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Study on the response of wheat to lead, cadmium and zinc

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Abstract—The effect of lead, cadmium and zinc on the transcriptions and structures of 5 DNA fragments was studied by RNA slot blot hybridization and the analysis of restriction fragment length polymorphism (RFLP). The seeds of three wheat strains (Yunmai29, 1257, 5118) which had grown in contaminated area, Huize Lead-Zinc Mine, Yunnan Province of China and in uncontaminated area were taken as the experimental materials. No obvious change of DNA structure was detected, but there were many differences in the DNA transcription levels. These results implied that lead, cadmium and zinc might inhibit DNA transcription and had much more effect on gene expression than structure in wheat, which might acclimate to metal pollution after having grown in pollution area for a long time and the interference of these metal ions in gene expression might be one of main mechanisms of metal toxicity and plant adaptation. The results also showed the microevolution of wheat in the lead-zinc mine.

Keywords: lead; cadmium; zinc; wheat; gene transcription.

1 Introduction

Lead, cadmium and zinc are important environmental pollutant, their concentration are increasing with industrialization. These metals have adverse detriments to plants. They interfere with the uptake and distribution of nutrient elements in plants (Breckle, 1992), inhibit cell elongation, destroy organic structure and cause retardation in plant growth (Aidid, 1992; Stegani, 1991), and also can affect gene express (Zhang, 1990; Meng, 1997; Shah, 1995). Many plants show some tolerance to toxic metals, e. g. some plants produced metal-binding complexes in response to metal-stress (Grill, 1988; Reddy, 1990). Metal-tolerances of plant can be genetically pre-determined (Aniol, 1990; Miller, 1996; Well, 1995) or be inducible (Baker, 1986; Von, 1993; Watmough, 1995) and the induced metal-tolerances may be more important in perennial plant. Under long-term metal-stress, how do plant microevolute and adapt? These question have been drawing the attentions of environmental scientists and ecologists.

Huize Lead-Zinc Mine, Yunnan Province of China, has been mined for several decades, as a result, the complex pollution of lead, cadmium and zinc was very serious. The yields and qualities of crops were very low in or near lead-zinc mine and most of improved varieties of crops degenerate fast. Some plants which have grown in this area for a long time even show an evident difference in morphology. They are good experimental materials for the study of heavy metal toxicity, plant adaptability and microevolution in metal mine. In this paper, we took the wheats which had grown

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in Huize Lead-Zinc Mine for a long time as the experimental materials, were concerned mainly with the changes of gene transcription levels of the wheats under the stress of lead, cadmium and zinc. We wanted to get some basic data on metal-toxicity, metal-tolerance and plant microevolution in metal mine by this research.

2 Materials and methods

2.1 Plant materials

The three strains wheat (*Triticum aestivum*. *L*)-Yunmai29, 1257 and 5118, the contaminated seeds were collected from the peasants in Huize Lead-Zinc Mine, Yunnan Province of China (they had been bred constantly in this area for over 8 years), and were identified by Wheat Division of Institute of Food Crops, Yunnan Academy of Agricultural Sciences. The uncontaminated seeds of relevant strain wheats were supplied by Wheat Division of Institute of Food Crops, Yunnan Academy of Agricultural Sciences. The contents of lead, cadmium and zinc in these seeds were determined using a Z8000 Atomic Absorption Spectrometer. The results showed that the contents of these metals in the contaminated wheat seeds were much higher than that in uncontaminated ones (Meng, 1998).

2.2 DNA probes

Two tobacco chloroplast probes (pTB29, pTBa5) were presented by Prof. Hu Zhong and Vice-Prof. Hu Yun-qian in Kunming Institute of Botany, The Chinese Academy of Sciences; three wheat nuclear DNA probes were cloned by authors from uncontaminated Yunmai29 wheat. The data about DNA probes are shown in Table 1.

