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Phytostabilization ability of the rice elite lines in cadmium-contaminated paddy soil

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Abstract One of the issues in rice-growing areas is the high concentration of heavy metals in rice paddy soils. Human beings, aquatic and terrestrial animals, and crops and other plants are all at risk from high cadmium concentrations in soils. Seedling vigor tests of the sixteen (16) rice elite lines were screened in the very severe (SVE) cadmium-contaminated soil (>1.60 mg/kg cadmium) to determine their cadmium resistance using the scales: 1=extra vigorous, 3=vigorous, 5=normal, 7=weak, and 9=very weak at 14 days after seeding. The identified resistant (extra vigorous) rice elite lines were planted in the cadmium-contaminated soil (10.23 mg/kg cadmium) under ~~a~~ controlled environment. The cadmium content of the unpolished and polished grains of the identified resistant rice elite lines was determined for their phytostabilization ability using the maximum allowable level of cadmium in the rice grains and biological accumulation coefficient (BAC). Three (3) rice lines, namely PR52643-B-5-1-1, PR51233-B-B-SAL106-2-2-1-Drt1, and PR51233-B-B-SAL106-2-2-1-Drt, were identified as extra vigorous based on scales of the seeding vigor test. PR52403ILR-6-1-1-1-6-B was identified as a rice elite line with phytostabilization ability because of its low accumulation of cadmium in the unpolished grain with 0.1 mg/kg cadmium and a BAC value of 0.01 and polished grain with 0.0 mg/kg cadmium and 0.00 BAC value. This rice elite line is recommended as parent material for rice breeding to create new rice variety(ies) with phytostabilization ability or rice cadmium excluder variety(ies) suitable to plant in the very severe cadmium-contaminated paddy soil.

Keywords: Contamination, Polished grain, Soil with heavy metals, Unpolished grain

Introduction

Even though heavy metals are undoubtedly present in soil, geologic processes and human activity increase their concentration to levels that are harmful to humans, animals, and plants (Alloway, 1990; Masindi and Muedi,

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2018). Humans are more likely to be exposed to heavy metals through bioaccumulation when they eat polluted plants. Because of their capacity to absorb and assimilate large concentrations of heavy metals, these plants are referred to as hyperaccumulating plants. Eating hyperaccumulating plants can cause metal accumulation in both humans and animals (Martin and Griswold, 2009). The development of lung insufficiency, renal disturbances (kidney illnesses), and osteomalacia (disease that weakens bones) have all been associated with excessive exposure to heavy metals, particularly cadmium. Additionally, several cancer types and hypertension have been linked to cadmium (Bernard and Lauwerys, 1986). A plant's root length and dry mass may decrease if the soil contains a higher concentration of cadmium (Ismael *et al*., 2019).

Phytostabilization is the process of stabilizing and lowering the bioavailability of pollutants by using plants that are inexpensive, low-impact, and environmentally benign (Jadia and Fulekar, 2009). According to Baker (1981), Krämer (2010), and Wei *et al.* (2005), "metal excluder plants" are plants that can grow normally in heavy metal-contaminated soil and maintain minimal uptakes of pollutants in the upper sections despite the high concentration of pollutants found in the root zone. Heavy metal absorption in the higher portions is limited, even though metal excluder plants can grow in contaminated soils without suffering negative consequences (Wei *et al.*, 2005).

One of the important crops in the world is the *Oryza sativa* (L.), commonly known as "rice" It is an essential diet for another half of the world's population. China, India, and the rest of Asia, including the Philippines, produce 92% of the world's rice (Gnanamanickam, 2009). The Philippines produced more over nineteen (19.07) million metric tons of rice, which is a staple grain for many Filipinos (Philippine Statistics Authority, 2019). Moreover, the problem in rice grain produced is cadmium (Cd), which is still a heavy metal present in rice in countries that eat it. Finding the amounts of dangerous and necessary heavy metals in rice grains and other key cereals is critical due to their impact on human health (Shimbo *et al.*, 2021).

