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Risk assessment of cadmium-contaminated soil on plant DNA damage using RAPD and physiological indices

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ABSTRACT

Impact assessment of contaminants in soil is an important issue in environmental quality study and remediation of contaminated land. A random amplified polymorphic DNA (RAPD) 'fingerprinting' technique was exhibited to detect genotoxin-induced DNA damage of plants from heavy metal contaminated soil. This study compared the effects occurring at molecular and population levels in barley seedlings exposed to cadmium (Cd) contamination in soil. Results indicate that reduction of root growth and increase of total soluble protein level in the root tips of barley seedlings occurred with the ascending Cd concentrations. For the RAPD analyses, nine 10-base pair (bp) random RAPD primers (decamers) with 60–70% GC content were found to produce unique polymorphic band patterns and subsequently were used to produce a total of 129 RAPD fragments of 144–2639 base pair in molecular size in the root tips of control seedlings. Results produced from nine primers indicate that the changes occurring in RAPD profiles of the root tips following Cd treatment included alterations in band intensity as well as gain or loss of bands compared with the control seedlings. New amplified fragments at molecular size from approximately 154 to 2245 bp appeared almost for 10, 20 and 40 mg L^{-1} Cd with 9 primers (one–four new polymerase chain reaction, (PCR) products), and the number of missing bands enhanced with the increasing Cd concentration for nine primers. These results suggest that genomic template stability reflecting changes in RAPD profiles were significantly affected and it compared favourably with the traditional indices such as growth and soluble protein level at the above Cd concentrations. The DNA polymorphisms detected by RAPD can be applied as a suitable biomarker assay for detection of the genotoxic effects of Cd stress in soil on plants. As a tool in risk assessment the RAPD assay can be used in characterisation of Cd hazard in soil.

1. Introduction

Along with the rapid development of modern industry, soil contaminated by heavy metals, e.g. Cd and Cr, is commonly identified in nowadays world. Cd has been released into the environment largely found in the water and soil through anthropogenic activities, such as mining deposits, aerial fallout from smelters and industrial processes etc. Cd has a capability not only to cause morbidity or/and mortality in the exposed organisms, but may potentially result in higher order changes such as alterations to population dynamics and change to biological diversity at both intra- and inter-species levels [1–4]. Such changes may initiate direct and adverse ecological consequences. Genotoxicity of Cd is directly related to its effect on structure and function of DNA, which may be determined using a number of laboratory methods [5,6]. However, there have been few direct experimental demonstrations of the wider relationships between DNA effects and their subsequent consequences at higher levels of biological organization in soil [7–11]. To address this issue, it is necessary to develop reliable and reproducible genotoxicity assays that can then be used in conjunction with traditional assays for detecting any impairment of population parameters (e.g. growth, reproduction, and viability of offspring).

In the soil genotoxicity study, advances in molecular biology have led to the development of a number of selective and sensitive assays for DNA analysis. Classical polymerase chain reaction (PCR) assays, simply requiring a target DNA sequence and two synthetic oligonucleotide primers complementary to opposite strands of the target DNA, have been used to detect mutations and DNA damage. Indeed, mutations may affect the annealing of the primers whereas DNA damage may interfere with the DNA polymerase activity, thus altering the number of newly synthesized amplicons [12]. PCR-based protocols have also been used to detect DNA damage and

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repair in specific genes and, under appropriate conditions, quantitative data can be obtained [10,13,14].

Despite the advantages of using traditional PCR assays for detecting DNA alterations, there are some potential difficulties: (1) the nucleotide sequence flanking upstream and downstream the target DNA needs to be determined; (2) the size of PCR products plays a crucial role in the detection of DNA damage because the amplification of short fragments (e.g. less than 300 bp) may not be possibly inhibited at all even in the case of extensive DNA damage; (3) it is conceivable that any reduction in the intensity of PCR amplicons could be due to a partial inhibition of PCR reaction caused by factors other than DNA damage such as residual phenol remaining after DNA extraction [15].

Such disadvantages can be eliminated by using the random amplified polymorphic DNA (RAPD) 'fingerprinting' technique, which is based on the amplification of genomic DNA with 10 bp primers of arbitrary nucleotide sequence which anneals to multiple regions of the genomic DNA [9,16]. The PCR reactions thus generate many amplicons of variable lengths (e.g. between 100 and 4000 bp for RAPD) which can be separated by gel electrophoresis to obtain DNA fingerprint. RAPD assays were successfully used to detect genetic instability in DNA alterations in animals, bacteria and plants induced by low doses of pollutants, mainly in the aquatic systems [7,9,12,15,17,18,19]; while a few of the research on DNA alterations in plants induced by soil contamination stress have been reported [6,11,18,20]. The objectives of this study were to detect DNA damage induced by Cd-contaminated soil using the RAPD technique, to compare changes in RAPD profiles with root growth and total soluble protein level in the root tips of barley seedlings measured under laboratory conditions, and to evaluate the usefulness of the RAPD assay in risk assessment of contaminated soil.

