

Full Length Research Paper

Antioxidant enzymes as bio-markers for copper tolerance in safflower (*Carthamus tinctorius* L.)

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Activities of antioxidant enzymes were investigated in order to evaluate protective mechanism of plants against oxidative stress induced by high concentration of copper. Safflower (*Carthamus tinctorius* L.) plants were exposed to 100 μ M copper (Cu) for two weeks under controlled environmental conditions using hydroponic culture. Cu induced changes in chlorophyll and carotenoids pigments. More Cu accumulated in the roots as compared with the leaves. Level of lipid peroxidation and antioxidant enzyme activities (peroxidase, catalase and superoxide dismutase) also correlated with the Cu content of the plant tissues. However, restricted transfer of the metal to the foliage and enhanced activity of peroxidase, catalase and superoxide dismutase may be of great significance for scavenging oxidative stress caused by excessive copper in safflower plants. Thus, these antioxidant enzymes served as good predictors for the evaluation of heavy metal tolerance.

Key words: *Carthamus tinctorius*, copper stress, oxidative damage, antioxidant defense.

INTRODUCTION

The accumulation of heavy metals in the environment is a serious concern for agriculture, animal and human health. Among heavy metals, copper (Cu) has been classified as a non biodegradable metal pollutant which enters the environment through various anthropogenic activities such as from pesticide, fungicides and municipal sewage (Ross, 1994). Cu is an essential micronutrient at low concentration for normal plant growth and development. However, excessive levels are toxic and are known to cause oxidative stress in plants (Stoys and Bagchi, 1995). Excessive Cu ions promote incomplete reduction of molecular oxygen leading to the generation of free radicals.

Consequently, enhanced production of reactive oxygen species (ROS) can damage biological membranes and other classes of macromolecules (Mittler, 2002; Blikhina et al., 2003; Andrade et al., 2010). Malondialdehyde (MDA) is the final product of lipid peroxidation and is widely used as an indication of tissue damage (Dhir et al., 2004). Similarly, excessive Cu ions can induce inhibition of pigments biosynthesis or degradation of their molecules (Luna et al., 1994). Accumulation of ROS activates anti-oxidative defense mechanisms in plants. The protection against oxidative stress is achieved by the production of enzymatic antioxidants which comprised of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) while glutathione, carotenoids and ascorbate represent non enzymatic components (Hall, 2002; Caregnato et al., 2008). As such, these biochemical attributes served as an index of metal sensitivity or tolerance in different groups of plants (Li et al., 2006; Srivastava et al., 2004). Therefore, in the present study, we aimed to identify useful bio-markers for Cu tolerance in safflower (*Carthamus tinctorius* L.). We investigated changes in chlorophyll,

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Abbreviations: ROS, Reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; TBA, thiobarbituric acid; NBT, nitro-blue tetrazolium.

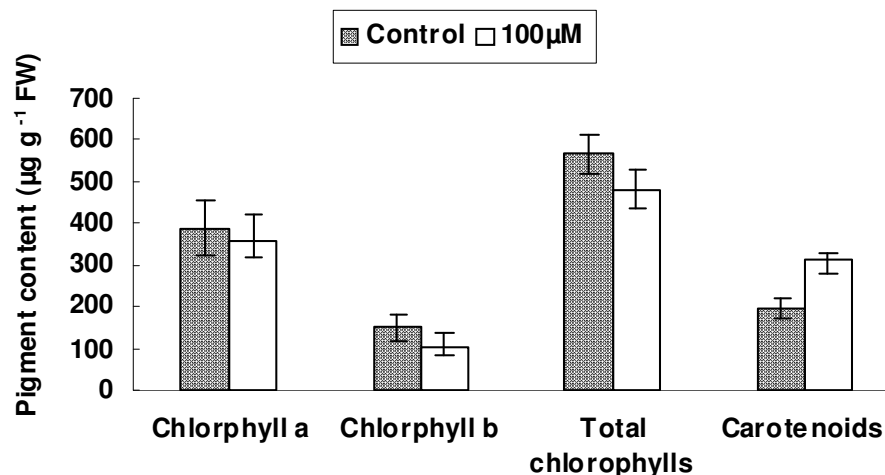


Figure 1. Changes in chlorophylls and carotenoids in leaves of *C. tinctorius* L. after two weeks exposure to copper. Values are mean of three replicates, bars indicate \pm standard errors.

carotenoids, MDA, SOD, POD and CAT levels in relation to bioaccumulation of Cu in plant tissues of safflower plants after their exposure to excessive copper.

