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Impact of Cadmium and Copper Excess on Cell Wall Peroxidases in Pea Stems

A. Chaoui and E. El Ferjani

Département des Sciences de la vie, Faculté des Sciences de Bizerte,
Bio-Physiologie Cellulaires, 7021-Zarzouna, Tunisie

Abstract: Supply of Cd or Cu to culture medium of 12-day-old seedlings of pea (*Pisum sativum* L. cv. douce province) was conducted for 4 d. Stems lost growth and increased their cell wall peroxidase activity. Elevation in capacities of NADH-malate dehydrogenase, H_2O_2 -generating NADH oxidase and coniferyl alcohol peroxidase, which successively act in monolignols polymerization, confirmed the stimulation of lignification.

Key words: Ascorbate, heavy metals, hydrogen peroxide, tissues ageing

INTRODUCTION

Heavy metals toxicity results from complex interaction of metal ions with several metabolic processes^[1-4]. It is not easy to purpose an absolute scheme about the mechanisms leading to growth loss. However, negative effects of heavy metals may be related to increase of some enzymatic activities which limit cell growth and, consequently, accelerate tissues ageing^[4-7]. Particularly, cell wall stiffening may control cell growth, through reduction of extensibility. This can be done by cell wall peroxidases implicated in lignification: oxidative polymerization of monolignols (sinapyl, coumaryl and coniferyl alcohols) in presence of hydrogen peroxide which is generated by NADH oxidase, itself receive NADH from malate dehydrogenase^[8-11]. The present work was undertaken to find out the cell wall peroxidase changes associated with pea poisoning by Cd and Cu. Capacities of malate dehydrogenase and NADH oxidase, which act upstream of final stage of monolignols polymerization, were determined also in stems.

MATERIALS AND METHODS

Seeds of pea (*Pisum sativum* L. cv. douce province) were germinated between wet paper towels at 25°C in the dark for 3 d. Seedlings were grown in nutrient solution (pH 5.7) containing: $Ca(NO_3)_2$ (2.5 mM), KNO_3 (2 mM), KH_2PO_4 (1 mM), $MgSO_4$ (1 mM), Fe-K-EDTA (50 µM), H_3BO_3 (30 µM), $MnSO_4$ (10 µM), $ZnSO_4$ (1 µM), $CuSO_4$ (1 µM) and $(NH_4)_6Mo_7O_{24}$ (0.03 µM), under mercury lamps providing a light intensity of $150 \mu mol m^{-2} s^{-1}$, day/night temperature of 25/20°C and 65 (±5)% relative humidity. After 12 d, treatments were performed, for 4 d, by adding

0 (control), $Cd(NO_3)_2$ or $CuSO_4$ to the above-mentioned solution. Extraction of ionically and covalently bound cell wall proteins was performed as described previously^[12]. The enzyme activities were determined according to following methods: guaiacol peroxidase^[13], ascorbate peroxidase^[14], coniferyl alcohol peroxidase^[11], NADH oxidase^[15] and malate dehydrogenase^[16]. One unit of enzyme was defined as the amount necessary to decompose or produce 1 µmol of substrate or product, respectively, per min at 25°C.

RESULTS AND DISCUSSION

Metal treatments reduce the growth of pea stems, except for 20 µM Cu (Table 1). This dose seems to be not toxic, as evidenced by the activity of unspecific guaiacol peroxidase, previously used as heavy metals stress-related marker^[2,17-19]. In fact, enzyme capacity was not significantly modified after exposure to 20 µM Cu, whereas the other treatments markedly enhanced it in the two protein fractions of cell wall, especially in ionically bound one (Table 1). The same results were found when peroxidase activity was assayed with other specific substrates: coniferyl alcohol and ascorbate (Table 1). Capacities of (1) lignifying peroxidases, measured as hydrogen peroxide-dependent oxidation of coniferyl alcohol, a lignin monomer, (2) NADH oxidase and (3) malate dehydrogenase were strongly increased in ionically bound cell wall fraction after metals exposure (Table 1). Deposit of insoluble phenols, as lignin, in cell wall has been observed in heavy metals-poisoned plants^[6] and might be related to induction of lignifying peroxidases^[5]. Lignification reduces cell wall extensibility and, then, decreases cell growth^[4]. Thus, increase in

