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Physiological responses and tolerance of kenaf (*Hibiscus cannabinus* L.) exposed to chromium

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ABSTRACT

Selection of kenaf species with chromium (Cr) tolerance and exploring the physiological mechanisms involved in Cr tolerance are crucial for application of these species to phyto-remediation. In the present study, a hydroponic experiment was conducted to investigate the variation in two kenaf cultivars, K39-2 and Zhe50-3 under Cr stress. At the same Cr concentration, the tolerance index (TI) of K39-2 was higher than that of Zhe50-3, indicating that K39-2 may be more tolerant to Cr than Zhe50-3. It was also observed that high concentration of chromium was accumulated both in the shoots and the roots of *Hibiscus cannabinus* L. The leaves of K39-2 accumulated 4760.28 mg kg^{-1} of dry weight under 1.50 mM Cr stress, and the roots accumulated 11,958.33 mg kg^{-1}. Physiological response shows that the antioxidant enzymes' superoxide dismutase (SOD), catalase activity (CAT) and peroxidase (POD) activities increased in the leaves and decreased in roots of the Cr-stressed plants nearly compared to the control. Moreover, the variation of antioxidant enzymes activities indicated Zhe50-3 was more vulnerable than K39-2, and the contents of the non-protein thiol pool (GSH, NPT and PCs) were higher in K39-2 than Zhe50-3 with the increased Cr concentration. Based on the observations above, it can be concluded that the well-coordinated physiological changes confer a greater Cr tolerance to K39-2 than Zhe50-3 under Cr exposure, and *Hibiscus cannabinus* L. has a great accumulation capacity for chromium.

1. Introduction

Contamination of soil caused by heavy metals, which has become a severe environmental problem around the world, has attracted extensive attention (Lin and Aarts, 2012; Chen et al., 2015). Chromium (Cr) is the second most common heavy metal that causes contamination in ground, water, soil, and sediments (Shrestha et al., 2007). The occurrence of Cr in the environment is primarily due to anthropogenic activities, particularly their widespread use in industries for electroplating, leather tanning, metal finishing, steel production, and pigment manufacturing (Ali et al., 2013c). In some Asian countries, 2000–3200 tons of elemental Cr were released into the environment yearly (Chandra et al., 1997), and very high levels of Cr contamination, 14,800 mg/L in ground water and 25,900 mg/L in soil, have been reported (Krishnamurthy and Wilkens, 1994). Cr accumulation in soil has become an essential environmental concern around the world due to its deleterious effects on crop growth and human health (Tiwari et al., 2009). It has been demonstrated that Cr toxicity caused stunting plant growth, chlorosis in newly leaves, damage to the roots, and decreased grain yield (Scoccianti et al., 2006; Ali et al., 2013a). In addition, plants growing in a Cr stressed environment also trigger the formation of reactive oxygen species (ROS) like H_2O_2, OH^-, and O_2^{-}·, which can damage the production of biomolecules (Gill and Tuteja, 2010) and resulting in membrane damage and electrolyte leakage (Ali et al., 2011, 2015a, 2015b). Malondialdehyde (MDA) is one of the ultimate products as a result of lipid peroxidation damage by free radicals and is an indicator of free radical production and the consequent tissue damage.

In the plants there is a well-organized and intricate antioxidant mechanism which can scavenge reactive oxygen species and alleviate their deleterious effects (Mittler, 2002). The defense mechanism includes various antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) (Dazy et al., 2009) and non-enzymatic antioxidants such as ascorbic acid (AsA), glutathione (GSH), and phytochelatins (PCs) (Apel and Hirt, 2004). SOD, the first-line enzyme defense against oxyradicals, is a crucial criterion for quenching ROS (Bowler et al., 1992). POD urges H_2O_2-dependent oxidation of the substrate, while CAT removes H_2O_2 by decomposing it directly to evolve water and oxygen (Bashir et al., 2013). The AsA–GSH pathway is a key part of the network of reactions involving important metabolites with redox properties for

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the efficient elimination of the removal of H_2O_2 in different cellular compartments (Noctor and Foyer, 1998). In fact, GSH, which is an important non-enzymatic antioxidant in the cellular milieu, can scavenge ROS and alleviate membrane damage through the ascorbate AsA–GSH cycle (Noctor et al., 2012). It is also considered to be an element of GSH S-transferase (GST)-based detoxification mechanisms (Rausch and Wachter, 2005) and as a substrate for the biosynthesis of PCs (Lavoie et al., 2009). PCs comprise a family of peptides with the general structure (g-Glu-Cys) *n*-Gly, where n=number of repetitions of the g-Glu-Cys unit, which can vary from 2 to 11 (more commonly from 2 to 5) (Grill et al., 1985). PCs possessing a high antioxidant capacity can sequester metals into the vacuole and play a part in buffering cytosolic metal-ion concentration (Cobbett and Goldsbrough, 2002; Song et al., 2010).

