraguchi *et al.* 2009). After 2–4 years' cultivation of 'Cho-ko-koku', the Cd concentration in the soil and grains of subsequent cultivars were reported to be lower than the levels in a control field (Ibaraki *et al.* 2009, 2014, Murakami *et al.* 2009). These results indicate that high-Cd-accumulating rice cultivars are effective for Cd phytoremediation.

Heavy metal ATPase (HMA) is a metal transporter, and some of the HMA in rice can transport Cd (Takahashi *et al.* 2012, Uraguchi and Fujiwara 2012, 2013). OsHMA3 functions to enable the sequestration of Cd into vacuoles in the root cells (Miyadate *et al.* 2011, Ueno *et al.* 2010). The responsive gene of quantitative trait loci (QTL) for the Cd concentration in the shoots of 'Cho-ko-koku', 'Jarjan', and 'Anjana Dhan' was identified as *OsHMA3* (Ueno *et al.* 2010, 2011, Tezuka *et al.* 2010). *OsHMA3* from these cultivars cannot transport Cd into vacuoles, resulting in the direct translocation of absorbed Cd from roots to shoots

Communicated by Toshio Yamamoto
Received June 16, 2020. Accepted September 8, 2020.
First Published Online in J-STAGE on November 7, 2020.
*Corresponding author (e-mail: takahashi-ryuichi@pref.akita.lg.jp)

(Ishikawa *et al.* 2011). Backcross inbred lines (BILs) containing the QTL for the Cd concentration from the 'Jarjan' allele reduced the Cd concentration in the soil and the shoots of subsequently grown rice (Abe *et al.* 2011). The loss of function of OsHMA3 causes high Cd accumulation in the shoots and grains (Yan *et al.* 2016). This indicates that OsHMA3 plays an important role in Cd accumulation in the shoots of rice.

OsNRAMP5 is a metal transporter involved in Cd uptake by roots (Ishimaru *et al.* 2012, Sasaki *et al.* 2012). *OsNRAMP5* knockdown plants have been reported to accumulate higher levels of Cd in shoots than wild-type plants due to enhanced Cd translocation to shoots; this process is associated with the expression of genes related to metal transport, such as *OsNRAMP1* and *OsIRT2* (Ishimaru *et al.* 2012). Higher Cd accumulation levels have been observed in the *OsNRAMP5* knockdown mutant of 'Anjana Dhan' than in wild-type 'Anjana Dhan', even in a Cd-contaminated field (Takahashi *et al.* 2014). However, high Cd-accumulating rice cultivars such as 'Cho-ko-koku', 'Jarjan', and 'Anjana Dhan' are difficult to cultivate because they tend to shatter and are easily lodged.

Abe *et al.* (2013, 2017) produced the rice line 'MJ3', 'MA22', and 'TJTT8' for Cd phytoremediation. 'MJ3' and 'MA22' were obtained by a gamma ray mutation of 'Jarjan' and 'Anjana Dhan', respectively. 'TJTT8' was obtained by backcrosses of 'Tachisugata', a rice variety used as a livestock feed after it is crossed with 'Jarjan'. 'MJ3' and 'TJTT8' accumulate high levels of Cd, with both varieties having a non-shattering habit and lodging resistance (Abe *et al.* 2013, 2017). However, the heading and maturing day of 'MJ3' are too late for it to be grown in Akita Prefecture, and it is difficult to obtain 'TJTT8' seeds due to sterility. Therefore, it is difficult to cultivate 'MJ3' and 'TJTT8' in northern regions of Japan. Previously, we released 'Akita 110' for Cd phytoremediation in northern regions of Japan (Takahashi *et al.* 2016). However, Cd accumulation in 'Akita 110' was lower than that in 'Cho-ko-koku' depending on field conditions. To improve the stability of Cd accumulation in the shoots, we produced a new rice line, 'Akita 119', for Cd phytoremediation.