Table 1: Fresh weight [g±SD, n = 7] and activities of ionically (I) and covalently (C) bound cell wall enzymes [U g⁻¹ (fresh weight)±SD, n = 4] of stems of 16-day-old pea plants grown in control nutrient solution or supplemented with metals for 4 d

		Control	20 µM Cd	100 µM Cd	20 µM Cu*	100 µM Cu
Fresh weight		0.44±0.02	0.35±0.03	0.30±0.04	0.45±0.04	0.34±0.04
Guaiacol peroxidase	I	2.27±0.45	15.67±1.86	17.39±1.49	2.45±0.51	8.84±1.19
	C	1.85±0.22	5.98±0.17	5.84±0.48	1.87±0.14	4.17±0.41
Coniferyl alcohol peroxidase	I	68.18±11.35	203.63±37.55	260.33±43.65	70.40±7.76	288.50±35.55
	C	47.03±10.88	45.45±3.33	42.53±4.92	46.58±9.50	47.03±7.14
NADH oxidase	I	3.97±0.76	22.50±4.66	38.05±5.24	3.79±0.72	24.99±2.28
	C	2.88±0.21	2.74±0.15	3.04±0.38	3.10±0.16	2.96±0.17
Malate dehydrogenase [x 10 ⁻³]	I	0.47±0.10	1.07±0.13	1.40±0.22	0.48±0.08	0.88±0.06
	C	22.08±1.99	25.50±4.26	20.38±2.69	20.40±1.55	22.13±2.06
Ascorbate peroxidase	I	0.26±0.04	0.62±0.04	1.10±0.21	0.27±0.04	0.57±0.04
	C	0.22±0.02	0.25±0.04	0.24±0.03	0.23±0.02	0.21±0.02

*All values corresponding to 20 µM Cu treatment are not significantly different from controls at the 0.05 level of probability

activities of lignifying enzymes in ionically bound cell wall fraction might have a role in loss of cell wall plasticity and, thereby, decrease of stems growth in pea stressed by Cd and 100 µM Cu. This appears to be corroborated by the lack of effect of 20 µM Cu on growth and enzymes activity (Table 1).

It has been realized that heavy metals stress results in accumulation of H₂O₂ which would act as signaling molecule initiating secondary reactions, such as lignification^[4,7]. However, improvement in activity of ionically bound cell wall ascorbate peroxidase after metals exposure (Table 1) should be in opposite to this scheme, since antioxidant peroxidase would detoxicate H₂O₂. The destruction of H₂O₂ has been shown to be an important function of plant peroxidases that use ascorbate as the hydrogen donor^[20]. Moreover, although the efflux from the symplastic into the apoplastic compartment is low, ascorbate appears to be a normal constituent of the apoplastic space, where it acts in competition with coniferyl alcohol regarding H₂O₂ consumption by cell wall peroxidases^[21]. A second hypothesis is that the pronounced metals-elevation of NADH oxidase activity (about 6 to 10-fold higher than control; Table 1) generates an over accumulation of hydrogen peroxide, which could be widely sufficient for peroxidase capacities using ascorbate or monolignols. A third mechanism supposes that the increase of lignification might be directly ascribed to a metals-stimulation of the prooxidant nature of endogenous phenolic compounds^[22]. Phenolic, including intermediates of lignin biosynthesis, can be oxidized to phenoxyl radicals by peroxidases to form polymers such as lignin in apoplast^[21].

In conclusion, cell wall peroxidases of pea stems can increase both their antioxidant and lignifying capacities after exposure to cadmium and copper excess. These responses seem to be restricted to ionically bound cell wall enzymes. Metals-induced lignification may cause cell wall strengthening and, then, tissues ageing, as evidenced by the decrease of growth.