Phytoremediation is an emerging green technology that has been considered for remediation of contaminated sites because of its low cost, esthetic advantages, and long term applicability (Zhao and McGrath, 2009). A large amount of research in China has been devoted to hyperaccumulation of heavy metals such as zinc (Yang et al., 2002), arsenic (Chen et al., 2003), manganese (Xue et al., 2004), and cadmium (Liu et al., 2004; Wei et al., 2005). However, there are rarely studies about chromium accumulation by the hyperaccumulator. Up till the present moment, only about three species could be qualified as chromium hyperaccumulators, i.e., *Dicoma niccolifera* Wild (Wild, 1974), *Sutera fodina* Wild (Baker and Brooks, 1989) in Zimbabwe, and *Leersia hexandra* Swartz (Zhang et al., 2007) in China. Kenaf (*Hibiscus cannabinus* L.) is an annual herbaceous plant from the Malvaceae family whose bast and core fibers are used in building materials, adsorbents, textiles, automobiles, and fibers in new and recycled plastics (Webber et al., 2002). It has been demonstrated that kenaf is able to extract metals from contaminated soil and accumulate it in the stem (Carlson et al., 1982; Cartoga et al., 2005). Kenaf had also been used for phytoremediation in the tropics with a combined purpose of biomass production to enhance the ecological and economic value of degraded areas (Ho et al., 2008; Arbaoui et al., 2013).

Kenaf is now commercially cultivated in more than 20 countries, particularly in China, India, and Thailand (FAO 1998), which accounts for 90% of the global area of kenaf cultivation and more than 95% of the global kenaf production (FAO 2003). In 2014, the global output of jute, kenaf, and allied fibers increased to 3.74 million tones. However, little is known about the physiological and biochemical mechanisms in kenaf under chromium stress. In the present study, a hydroponic experiment was conducted to investigate the potential of kenaf resistance against Cr stress. We examined the physiological changes in two kenaf seedlings after exposure to different Cr concentrations, and explored the accumulation and tolerance of chromium and the role of cellular antioxidant activities in protecting the plants from Cr-toxicity.

2. Materials and methods

2.1. Plant material and growth conditions

The seeds of ten cultivars of kenaf (*Hibiscus cannabinus* L.): Zhe 50-3, H032, H101b, K80, Jinguang Wuci, Liao 55B, Zhe 134-20, ZB90, K39-2, and 85-239 were obtained from the Research Institute of Bast Fiber Crops, Hunan Province, China. Healthy and equally sized seeds were screened out and surface sterilized in 75% ethanol for 10 min, then rinsed with sterile water. The seeds were sown in plastic pots (200 cm × 130 cm × 60 cm, length, breadth, and depth) containing nutrient soil for 6 days. Afterwards uniform seedlings were transplanted into plastic pots (15 plants per pot) filled with 500 mL of Hoagland nutrient solution (Hoagland and Arnon, 1950). The solution was adjusted to pH 6.5 and renewed every 3 days. Seven days later, ten genotypes seedlings were randomly assigned to 1.6 mM Cr (III) treatment, K39-2 and Zhe50-3 through screening and were exposed to four different Cr (III) treatments: 0, 0.5, 1.0, and 1.5 mM $CrCl_3$. The seedlings were maintained in a cultivation chamber under controlled conditions, with a light/dark photoperiod of 14/10 h, a photon flux density of 150 μmol/m^2 s at the leaf level, a day/night temperature of 25 °C/18 °C, and a relative humidity of 65–75%. After 4 days of exposure to Cr, the plants were harvested and washed with 20 mM Na_2-EDTA for 30 min (Yang et al., 2004) followed by distilled water. There were three replicates for each treatment. The sampled plants were separated into shoots and roots and dried at 80 °C in an oven to a constant weight for biomass determination.

2.2. Cr and chlorophyll contents in plant tissues

Approximately 0.1 g of dried fine powder from the roots and shoots was digested with a mixture of HNO_3 and $HClO_4$ (v/v=4:1) at 220 °C until the liquid became clear and there was no white smoke come out. Deionized water was used in measuring Cr content. The content of Cr was determined by flame atomic absorbance spectrometry (Hitachi 180-80, Japan) and Cr content was expressed as milligram per kilogram DW. Chlorophyll content was determined according to Arnon (1949).

2.3. Bio-concentration factor, translocation factor, and tolerance index

The bio-concentration factor (BCF) was defined as the ratio of metal concentration in plant roots or aerial tissues to that in the soil or solution. The translocation factor (TF) indicated the ability of plants to translocate chromium from the roots to the shoots. BCF, TF, and Tolerance Index (TI) were calculated as following (Shi et al., 2009):

$$BCF = \frac{Croot}{Csolution} \quad (1)$$

where Croot is the concentration of Cr in root and Csolution is the concentration of total Cr in solution.

$$TF = \frac{Caerial}{Croot} \quad (2)$$

where Caerial is the concentration of Cr in aerial parts.

$$TI(\%) = \frac{Plant\ root\ or\ shoot\ biomass\ in\ solution\ with\ Cr}{Plant\ root\ or\ shoot\ biomass\ in\ solution\ without\ Cr} \times 100 \quad (3)$$

2.4. Analysis of lipid peroxidation and propidium iodide staining

Lipid peroxidation was measured in terms of malondialdehyde (MDA), which was analyzed according to Lei et al. (2007). Propidium iodide, a membrane-impermeable dye, binds to nucleotides and is generally excluded from living cells. The nucleus of cells with of a loss of membrane integrity was labeled. Root tips were incubated for 5 min in 5 μg mL^{-1} propidium iodide dissolved in distilled water. After washing, samples were observed under a fluorescence microscope (Leica DM5000 B, Germany) using excitation at 546 nm (De Cnodder et al., 2005).

2.5. Assays of enzymes and metabolites of the antioxidant system

The soluble protein content in the samples was measured according to Bradford, (1976) using bovine serum albumin (BSA) as the standard protein. 0.5 g Fresh leaves were ground in 5 mL of 50 mM cold phosphate buffer (pH 7.0) containing 1% (w/v) soluble polyvinylpyrrolidone and centrifuged at 15,000*g* for 20 min at 4 °C. The