The sediments and heavy metals through the irrigation system from Agno River has lowered the soil quality with a resulting impact on agriculture of lowland plains because of the rapid deforestation, land conversion, and extensive mining is the cause of the heavy erosion and high load of sediment from the upper part of Agno River (Baluyut, 1985). In 2012, the tailings ponds of the mining company collapsed due to heavy rains and mine tails were deposited in the Balog Creek and parts of the Agno River. The amount of Cd and other heavy metals in the waters of Agno River were high during the time of the leak (Baluyut, 1985; de Jesus-Abejero, 2015).

Rice lines with phytostabilization ability are used as parent materials in rice breeding to produce new varieties of rice known as 'rice cadmium excluder variety.' The future creation of rice cadmium excluder variety(ies) would guarantee that the rice grain (unpolished and polished) produced by the local rice farmers is safe to eat and healthy.

The objective of the study was to identify rice elite lines as cadmium excluder rice in their unpolished and polished grains using seedling vigor test, maximum allowable level of cadmium in rice grain, and biological accumulation coefficient (BAC).

Materials and methods

Collection and analysis of cadmium concentration of the soil samples

Soil sampling

Soil samples were collected at 20cm depth in sampling sites in San Manuel, Pangasinan with 16.074947 N, 120.668934 E coordinates (Figure 1). Soil samples were analyzed with their levels of heavy metals contamination. Moreover, selected and categorized soils were used in the experiment based on the results of their levels of heavy metals concentration.

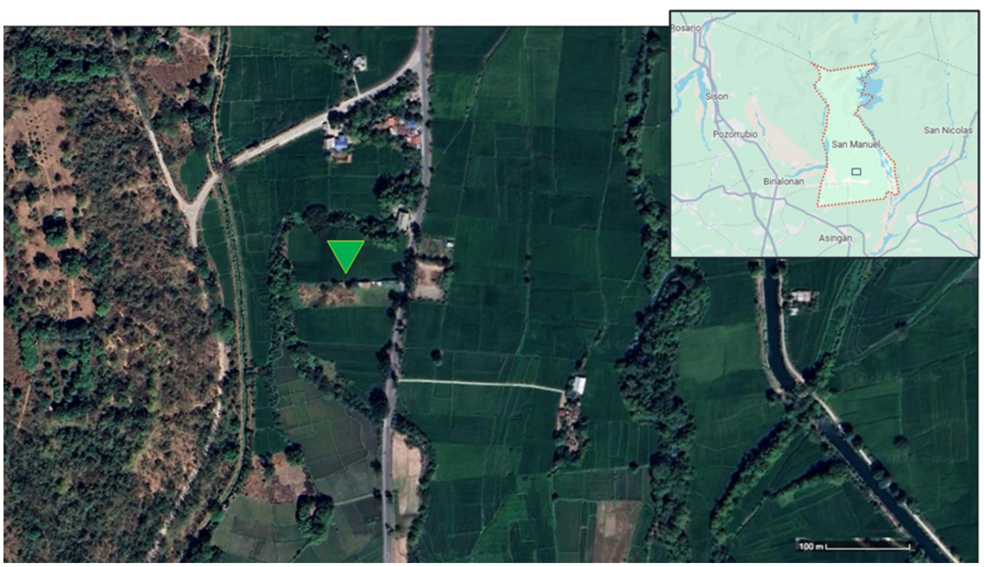

Figure 1. Map site of the collected soil samples at the municipality of San Manuel, province of Pangasinan (Google Maps, 2024)

Soil analysis

The method for extraction of soil samples was aqua regia or a combination of concentrated HNO_3 and concentrated HCl with a 1:3 ratio. The concentration of heavy metal specifically cadmium in the soil samples was analyzed through analytical methods of inductively coupled plasma–optical emission spectroscopy (ICP – OES).

The contamination/pollution index (C/P) by Chiroma *et al.* (2014) was used to determine the levels of soil contamination. The qualified soil sample was at a very severe (VSE) level of contamination for cadmium in the experiment (Table 1).