Table 1 The data about DNA probes

Probes	Sources	Vectors	Length of inset, kb
pWH58	Wheat DNA	PBR322	12.6
pWH63	Wheat DNA	PBR322	3.9
pWH98	Wheat DNA	PBR322	3.0
pTB29	Tobacco ctDNA	PBR322	3.4
pTBa5	Tobacco ctDNA	PBR322	10.9

2.3 The wheat culture

After all wheats had grown up to one-leaf stage in plate with distilled water, they were cultured under two different conditions: polluted or unpolluted conditions. The pollution solution contained: 0.1 mmol/L $Pb(NO_3)_2$, 0.15 mmol/L $Zn(NO_3)_2$, 0.10 mmol $CdCl_2$; uncontaminated solution is distilled water. The wheats were cultured under natural light at room temperature, and the solution were replaced everyday. After 7 days, the whole plant of wheat was taken as experimental material.

2.4 Preparation of total RNAs

Total RNAs were prepared according to the procedure of Chomazynski P. (Chomazynski, 1987). Placed about 1g fresh excised wheat tissues in a baked glass homogenizer, added about 10 fold volumes homogenization buffer [0.1% SDS, 0.7% β-mercapatoethanol, 20 mmol/L sodium acetate (pH 5.2), 8 mol/L guanidine HCl], and immediately homogenized until the tissue was thoroughly dispersed. Transferred the homogenatant into 50 ml centrifuge tube, added 1/10-volume 2mol/L sodium acetate (pH 5.2), mixed well, then added equal volume acid-phenol-chloroform (4:1), covered the top of centrifuge tube, and mixed the contents by shaking the tube intensely for 15 seconds, stored the tube on ice for 15 minutes. Centrifuged the tube at 8000 r/min at 4℃ for 15 minutes. Transferred the supernatant to a clean 50 ml centrifuge tube, repeated acid-phenol-chloroform (4:1) extracting, till it was clear at the interface, precipitated the RNA with equal volume of isopropanol, mixed well and stored the tube for 10 minutes at −20℃. Recovered the RNA by centrifugation at 10000 r/min for 15 minutes at 4℃. Dissolved the pellet of RNA in 3 ml 20 mmol/L EDTA, then added 3-fold volume 4 mol/L sodium acetate (pH 7.0), mixed well and centrifuged at 10000 r/min for 15 minutes. Decant the supernatant, washed the pellet of RNA with 75% ethanol (in DEPC-treated water) for 2—3 times and dissolved the pellet of RNA in DEPC-treated water. In the end, the RNAs were determined and quantitated by spectrophotometry.

2.5 Slot blot hybridization of wheat RNA

20 μg total denatured RNA per slot were transferred to nitrocellulose filters with Bio-Dot S F Microfiltration Apparatus (Bio-RAD). Slot hybridization was done with DIG-DNA Labeling and Detection Kit (Boehring Manheim) and conducted according to the Applications Manual (this experiment was repeated twice).

2.6 The analysis of wheat DNAs' RFLP

2.6.1 The preparation of wheat DNA

Placed fresh excised materials into ZK High Speed Tissue Blenser (made in China) and added 5—10 fold volume ice-cold blend buffer (1.5% citric acid), blended at 10000 r/min, 5 seconds each time, for three times. Filtered the homogenatant through two layers of cheese cloth into 50 ml centrifuge tube and collected the cell by centrifugation at 5000 r/min for 10 minutes at 4℃, resuspended the precipitate with extraction buffer [10 mmol/L Tri. Cl (pH 8.0), 0.1 mol/L EDTA (pH8.0), 20μg/ml pancreatic RNAase, 0.5% SDS] 2 ml/g material. The following procedure was conducted according to Blin and Stafford's (1976; Sambrok, 1989). The isolated DNA was dissolved in 1 × TE buffer [10 mmol/L Tri. Cl, 1 mmol/L EDTA (pH 8.0)], determined and quantitated by Spectrophotometry.

2.6.2 Restriction enzymes digestion of the wheat DNA and southern hybridization

In this paper, we adopted three restriction enzymes: EcoR I; Hind III; BamH I. 10 μg total DNA per lane was digested with 5u/μg restriction enzymes at 37℃ over night and transferred into nitrocellulose filters (Sambrok, 1989). Southern hybridization was manipulated using DIG DNA Labeled and Detection Kit (Boehrginer Mannheim).

