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Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*)

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Abstract

The effects of multiple heavy metal stress on the activity of antioxidative enzymes and lipid peroxidation were studied in leaves and roots of two mangrove plants, *Kandelia candel* and *Bruguiera gymnorrhiza*, grown under control (10‰ NaCl nutrient solution) or five levels of multiple heavy metal stress (10‰ NaCl nutrient solution containing different concentration of Pb^{2+}, Cd^{2+}, and Hg^{2+}). Leaves and roots of control and heavy metal-stressed plants were harvested after two months. In leaves of heavy metal-stressed plants superoxide dismutase (SOD) and peroxidase (POD) activities fluctuated in different stress levels compared to the control, while catalase (CAT) activity increased with stress levels in *K. candel*, but remained unchanged in leaves of *B. gymnorrhiza*. In comparison with the control, the dynamic tendency of SOD, CAT, and POD activities in roots of heavy metal-stressed plants all ascended, and then declined. The increase in enzyme activities demonstrated that *K. candel* is more tolerant to heavy metals than *B. gymnorrhiza*. Lipid peroxidation was enhanced only in leaves of heavy metal-stressed *B. gymnorrhiza*. These results indicate that in heavy-metal stress antioxidative activities may play an important role in *K. candel* and *B. gymnorrhiza* and that cell membrane in leaves and roots of *K. candel* have greater stability than those of *B. gymnorrhiza*. For pollution monitoring purposes, POD activity in roots and leaves maybe serve as a biomarker of heavy metal stress in *K. candel*, while lipid peroxidation maybe serve as biomarker in *B. gymnorrhiza*.

Keywords: Heavy metal stress; Superoxide dismutase (SOD); Catalase (CAT); Peroxidase (POD); Lead; Cadmium; Mercury; Mangrove pollution

1. Introduction

Mangrove ecosystems, possessing great ecological and commercial value, are diverse communities in inter-tidal zones of tropical and subtropical coastal rivers, estuaries and bays. Like other wetlands, the mangrove ecosystem has been widely used as sites where effluents are discharged and solid wastes are dumped, including metallic anthropogenic wastes (Saenger et al., 1990; Peters et al., 1997), and has a large capacity in retaining heavy metals and nutrients (Roberson and Phillips, 1995; Tam and Wong, 1996). Mangrove ecosystems can act as sinks for heavy metals, which can become pollution sources to plants. Some mangrove plants appear to possess a great tolerance to high levels of heavy metal pollution (Peters et al., 1997), but in excessive heavy metal contamination, mangrove plants may initiate a variety of subcellular responses, i.e. metabolic reactions, which can cause damage at the cellular level or lead to wider phytotoxic responses (Vangronsveld and Clijsters, 1994).

Toxic levels of heavy metal affect a variety of processes in plants (Maksymiec, 1997; Siedlecka et al., 2001). One of

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the major consequences is the enhanced production of reactive oxygen species (ROS), which damage cell membranes, nucleic acids and chloroplast pigments (Somashekaraiah et al., 1992; Chaoui et al., 1997; Weckx and Clijsters, 1997; Fang and Kao, 2000; Tewari et al., 2002). Accumulation of ROS may be the consequence of disruption of the balance between their production and the antioxidative system activity, composed of enzymic antioxidants such as catalase (CAT), peroxidases (POD) and superoxide dismutases (SOD), and non-enzymic scavengers, e.g. glutathione, carotenoids and ascorbate (Tukendorf and Rauser, 1990; Vangronsveld and Clijsters, 1994; Noctor and Foyer, 1998; Xiang and Oliver, 1998; Srivastava et al., 2004). SOD is the major $O_2^{\cdot-}$ scavenger and its enzymatic action results in H_2O_2 and O_2 formation. CAT and several classes of peroxidases then scavenge the H_2O_2 produced. CAT dismutates H_2O_2 into H_2O and O_2, which is found in peroxisomes, cytosol and mitochondria (McKersie and Leshem, 1994). POD decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and/or antioxidants (Blikhina et al., 2003). Under normal circumstances, concentration of oxygen radicals remains low because of the activity of these antioxidative enzymes (Asada, 1984). In stress condition, the free radical species (forms of active oxygen) may be increased, which will enhance the activities of these detoxifying enzymes. The activities of SOD, CAT, and POD are induced in plants species by heavy metals (Bhattacharjee, 1997–98; Gallego et al., 1999; Pereira et al., 2002; Fornazier et al., 2002b; Lee and Shin, 2003; Skorzýnska-Polit et al., 2003–2004; Li et al., 2006). Malondialdehyde (MDA) is a cytotoxic product of lipid peroxidation and an indicator of free radical production and consequent tissue damage (Ohkawa et al., 1979). Thus, cell membrane stability has widely been utilized to study effects of stress on plants.