Table 1. Levels of contamination in soil based on the maximum permissible addition of heavy metals in soil and contamination/pollution index (C/P)

MPC for cadmium in soil[1] (mg/kg)	Contamination/pollution index (C/P) of cadmium (mg/ kg)			
	Slight (SL)	Moderate (MO)	Severe (SE)	Very severe (VSE)
1.6	0.16 – 0.40	0.41 – 0.80	0.81 – 1.20	1.21 – 1.60

[1]/ Maximum permissible concentration for cadmium in soil by Crommentuijn *et al.*, 2000

Physicochemical data

The compilation book ‘Pedological Characterization of Lowland Areas in the Philippines’ contains the physicochemical data of the soil samples (Miura *et al.*, 1995). The Kjeldahl method for total N (Kirk, 1950), the Olsen method for available P (Sims, 2000), and the flame photometry method for available K (Pratt, 1965) were used to determine the nutrients in the soil. We measured pH and electrical conductivity in a 1:1 solution with water. The Walkey-Black technique was used to calculate the organic carbon (Mylavarapu *et al.*, 2014).

Seedling vigor screening of the rice elite lines

Sixteen rice elite lines of indica group such as cool elevated (CE), saline (Sal), irrigated lowland (IR), and direct-seeded rice (DSR) were screened for their seedling vigor at 14 days after sowing (DAS) in very severe (VSE) cadmium-contaminated soil. The seeds used in the experiment were from the Plant Breeding and Biotechnology Division at the Philippine Rice Research Institute (PhilRice). The facility consisted of plastic boxes measuring 60 x 45 x 20 cm in length, width, and depth (LWD). Each plastic box contained soils with different levels of concentration of heavy metals from the municipality of San Manuel, province of Pangasinan. The first row was 10 cm apart along the width of the plastic box. Rows 5 cm apart were dibbled with the pre-germinated seeds for each entry (rice elite line) along the row. At 14 DAS, the 16 entries (rice elite lines) were tested to contaminated soil for their seedling vigor and scored shown in Table 2.

Table 2. Scoring for seedling vigor screening at 14 days after seeding

Scale	Parameter
1	Extra vigorous (very fast growing; plants at 5-6 leaf stage have 2 or more tillers in the majority of the population)
3	Vigorous (fast-growing; plants at the 4-5 leaf stage have 1-2 tillers in the majority of the population)
5	Normal (plants at the 4-leaf stage)
7	Weak (plants somewhat stunted; 3-4 leaves; thin population; no tiller formation)
9	Very weak (stunted growth; yellowing of leaves)

Standard Evaluation System (SES) for Rice (IRRI, 2013)

Rice elite lines with scales of 1 and 3 seedling vigor (1= extra vigorous; 3= vigorous) were used for the uptake and accumulation of cadmium in unpolished and polished grains of the rice varieties.

Cadmium accumulation in unpolished and polished grains of the cadmium-resistant rice elite lines

Experiment under controlled environment

All screened rice elite lines with 1 and 3 scores using scoring for seedling vigor (Table 2) were used for the experiment. Collected contaminated soil with a weight of 500 kg was placed and soaked with water in a wooden box measuring 25 cm depth x 1 m with x 5 m length. The wooden box was not leaked because of covered with a plastic sheet. After a week of soaking with water, the soil was mixed thoroughly until the saturation state was achieved. Healthy seedlings (10 days old) were planted into the wooden box and were cared for until three days after transplanting (3 DAS), and a 3 cm water level was maintained (Figure 2).

Fertilization of the experiment was computed at 120-60-90 kg of nitrogen, phosphorus, and potassium during the dry season. At the maturity stage, grains of the entries (rice elite lines) were separated and processed into unpolished and polished.

Figure 2. Experimental set-up using wooden box contains contaminated soil with cadmium

Analysis of cadmium concentration of the plant samples

Dried and pulverized plant samples (200 g) were baked at 300°C for three hours and then raised to another 500°C for two hours until the ash turns white. The 5N HNO_3 (3 ml) was mixed with the ashed plant samples. The plant samples were baked until dry on the heat-regulated hot plate. The concentrated HCl (3 ml) was mixed into ashed plant samples until dry and baked for one hour to dehydrate the silica. Ashed plant samples were removed from the hot plate and cooled down. The 2N HNO_3 (5 ml) was added and stirred with a rubber stirrer to dissolve the residue of salts. Plant tissue extract was filtered and kept in polypropylene. Plant tissue extract was analyzed through the test methods of the ICP–OES.