Materials and Methods

Plant growth conditions

Seeds of 'Akita 119', 'Cho-ko-koku', 'Akitakomachi', and 'Akita 110' were germinated and grown as described previously (Takahashi *et al.* 2016). 'Akitakomachi' is a general japonica cultivar and is the leading variety grown in Akita Prefecture, Japan. Seedlings were grown in plastic greenhouses in Akita Prefectural Agricultural Experiment Station, Akita Prefecture, Japan from mid-April to May. For line selection, seedlings were transplanted one by one into planting lines in a paddy field at Akita Prefectural Agricultural Experiment Station. For DNA marker selection, a technique reported previously was applied (Miyadate *et al.* 2011). To determine agronomic traits, yield, and Cd extraction, four seedlings were transplanted as one hill. About 120 hills were planted per experimental plot, with two replicates. In each experiment, seedlings were transplanted into 22 × 22-cm areas, with a planting density of 20.7 hills m^{-2}. Experimental plots were established in Cd-contaminated paddy fields located in Akita Prefecture. The soil type of the paddy fields was classified as gray lowland soil, and the soil pH was around 5.5. The amount of base dressing fertilizer applied and the soil Cd concentration (before planting, extraction with 0.1 M HCl) of each experimental field are indicated in **Table 1**. No additional fertilizer was supplied. Flood irrigation was conducted until the heading stage. When the plants entered the heading stage, irrigation was stopped, and drainage was maintained until harvest.

Yield and agronomic trait tests

Yield and agronomic trait tests were performed based on the Rice Cultivation Guidebook for Akita Prefecture (Akita Prefecture 2010). For yield tests, harvesting was performed on 64 hills in two experimental plots for each cultivar. Culm length, panicle length, and panicle number were measured at the maturing stage for 10 hills per experimental plot. Shoot dry weight, unhulled rice grain weight, and thousand-grain weight were measured as described previously (Takahashi *et al.* 2016). Heading date and lodging were estimated from observations of experimental plots within the growing fields. Days to heading was calculated as the period from transplanting day to heading day. Lodging was evaluated on an ordinal scale from 0 (not lodged) to 5 (completely lodged). The shattering habit was evaluated by shedding rate after grasping panicles in the maturing stage.

Measurement of Cd concentration

To measure the Cd concentration of test plants, two hills were harvested from three locations in the experimental plots. Harvested plants were separated into shoots and panicles and dried at 80°C for 2 days. Sample grinding and

Table 1. Amount of base dressing fertilizer applied and soil cadmium (Cd) concentrations in experimental fields

	Field A	Field B	Field C	Field D	Field E	Field F
(Year)	2011	2012	2013	2014	2015	2016
Base dressing fertilizers (gN m^{-2})	11.0	8.0	4.0	4.0	6.0	6.0
Cd concentration (mg kg^{-1})	0.55 ± 0.09	1.47 ± 0.07	0.73 ± 0.13	0.96 ± 0.08	0.75 ± 0.09	0.55 ± 0.08

Table 2. The ‘Akita 119’ breeding process

Year	Generation	Process
2005	M_1	Mutation processing to seed of ‘Cho-ko-koku’ by soft X-ray
2006	M_2	Initial screening by non-shattering habit and heading date (35,000 plants→45 plants)
2007	M_3	Line selection
2008	M_4	↓
2009	M_5	↓ Agronomic traits tests
2011	M_6	↓ Marker check (*OsHMA3*) Named ‘Akikei 697’; ↓ Yield tests, Cd extraction tests
2012	M_7	↓ ↓ ↓
2013	M_8	↓ ↓ ↓
2014	M_9	↓ ↓ ↓
2015	M_{10}	↓ ↓ ↓
2016	M_{11}	↓ Named ‘Akita 119’ ↓ ↓
2017	M_{12}	Check genetic fixation

digestion were performed as described previously (Takahashi *et al.* 2016). To measure the soil Cd concentration, soil was collected from five or six locations in each field (fields A–F). The Cd concentration was measured using inductively coupled plasma optical emission spectrometry (ICP-OES: Vista-PRO; Varian, Australia). The Cd extraction was calculated as Cd concentration in the shoots × shoots dry weight per area.

