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Toxicological effects of rare earth yttrium on wheat seedlings (*Triticum aestivum*)

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Abstract: This study examined the biochemical responses of wheat (*Triticum aestivum*) to the stress of rare earth yttrium (Y) and showed that 25–100 mg/kg Y treatments evidently increased the biomass (root mass, shoot mass and leaf mass), accompanied by a significant (p<0.05) increase in the chlorophyll (CHL) content in wheat leaves. Increased malondialdehyde (MDA) levels were detected in wheat shoots (stem and leaf) and roots too, indicating the presence of poisoning active oxygen species (AOS). The MDA content in wheat roots increased with the augmentation of Y concentration. These results indicated that there was a dose-dependent effect of Y on the changes of MDA content in wheat roots. Although the activities of superoxide dismutases (SOD), peroxidases (POD) and catalases (CAT) in wheat shoots and roots irregularly fluctuated with the increase in Y concentration, 25–100 mg/kg Y significantly (p<0.01) increased the activities of SOD and POD. In general, the dose-dependent effects of Y on the activity of antioxidant enzymes were insignificant. Our data also indicated that the increase in SOD and POD activities could be used as a good biomarker for the stress induced by low concentrations of Y.

Keywords: rare earth elements (REEs); toxicological effects; oxidative stress; wheat (*Triticum aestivum*)

Rare earth elements (REEs), Y, lanthanum (La) and 14 lanthanides, naturally present in the environment, form a chemically uniform group[1]. Although some of them, mainly La and Ce, are fairly abundant in nature, they occur only in trace amounts in biological systems. In recent years, REEs, owing to their specific characteristics, are attracting more and more attention in industry and agriculture[2]. They are currently used mainly in industrial production of technological devices. The world consumption of REEs in industry was 84,000 tons of rare earth oxides in 2003[3]. Discoveries that yield and quality of crop could be significantly improved by using rare earth microfertilizers have led to a large-scale application of rare earth microfertilizers in crop production. REEs-containing fertilizers are applied to over 6 million hectares of farmlands per year[4]. With their wide use in industry and agriculture, the entry of REEs into the environment is increasing. As a result, more attention has been paid to the environmental and ecological effects of REEs[5]. Although the toxicological effects of light REEs were investigated, the toxicological effects of heavy REE, Y, remained largely unknown[6,7].

Plants are one of the key producers in ecosystems with important roles in sustaining the integrity of ecosystem. Even under natural conditions of growth and development, plants are unavoidably exposed to different stresses. Oxidative stress has been demonstrated as one of the underlying factors causing tissue injury after exposing the plants to a wide variety of stress conditions[8–10]. Oxidative stress is a condition when the defenses of an organism can no longer get rid of all the undesired radicals and other AOS, which can result in damages to the lipids, proteins and DNA[11]. Plants have evolved various protective mechanisms to eliminate or reduce the AOS caused by damage. Enzymatic antioxidant system is one of the protective mechanisms, operating with sequential and simultaneous actions by a series of enzymes including SOD, POD and CAT[12]. The SOD decomposes superoxide radical to oxygen (O_2) and hydrogen peroxide (H_2O_2) through the process of dismutation: $2O_2+2H^+ \rightarrow H_2O_2+O_2$; the H_2O_2 produced from this process can be further eliminated by various antioxidant enzymes such as CAT and POD. While the CAT can remove most of the photorespiratory and respiratory H_2O_2, the POD participates in lignin biosynthesis and converts H_2O_2 to water[13]. Enzyme activities are considered to be sensitive to pollution and have the further advantage of easy detection without the needs for expen-

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sive, sophisticated instruments[14]. While some plant species are commonly used as biomonitors to determine the toxic effects of pollutants[15,16], wheat has been validated as an important crop of ecotoxicological significance[17]. Therefore, the present study investigated the toxic effects on antioxidant enzyme activity and CHL content in wheat seedlings under the stress of Y in order to seek sensitive biomarkers for diagnosing potential adverse effects of Y on plants at biochemical levels. Our results also showed that the concentration of NO_3^- in soil reached 3 g/kg, affecting the growth of wheat, the effect of which, however, was not considered in this study.

