Quantitative and qualitative changes in peroxidase of *Cucurbita pepo* cultivars stressed with heavy metals

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Abstract

Seedlings of two cultivars of zucchini (*Cucurbita pepo* L.) Courgette d'Italie (CI) and Courgette d'Alger (CA) were pre-treated with various concentrations of cadmium, copper and zinc for 30 d. High accumulation of heavy metals especially in the roots was showed. Peroxidase activity was affected according to the type of metal added, concentration, and the plant cultivar used. In leaves and roots of the CI control plants peroxidase activities were 50 and 17 % higher than in the CA control plants. Treatment with Cd (5 μg g^{-1}), Cu (200 μg g^{-1}), and Zn (500 μg g^{-1}) increased peroxidase activities in CA but decreased it in CI both in leaves and roots. Heavy metals tested lead also to some qualitative changes characterized by appearance of new isoforms of peroxidase. The results show the possibility to use the activities of peroxidase as biomarkers for Cd, Cu and Zn stresses.

Additional key words: cadmium, copper, peroxidase isoforms, zinc, zucchini.

Introduction

Contamination of soil used for food chain crops requires careful management of heavy metals that pose a potential health hazard to human and other consumers of the plant. Before any phytotoxic symptom was detected, elevated concentrations of heavy metals in plant tissue may result in enzyme inhibition as well as induction of new enzymes (Van Assche and Clijster 1990). Peroxidases produced by plants are present in multiple isoenzymic forms. These have various physiological roles in plant cells and participate in many reactions including lignification, cross-linking of cell wall polysaccharides, oxidation of indole-3-acetic acid, regulation of cell elongation, phenol oxidation (*e.g.* Gaspar *et al.* 1991). There is also evidence that increase in

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peroxidase activity can appear as a metabolic response to various stresses conditions resulting in the pollution of soil by heavy metals (De Vos *et al.* 1993). This greenhouse experiment was undertaken to compare the effect of Zn, Cu and Cd on peroxidase activity and isoenzyme pattern of two zucchini (*Cucurbita pepo* L.) cultivars (cv. Courgette d'Alger and cv. Courgette d'Italie) with the aim to find biochemical markers for the heavy metal effects on vegetables.

Materials and methods

Plants: Seeds of zucchini (*Cucurbita pepo* L.) cv. Courgette d'Alger (CA) and Courgette d'Italie (CI) were germinated on moist vermiculite. After 10 d of germination, homogeneous seedlings were transferred to pots containing sand and cadmium as 5 or 20 µg[$Cd(NO_3)_2$. 4 H_2O] g^{-1}(dry sand), copper as 200 or 1000 µg ($CuSO_4$. 5 H_2O) g^{-1} and zinc as 500 and 2000 µg($ZnSO_4$. 7 H_2O) g^{-1} was added. Control contained 0.09 µg g^{-1} Cd, 10 µg g^{-1} Cu and 32 µg g^{-1} Zn. Plants were grown for 30 d at temperature of 25 °C, 16-h photoperiod (irradiance of 185 µmol m^{-2} s^{-1}) and air humidity of 70 %. Pots were irrigated with Hoagland nutrient solution.

Enzyme extraction and peroxidase assay: After 30 d of culture, 0.2 g (f.m.) of leaf or root tissue was homogenized in an ice cold porcelain mortar in 1.5 cm^3 of 5 mM Tris HCl buffer (pH 7.2) containing 0.25 M sucrose and 1 mM $MgCl_2$. The homogenates were centrifuged for 7 min at 9 000 *g* and the supernatants collected corresponded to soluble enzyme fractions.

Whole peroxidase activity was analyzed using guaiacol as an electron donor, in a reaction mixture composed of 0.05 M acetate buffer (pH 5.0), 6 mM guaiacol and 0.010 cm^3 of enzyme extract. The hydrogen peroxide dependent oxidation of guaiacol was followed by an increase in the absorbance at 470 nm. Enzyme activity was expressed in arbitrary units which corresponded to change in absorbance equal to 0.1 per minute.

Electrophoresis: Acidic isoforms of peroxidase were separated by electrophoresis using 6 - 15 % gradient polyacrylamide gels stabilized by 43 % glycerol. The electrophoretic buffer (pH 8.3) was 0.025 M Tris, 0.19 M glycine and 1 % sodium dodecyl sulphate (SDS). Gel were stained for peroxidase isoforms by incubation in 0.1 M acetate buffer (pH 5.0) containing 0.1 % (m/v) benzidine and 0.01 % hydrogen peroxide.