Results

Breeding process and agronomic characteristics

‘Akita 119’ is a rice line developed at the Akita Prefectural Agricultural Experiment Station. We used soft X-rays to irradiate seeds of a naturally high-Cd-accumulating rice cultivar, ‘Cho-ko-koku’, in 2005 (**Table 2**). Forty-five M_2 plants were identified from 35,000 plants in the initial screening based on their non-shattering habit and suitable heading date for Akita Prefecture. After the M_3 generation, we selected rice lines based on the Cd concentration in the aerial parts of the plants. In the M_6 generation, we identified plants that possessed the *OsHMA3* allele of ‘Cho-ko-koku’. In the M_6 generation (2011), one of the promising breeding lines was designated ‘Akikei 697’, and we determined agronomic traits, yield, and Cd extraction until the M_{11} generation (2016). ‘Akikei 697’ was eventually evaluated as a promising candidate rice line for Cd phytoremediation and was redesignated ‘Akita 119’. The heading date of ‘Akita 119’ was around 2 weeks later than that of ‘Akitakomachi’ (**Table 3**). ‘Akita 119’ had a short culm length and large panicle numbers (**Fig. 1A**). The culm of ‘Akita 119’ was around 30 cm shorter than that of ‘Cho-ko-koku’. The shattering resistance of ‘Akita 119’ was strong, and the lodging resistance of ‘Akita 119’ was improved compared to ‘Cho-ko-koku’. The thousand-grain weight of ‘Akita 119’ was slightly lower than that of ‘Cho-ko-koku’ and much lower than that of ‘Akitakomachi’. The grain size and shape of ‘Akita 119’ were different from those of ‘Akitakomachi’, and its grains could be easily distinguished from general japonica cultivars (**Table 4**, **Fig. 1B**, **1C**). In the M_{12} generation (2017), the plant type of ‘Akita 119’ was investigated using 30 plants from six lines to determine the genetic fixation. Culm length in most ‘Akita 119’ plants

Table 3. Characteristics of ‘Akita 119’. The data shown are averages calculated for the period 2011–2016. Lodging was evaluated on an ordinal scale from 0 (not lodged) to 5 (completely lodged)

	Akita 119	Cho-ko-koku	Akita 110	Akita-komachi
Heading date (m/d)	8/19	8/16	8/20	8/3
Days to heading (d)	85	82	86	69
Culm length (cm)	68.0	99.0	81.8	78.0
Panicle length (cm)	21.0	22.6	16.4	17.7
Panicle number (m^{-2})	578	458	457	419
Shoots DW ($g\ m^{-2}$)	749	703	729	579
Grains DW ($g\ m^{-2}$)	391	337	327	667
Thousand grain weight (g)	11.6	13.1	15.7	21.7
Lodging (0 – 5)	0.4	2.3	0.2	0.6
Shattering resistance	strong	weak	strong	strong

Fig. 1. (A) ‘Akita 119’ in the maturing stage. White bars indicate 30 cm. (B) ‘Akita 119’ grains. (C) ‘Akita 119’ decorticated grains.

Table 4. Size and shape of decorticated grains of 'Akita 119'. Twenty grains were investigated per experimental plot, with two replicates in 2017

	length (mm)	width (mm)	thickness (mm)	length/width	shape
Akita 119	5.80	1.80	1.47	3.23	long spindle-shaped
Cho-ko-koku	6.06	1.78	1.58	3.41	long spindle-shaped
Akita 110	5.67	2.17	1.52	2.61	spindle-shaped
Akitakomachi	4.90	2.87	2.08	1.71	semi-round

Table 5. Culm length (A), panicle length (B), and panicle number (C) of 'Akita 119'. Thirty plants of six lines were investigated in 2017. Thirty 'Cho-ko-koku' and 'Akitakomachi' plants of were also investigated. Squares indicate the maximum value in each line or cultivar. SD and CV indicate standard deviation and coefficient of variation, respectively

(A) Culm length

	line No.	Average ± SD	CV (%)	Culm length (cm)												
				~40	~45	~50	~55	~60	~65	~70	~75	~80	~85	~90	~95	Total
Akita 119	1	50.1 ± 4.8	9.6	1	4	12	10	3	0	0	0	0	0	0	0	30
	2	48.8 ± 4.9	10.0	0	11	8	9	1	1	0	0	0	0	0	0	30
	3	51.0 ± 5.2	10.2	0	5	9	9	7	0	0	0	0	0	0	0	30
	4	50.6 ± 4.0	7.9	0	3	11	13	2	1	0	0	0	0	0	0	30
	5	53.4 ± 3.9	7.3	0	0	5	19	5	1	0	0	0	0	0	0	30
	6	52.4 ± 4.5	8.6	0	1	10	11	7	1	0	0	0	0	0	0	30
Cho-ko-koku		82.9 ± 4.6	5.5	0	0	0	0	0	0	0	2	5	14	8	1	30
Akitakomachi		69.6 ± 4.2	6.0	0	0	0	0	0	8	10	8	4	0	0	0	30

