

Cadmium Phytoremediation Using Rice

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Cadmium (Cd) is a toxic heavy metal that causes severe health issues in humans. Cd accumulates in the human body when foods produced in Cd-contaminated fields are eaten. Therefore, soil remediation of contaminated fields is necessary to provide safe foods. Rice is one of the primary candidates for phytoremediation. There is a genotypic variation of Cd concentration in the shoots and grains of rice. Using the world rice core collection, 'Jarjan', 'Anjana Dhan', and 'Cho-ko-koku' were observed with a significantly higher level of Cd accumulation in the shoots and grains. Moreover, OSHMA3, a heavy metal transporter, was identified as a responsive gene of quantitative trait locus (QTL) for high Cd concentration in the shoots of these three varieties likewise. However, it is difficult to apply practical phytoremediation to these varieties because of their unfavorable agricultural traits, such as shatter and easily lodged. New rice varieties and lines were bred for Cd phytoremediation using OSHMA3 as a DNA marker selection. All of them accumulated Cd in the shoots equal to or higher than 'Cho-ko-koku' with improved cultivation traits. Therefore, they can be used for practical Cd phytoremediation.

Keywords: cadmium ; paddy field ; phytoremediation ; quantitative trait locus (QTL) ; rice ; transporter

1. Introduction

Cadmium (Cd) is a toxic heavy metal that causes severe health issues in humans ^[1]. Cd may accumulate in the human body when it enters the food chain. The Codex Alimentarius Commission established a Cd limit in some agricultural products to provide safe food for human intake, e.g., 0.4 mg kg⁻¹ for polished rice ^[2]. However, agricultural products harvested from Cd-contaminated fields can lead to Cd levels that exceed the Cd limit ^{[3][4][5]}. This high level of Cd can become a significant problem. Cd pollution of agricultural fields is mainly caused by the influx of Cd filled wastewater from mines and factories into agricultural fields. Cd pollution has an especially large impact on paddy fields. In addition, rice is the primary source of dietary Cd intake among Asians ^{[3][4][5]}. Therefore, soil remediation of contaminated fields, especially paddy fields, is necessary to provide safe foods for human health.

Phytoremediation is a soil remediation method that removes pollutants using plants. Phytoremediation is superior to other methods regarding its low cost and its ability to effectively purify large areas of field without any facilities. Generally, plants with large biomass and plants that accumulate high Cd levels in the aerial parts (shoots and grain) are required for effective phytoextraction. Cd hyperaccumulators, such as *Solanum nigrum*, *Pterocypsela laciniata*, *Sedum plumbizincicola*, are some of the candidates for soil remediation because of the high levels of Cd accumulation in the aerial parts ^{[6][7][8]}. However, most Cd hyperaccumulator have a small biomass which makes harvesting work difficult. Furthermore, a practical problem is the concern that they will spread as weed in the agricultural field after phytoremediation. On the other hand, some particular plant species, such as *Vigna unguiculata*, *Solanum melonaena*, *Momordica charantia*, *Nicotiana tabacum*, have also been reported for use with Cd phytoremediation ^[9]. In addition to these plants, rice (*Oryza sativa* L.) is also one of the primary candidates for soil remediation ^[10]. There is a genotypic variation of concentration of Cd ions in the shoots and rice grains, and some rice varieties showed a significantly high Cd accumulation in the shoots ^{[11][12][13]}. In addition, rice has large biomass. Furthermore, in the case of rice, there is a fully established cultivation and harvesting method, which means farmers can easily continue to work on soil remediation as they always have when cultivating and harvesting rice. Therefore, it is possible to perform phytoremediation by selecting high Cd-accumulating varieties to be cultivated without any special method or equipment.

2. Phytoremediation Using Local Rice Varieties

Among rice species, *Oryza sativa* subsp. *indica* and hybrid varieties of *O. sativa* subsp. *japonica* and *indica* (*japonica* - *indica*) exhibit a relatively high Cd accumulation in the shoots compared to the *O. sativa* subsp. *japonica* varieties ^[14]. Therefore, the candidate varieties for Cd phytoremediation were initially selected among *indica* or *japonica* - *indica* hybrid varieties. 'Milyang 23' is a *japonica* - *indica* variety that exhibits a relatively high Cd accumulation in the shoots ^[11]. When 'Milyang 23' was grown in Cd contaminated soil, it accumulated 10–15% of soil Cd in its shoots, and the decrease in Cd

concentration in the soil after cultivation was the largest among the major crops, including the japonica rice variety 'Nipponbare' [15]. The Cd contents of soybean seeds cultivated in the same field after 'Milyang 23' was grown were less than those cultivated using untreated soil [16]. In a field experiment, the indica variety 'Moretsu' exhibited approximately 2.5-fold higher Cd accumulation than 'Milyang 23', and 'IR-8' (indica variety) also showed a high level of Cd accumulation in the shoots [17]. After performing phytoremediation using these two varieties for two years, the Cd concentration in the soil decreased by 18% compared with the soil before cultivation. Furthermore, the Cd contents in the grains of subsequently grown japonica rice were lower than those grown in a field without phytoremediation [17]. These results indicate that remediation using high Cd-accumulating rice is effective not only in the paddy field but also in the converted upland field.