Data gathered

Phytostabilization ability of the rice varieties was measured using maximum allowable levels of cadmium in rice grain shown in Table 3. All rice elite lines with lower or zero value than the maximum allowable level of cadmium in rice grain is considered as phytostabilizers or heavy metal excluders specifically in cadmium (Fu *et al.*, 2015).

Table 3. Scoring for seedling vigor screening at 14 days after seeding

Standard	Cadmium (mg/kg)
Maximum allowable level of cadmium in rice grain (Fu *et al.*, 2015)	0.15

The biological accumulation coefficient (BAC) of the unpolished and polished grains from rice varieties was determined using the following formula:

$$BAC = \frac{\text{Cadmium concentration in grain (mg/kg)}}{\text{Cadmium concentration in soil (mg/kg)}}$$

Unpolished and polished grains from rice elite lines with BAC <1.0 are considered phytostabilizers or heavy metal excluders while BAC >1.0 is considered a phytoextractor or hyperaccumulator (Hakeem *et al.*, 2015).

Statistical analysis

The mean ± standard error was used to present all data. One-way ANOVA was used for statistical analysis, and the Tukey's Honest Significant Difference (HSD) Test was used to compare the means at the 5% level of significance.

Results

Nutrients and physicochemical properties of the soil samples

The soil sample is composed of 1.34% organic matter with 0.069% mg/kg total nitrogen (N), 5.0 mg/kg available phosphorus (P), and 0.40 cmol(+)/kg available potassium. The soil texture of the soil sample is silty clay composed of 48.3% clay, 40.7% silt and 11.0% sand. Cation exchange capacity (CEC) of the soil sample is 32.51 cmol(+)/kg. Cadmium content of the soil sample is 10.23 mg/kg (Table 4).

Table 4. Nutrients, physical, and chemical properties of the soil used in the experiment

Parameter	Value
Soil pH[1]	6.9
Organic matter (OM)[1]	1.34%
Soil texture[1]	Silty clay
Sand	11%
Clay	48.3%
Silt	40.7%
Cation exchange capacity (CEC)[1]	32.51 cmol(+)/kg
Total nitrogen (N)[1]	0.069%
Available phosphorus (P)[1]	5.0 mg kg^{-1}
Available potassium (K)[1]	0.40 cmol(+)/kg
Cadmium[2]	10.23 mg/kg
Maximum permissible concentration[1]	1.60 g/kg

1/ Miura *et al.*, 1995
2/ Crommentuijn *et al.*, 2000

Evaluation and screening of rice lines and varieties with their seedling vigor

The seedling vigor test was used to determine the resistance to the very severe (VSE) cadmium-contamination of the different rice elite lines in soil contaminated with cadmium. Rice elite lines with a score of 1 (based on the SES for rice by IRRI, 2013) were identified as vigorous rice elite lines and qualified for screening study of rice elite lines with phytostabilization ability. Out of 16 rice elite lines evaluated, three (3) were qualified for the screening of phytostabilization ability namely: PR52643-B-5-1-1 (cool-elevated ecosystem), PR51233-B-B-SAL106-2-2-1-Drt1 (saline ecosystem) and PR52403ILR-6-1-1-1-6-B (direct-seeded rice under irrigated lowland ecosystem). These rice elite lines produced two tillers with five leaves and categorized as extra vigorous (score 1) rice plant. Rice elite lines with scores 7 and 9 produced no tillers with 2 leaves (Table 5).

Table 5. Ecosystem, tiller count, leaf count, and score of seedlings of the elite rice lines at 14 days after sowing (DAS)