3 Results

3.1 Wheat RNA's slot blot hybridization

Under unpolluted condition, the transcription levels of the 5 DNA fragments in the wheats from contaminated seeds were much lower than that in the wheats from uncontaminated seeds and all of the three strains wheats, the 5 DNA fragments showed so (Fig.1). Among the three strain wheats, the drop degree of transcription levels of 3 nuclear DNA fragments was the highest in

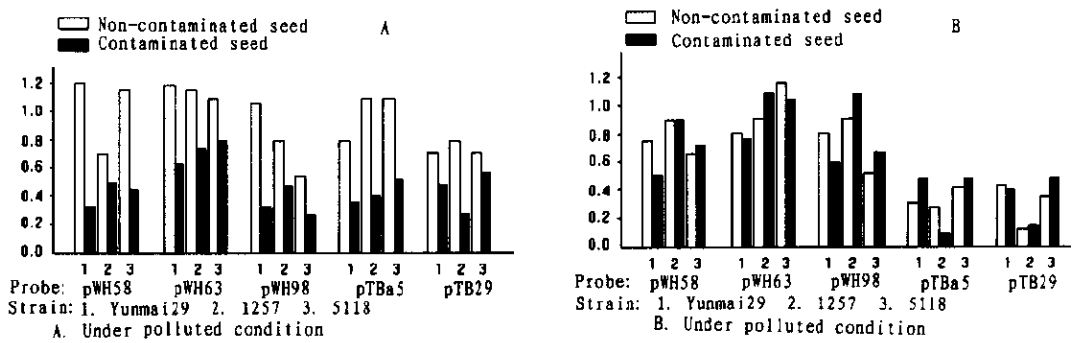

Fig.1 The gene transcription levels of the wheats from uncontaminated seeds and those from contaminated seeds

Table 2 Under unpolluted condition, the difference rates of genes transcription levels of the wheats from contaminated seeds to that of the wheats from uncontaminated seeds, %

Probes	Yunmai29	1257	5118
pWH58	(-)70.83	(-)28.58	(-)60.87
pWH63	(-)50.00	(-)34.78	(-)27.27
pWH98	(-)66.67	(-)37.50	(-)45.45
pTBa5	(-)50.00	(-)59.09	(-)50.00
pTB29	(-)28.57	(-)62.50	(-)14.29

Notes: (+) higher; (-) lower

Yunmai29, the lowest in 1257; but the drop degree of transcription levels of 2 ctDNA fragments was highest in 1257, lowest in 5118 (Table 2). Under polluted condition, there is no regular difference in the transcription levels of the 5 DNA fragments between the wheats from uncontaminated seeds and the relevant strain wheat from contaminated seeds (Fig.1) and Fig. 1 also shows that the transcriptions of 2 ctDNA fragments were very low under polluted condition in all the wheats (Table 3). To the wheat from uncontaminated seeds, their transcription levels of almost all these DNA fragments descended evidently under polluted condition in contrast with that of these wheats under unpolluted condition (Fig. 1) and the transcriptions of the two ctDNA fragments dropped smartly, especially it was wheat 1257 that the drop rate of its transcription levels of pTB29 fragment was 81.25% (Table 4). As far as the wheats from contaminated seeds were concerned, their transcription levels of the three nuclear DNA fragments ascended smartly

under polluted condition in comparison with that of these wheats under unpolluted condition (Fig. 1). Among these wheats, the increase degree was highest in wheat 1257, smallest in wheat Yunmai29(Table 5).

Table 3 Under polluted condition, the difference rates of genes transcription levels of the wheats from contaminated seeds to that of the wheats from uncontaminated seeds, %

Probes	Yunmai29	1257	5118
pWH58	(-)33.33	0.00	(+)15.38
pWH63	(-)6.25	(+)22.22	(-)8.69
pWH98	(-)25.00	(+)22.22	(-)30.00
pTBa5	(+)42.86	(-)66.67	(+)11.11
pTB29	(-)11.10	(+)33.33	(+)42.86

Notes: (+) higher; (-) lower

Table 4 The alteration rates of genes transcription levels of the wheats from uncontaminated seeds under polluted condition to that of these wheats under unpolluted condition, %