(B) Panicle length

	line No.	Average ± SD	CV (%)	Panicle length (cm)						
				~16	~18	~20	~22	~24	~26	Total
Akita 119	1	19.8 ± 1.0	5.1	0	2	17	10	1	0	30
	2	19.7 ± 1.0	5.1	0	0	18	12	0	0	30
	3	20.0 ± 0.9	4.5	0	0	14	16	0	0	30
	4	19.9 ± 1.1	5.5	0	1	14	14	1	0	30
	5	19.9 ± 0.9	4.5	0	0	17	12	1	0	30
	6	19.5 ± 1.1	5.6	0	5	14	11	0	0	30
Cho-ko-koku		20.6 ± 1.0	4.9	0	0	13	15	2	0	30
Akitakomachi		17.7 ± 1.1	6.2	2	19	9	0	0	0	30

(C) Panicle number

	line No.	Average ± SD	CV (%)	Panicle number per hill						
				~6	~9	~12	~15	~18	~21	Total
Akita 119	1	13.0 ± 2.8	21.5	0	2	13	10	3	2	30
	2	11.5 ± 2.5	21.7	0	4	17	8	0	1	30
	3	12.6 ± 2.4	19.0	0	1	15	9	5	0	30
	4	12.5 ± 2.5	20.0	0	3	12	11	4	0	30
	5	13.2 ± 2.7	20.5	0	1	15	6	7	1	30
	6	12.8 ± 2.9	22.7	0	3	10	12	4	1	30
Cho-ko-koku		12.7 ± 2.4	18.9	0	2	14	11	2	1	30
Akitakomachi		8.6 ± 1.5	17.4	1	20	9	0	0	0	30

was between 45 and 55 cm, which was short compared with 'Cho-ko-koku' and 'Akitakomachi' (**Table 5**). The panicle length of 'Akita 119' was slightly shorter than that of 'Cho-ko-koku'. The panicle number was almost the same as that of 'Cho-ko-koku', although much higher panicle numbers were observed in the agronomic trait and yield tests (**Table 3**).

Cadmium extraction capacity of 'Akita 119'

To investigate the Cd extraction capacity, 'Akita 119' was grown in a Cd-contaminated field located in Akita Prefecture from 2011 to 2016. Both the shoot dry weight and Cd concentration of 'Akita 119' were similar to those of 'Cho-ko-koku' in each field (**Table 6**). Although the 'Akita 119' culm was much shorter than that of 'Cho-ko-koku', shoot dry weight did not differ due to the many tillers of

Table 6. Shoot dry weight (DW), cadmium (Cd) concentration in the shoots, and Cd extraction from ‘Akita 119’. Different letters indicate statistically significant differences between cultivars (Tukey–Kramer test, $P < 0.05$)

	Field A			Field B			Field C		
	Shoots DW ($g\ m^{-2}$)	Cd conc. ($mg\ kg^{-1}$)	Cd extraction ($mg\ m^{-2}$)	Shoots DW ($g\ m^{-2}$)	Cd conc. ($mg\ kg^{-1}$)	Cd extraction ($mg\ m^{-2}$)	Shoots DW ($g\ m^{-2}$)	Cd conc. ($mg\ kg^{-1}$)	Cd extraction ($mg\ m^{-2}$)
Akita 119	726.6^a	16.6^a	11.9^a	830.1^a	84.4^a	70.0^a	786.6^a	15.2^a	14.9^a
Cho-ko-koku	647.9^a	17.0^a	10.8^a	894.2^a	76.4^{ab}	68.5^a	666.5^{ab}	14.5^a	12.2^a
Akita 110	883.9^a	14.2^a	12.7^a	830.1^a	59.8^b	48.7^a	561.0^b	11.1^a	7.6^b
Akitakomachi	689.3^a	4.1^b	2.8^b	660.3^a	18.0^c	11.9^b	472.0^b	2.9^b	1.6^c