Elite line	Ecosystem[1]	Tiller count	Leaf Count	Score (Seedling vigor)[2]
PR52673-B-20-1-1	Ind, CE	0	2	9
PR52637-B-2-1-1-2	Ind, CE	0	2	7
PR52654-B-1-1-1	Ind, CE	0	2	7
PR52643-B-5-1-1	**Ind, CE**	**2**	**5**	**1**
PR52654-B-3-1-1	Ind, CE	0	2	7
PR52656-B-1-1-1	Ind, CE	0	2	7
PR51237-Drt91-2-2-Sal1-1-Drt1	Ind, Sal, IL	0	2	7
PR51254-Sal31-2-1-Sal3-1-Drt1	Ind, Sal, IL	0	2	7
PR51233-B-B-SAL106-2-2-1-Drt1	**Ind, Sal**	**2**	**5**	**1**
PR51234-B-B-SAL7-3-1-2-Drt1	Ind, Sal	0	2	7
PR51237-Drt102-2-2-3-Sal1-1	Ind, Sal	0	2	7
PR52872ILR-30-1-2-B	Ind, DSR, IL	0	2	7
PR52893ILR-2-1-2-B	Ind, DSR, IL	0	2	7
PR52403ILR-6-1-1-1-3-B	Ind, DSR, IL	0	2	7
PR52403ILR-6-1-1-1-6-B	**Ind, DSR, IL**	**2**	**5**	**1**
PR52397ILR-10-1-2-1-3-B	Ind, DSR, IL	0	2	7

1/ Legend: Ind = Indica, Sal = Saline, CE = Cool Elevated, DSR = Direct Seeded Rice, IL = Irrigated Lowland

2/ Seedling vigor score: 1 = extra vigorous, 3 = vigorous, 5 = normal, 7 = weak, 9 = very weak

Cadmium accumulation in the unpolished and polished grains

The amount of cadmium present in the polished and unpolished grains is shown in Table 6. It can be noted that the polished grains registered lower cadmium content compared to unpolished grains. In unpolished grains, PR52403ILR-6-1-1-1-6-B line registered significantly lower of cadmium content with a mean 0.1 mg/kg while PR52643-B-5-1-1 line recorded significantly higher cadmium content with a mean of 0.2 mg/kg. In the polished rice, no trace of cadmium was noted in PR52403ILR-6-1-1-1-6-B line. On the other hand, higher cadmium content was obtained in PR52643-B-5-1-1 elite line and PR52643-B-5-1-1 with a mean of 0.01 mg/kg.

Table 6. Amount of cadmium in unpolished and polished grain of the rice elite lines

Elite line	Cadmium accumulation in grain (mg/kg)	
	Unpolished	**Polished**
PR52643-B-5-1-1	0.2 ± 0.010 [b]	0.1 ± 0.010 [a]
PR51233-B-B-SAL106-2-2-1-Drt1	0.4 ± 0.006 [a]	0.1 ± 0.010 [a]
PR52403ILR-6-1-1-1-6-B	0.1 ± 0.000 [c]	0.0 ± 0.000 [b]
Maximum allowable level of cadmium in rice grain (Fu *et al.,* 2015)	0.15	

Values are mean ± SD (n=3). Means with the same letter are not significantly different using the Tukey's Honest Significant Difference (HSD) Test ($p < 0.01$)

Biological accumulation coefficient (BAC) in the unpolished and polished grains

The BAC of the polished and unpolished grains of different elite lines is presented in Table 7. In the unpolished grains, PR52403ILR-6-1-1-1-6-B recorded significantly lower BAC with a mean value of 0.01 while significantly higher BAC was noted in PR51233-B-B-SAL106-2-2-1-Drt1elite line with a mean value of 0.04. Meanwhile, in polished grains, PR52403ILR-6-1-1-1-6-B elite line showed no BAC value. On the other hand, significantly higher BAC was noted in PR52643-B-5-1-1 elite line and PR51233-B-B-SAL106-2-2-1-Drt1 with a mean value of 0.01.

Table 7. Biological accumulation coefficient (BAC) for the cadmium accumulation in unpolished and polished grain of the rice elite lines

Elite line	Cadmium accumulation in grain (mg/kg)	
	Unpolished	**Polished**
PR52643-B-5-1-1	0.02 ± 0.0010[b]	0.01 ± 0.0010[a]
PR51233-B-B-SAL106-2-2-1-Drt1	0.04 ± 0.0006[a]	0.01 ± 0.0006[a]
PR52403ILR-6-1-1-1-6-B	0.01 ± 0.0006[c]	0.00 ± 0.0000[b]
BAC for cadmium excluder rice (Zu *et al.,* 2005)	<1.00	

Values are mean ± SD (n=3). Means with the same letter are not significantly different using the Tukey's Honest Significant Difference (HSD) Test ($p < 0.01$)

Discussion

The present study identified cadmium-resistant rice elite lines with low grain cadmium accumulation, also known as 'cadmium-excluding rice.' The seedling vigor test, cadmium accumulation in the grain and the biological accumulation coefficient were used to identify these rice elite lines.

