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Original Research

Effect of Cadmium on DNA Changes in *Ipomoea aquatica* Forssk.

Tawatchai Tanee[1, 2], Runglawan Sudmoon[2, 3], Penkhae Thamsenanupap[1], Arunrat Chaveerach[2, 3]*

[1]Faculty of Environment and Resource Studies, Mahasarakham University, Maha Sarakham 44150, Thailand
[2]Genetics and Environmental Toxicology (GET) Research Group, Khon Kaen University, Khon Kaen 40002, Thailand
[3]Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand

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Abstract

Cadmium (Cd) contamination in plants fertilized with inorganic fertilizers is a major risk to human health. *Ipomoea aquatica* Forssk., which is one of the most popular vegetables used in Thai cuisine, is also vulnerable to Cd contamination. This study aimed to investigate Cd accumulation in *Ipomoea aquatica* Forssk. and the associated changes in its DNA. The plant was grown in soil supplemented with Cd at 0, 15, 30, 60, and 120 mg/kg. After 21 days, accumulation in the roots, stems, and leaves was analyzed using atomic absorption spectrophotometry (AAS), and the bioconcentration (BCF) and translocation factors (TF) were analyzed. DNA changes were assessed by a combination of random amplified polymorphic DNA (RAPD) and genomic template stability (GTS) tests. Cd concentrations in the roots, stems, and leaves ranged from 0 to 12,333 mg/kg, 0 to 5,909.27 mg/kg, and 0 to 1,653.26 mg/kg, respectively. The BCF and TF values ranged from 0 to 21.15 and 0 to 1.21, respectively. From the RAPD profiles, the GTS values ranged from 52.3 to 91.1%. Taken together, these results indicate that *I. aquatica* is a Cd-hyperaccumulator; therefore, consuming *I. aquatica* plants grown in Cd-polluted areas is a health risk.

Keywords: cadmium, *Ipomoea aquatica* Forssk., bioconcentration factor, translocation factor, genotoxicity

Introduction

Cadmium (Cd) is a heavy metal that has been used extensively in the agricultural and chemical industries as a component of inorganic fertilizers, pesticides, and paints, and it can be released into the soil and water. Humans are mainly exposed to Cd via the food supply. Cd accumulation in the food chain could pose a direct threat to human health and can cause disease (e.g., itai-itai disease, cancer), damage to the skeletal system, high blood pressure, adverse cardiovascular events, enzyme inhibition, and DNA damage [1]. Cd also causes chronic toxicity in animals [2]. The World Health Organization

*e-mail: raccha@kku.ac.th

[3] has established a provisional tolerable weekly intake of 7 µg of Cd per kg of body weight, whereas the Cd concentration in the soil should be less than 37 mg/kg [4], and the concentration in the leaves of edible plants should be < 0.2 mg/kg [5].

In Cd-contaminated soil, plants can take up the metal through the roots and then translocate it to the stems and leaves via physio-biochemical mechanisms. Cd contamination in agricultural soils is unlikely to affect plant growth, but Cd is easily transferred from soil to the human food chain. Moreover, an excess of toxic heavy metal ions induces several cellular stress responses and damages different cellular components of the plant, such as membranes, proteins, and DNA [1, 6]. *Ipomoea aquatica* is a popular vegetable in northeastern Thailand and other Asian countries. Knowledge regarding Cd accumulation and DNA damage in edible plant species should be expanded.

Recently, the development of molecular technology has provided suitable tools for DNA analysis in the field of genotoxicology. In many studies, random amplified polymorphic DNA (RAPD) has been used to detect various types of DNA damage and mutations (point mutations, rearrangements, and small deletions or insertions). Furthermore, RAPD banding profiles can be scored for genomic template stability (GTS) analysis to detect changes in DNA. This technique has been successfully applied to the study of DNA damage and mutation caused by heavy metals in plants and animals [6-11].

The aims of this study were to determine the Cd content in different plant parts, including the roots, stems, and leaves, and to detect Cd-induced changes in *Ipomoea aquatica* DNA using RAPD markers.