Metal content in plants: Dry matter of harvested plants was put in a muffle oven for 4 h at 450 °C. After cooling, remaining matter was taken by 5 cm^3 of concentrated HCl and then evaporated till dryness. The residue is then taken by small volume of 5 % HCl, filtered and completed up to 10 cm^3. Metal analysis was carried out by atomic absorption spectrophotometry in an acetylen-air flame using a *Unicam 929* spectrophotometer.

Results

Plants growing in presence of heavy metals showed very high levels of selected metals compared to controls with a marked difference between leaves and root (Table 1).

Table 1. Cadmium, copper and zinc contents [$\mu g\ g^{-1}$(d.m.)] in leaves and roots of two zucchini cultivars Courgette d'Italie (CI) and Courgette d'Alger (CA) after 30 d treatment. For each metal, differences between data followed by the same letter are not significant (Newman-Keuls test at 5 % level).

Metal	Conc. [$\mu g\ g^{-1}$]	CI leaves	CI roots	CA leaves	CA roots
Cadmium	0.1	0.03a	1.3d	0.06g	1.8j
	5	4.2 b	249 e	13.5 h	326 k
	20	36 c	1033 f	50 i	1474 l
Copper	10	6.2 a	27 d	6.7 a	31 d
	200	52 b	608 e	46 b	616 e
	1000	203 c	1212 f	179 g	1064 h
Zinc	32	43 a	195 d	61 g	281 h
	500	602 b	2687 e	637 b	3011 i
	2000	1315 c	4804 f	1293 c	5463 j

Activities of peroxidase in leaves and roots in CI was 50 % and 17 % higher than those of CA respectively. In both cultivars, root showed higher peroxidase activities than leaves. The treatment with 5 $\mu g\ g^{-1}$ Cd decreased peroxidase activity in cv. CI (36 % in leaves and 13 % in roots), whereas in CA, peroxidase activity increased of about 30 % in leaves and 13 % in roots (Fig. 1). Peroxidase activity decreased with increasing the Cd concentration (20 $\mu g\ g^{-1}$). The treatment with Cu led to the similar changes in CI and CA. However, the treatment with 1000 $\mu g\ g^{-1}$ Cu which induced a 70 % decline in peroxidase activity in leaves increased its activity of about 25 % in roots (Fig. 1). The treatment with Zn induces changes similar to those obtained for Cd with marked difference between leaves and roots of CA.

Heavy metals led also to some qualitative modifications which affect peroxidase zymogram (Fig. 2). New isoforms of peroxidase appeared in CA leaves of plants treated with Cu (1000 $\mu g\ g^{-1}$) and Cd (5 $\mu g\ g^{-1}$). Electrophoretic pattern of root samples showed differences which depended on the type of metal tested, its concentration and the plant cultivar. Most important changes are characterized by disappearance of the isoform characterized by a Rf of 0.72 at all treatments used in CA and the appearance of isoforms which have a Rf of 0.58 in roots of both cultivars treated with Cu (1000 $\mu g\ g^{-1}$). Another isoform (Rf = 0.47) appears in the CI roots after 30 d of culture. The isozymic pattern showed intensification of some bands especially those characterized by a Rf of 0.48 in CA leaves at Cu (200 or 1000 $\mu g\ g^{-1}$) and Cd (5 $\mu g\ g^{-1}$). The same observations concern the band characterized by a Rf of 0.74 in CI root at the treatment with Zn (2000 $\mu g\ g^{-1}$), Cu (1000 $\mu g\ g^{-1}$), and Cd (5 and 20 $\mu g\ g^{-1}$).

Discussion

Treatment with Cd, Cu and Zn declined dry mass production of two zucchini cultivars. Cultivar CA was more tolerant in comparison to CI. Toxicity of Cd has been more expressed in roots. Differences between the two cultivars of zucchini were characterized particularly by accumulation rate of Cd which was higher in CA. Transfer of Cu and Zn showed lower differences between the two cultivars in comparison to those observed for Cd. Similar differences in accumulation in relation to plant cultivar have been noticed by John and Laerhoven (1978).