	Field D			Field E			Field F		
	Shoots DW ($g\ m^{-2}$)	Cd conc. ($mg\ kg^{-1}$)	Cd extraction ($mg\ m^{-2}$)	Shoots DW ($g\ m^{-2}$)	Cd conc. ($mg\ kg^{-1}$)	Cd extraction ($mg\ m^{-2}$)	Shoots DW ($g\ m^{-2}$)	Cd conc. ($mg\ kg^{-1}$)	Cd extraction ($mg\ m^{-2}$)
Akita 119	672.8^a	17.6^a	13.9^a	768.0^a	15.2^a	15.2^a	534.1^a	11.0^a	8.2^a
Cho-ko-koku	643.8^a	19.7^a	15.1^a	610.7^a	16.1^a	13.1^a	469.9^{ab}	12.8^a	8.2^a
Akita 110	751.4^a	18.7^a	15.7^a	621.0^a	11.1^b	8.5^b	451.3^{ab}	14.5^a	8.1^a
Akitakomachi	641.7^a	4.7^b	3.4^b	623.1^a	3.0^c	2.2^c	368.5^b	2.5^b	1.1^b

‘Akita 119’. As a result, Cd extraction using ‘Akita 119’ was similar to that achieved by ‘Cho-ko-koku’ in all fields, whereas Cd extraction using ‘Akita 110’ was lower than that achieved by ‘Cho-ko-koku’ in fields C and E.

Discussion

Rice is an effective plant for practical Cd phytoremediation of paddy fields (Ishikawa *et al.* 2006). A rice variety used for Cd phytoremediation is required to have excellent agronomic characteristics and high Cd-extraction capacity. The lodging resistance of ‘Akita 119’ was stronger than that of ‘Cho-ko-koku’ because of the shorter culm length, and ‘Akita 119’ also had strong shattering resistance (**Table 3**). ‘Akita 119’ had high tiller numbers, and its biomass was as great as that of ‘Cho-ko-koku’ (**Table 6**). These results demonstrated that ‘Akita 119’ exhibited improved agronomic characteristics compared to ‘Cho-ko-koku’.

Cd extraction rates depend on shoot Cd concentration and shoot dry weight. ‘Akita 119’ attained the same Cd concentration and shoot dry weight as ‘Cho-ko-koku’ in all fields (**Table 6**). As a result, their Cd extraction rates were similar. However, less Cd was sometimes extracted from ‘Akita 110’ than from ‘Cho-ko-koku’ (**Table 6**). The Cd concentration of ‘Akita 110’ was lower than that of ‘Cho-ko-koku’ in fields B and E, and the shoot dry weight of ‘Akita 110’ was lower than that of ‘Cho-ko-koku’ in field C. Cadmium absorption from the soil to rice roots is dependent on soil conditions after the heading stage, and it is maximized under drained and oxidative soil conditions (Ibaraki *et al.* 2009, Murakami *et al.* 2009). This result suggested that Cd phytoremediation by ‘Akita 119’ was less susceptible to soil conditions than ‘Akita 110’ would be. As observed for ‘Cho-ko-koku’, the gene responsible for Cd concentration in ‘Akita 119’ and ‘Akita 110’ shoots is *OsHMA3*, which suggests that they may have very similar Cd translocation ability from root to shoot. It is possible that the much higher panicle numbers observed in ‘Akita 119’ cause stably high Cd extraction ability compared to ‘Akita 110’. ‘Akita 119’ panicle numbers were found to be nearly identical to those of ‘Cho-ko-koku’ in a genetic fixation test (**Table 5**), whereas far higher panicle numbers were observed in agronomic trait and yield tests (**Table 3**). In 2017, it was not possible to increase the tiller number in the early stage because of the low temperature and sunshine duration in June in Akita (data not shown). It is possible that the tiller number of ‘Akita 119’ was affected by weather conditions in the early stage.

In conclusion, compared to ‘Cho-ko-koku’, ‘Akita 119’ exhibited improved agronomic characteristics and improved stability of Cd accumulation in shoots. ‘Akita 119’ also exhibited improved stability of Cd accumulation in shoots compared to ‘Akita 110’. The small grain size of ‘Akita 119’ requires only a short maturing period, and seed production can be stable even in northern parts of Japan. Therefore, ‘Akita 119’ is a promising candidate rice line for Cd phytoremediation of paddy fields in northern parts of Japan.