Materials and Methods

Soil Collection and Experimental Design

The tested soil was collected from an agricultural field in Maha Sarakham Province, Thailand, and analyzed to determine the Cd concentration before initiating the experiments. The collected surface soil samples (depth of 0-30 cm) were supplemented with Cd ($CdCl_2 \cdot 2.5H_2O$; Univar, Australia) at five concentrations: 0 (no supplementation), 15, 30, 60, and 120 mg/kg. Samples (1 kg) of the supplemented soils were placed into individual plastic pots. Seeds of *I. aquatica* (Chia Tai Seed Co. Ltd., Thailand) were sown to obtain five seedlings in each pot. Each treatment was replicated three times and arranged in a completely randomized design. To simulate field conditions, the plants were grown under open field conditions with added organic fertilizers. After 21 days of experimentation, the leaves were collected for DNA analysis. All of the plants and soil samples were used to determine Cd accumulation.

The field-collected soil samples (collected before the experiments) and the soil samples collected from each pot (collected after the experiments) were dried in an oven (Binder, USA) at 105ºC for 24 h. The plants were dissected into roots, stems, and leaves and then rinsed thoroughly with distilled water to remove any surface materials. After complete drying, the dried samples were ground with a mortar and pestle. One gram of each dried sample (soils and plant parts) was added to 12 ml of an $HClO_4$:HNO_3 mixture (1:3) and boiled at 100ºC [12]. The solutions were filtered to obtain clear liquids, which were brought up to a volume of 50 ml in a volumetric flask with deionized-distilled water. The digested samples were analyzed for Cd using an AA 6200 atomic absorption spectrophotometer (Shimadzu, Japan). All analyses were performed in triplicate.

DNA Extraction

Total genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method according to Porebski et al. [13]. Briefly, 50 mg of each leaf sample was finely ground in 600 µl of warm (65ºC) extraction buffer (100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2% CTAB) with a mortar and pestle. The homogenate was transferred to a 1.5-ml microcentrifuge tube; subsequently, 5 µl of 10 mg/ml RNase A was added, and the samples were incubated at 65°C for 30 min. An equal volume of chloroform-isoamyl alcohol (24:1 v/v) was then added. The tube was centrifuged at 8,000 ×g for 10 min, and the aqueous phase was transferred to a new tube. Finally, genomic DNA was precipitated with an equal volume of cold (-20ºC) 2-propanol for 30 min and then centrifuged. The precipitate was washed with 70% ethanol and resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Extracted DNA was examined by 0.8% agarose gel electrophoresis and ethidium bromide staining.

RAPD Procedures

RAPD amplifications were carried out in 25-µl reactions consisting of GoTaq Green Master Mix (Promega, USA), 0.5 µM primers (Invitrogen, USA), and 20 ng of DNA template. Eighteen RAPD primers were screened, and the 10 primers that successfully amplified clear bands were as follows (5′-3′): GCGGCTGGAG, GTGACGCCGC, CTCGGGTGGG, TTCCGCGGGC, GTAGACGAGC, GTGCGTCCTC, CTGGCGGCTG, CGTGGGCAGG, AGCAGCGTGG, and GCCTGGTTGC. In a Swift Maxi Thermal Cycler (Esco Micro Pte. Ltd., Singapore), the reaction mixture was denatured at 94ºC for 3 min, and amplification was performed by 35 cycles of the following: denaturation for 30 s at 94ºC, annealing for 30 s at 40ºC, and extension for 2 min at 72ºC followed by a 7-min final extension at 72ºC. The amplified products were separated by 1.2% agarose gel electrophoresis in TAE buffer and visualized using ethidium bromide staining. The resulting RAPD bands were used to analyze the percentage of GTS.

Data Analysis

All experiments were replicated three times. The means and standard deviations (SD) of Cd in the soil and plant parts were calculated using Microsoft Office Excel 2010. The bioconcentration factor (BCF) was calculated as the ratio of the Cd concentration in the plant root to that in the soil, whereas the translocation factor (TF) was calculated as the ratio of the Cd concentration in the plant shoot to that in the root [14]. Cd-hyperaccumulating plants were defined based on the following standards:

1) accumulation capability corresponding to a threshold shoot metal concentration greater than 100 mg/kg shoot dry weight,
2) a BCF index greater than 1.0, sometimes reaching 50-100,
3) a TF index used to measure a plant's ability to translocate metal from the roots to the shoots [15-17], greater than 1.0.

A GTS test was performed using the following equation: GTS (%) = (1-a/n) × 100, where 'a' is the number of polymorphic bands detected in each treated sample and 'n' is the number of total bands in the control [9]. The polymorphisms observed in the RAPD profiles included the disappearance of a normal band and the appearance of a new band when compared with the control RAPD profiles.

