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Growth parameters and photosynthetic pigments in leaf segments of *Zea mays* exposed to cadmium, as related to protection mechanisms

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Summary

The influence of cadmium on growth parameters and photosynthetic pigment content was studied in maize leaf segments differing in tissue maturity. Experiments were carried out with maize seedlings *Zea mays* L. cv. Hidosil treated with 25, 50, 100, 150 and 200 μM Cd for 14 days under low light conditions. Tissue age-dependent decrease of fresh mass, dry mass and area of the leaf segments was correlated with Cd concentration in the growth medium. Cd induced changes in chlorophyll and carotenoid contents, and the specific areas and densities of the leaf segments were dependent on age and metal concentration. Results are discussed with respect to the protection mechanisms in the leaf segments.

Introduction

Monocotyledons are well suited for studies of the leaf growth process because growth is predominantly unidirectional. Cells are dislocated from the basal meristem as a result of continued production and elongation of new cells. As cells are displaced in the basal part of a growing leaf, they expand and differentiate. The growth process of these plants results in the formation of a spatial gradient of increasing cell length with the distance from the base of an elongating leaf (Leech et al., 1973; Piquery et al., 2002 and references therein). Division of leaf cells of graminaceous plants in the basal meristem causes older cells to be displaced by younger cells below them (Nelson and Langdale, 1989). Monocotyledons have often been used in studies on the development of the structure and physiological processes in growing tissues (Baszyński, 1971; Baker and Leech, 1977; Nelson and

Abbreviations: Chl, chlorophyll; DM, dry mass; FM, fresh mass; GSH, glutathione; PCs, phytochelatins; PPFD, photosynthetic photon flux density; ROS, reactive oxygen species; *x+c*; carotenoids

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Langdale, 1992; Babani and Lichtenthaler, 1996; Piquery et al., 2002; Rodríguez et al., 2002; Wagner et al., 2003).

Various environmental stresses can considerably alter leaf structure and metabolism. The stress dynamics interact with the developmental dynamics of structure and function in growing tissues, resulting in very different responses to the stress in the leaves at particular development stages (Delpérée et al., 2003 and references therein). The responses of plants to heavy metal stress, depending on the growth stages at which they were exposed to a stress factor, have been shown both in dicotyledons (Barceló et al., 1988; Maksymiec et al., 1994, 1995; Skórzyńska-Polit and Baszyński, 1995, 1997; Skórzyńska-Polit et al., 1995; Maksymiec and Baszyński, 1996a, b) and monocotyledons, C_3 (Krupa and Moniak, 1998) and C_4 plants (Rascio et al., 1993; Drążkiewicz et al., 2003).

Studies on the age-dependent response of maize plants to Cd stress have shown that the metal distribution in leaf segments depends on their age, as well as Cd concentration in the growth medium (Drążkiewicz et al., 2003). Chlorophyll level was diminished in the mature region of maize exposed to high Cd concentrations (Rascio et al., 1993). In mature leaf tissues, the chloroplast ultrastructure was damaged under Cd treatment, while in younger tissues it was unaltered (Rascio et al., 1993). Cd reduced the photochemical processes more in older than in younger leaf segments, but the functional status of the dark phase of photosynthesis (R_{fd}) was diminished more strongly in younger ones (Drążkiewicz et al., 2003).

To counteract Cd toxicity, maize has developed mechanisms of metal detoxification, which involve, among other things, intracellular thiol-containing compounds such as glutathione (GSH) and phytochelatins (PCs). As shown previously, GSH content both in control and Cd-treated plants increased with the age of leaf tissue, and PC accumulation was also age-dependent (Drążkiewicz et al., 2003). Some of these compounds could influence photosynthesis in the maize leaf segments (Drążkiewicz et al., 2003).

The aim of this paper was to the characterize changes in growth parameters and photosynthetic active pigments in maize leaf segments of different tissue maturity, and to relate them to defence strategies of this plant against Cd toxicity.

Materials and methods

Maize plants (*Zea mays* L. cv. Hidosil) were germinated from seeds on wet filter paper in a thermostat-controlled, darkened chamber at 95% relative humidity and 25 °C for 3 days. The seedlings were transferred into Hoagland full strength nutrient solution containing 0, 25, 50, 100, 150 and 200 μM Cd (in the form of $3CdSO_4 \cdot 8H_2O$) and grown for 14 days at PPFD of 100 μmol m^{-2} s^{-1} under a day/night regime of 14/10 h and 25/18 °C. The nutrient solution was replaced weekly. The third leaf was sampled and divided into three sections of equal length from the basal (youngest), through the middle (mature), to the apical (oldest) parts, and used for all subsequent analyses.

Measurements

In preliminary experiments the lengths of roots and shoots was measured, and the tolerance index was calculated according to Wilkins (1957).

Pigments were extracted from the leaf segments with chilled 80% acetone. Estimation of chlorophylls and carotenoids was performed in a spectrophotometer (Shimadzu UV-160A, Kyoto, Japan) after Lichtenthaler and Wellburn (1983), and calculated per area of the leaf segments.

The leaf segment area was measured with a GeniScan GTS-4500 scanner (Genius, Taiwan) and with the computer software manufactured by Witra (Poland, Warsow). The leaf segment density was defined as dry mass (DM) per area unit, and the specific area of leaf segments as leaf area per fresh mass (FM) unit.

Statistical analysis

Mean values were examined statistically by using the SigmaStat 3.0 software. A correlation analysis was conducted to determine the relationships between the different variables. Three independent experiments were carried out. Three to eight leaves were analysed for individual growth parameters and pigments in each experiment.

Results and discussion

Inhibition of root elongation is among the most sensitive responses to Cd exposure, and occurs faster than most of other physiological reactions (Schűtzendűbel et al., 2001). Therefore, in maize seedlings treated with Cd, the tolerance index for roots was lower than that for shoots, and it declined with increasing Cd concentration in the growth medium and exposition time (Fig. 1A and B). This could result from a higher Cd accumulation