Probes	Yunmai29	1257	5118
pWH58	(-)37.50	(+)28.57	(-)43.48
pWH63	(-)33.33	(-)21.74	(+)4.54
pWH98	(-)23.81	(+)12.50	(-)9.09
pTBa5	(-)56.25	( )42.76	( )59.09
pTB29	(-)35.71	(-)81.25	(-)50.00

Notes: (+) increase; (-) decrease

Table 5 The alteration rates of genes transcription levels of the wheats from contaminated seeds under polluted condition to that of these wheats under unpolluted condition, %

Probes	Yunmai29	1257	5118
pWH58	(+)42.86	(+)80.00	(+)77.78
pWH63	(+)25.00	(+)46.67	(+)31.25
pWH98	(+)71.43	(+)120.00	(+)116.67
pTBa5	(+)25.00	(-)77.78	(-)9.09
pTB29	(-)20.00	(-)33.33	(-)16.67

Notes: (+) increase; (-) decrease

3.2 The analysis of wheat DNA's RFLP

After by restriction enzymes EcoR I, Hind III or BamH I digestion no obvious restriction fragments difference of the 5 DNA fragments was detected. The wheat from contaminated seeds had the same restriction fragments of the 5 DNA as the one from uncontaminated seeds under polluted condition or unpolluted condition. The same source wheat also had the same restriction fragments between unpolluted condition and polluted condition.

4 Discussion

The transcriptions of the wheats from uncontaminated seeds decreased under the mixture of lead, cadmium and zinc, it suggested that these metal ions had inhibition on DNA transcription of wheat. However, the transcriptions of the wheats from contaminated seeds increased smartly under the same polluted condition and these contaminated wheat had a very low transcription levels under unpolluted condition. This was a very odd result, but three strain wheats, 3 nuclear DNA fragments and two repeated experiments, all showed this result. Analyzing the causes of this result, we thought that it might be: (1) the activities of the wheats from contaminated seeds dropped after a long-term polluted-stress; (2) with the growing of wheat, the lead, cadmium and zinc in seed were transferred into plant and inhibited gene transcription of the wheats under unpolluted condition. Namely, internal heavy metals might inhibit gene transcription, consequently, the contaminated wheats had low transcription levels under unpolluted condition; (3) the stress of lead, cadmium and zinc for a long time might interfere or even change the regulation mechanism of gene transcription in wheats, and the wheat had grown in lead-zinc mine for a long-term might have adapted this metal-contaminated environment, even their normal gene transcription need definite contents of these metal ions, in other words, the contaminated wheats have gained some adaptability to these metals in gene expression after a long-term metal-stress. This result showed the microevolution of wheat in lead-zinc mine, and also implied that wheat could gain some adaptability to toxic metals after a long-term metal-stress. But the transcription levels of two chloroplast DNA fragments were very low under polluted condition in all the wheats, especially in 1257 wheat. The pTB29 fragment contains rbcl gene it codes the large unit of enzyme RUBP (ribulose bisphosphate carboxylase oxygenase; Sugiura, 1986), so the drops of the gene transcription levels in the two ctDNA fragments might drop the photosynthesis of wheat under polluted condition. This result implied that these metals might have especial toxicity in chloroplast, and the inhibition on gene transcription of ctDNA might be one of main mechanisms of photosynthetic toxicity of heavy metals.

In this paper, we studied the effect of lead, cadmium and zinc on the transcription, also on the structure of DNA in wheat using the same wheat materials and DNA probes. In the DNA structure, no obvious change was detected, but intranscription level of DNA, there were many differences between the wheats from contaminated seeds and those from uncontaminated seeds, under polluted and unpolluted conditions. Moreover, we yet found that these metals had strong influence on the gene expression of seed proteins of these wheats (Meng, 1998) and a lot of other study on metal toxicity had shown that heavy metals had very widespread influence on gene expression (Zheng, 1990; Meng, 1998; Shah, 1995), so we thought that lead, cadmium and zinc had more effect on gene expression than on gene structure. These results also displayed the microevolution and acclimation of wheat in lead-zinc mine.

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