Results and Discussion

In the present investigation, plant heights ranged from 1.6 to 9.7 cm. Before the experiments, the field soils contained 14.92±1.18 mg Cd/kg soil. The amount and distribution of Cd in the *I. aquatica* parts treated with different concentrations (0, 15, 30, 60, 120 mg Cd/kg) are shown in Table 1. Cd concentrations in the roots, stems, and leaves ranged from 0 to 12,333 mg/kg, 0 to 5,909.27 mg/kg, and 0 to 1,653.26 mg/kg, respectively. The mean Cd concentration in different parts of the plant increased in the following sequence: leaves < stems < roots. This result is concordant with previous research showing that roots can accumulate Cd from the soil more robustly than stems and leaves [18]. The concentration of Cd in all parts of the plant increased with increasing concentrations of Cd supplemented in the soils, and there was a positive linear correlation between the root, stem, and leaf Cd uptake and Cd concentrations in the soils. The corresponding regression equations can be expressed as follows:

$$Y_R = 101.64X + 306.31 \quad (R^2 = 0.99) \qquad (1)$$

$$Y_S = 38.539X + 946.76 \quad (R^2 = 0.87) \qquad (2)$$

$$Y_L = 10.183X + 505.63 \quad (R^2 = 0.91) \qquad (3)$$

…where Y_R, Y_S, and Y_L are the Cd concentrations in the roots, stems, and leaves, respectively, and X is the concentration of Cd in the soil.

The BCF value represents a plant's ability to accumulate Cd from the soil, and the TF value represents a plant's ability to translocate Cd from the roots to the shoots. In the present investigation, BCF and TF ranged from 0 to 21.15 and from 0 to 1.21, respectively (Table 1). Thus, the concentration in the shoots increased with an increasing Cd concentration in the soil. These results are concordant with previous research showing that plants can accumulate Cd from the soil [18-20]. Cd-hyperaccumulator plants can contain > 100 mg Cd/kg tissue, whereas the normal level of Cd in most plants is only 0.1 mg/kg. In addition to the total Cd content, the BCF and TF indices must also be considered when evaluating hyperaccumulators, and hyperaccumulating plants should have a BCF and TF > 1 [15-17]. In this study, the concentration of Cd in both stems and leaves ranged from 491 to 5,909 mg/kg, and the BCF and TF indices were higher than 1 (Table 1). All of the values exceeded critical levels; therefore, *I. aquatica* can be defined as a Cd-hyperaccumulator.

Cd accumulation in edible plants is a serious problem not only because it could reduce crop yield but also because it is a hazard to human health through the food chain. *Ipomoea aquatica* is one of the most popular vegetables used in Thai cuisine. Based on the high level of Cd accumulation in *I. aquatica*, plants that grow in contaminated soils are not recommended for human consumption. Cd toxicity can pose direct threats to human health via the food chain, and it can cause disease (e.g., itai-itai disease, cancer), damage to the skeletal system,

Table 1. Cadmium accumulation, bioconcentration factor (BCF), translocation factor (TF), and genomic template stability (GTS) of *Ipomoea aquatica* under various Cd treatments.

Cd conc. (mg/kg)	Accumulated Cd (mg/kg) in plant parts (mean±SD)			BCF	TF	GTS (%)
	Root	Stem	Leaf			
0 (control)	0	0	0	0	0	-
15	1,873.55±918.20	1,612.50±825.62	491.16±266.53	12.58	1.12	91.1
30	2,950.83±1,817.87	2,647.13±1,573.62	913.23±448.51	15.85	1.21	76.6
60	6,937.45±3,003.51	2,289.40±1,235.15	1,256.13±643.78	21.15	0.51	55.5
120	12,333.00±6,413.73	5,909.27±2,854.82	1,653.26±748.66	16.84	0.61	52.3