Author Contribution Statement

Breeding ‘Akita 119’ including yield and agronomic traits tests was performed by RT, KK, IK, SS, KS, RT, SM and TK. Measurement of Cd concentrations was performed by MI.

Acknowledgments

This work was supported by a grant from the Ministry of Agriculture, Forestry, and Fisheries of Japan (Regulatory Research Projects for Food Safety, Animal Health and Plant Protection, no. 2603).

Literature Cited

Abe, T., F. Taguchi-Shiobara, Y. Kojima, T. Ebitani, M. Kuramata, T. Yamamoto, M. Yano and S. Ishikawa (2011) Detection of a QTL for accumulating Cd in rice that enables efficient Cd phytoextraction from soil. Breed. Sci. 61: 43–51.

Abe, T., M. Kuramata, M. Iwasaki, T. Honma, T. Ibaraki, T. Yamamoto, M. Yano, M. Murakami and S. Ishikawa (2013) "MJ3" and "MA22", new high-cadmium-accumulating practical rice lines with non-shattering derived from gamm ray mutation. Breed. Res. 15: 17–24 (in Japanese).

Abe, T., M. Ito, R. Takahashi, T. Honma, N. Sekiya, K. Shirao, M. Kuramata, M. Murakami and S. Ishikawa (2017) Breeding of a practical rice line 'TJTT8' for phytoextraction of cadmium contamination paddy fields. Soil Sci. Plant Nutr. 63: 388–395.

Akita Prefecture (2010) Rice Cultivation Guidebook, pp. 116–132 (in Japanese).

Arao, T. and N. Ae (2003) Genotypic variations in cadmium levels of rice grain. Soil Sci. Plant Nutr. 49: 473–479.

CODEX (2006) Report of the 38th session of the CODEX committee on Food Additives and Contaminants. Codex Alimentarius Commission, ALINORM 06/29/12, 1–12.

Horiguchi, H., E. Oguma, S. Sasaki, H. Okubo, K. Murakami, K. Miyamoto, Y. Hosoi, K. Murata and F. Kayama (2013) Age-relevant renal effects of cadmium exposure through consumption of home-harvested rice in female Japanese farmers. Environ. Int. 56: 1–9.

Ibaraki, T., N. Kuroyanagi and M. Murakami (2009) Practical phytoextraction in cadmium-polluted paddy fields using a high cadmium accumulating rice plant cultured by early drainage of irrigation water. Soil Sci. Plant Nutr. 55: 421–427.

Ibaraki, T., S. Fujitomi, A. Ishitsuka and M. Yanaka (2014) Phytoextraction by high-Cd-accumulating rice to reduce Cd in wheat grains grown in Cd-polluted fields. Soil Sci. Plant Nutr. 60: 266–275.

Ishikawa, S., N. Ae, M. Sugiyama, M. Murakami and T. Arao (2005) Genotypic variation in shoot cadmium concentration in rice and soybean in soils with different levels of cadmium contamination. Soil Sci. Plant Nutr. 51: 101–108.

Ishikawa, S., N. Ae, M. Murakami and T. Wagatsuma (2006) Is *Brassica juncea* a suitable plant for phytoremediation of cadmium in soils with moderately low cadmium contamination?—Possibility of using other plant species for Cd-phytoextraction. Soil Sci. Plant Nutr. 52: 32–42.

Ishikawa, S., N. Suzui, S. Ito-Tanabata, S. Ishii, M. Igura, T. Abe, M. Kuramata, N. Kawachi and S. Fujimaki (2011) Real-time imaging and analysis of differences in cadmium dynamics in rice cultivars (*Oryza sativa*) using positron-emitting ^{107}Cd tracer. BMC Plant Biol. 11: 172.

Ishimaru, Y., R. Takahashi, K. Bashir, H. Shimo, T. Senoura, K. Sugimoto, K. Ono, M. Yano, S. Ishikawa, T. Arao *et al.* (2012) Characterizing the role of rice NRAMP5 in manganese, iron and cadmiun transport. Sci. Rep. 2: 286.