1 Experimental

1.1 Tested chemicals and soil

The tested form of Y, $Y(NO_3)_3{\cdot}6H_2O$, was purchased from A&C Rare Earth Materials Center. Riboflavin, guaiacol, nitro blue tetrazolium, L-methionine and trichloroacetic acid were bought from the Acros Company (USA), and thiobarbituric acid was from the Sigma Company (USA). All chemicals used were of analytical grade with chemical purity of 97.0%–99.0%.

The tested soil samples were collected from the surface layer (0–20 cm) of an uncultivated and unpolluted field in an arboretum of Ganzhou, China. The fresh soil samples were air-dried and ground to pass a 2.0-mm sieve before use. The general chemical properties and the calculation of total rare earth oxides in the tested soil are shown in Tables 1 and 2.

1.2 Plant culture

Well-developed wheat seeds were soaked for 5 min in 3.0 vol.% sodium hypochlorite, washed several times with sterilized deionized water, and germinated in darkness at 25 °C for 72 h. Then 50.0 g of air-dried soil samples were put into a culture dish with a depth of 10 cm. After Y solution was sprinkled on the surface of soil samples, various concentrations of Y were homogeneously mixed. The final humidity of the tested soil was about 30%. The concentrations of Y were 0, 25, 50, 100, 200 and 400 mg/kg dry mass of soil, respectively, which corresponded to the respective inhibitory rates of root elongation of 0%, 12%, 20%, 30%, 50%, and 60%, according to the results of germination experiments. On each culture dish, 15 germinated seeds were scattered and covered. Then the culture dishes were put into a culturing box (PQX-350H, China) which was operated with a photoperiod of 14 h and a constant temperature of 25 °C. Each treatment had 3 replicates. After incubation for 7 d, the seedlings were harvested. The paddy wheat was collected with roots at 7th day and rinsed with tap water and distilled water respectively. After being dried with filter paper, the whole plant in each exposure group was weighted. Then the root, stem and leaf were separated by scissors, and weighted separately to determine the fresh weight (FW) of each part.

1.3 Determination of CHL and lipid peroxidation

The CHL content in wheat leaves was determined in 80 vol.% acetone extract of 0.1 g of leaf as described by Hegedüs et al.[13] and expressed as mg/g FW. The MDA content was also determined as described by Hegedüs et al.[13] and expressed as mol/g FW.

1.4 Preparation of tissue extracts and assays of enzyme activity

Tissue samples were homogenized in buffer solution (50 mmol/L Na-phosphate buffer solution, pH 7.8; with 1 vol.% polyvinylpyrrolidone to protect the enzymes) using a tissue homogenizer. Approximately 0.1 g of plant tissue was ground with 1.5 mL of buffer solution. The filtered tissue extract was further centrifuged at 15000 r/min for 15 min at 4 °C. The supernatant was retained, aliquoted, and stored at –80 °C for further enzyme analysis.

The POD activity was determined using guaiacol substrates as described by Wu and von Tiedemann[18]. Crude enzyme extracts (20 μL) and reagent mixtures (200 μL) were incubated for 10 min at room temperature in the dark. The absorbance of the brown guaiacol reaction was immediately measured at 450 nm. The SOD activity was also determined as described by Wu and von Tiedemann[18]. The enzyme extracts and reagent mixtures were incubated for 30 min at room temperature, and the activity of CAT was assayed by the decrease in absorbance at 240 nm, following H_2O_2 consumption[18]. An incubation mixture without enzyme extracts served as the maximum absorbance control. The activity of enzymes was expressed as U/g FW. One enzyme unit was defined as 50% inhibition of the colorimetric reaction.

Table 1 Normal parameter of natural soil (wt.%)

pH	Redder	Powder	Clay	Hydrolysable nitrogen	Available phosphorus	Available kalium
6.0	13.5	17.6	68.9	0.21	0.02	0.003

Table 2 Contents of REEs in natural soil (REO, mg/kg)

Element	Lu	Tm	La	Er	Gd	Ho	Tb	Dy	Sm	Yb	Y	Eu	Pr	Nd	Ce
Content	0.00750	0.03246	0.04992	0.00001	0.01498	0.00481	0.01099	0.01827	0.04349	0.02000	0.21237	0.01289	0.07520	0.10423	0.46200

REO—The calculation of total rare earth oxides