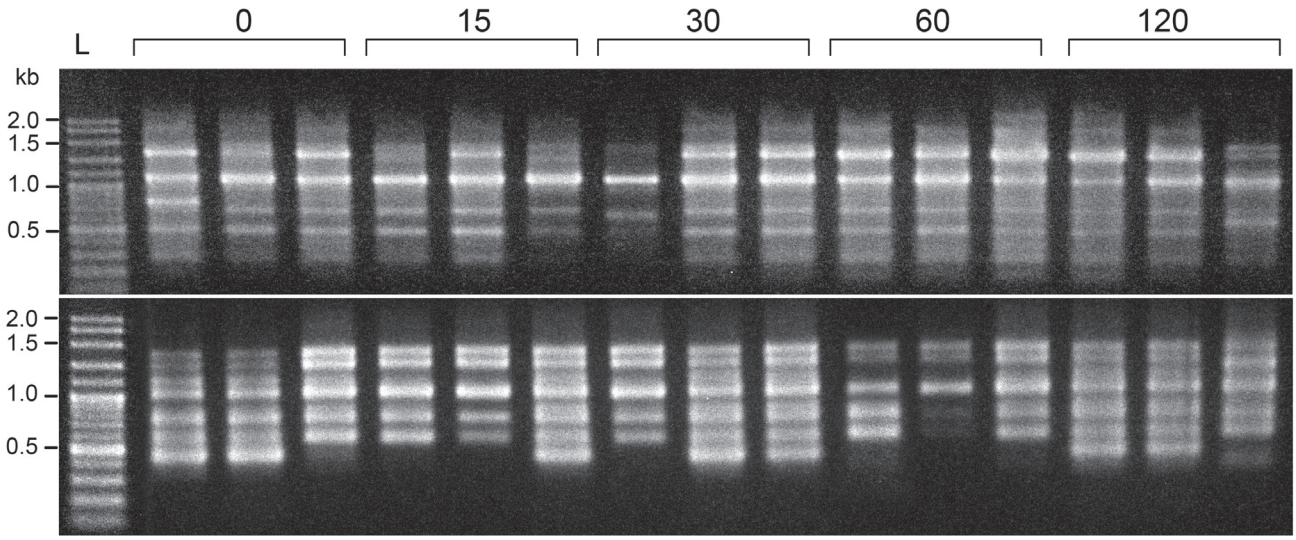

Fig. 1. RAPD profiles using primers CGTGGGCAGG (top) and CTGGCGGCTG (bottom) of *Ipomoea aquatica* exposed to different concentrations of Cd: 0 (control), 15, 30, 60, and 120 mg/kg.

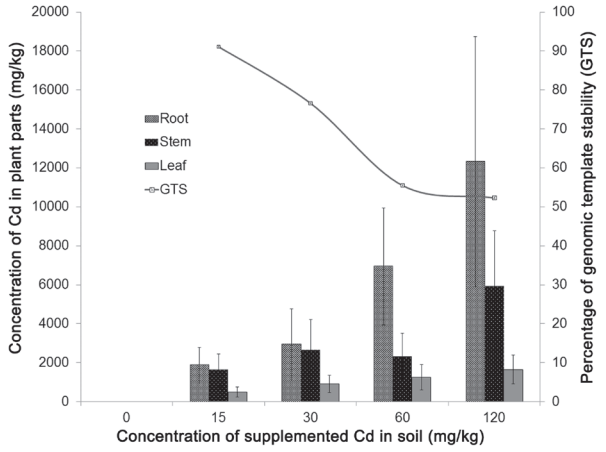

Fig. 2. Comparison of Cd accumulation and GTS values in *Ipomoea aquatica*.

adverse cardiovascular events, enzyme inhibition, DNA damage in humans [1], and chronic toxicity in animals [2].

An example of the RAPD banding patterns observed in RAPD fingerprinting experiments is shown in Fig. 1. There were substantial differences in the RAPD profiles between the control and treated plants, with apparent changes (disappearance and/or appearance) in the number of DNA bands produced by each primer. The GTS values ranged from 52.3 to 91.1% (Table 1). The highest concentration of Cd supplementation (120 mg/kg) caused the most extensive changes in the plant DNA (GTS = 52.3%). These results indicate that the GTS value in *I. aquatica* was affected by Cd exposure. A decrease in GTS values was observed with an increase in the Cd concentration (Fig. 2 and Table 1). These results correlated with previous data suggesting that Cd could induce changes in plant DNA [6, 8, 10, 18].

Conclusion

Cd accumulation in plant species can affect growth. In the current study, Cd-induced DNA damage in *I. aquatica* was evidenced by the results of the RAPD assay. *Ipomoea aquatica* can accumulate Cd at concentrations greater than 100 mg/kg, whereas the normal level of Cd in most plants is only 0.1 mg/kg. This high level of accumulation, in addition to the high values of BCF and TF, suggests that *I. aquatica* is a Cd-hyperaccumulator. Therefore, consumers should be concerned that consumption of plants grown in Cd-polluted areas poses a health risk. The results of this study can be used as a basis for the development of food safety guidelines for farmers and others, especially guidelines pertaining to the use of pesticides, herbicides, or fertilizers.

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