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Subcellular distribution and chemical forms of cadmium in *Bechmeria nivea* (L.) Gaud.

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Abstract

Bechmeria nivea (L.) Gaud. (Ramie) is a promising species for Cd phytoextraction with large biomass and fast growth rate. Nevertheless, little information is available on its tolerance mechanisms towards Cd. Determination of Cd distribution and chemical speciation in ramie is essential for understanding the mechanisms involved in Cd accumulation, transportation and detoxification. In the present study, ramie plants were grown in hydroponics with increasing Cd concentrations (0, 1, 3, 7 mg l^{-1}). The subcellular distribution and chemical forms of Cd in different tissues were determined after 20 days exposure to this metal. To assess the effect of Cd uptake on plant performance, nitrate reductase activity in leaves and root activity were analyzed during the entire experimental period. Increased Cd level in the medium caused a proportional increase in Cd uptake, and the highest Cd concentration occurred in roots, followed by stems and leaves. Subcellular fractionation of Cd-containing tissues indicated that about 48.2–61.9% of the element was localized in cell walls and 30.2–38.1% in soluble fraction, and the lowest in cellular organelles. Cd taken up by ramie rapidly equilibrated among different chemical forms. Results showed that the greatest amount of Cd was found in the extraction of 1 M NaCl and 2% HAC, and the least in residues in all test tissues. In roots, the subdominant amount of Cd was extracted by d-H_2O and 80% ethanol, followed by 0.6 M HCl. While in stems and leaves, the amount of 0.6 M HCl-extractable Cd was comparable with that extracted by 80% ethanol or d-H_2O. 1 mg l^{-1} Cd stimulated nitrate reductase activity in leaves and root activity, while a concentration-dependent inhibitory effect was observed with increasing Cd concentration, particularly at 7 mg l^{-1} Cd. It could be suggested that the protective mechanisms evolved by ramie play an important role in Cd detoxification at relatively low Cd concentrations (below 3 mg l^{-1} Cd) but become restricted to maintain internal homeostasis with higher Cd stress.

Keywords: Cadmium; Ramie; Subcellular distribution; Chemical form; Nitrate reductase activity; Root activity

1. Introduction

The accumulation of Cd within soils as a consequence of industrial and agricultural activities is becoming a major environmental problem due to the great toxicity of Cd and its high mobility from soil to plants and further to the food chain (Vig et al., 2003). Excess Cd induces nutritional deficiency (Yoshihara et al., 2006) and disturbs a series of physiological metabolisms in plants such as transpiration (Barcelo and Poschenrieder, 1990), respiration, photosynthesis, and nitrogen assimilation (Sanita di Toppi and Gabbrielli, 1999).

In order to survive and reproduce in heavy metal-contaminated environment, plants have evolved a wide range of defense mechanisms (Vogeli-Lange and Wagner, 1990; Sanita di Toppi and Gabbrielli, 1999) including metal exclusion, active excretion, restricted distribution of toxic metal in sensitive tissues, metal binding to the cell wall, chelation by organic molecules and compartmentalization in vacuoles. Plant species vary greatly in their capacity for Cd accumulation and tolerance, which is mainly attributed to the different strategies employed by plants under Cd stress.

First of all, specific capacity of plant to accumulate different amount of Cd in different tissues appears to play a predominant role in its adaptation to Cd stress. For instance, lettuces displayed high potential for Cd uptake and translocation, with approximately 50% of the total Cd taken up being distributed in the above ground parts (Ramos et al., 2002). While in

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Cd-sensitive bean plants about 98% of the total Cd was retained in roots to avoid perturbation of the vital metabolic processes in shoots (Ouariti et al., 1997). Thus tight control of Cd in sensitive tissues may act as the first defense line of plants to counteract Cd toxicity.

However, Lozano-Rodríguez et al. (1997) have found that although Cd concentration was similar in the root and shoot tissues of maize and pea plants, the latter accumulated larger amount of lipid peroxidation products and exhibited more severe toxic symptoms. Thus, the difference in Cd accumulation among tissues cannot comprehensively elucidate the distinctness in Cd tolerance among plant species. According to Van Assche and Clijsters (1987) only a part of the heavy metal entering into plants is phytotoxic. In other words, the phytotoxicity of heavy metal is partially determined by its biological activity in plants, which is associated with the subcellular distribution and chemical forms of heavy metal in plant cells. For instance, it was observed that a Cd-sensitive barley genotype had higher Cd concentration in the organelle containing fractions, and a larger amount of Cd in inorganic and water-soluble forms as compared with the Cd-resistant genotypes (Wu et al., 2005). Therefore, the pattern of Cd subcellular distribution and chemical forms in plants are also considered as important factors influencing the characteristics of Cd migration, accumulation and phytotoxicity degree in different species or genotypes.