REFERENCES

1. Woolhouse, H.W., 1983. Toxicity and Tolerance in the Responses of Plants to Metals. In: Lange, O.L., P.S. Nobel, C.B. Osmond and H. Ziegler (eds.): Encyclopaedia of Plant Physiology. New Series 12C, Springer-Verlag, Berlin, pp: 245-300
2. Van Assche, F. and H. Clijsters, 1990. Effects of metals on enzyme activity in plants. Plant Cell Environ., 13: 195-206
3. Fernandes, J.C. and F.S. Henriques, 1991. Biochemical, physiological and structural effects of excess copper in plants. Bot. Rev., 57: 246-273
4. Schützendübel, A. and A. Polle, 2002. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J. Exp. Bot., 53: 1351-1365
5. Pandolfini, T., R. Gabbriellini and C. Comparini, 1992. Nickel toxicity and peroxidase activity in seedlings of *Triticum aestivum* L. Plant Cell Environ., 15: 719-725
6. Sasaki, M., Y. Yamamoto and H. Matsumoto, 1996. Lignin deposition induced by aluminium in wheat (*Triticum aestivum*) roots. Physiol. Plant, 96: 193-198
7. Schützendübel, A., P. Schwanz, T. Teichmann, K. Gross, R. Langenfeld-Heyser, O.L. Godbold and A. Polle, 2001. Cadmium-induced changes in antioxidative systems, H₂O₂ content and differentiation in pine (*Pinus sylvestris*) roots. Plant Physiol., 127: 887-892
8. Gross, G.G., C. Janse and E.F. Elstner, 1977. Involvement of malate, monophenols and the superoxide radical in hydrogen peroxide formation by isolated cell walls from horseradish (*Armoracia lapathifolia* Gilib.). Planta, 136: 271-276
9. Mäder, M., J. Ungemach and P. Schlob, 1980. The role of peroxidase isoenzyme groups of *Nicotiana tabacum* in hydrogen peroxide formation. Planta, 147: 467-470

10. Goldberg, R., A. Imbert, M. Liberman and R. Prat, 1986. Relationships between Peroxidasic Activities and Cell Wall Plasticity. In: Greppin, H., C. Penel and T. Gaspar (eds.): Molecular and Physiological Aspects of Plant Peroxidases. University of Geneva Press, Geneva, pp: 209-220
11. Pedreno, M.A., A. Ros Barcelo, F. Sabater and R. Munoz, 1989. Control by pH of cell wall peroxidase activity involved in lignification. Plant Cell Physiol., 30: 237-241
12. De Jaegher, G., N. Boyer and T. Gaspar, 1985. Thigmomorphogenesis in *Bryonia dioica*: Changes in soluble and wall peroxidases, phenylalanine ammonia-lyase activity, cellulose, lignin content and monomeric constituents. Plant Growth Regul., 3: 133-148
13. Fielding, J.L. and J.L. Hall, 1978. A biochemical and cytochemical study of peroxidase activity in roots of *Pisum sativum*: I. A comparison of DAB-peroxidase and guaiacol-peroxidase with particular emphasis on the properties of cell wall activity. J. Exp. Bot., 29: 969-981
14. Nakano, Y. and K. Asada, 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol., 22: 867-880
15. Ishida, A., K. Ookubo and K. Ono, 1987. Formation of hydrogen peroxide by NAD (P)H oxidation with isolated cell wall-associated peroxidase from cultured liverwort cells, *Marchantia polymorpha* L. Plant Cell Physiol., 28: 723-726
16. Bergmeyer, H.U., K. Gaweh and M. Grassl, 1974. Enzymes as Biochemical Reagents. In: Bergmeyer, H.U. (ed.): Methods in Enzymatic Analysis, pp: 425-522
17. Karataglis, S., M. Moustakas and L. Symeonidis, 1991. Effects of heavy metals on isoperoxidases of wheat. Biol. Plant, 33: 3-9
18. Mocquot, B., J. Vangronsveld, H. Clijsters and M. Mench, 1996. Copper toxicity in young maize (*Zea mays* L.) plants: effects on growth, mineral and chlorophyll contents and enzyme activities. Plant Soil, 128: 287-300
19. Tahlil, N., A. Rada, M. Boaziz, J.L. Morel, M. El-Meray and M. El-Aatmani, 1999. Quantitative and qualitative changes in peroxidase of *Cucurbita pepo* cultivars stressed with heavy metals. Biol. Plant, 42: 75-80
20. Asada, K., 1994. Production and Action of Active Oxygen Species in Photosynthetic Tissues. In: Foyer, C.H. and P.M. Mullineaux (eds.): Causes of Photooxidative Stress and Amelioration of Defence Systems in Plants. CRC Press, Boca Raton, Florida, pp: 77-104
21. Takahama, U. and T. Oniki, 1992. Regulation of peroxidase-dependent oxidation of phenolics in the apoplast of spinach leaves by ascorbate. Plant cell Physiol., 33: 379-387
22. Sakihama, Y., M.F. Cohen, S.C. Grace and H. Yamasaki, 2002. Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. Toxicology, 177: 67-80