Ministry of the Environment (2017) http://www.env.go.jp/press/files/jp/107776.pdf (Dec. 2017, in Japanese).

Miyadate, H., S. Adachi, A. Hiraizumi, K. Tezuka, N. Nakazawa, T. Kawamoto, K. Katou, I. Kodama, K. Sakurai, H. Takahashi *et al.* (2011) OsHMA3, a P_{1B}-type of ATPase affects root-to-shoot cadmium translocation in rice by mediating efflux into vacuoles. New Phytol. 189: 190–199.

Murakami, M., F. Nakagawa, N. Ae, M. Ito and T. Arao (2009) Phytoextraction by rice capable of accumulating Cd at high levels: Reduction of Cd content of rice grain. Environ. Sci. Technol. 43: 5878–5883.

Sasaki, A., N. Yamaji, K. Yokosho and J.F. Ma (2012) Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. Plant Cell 24: 2155–2167.

Takahashi, R., Y. Ishimaru, T. Senoura, H. Shimo, S. Ishikawa, T. Arao, H. Nakanishi and N.K. Nishizawa (2011) The OsNRAMP1 iron transporter is involved in Cd accumulation in rice. J. Exp. Bot. 62: 4843–4850.

Takahashi, R., K. Bashir, Y. Ishimaru, N.K. Nishizawa and H. Nakanishi (2012) The role of heavy metal ATPases, HMAs, in zinc and cadmium transport in rice. Plant Signal. Behav. 7: 1605–1607.

Takahashi, R., Y. Ishimaru, H. Shimo, K. Bashir, T. Senoura, K. Sugimoto, K. Ono, N. Suzui, N. Kawachi, S. Ishii *et al.* (2014) From laboratory to field: *OsNRAMP5*-knockdown rice is a promising candidate for Cd phytoremediation in paddy fields. PLoS ONE 9: e98816.

Takahashi, R., M. Ito, K. Katou, K. Sato, S. Nakagawa, K. Tezuka, H. Akagi and T. Kawamoto (2016) Breeding and characterization of the rice (*Oryza sativa* L.) line "Akita 110" for cadmium phytoremediation. Soil Sci. Plant Nutr. 62: 373–378.

Tezuka, K., H. Miyadate, K. Katou, I. Kodama, S. Matsumoto, T. Kawamoto, S. Masaki, H. Satoh, M. Yamaguchi, K. Sakurai *et al.* (2010) A single recessive gene controls cadmium translocation in the cadmium hyperaccumulating rice cultivar 'Cho-Ko-Koku'. Theor. Appl. Genet. 120: 1175–1182.

Trzcinka-Ochocka, M., M. Jakubowski, W. Szymczak, B. Janasik and R. Brodzka (2010) The effects of low environmental cadmium exposure on bone density. Environ. Res. 110: 286–293.

Ueno, D., N. Yamaji, I. Kono, C.F. Huang, T. Ando, M. Yano and J.F. Ma (2010) Gene limiting cadmium accumulation in rice. Proc. Natl. Acad. Sci. USA 107: 16500–16505.

Ueno, D., E. Koyama, N. Yamaji and J.F. Ma (2011) Physiological, genetic, and molecular characterization of a high-Cd-accumulating rice cultivar, Jarjan. J. Exp. Bot. 62: 2265–2272.

Uraguchi, S., S. Mori, M. Kuramata, A. Kawasaki, T. Arao and S. Ishikawa (2009) Root-to-shoot Cd translocation via the xylem is the major process determining shoot and grain cadmium accumulation in rice. J. Exp. Bot. 60: 2677–2688.

Uraguchi, S. and T. Fujiwara (2012) Cadmium transport and tolerance in rice: perspectives for reducing grain cadmium accumulation. Rice (N Y) 5: 5.

Uraguchi, S. and T. Fujiwara (2013) Rice breaks ground for cadmium-free cereals. Curr. Opin. Plant Biol. 16: 328–334.

Yan, J., P. Wang, P. Wang, M. Yang, X. Lian, Z. Tang, C.-F. Huang, D.E. Salt and F.J. Zhao (2016) A loss-of-function allele of *OsHMA3* associated with high cadmium accumulation in shoots and grain of *Japonica* rice cultivars. Plant Cell Environ. 39: 1941–1954.