2. Materials and methods

2.1. Plant material, growth and total soluble protein level test

We designed experiments to study the effect of soil contamination by Cd, at various concentrations, on plant (barley, *Hordeum vulgare*). Dry mature seeds of barley (cultivar Kepin No. 7 from Nanjing University) were soaked in distilled water at 4 °C for three days and germinated to radicle lengths of 2 mm in a petri dish (diameter 12 cm) containing three pieces of filter paper at 15 °C under dark condition, so simulate the soil condition. Uniformly 20 germinated seeds were selected and transferred to Petri pots (diameter 9.5 cm and height 14 cm) containing soil of quartz sands at sizes of 1.2–2.2 mm (about 40%) and 0.6–1.2 mm (60%), saturated with 400 mL of distilled water or test solution. The growth inhibition test was performed with the above barley plantlets exposed to 0, 10, 20, and 40 mg L^{-1} Cd, respectively (in the form of $CdCl_2$ $2H_2O$ with purity 99.5%) for 10 days. The test solution in each container was checked into target concentrations of Cd as above every other day. Petri pots were incubated in a growth chamber at 21 ± 1 °C and a 16–8 h day–night photoperiod with a light intensity approximately of 8000 lux. Each treatment was replicated three times.

After the 10 days of incubation, the root length was measured using a ruler, and total soluble protein level of root tips in barley seedlings was measured. Inhibitory rate (IR, %) of the above indices was calculated by the following formula:

$$\mathrm{IR} = \left(1 - \frac{x}{y}\right) \times 100 \quad (1)$$

where x and y are the average values detected in the control and each sample treated, respectively.

2.2. DNA isolation, RAPD procedures and estimate of genomic template stability

Approximately 1.5 cm root tips of 20 seedlings were collected, ground in liquid nitrogen, and total DNA from the root tips was extracted using CTAB protocol in previous study [11]. DNA concentration of each sample was quantified fluorimetrically by a Biophotometer. The condition of DNA amplification was optimized following procedure of Conte et al. [17] with some modifications. PCRs were performed in reaction mixtures of 25 μL containing approximately 80 ng of genomic DNA, 1.7 μM primer, 200 μM dNTPs (50 μM each) and 1× reaction buffer. Sequences (5′ → 3′) from primers 1–9 were presented as follows: CTGGCGAACT; TCCGATGCTG; CTGCGCTGGA; CTGAGGTCTC; CTGGGGCTGA; TCATCCGAGG; TC TCCGCCCT; AAAGTGCGGC; ACCTTTGCGG; respectively. Reactions were hot-started at 94 °C for 5 min before the addition of 2.2 U of Taq DNA polymerase. Amplification was carried out in a thermocycler (Little Genius, China) with heated lid for 35 cycles (30 s at 94 °C, 60 s at 38 °C, then 60 s at 72 °C), followed by a final 10 min extension at 74 °C. Control PCRs lacking genomic DNA were run with every set of samples. PCR products were resolved electrophoretically in a 1.4% agarose gel (12 cm × 12 cm × 0.5 cm) run on 0.5× TBE buffer at 0 °C at 100 V for about 3 h and visualized after ethidium bromide staining using the Bio Image Analyzer system (Vendor). Chemicals were ordered from TaKaRa Biotechnology Ltd. (PR China).

Genomic template stability (GTS, %) was calculated as following:

$$\mathrm{GTS} = \left(1 - \frac{a}{n}\right) \times 100 \quad (2)$$

where a is the average number of polymorphic bands detected in each treated sample and n the number of total bands in the control. Polymorphism in RAPD profiles included disappearance of a normal band and appearance of a new band in comparison to control. The average was calculated for each experimental group exposed to different Cd treatments. To compare the sensitivity of each parameter, changes in these values were calculated as a percentage of their control (set to 100%).

2.3. Statistical analysis

The statistical analyses were carried out using the package software SPSS 10.0 for Windows. Changes in total soluble protein level and root growth were tested statistically by performing one-way analysis of variance (ANOVA). The least significant differences (LSD) test was used to reveal statistical difference.

3. Results

3.1. Effect of Cd on root growth and total soluble protein level

This test was performed to evaluate the inhibitory effect of Cd-contaminated soil on root growth of barley seedlings at various concentrations used in this experiment. Data (Table 1) obtained suggest that root lengths were substantially inhibited with the increase of 10, 20, and 40 mg L^{-1} Cd concentration after the 10-day exposure ($P < 0.05$, <0.01, <0.01, respectively) compared with the control plantlets, indicating a dose-dependent response. These results confirmed that Cd is indeed a toxic agent for barley plants as described previously by Singh and Tewari [21] and Enan [20].

The results obtained for the total soluble protein level in the root tips of barley seedlings in response to Cd pollutant are also shown in Table 1. Total soluble protein levels in seedlings increased slightly ($P > 0.05$) at 10 mg L^{-1} Cd and increased significantly ($P < 0.05$ and $P < 0.01$) along with the increase of Cd concentration in comparison