As for mangrove plants, previous studies have concentrated on their response to salt stress and waterlogging, distribution and accumulation of metals, and effect on photosynthesis of a single heavy metal (Macfarlae and Burchett, 1999, 2001; Yim and Tam, 1999; Takemura et al., 2000; Ye et al., 2003). Little information exists on their physiological and biochemical mechanisms under multiple heavy metal stress. In fact, mangrove plants are growing in a complicated environment including multiple heavy metals. Hence, it is necessary to study the correlation between mangrove plants and heavy metals for the purpose of improving mangrove ecosystem.

Kandelia candel and *Bruguiera gymnorrhiza* are two major mangrove species of the eastern group and are dominant along South China coast. Their responses and tolerance to multiple heavy metals have not been studied. Furthermore, differences between these two species in resistance to heavy metals are not well known. This study investigated the effects on lipid peroxidation and SOD, CAT, and POD antioxidant enzymes activities in leaves and roots of *K. candel* and *B. gymnorrhiza* during exposure to Pb^{2+}, Cd^{2+}, and Hg^{2+}.

2. Materials and methods

2.1. Plant growth and metal treatment

Viviparous seeds of *K. candel* and *B. gymnorrhiza* were collected from a national nature reserve in Guangdon Province and from the Bay of SangYa in HaiNan province, respectively. Only complete, undamaged propagules with testa and no emergent hypocotyls or radicle were selected. Propagules chosen for germination were those collected in the most abundant weight class, 35–36.5 g fresh weights. Seeds were planted in free-metal plastic pots (five seeds in each pot) with sand washed with sterilized water. These plants were kept in a greenhouse with temperature of 25 ± 5 °C and light intensity of 480 μmol m^{-2} from natural sunlight. Each pot was irrigated with 0.5 l of liquid fertilizer (half strength Hoagland's solution with 10‰NaCl) every 3 days. After two leaves had been developed, the seedlings were divided into five groups (4 in each group). Four groups of both *K. candel* and *B. gymnorrhiza* were irrigated with liquid fertilizer (as described above) containing heavy metals at four levels. In level 1HM, the irrigation medium contained 1.0 mg/l Pb^{2+}, and 0.2 mg/l Cd^{2+} and Hg^{2+}; levels 5HM, 10HM and 15HM contained metal concentrations that were 5×,10× and 15× higher than 1HM, respectively. One group of each species served as control (C). Each pot was irrigated with 1 l of corresponding liquid twice a week. After two months, leaves and roots were harvested for analysis of lipid peroxidation products and SOD, POD and CAT activities.

2.2. Enzymes extraction

All biochemical analyses were performed at 4 °C; 1.0 g of fresh leaves or roots were extracted in 3 ml of 50 mmol/l sodium phosphate buffer (pH 7.8) including 1.0 mmol/l EDTA and 2% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 5000*g* for 10 min, and the supernatant was used for the enzymatic assays. Proteins were determined according to Bradford (1976) using bovine serum albumin as the standard protein.

2.3. Enzymes assays

SOD activity was determined by the method of Beauchamp and Fridovich (1971) by following the photoreduction of nitroblue tetrazolium (NBT). The reaction mixture contained 50 mmol/l phosphate buffer (pH 7.8), 0.1 mmol/l EDTA, 13 mmol/l methionine, 75 μmol/l NBT, 2 μmol/l riboflavin and 100 μl of the supernatant. Riboflavin was added as the last component and the reaction was initiated by placing the tubes under two 15 W fluorescent lamps. The reaction was terminated after 10 min by removing the reaction tubes from the light source. Non-illuminated and illuminated reactions without supernatant served as calibration standards. The photoreduction of NBT (pro-