The world rice core collection (WRC) covered the genetic diversity of 32,000 genotypes of cultivated rice [18]. Within WRC, 'Jarjan' 'Anjana Dhan', and 'Cho-ko-koku' accumulated a significantly high level of Cd in the shoots and grains [13]. Cd uptake by 'Cho-ko-koku' was higher than 'IR-8' even in the field, and the Cd concentration in the field soil and grains of subsequent cultivars decreased more than those in the control field after the cultivation of 'Cho-ko-koku' for 2 or 4 years [19][20]. Phytoremediation capacity of Cd removal from soil depends on Cd concentration in the aerial parts and biomass of the plants. 'Jarjan', 'Anjana Dhan', and 'Cho-ko-koku' have a large biomass, and these varieties were considered advantageous for phytoremediation. However, 'Jarjan', 'Anjana Dhan', and 'Cho-ko-koku' presented some difficulties in regard to practical phytoremediation because of their unfavorable agricultural traits, such as shatter and easily lodged. Therefore, it was necessary to breed new rice varieties, which accumulate high levels of Cd like 'Jarjan', 'Anjana Dhan', and 'Cho-ko-koku'.

3. Breeding New Rice Varieties for Phytoremediation

Rice line 'MJ3' and 'MA22' were obtained by gamma-ray mutation of 'Jarjan' and 'Anjana Dhan', respectively [21]. 'MJ3' and 'MA22' showed the same level of Cd extraction as 'Jarjan' and 'Anjana Dhan', respectively, with a non-shattering habit. However, the culm length of 'MJ3' was shorter than 'Jarjan', and the lodging resistance of 'MJ3' was improved when compared with 'Jarjan'. On the other hand, 'MA22' showed almost the same culm length and was as easily lodged as 'Anjana Dhan'.

'TJTT8' was developed from BILs derived from 'Jarjan' and 'Tachisugata' [22]. 'Tachisugata' is a japonica - indica hybrid variety used as a livestock feed. It shows a large biomass and lodging resistance because of its thick and rigid culms [23]. The grains of 'TJTT8' are dark brown color and can easily be distinguished from the grains of general japonica varieties like 'MJ3'. 'TJTT8' was selected using the qCdp7 allele and showed the same level of Cd extraction as 'Jarjan' at several locations of Cd-contaminated paddy fields in Japan [22]. However, the heading date and maturing date of 'MJ3', 'MA22', and 'TJTT8' were too late in northern parts of Japan. Late heading date and maturing date characteristics may lead to difficulty in the harvest work due to insufficient drying of the aerial parts and the need for obtaining seeds for subsequent planting. Therefore, other varieties suitable for the cultivation conditions in northern parts of Japan were required.

'Akita 110' is a rice line developed by the Akita Prefectural Agricultural Experiment Station located in the northern part of Japan. 'Akita 110' was selected from a cross between 'Cho-ko-koku' and 'Akita 63' [24]. 'Akita 63' showed lodging resistance and large biomass of the aerial parts [25]. In the process of breeding 'Akita 110', OSHMA3 was used as a DNA marker to select plants that possessed this 'Cho-ko-koku' allele. As a result, the Cd extraction of 'Akita 110' was almost the same level as 'Cho-ko-koku'. In one year of a large-scale field trial, soil Cd concentrations in plots remediated with 'Akita 110' were reduced by 15.5%, whereas remediation with 'Cho-ko-koku' reduced soil Cd levels by 10.1% [24]. However, the Cd extraction of 'Akita 110' was sometimes lower than that of 'Cho-ko-koku', depending on the field conditions. Then, a new rice line, 'Akita 119', was developed to improve stable Cd accumulation in the aerial parts. 'Akita 119' was obtained by a soft X-ray mutation of 'Cho-ko-koku' [26]. 'Akita 119' was also selected by the OSHMA3 allele of 'Cho-ko-koku'. The culm length of 'Akita 119' was around 30 cm shorter than 'Cho-ko-koku', and the lodging resistance of 'Akita 119' was improved compared to 'Cho-ko-koku'. 'Akita 119' had many panicles, and its biomass was as great as that of 'Cho-ko-koku', even though the culm was short. The grains of 'Akita 119' are slender with a light brown color. The grains can also be distinguished from the grains of the general japonica varieties and do not mix in the distribution process if separated by a sieve.

When 'Akita 119' was cultivated in a Cd-contaminated paddy field in Akita Prefecture, the Cd extraction of 'Akita 119' was almost the same level as 'Cho-ko-koku' in all experiment fields from 2011 to 2016, whereas 'Akita 110' was lower than 'Cho-ko-koku' in some fields. Researchers also cultivated 'MJ3', 'MA22', and 'TJTT8' in the same Cd-contaminated paddy field in Akita Prefecture from 2014 to 2016. 'MA22' showed the highest Cd extraction among trial plants in 2014 but lodged

more severely than 'Cho-ko-koku.' On the other hand, Cd extraction of 'MJ3' and 'TJTT8' was about 1.5 times higher than that of 'Cho-ko-koku' on average, although some cultivating problems of these lines, such as late heading and maturing date, were revealed in northern parts of Japan.

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