Taking into consideration the dramatic bioaccumulation coefficient of Cd in plants, phytoextraction has been proposed to remediate Cd-contaminated soils. In order to select proper species, much research has been done with the emphasis on hyperaccumulator plants (Brown et al., 1995) and crops (Peralta-Videa et al., 2002). Unfortunately, many known hyperaccumulators exhibit slow growth rate and small biomass, leading to limited usefulness for phytoextraction of Cd-polluted sites (Ebbs and Kochian, 1997). Most crops, on the other hand, are susceptible to high Cd stress (Smith et al., 1985; Gouia et al., 2000). Furthermore, the employment of crops in phytoextraction would increase the risk of Cd entry into the food chain.

Recently, special attention has been paid to the inedible crops such as bioenergy and industrial crops with fast growth rate and large biomass (Arduini et al., 2006). Furthermore, they are safe and economically profitable after harvest, as is considered a priority in phytoremediation. Willow, poplar and hemp have been shown to be promising for Cd phytoextraction (Klang-Westin and Perttu, 2002; Linger et al., 2002; Pulford and Watson, 2003).

Bechmeria nivea (L.) Gaud. (Ramie) is a vigorous, high-yielding, perennial, Urticaceae species and cultivated widely as a fibre crop. However, to our best knowledge, little information is available on Cd uptake and distribution pattern in response to Cd stress in this economic plant species. Therefore, the aims of this study were to investigate the characteristics of Cd uptake, subcellular distribution and chemical forms in ramie and their roles in Cd tolerance. To evaluate the stress in tissues due to Cd uptake and allocation, nitrate reductase activity in leaves and root activity were determined accordingly.

2. Materials and methods

2.1. Plant growth and treatment

One-month-old ramie plants collected from a commercial greenhouse were grown in 3 l plastic pots (3 plants per pot) containing 1/4 strength Hoagland nutrient solution with aeration. After acclimatization for 2 weeks, the plants were divided into two groups and four treatments were applied for each group including 0 (T0), 1 (T1), 3 (T2) and 7 (T3) mg l^{-1} Cd as Cd $(NO_3)_2$. Experiments were carried out in a controlled room with 14 h light period at light intensity of 300 μmol m^{-2} s^{-1}, 25 °C/20 °C day/night temperature and 60–70% relative humidity. All nutrient solutions were renewed every two days and the pH values were maintained in the range of 6.0 ± 0.5. To analyze the nitrate reductase activity in leaves and root activity over the 20d experimental period, leaves and roots of ramie plants in one group were sampled once every day in the initial four days after Cd application and every other day thereafter till the end of the experiment. For Cd determination, ramie plants in the other group were harvested at the end of the experiment (after 20d exposure to Cd) and they were separated into roots, stems and leaves, and frozen in liquid N_2 until use.

2.2. Tissue fractionation

Frozen materials were homogenized in pre-cold extraction buffer containing 50 mM Tris–HCl (pH 7.5), 250 mM sucrose and 1.0 mM $C_4H_{10}O_2S_2$. Cells were separated into three fractions: cell wall, soluble fraction and organelle containing fraction using differential centrifugation technique as suggested by Weigel and Jäger (1980) with some modifications. The homogenate was centrifuged at 3000 r min^{-1} for 15 min and the precipitation was designated as 'cell wall fraction' mainly consisting of cell walls and cell wall debris. The resulting supernatant solution was further centrifuged at 15000 r min^{-1} for 30 min. The resultant deposition and supernatant solution were referred to as 'organelle containing fraction' and 'soluble fraction', respectively. All steps were performed at 4 °C. After digestion (for cell wall and organelle containing fractions) with concentrated HNO_3–$HClO_4$ (3:1, v/v) and proper dilution to constant volume, Cd concentrations in different fractions were analyzed by Atomic Absorption spectrophotometry (AAnalyst 300, Perkin-Elmer, Germany). In order to determine the total Cd concentrations in different tissues and calculate the percentage recovery of Cd, frozen tissues were subjected to acid digestion with HNO_3–$HClO_4$ (3:1, v/v) for Cd determination. Translocation Factor ($TF = (C_{srem} + C_{leaf})/C_{root}$) was calculated to identify the characteristic of Cd transportation from root to the aerial part.

2.3. Extraction of Cd in different chemical forms

Cd associated with different chemical forms was successively extracted by designated solutions in the following order (Yang et al., 1995):