MATERIALS AND METHODS

About 100 seeds of safflower (*C. tinctorius* L.) variety US -10 were germinated in Petri dishes in a growth chamber at 25°C, 12 h light /12 h dark period, (illumination of 2500 Lux, Philips T2 40W/33 lamp). Five seedlings (7 days old) of comparable size were transferred to each of 24 plastic beakers (250 ml) containing half strength Hoagland's solution (Hoagland and Arnon, 1938). Plants in half of the beakers were exposed to 100 µM Cu prepared from CuSO₄.5H₂O (Merck, Germany). The experiment continued for 14 days in the laboratory with 14 h photoperiod at 25 \pm 2°C. Solutions were adjusted daily with HCl and NaOH to pH 5.8, aerated every day and renewed once every 3 days. Twenty-one (21) days old plants were harvested and investigated for various biochemical attributes. Pigments were extracted from the leaves using 80% actone (v/v). Amount of chlorophyll was estimated following Arnon (1949), while carotenoids were determined according to Kirk and Allen (1965). The root and leaf samples were digested with a solution of 3:1 HNO₃:HClO₄ (v/v). Metal accumulation in plant parts was measured by atomic absorption spectrometry (Varian AAS, 1475, California, USA). The level of lipid peroxidation, expressed as MDA content, was determined as 2-thiobarbituric acid (TBA) reactive metabolites, according to Heath and Packer (1968). Enzyme extraction was carried out in a cold room at 4°C from 1.0 g of fresh roots and leaves. SOD activity was determined by using spectrophotometer (U-2000, Hitachi Instrument, Tokyo, Japan) at 560 nm based on photo-reduction of nitro-blue tetrazolium (NBT) following Beauchamp and Fridovich (1971). POD activity was assessed with guaiacol as described by Zhang et al. (1992) while, CAT activity was measured according to Aebi, (1984). All analyses were carried out in triplicate and results are presented as mean \pm S.E.

RESULTS AND DISCUSSION

Some chlorosis, root necrosis and browning of the roots

were observed in plants after their exposure to copper. Though, chlorosis reflected a decrease in chlorophyll, this decline was not too drastic (Figure 1). An increase in total carotenoids in response to the application of 100 µM Cu was observed. A significantly higher concentration of Cu was observed in roots as compared to leaves (Figure 2A). A greater accumulation of MDA, a product of lipid peroxidation, was observed in the roots of Cu treated plants (Figure 2B) which may be correlated to higher metal content of the tissue. A greater activity of antioxidant enzyme systems SOD (Figure 2C), POD (Figure 2D) and CAT (Figure 2E) was traced from roots of the plants at 100 µM copper treatment.

The study showed an overall restraining of total chlorophyll content of the plant that could be attributed to the inhibitory effect of Cu on their molecules (Xu et al., 2010). However, the increase in total carotenoids in response to the application of 100 µM Cu might be an indication of non-enzymatic antioxidant defense. The higher concentration of Cu in roots than in the leaves is in line with the findings of Baker (1981) who suggested that metal tolerant species absorbed and retained a large proportion of Cu in their roots but in non-tolerant plants root, endoderm act as a weak barrier thus allowing the transfer of metal ions to above ground plant tissues.

A noticeable increase in MDA signifies oxidative stress since excessive copper has been reported to enhance the activity of lipoxygenase (Fernandez and Henriquez, 1991) which catalyzes lipid peroxidation, indicated by an increase in MDA level. However, observed increase in the total activity of SOD, POD and CAT in the root tissues can be a manifestation of the initiation of antioxidant defense (Marquez-Garcia and Cordoba, 2010). SOD is one of the antioxidant enzymes that play a key role in cellular defense against ROS (Bowler et al., 1992). Similarly, CAT is considered to be the most important