The seedling vigor test was used for rapid results of the resistance of the rice elite lines in very severe (VSE) cadmium-contaminated soils. Essential phenotypic traits such as extra vigorous for growth and development, better leaf formation, and tillering ability that are suitable to plants in the identified environment (Mahender *et al.*, 2015). Yang *et al.* (2010) explained that better leaf formation and tillering ability of the rice elite lines are the main characteristics of vigorous seedlings. Meanwhile, Shi *et al.* (2020) reported that cadmium-resistant (extra vigorous) rice elite lines grow and develop better in the early or seedling stage than non-resistant rice elite lines. This is because the non-resistant rice elite lines' leaves are stunted and yellowing, and their shoots and roots are short and weak. The results of the present study indicate that the different elite lines used exhibited variation in their ability to grow in soil contaminated with cadmium. This could be attributed to the difference in their genetic make-up.

The cadmium content of the three elite lines is lower than the allowable level of cadmium in rice grain. The cadmium content obtained in polished rice in all elite lines are lower than the maximum allowable level of cadmium content. This result indicated that cadmium is presented in the seed coat of the unpolished rice which become the rice hull and rice bran after milling (Hakeem *et al.*, 2015; Shackira and Puthur, 2019). Hence, the polished rice contains negligible amount of cadmium. The variation in the accumulation of rice among the elite lines could be attributed to the difference in their genetic make. Variation does not only occur among species of rice but also within species or lines.

The relationship between the element's concentration in the rice shoot system and the soil overall cadmium content is known as the biological accumulation coefficient, or BAC (Drozdova *et al.*, 2019). It is important for understanding and assessing the environmental risk. It is the ability of the plant to absorb or accumulate heavy metals in the soil. The three rice elite lines (PR52643-B-5-1-1, PR51233-B-B-SAL106-2-2-1-Drt1, and PR52403ILR-6-1-1-1-6-B) recorded biological accumulation coefficients (BAC) below the permissible BAC for cadmium excluder rice. The allowed level of contamination in the rice grain, however, might not be reflected in the acceptable BAC value for cadmium excluder rice. Since the rice grain is the most vital component of the rice plant, only the grain—both unpolished and polished—was used in the study. This parameter provides valuable information in ability to accumulate or exclude the heavy metals, in the various parts of the rice plants. BAC is used as the basis in selecting plants with phytostabilization ability. Plants with BAC value greater than 1 are regarded as metal hyperaccumulating plants (Malik *et al.,* 2010) while those with lower BAC values (<1.00) are regarded as candidates for the selection for phytostabilization potential (Sun *et al.*, 2009; Malik *et al.*, 2010; Ramos *et al.*, 2025). In relation to the result of the present study, phytosiderophores is the reason for the decreasing cadmium uptake and boosting-resistant respiration. Low grain-cadmium accumulating elite lines has potential parent material for the breeding of low grain-cadmium-accumulating rice cultivars. One of the primary causes of the low grain-cadmium accumulation of the rice plant is the robust root-to-shoot cadmium transport pathway and the cadmium efflux capacity of rice roots. In addition, the buildup of cadmium in both polished and unpolished rice grains is being decreased by the increased expression of OsPCR1 in shoots (Wang *et al.*, 2015). Rice grain accumulation of heavy metals depends on genetic components of elite rice lines and soil characteristics. Additionally, the way that various elite rice lines react to Cd stress varies (Li *et al.*, 2019).

In summary, one rice elite line namely: PR52403ILR-6-1-1-1-6-B exhibited phytostabilization ability. This rice elite line can be used as parent material for rice breeding to create rice varieties with phytostabilization ability. However, many rice elite lines of different ecosystem will be screened as additional parent materials for rice breeding to create rice varieties with phytostabilization ability.

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Conflicts of interest

The authors declare no conflict of interest.

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