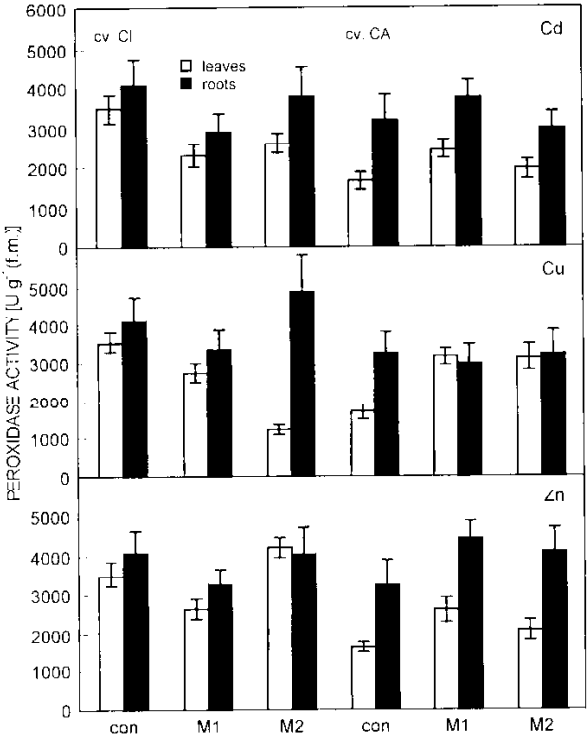

Fig. 1. Peroxidase activities in leaves and roots of two zucchini cultivars. Comparison of control plants (con) and those treated either with Cd (M1 - 5, M2 - 20 μg g^{-1}), Cu (M1 - 200, M2 - 1000 μg g^{-1}), or Zn (M1 - 500, M2 - 2000 μg g^{-1}). Means of 5 measurements ± S.E.

Peroxidase activity was higher in root than in leaves probably because the roots are in direct contact with the surrounding medium which contains heavy metals at high concentrations. This observation has been also reported by Blinda *et al.* (1996) in the case of cultured cells. On the other hand, the appearance of new bands in isozymic pattern indicates an induction in synthesis of new isoforms of peroxidase under heavy metal stresses particularly under Cu and Cd exposure in leaves and only

under Cu exposure in roots. Increase of peroxidase activity accompanied with synthesis of new isoforms, which may be the consequence of cell specific gene expression (Brune *et al.* 1994), are directed to protect the cell against oxidative damage and partially bypass metal-sensitive reactions. These mechanisms of detoxification were reported, *e.g.*, by Garcia *et al.* (1996).

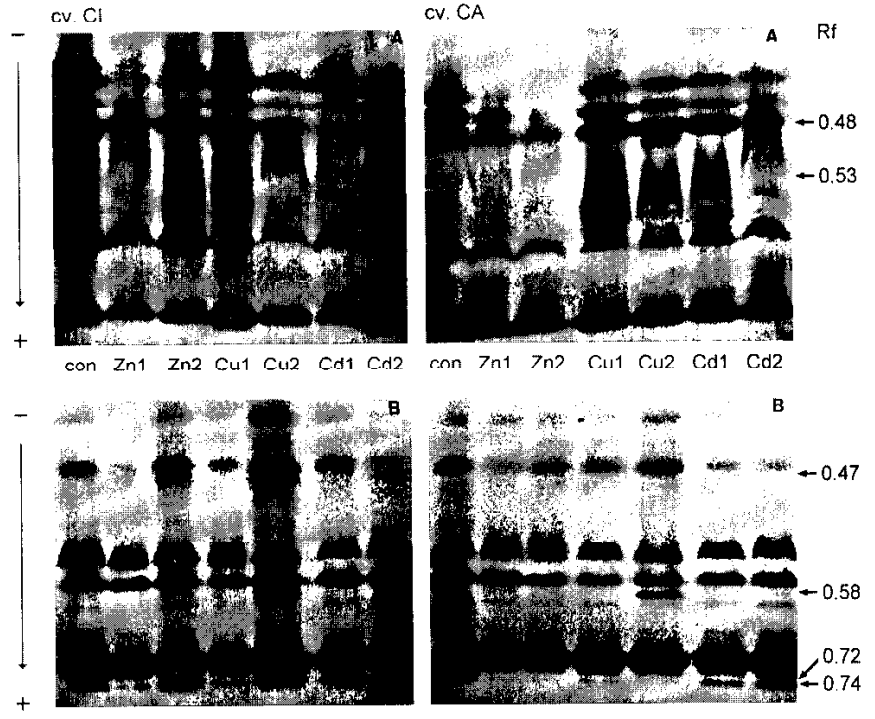

Fig. 2. Isoenzyme pattern of acid soluble peroxidase in leaves (*A*) and roots (*B*) of zucchini cvs. Cl and CA. Control plants (con) and plants stressed with zinc (Zn1- 500, Zn2 - 2000 μg g^{-1}), copper (Cu1 - 200, Cu2 - 1000 μg g^{-1}), and cadmium (Cd1 - 5, Cd2 - 20 μg g^{-1}) were compared.

In a simple approach, peroxidase activity might be used as general marker of stress caused by plant exposure to tested metals. The response depends on the type of metal, its concentration and plant species and cultivar. In addition, peroxidase activity can be used in experiments directed to evaluate toxicity of soils contaminated with heavy metals. Enzymic changes therefore can be used as markers of heavy metal pollution before the appearance of visible damage or a decrease in productivity.

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