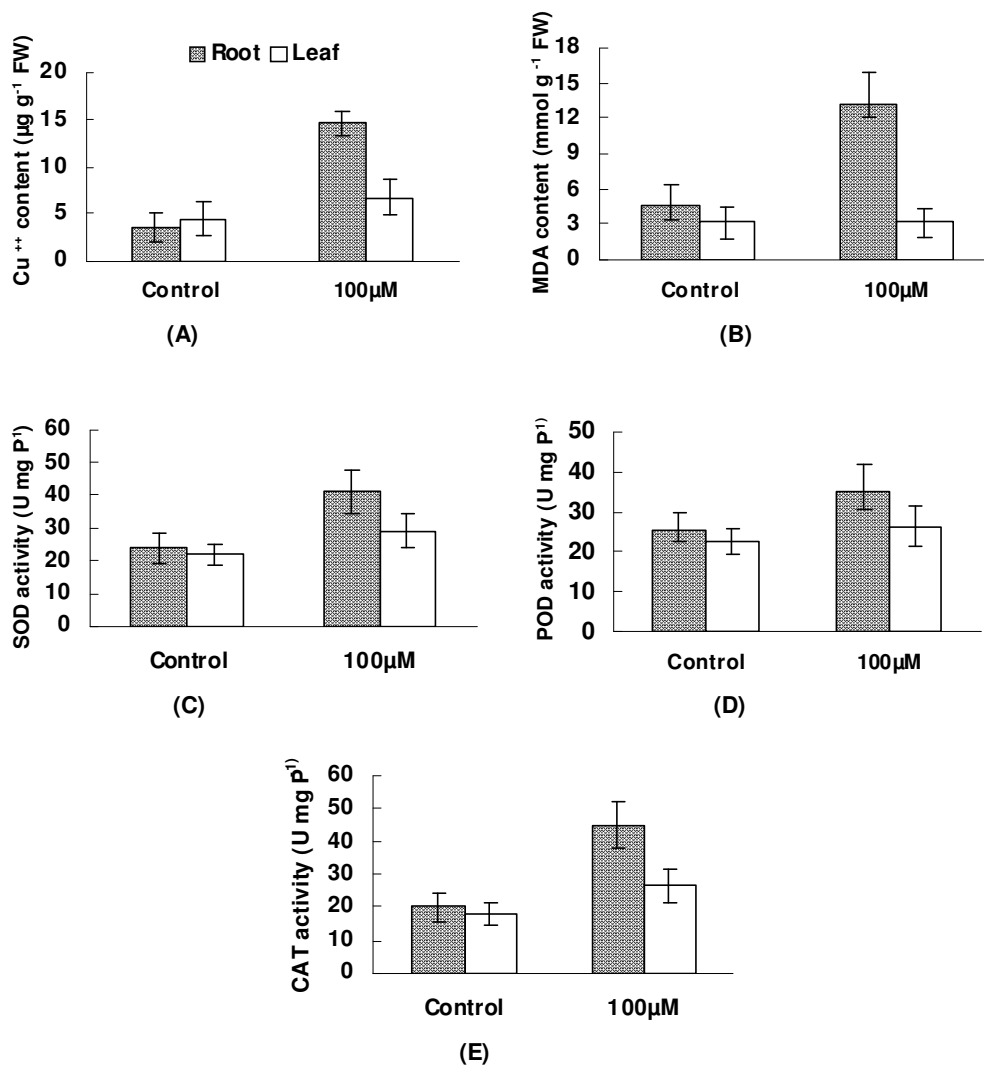


Figure 2. A. Bioaccumulation of metal, B. malondialdehyde (MDA), C. superoxide dimutase (SOD), D. peroxidase (POD), E. catalase (CAT) in the tissues of *C. tinctorius* L. after two weeks exposure to copper. Values are mean of three replicates, bars indicate \pm standard errors.

enzyme which eliminates H_2O_2 from the cells. The stimulation of SOD activity along with CAT seemed to play a protective role against membrane damage as Cu is particularly toxic to membranes (Mazhoudi et al., 1997). Although, an increase in POD activity is associated with the formation of necrotic spots under heavy metals stress, it also promotes lignin biosynthesis (Hegedus et al., 2001) and can build up a physical barrier against toxic heavy metals, thereby, strengthening the tissues against highly reactive free radicals that are injurious to cellular entities (Fang and Kao, 2000).

This study demonstrated some decline in green pigments, induction of lipid peroxidation and generation of oxidative stress in response to excessive copper. However, restricted transfer of the metal to foliage and increase in the three major antioxidant enzymes imply a defensive mechanism in the species to avoid Cu toxicity.

Thus, the enhanced production of SOD, POD and CAT may serve as useful biomarkers for Cu tolerance in *C. tinctorius*.

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