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Effect of Heavy Metals on Isoperoxidases of Wheat

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Abstract. The influence of increasing concentrations of copper, zinc, lead, nickel, chromium and cadmium on 14-day-old seedlings of wheat (*Triticum aestivum* L. cv. Vergina) was studied. Plants were grown in 1/10 strength Rorison's nutrient solution with increasing concentrations of each of the metals added separately. The toxicity of metals depressed shoot growth but the most evident symptoms were on roots. The concentration of each metal which caused inhibition of root growth was chosen to study the influence of metals on isoperoxidases of wheat shoots. The concentrations employed did not alter the number of peroxidase bands but almost in all cases enhanced the intensities of bands of pH 4.0–4.2 and 5.0–5.4, while they decreased the intensities of bands of pH 4.2–4.6 and 5.4–6.5.

The similar effects of the different heavy metals employed may suggest similarity in metal action on wheat isoperoxidases. The increased intensities of peroxidase bands may be considered as an indication of enhanced senescence caused by the heavy metal treatments.

Generally, our results suggest that the heavy metals employed have caused complex changes on the multiple forms of peroxidases.

Additional index words: *Triticum aestivum* L. cv. Vergina, toxicity, isoelectric focusing.

The heavy metals include about thirty-eight elements which have the common feature in relation to biological systems, that in excessive quantities they are poisonous and can cause the death of most living organisms (Antonovics *et al.* 1971).

The strong affinity of heavy metals for side-chain ligands of proteins suggests that enzymes are among the first molecules with which metals may interfere in higher plants (Weigel and Jäger 1980). Thus, heavy metal phytotoxicity is due to the interference of these elements in plant metabolism, inactivating some enzymes or increasing the activity of others (Agarwala *et al.* 1961, Lee *et al.* 1976, Maier 1978, Braber 1980, Kar and Feierabend 1984). A metabolic criterion should therefore be more appropriate for monitoring phytotoxicity (Van Assche and Clijsters 1987).

Peroxidase activity is known to increase in diseased or ethylene-treated leaves and

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reflects a general senescence response (Lee *et al.* 1976, Kar and Feierabend 1984). In contrast, Agarwala *et al.* (1961) have shown that heavy metals such as cobalt, nickel, molybdenum and chromium when supplied 1 mM or more depress peroxidase activity.

In this study, concentrations of copper, zinc, lead, nickel, chromium and cadmium which almost completely inhibited root growth have been chosen to study the influence of each metal on shoot isoperoxidases of wheat (*Triticum aestivum* L. cv. Vergina).

Among the isozyme systems, peroxidases were chosen since they are included in the defence system of the plants and they reflect the changes connected with stress.

MATERIALS AND METHODS

Plant Material

The seeds of *Triticum aestivum* cv. Vergina used in this study were derived from the Cereal Institute of Thessaloniki, Greece.

Growth Conditions

All the experiments were carried out in a growth room at 23 °C with a 16 h photoperiod in perspex chambers to maintain high humidity and minimize evaporation of culture solutions. Approximately 20 seeds were sown for 14 d on a raft of black alkathene beads floated at the surface of 1/10 strength Rorison's nutrient solution minus phosphate (Symeonidis *et al.* 1985) in each of three, 300 cm^3 plastic beakers (in total 60 seeds) with and without each one of the metals zinc, copper, lead, nickel, chromium and cadmium over a range of concentrations (Fig. 1). The metals were offered as $ZnSO_4 . 7 H_2O$, $CuSO_4 . 5 H_2O$, $Pb(NO_3)_2$, $Ni(NO_3)_2 . 6 H_2O$, $Cr_2(SO_4)_3 . 15 H_2O$, $3 CdSO_4 . 8 H_2O$. The pH of the solutions was adjusted to 7 ± 0.5 and the solutions were changed every 3 days to allow for aeration and to maintain the metal concentration (Wong and Bradshaw 1982).

Using the progressive inhibition of root growth in culture solutions brought about by increasing levels of heavy metals, an evaluation of the concentration of each metal in which root growth was minimized has been made. These concentrations were used to study the influence of heavy metals on the multiple forms of shoot peroxidases of *Triticum aestivum* cv. Vergina.

Enzyme Extraction and Electrophoresis

Crude extracts were obtained after crushing in distilled water at 4 °C shoots of 14-days old seedlings grown at the above mentioned concentrations. The electrophoretic procedure was carried out by applying the isoelectric focusing method (Moustakas *et al.* 1983). Equal amounts of protein, that is 80 μg of each sample was

used for isoelectric focusing. The protein concentration was determined for each preparation from a partial aliquot using the technique described by Lowry *et al.* (1951). Bovine albumin was used in order to establish the standard curve. A polyacrylamide gel containing 2.2 % m/v LKB carrier ampholite with pH range 4.0–6.5 was used. three gel replications were used in order to verify the reproducibility of the results. After 2.5 hours of isoelectric focusing, the gels were stained for peroxidases as described by Symeonidis *et al.* (1979). In order to determine the intensity of the various bands of the zymograms we obtained their absorbance at 375 μm by means of a Kipp and Zonen DD2 densitomer.

RESULTS AND DISCUSSION

The toxicity of metals was manifested by a depression of shoot growth but the most evident symptoms were on root growth. The length of the shoot and of the longest root of *Triticum aestivum* cv. Vergina seedlings at each metal concentration used in the experiment, are presented in Fig. 1. It can be observed that the degree of

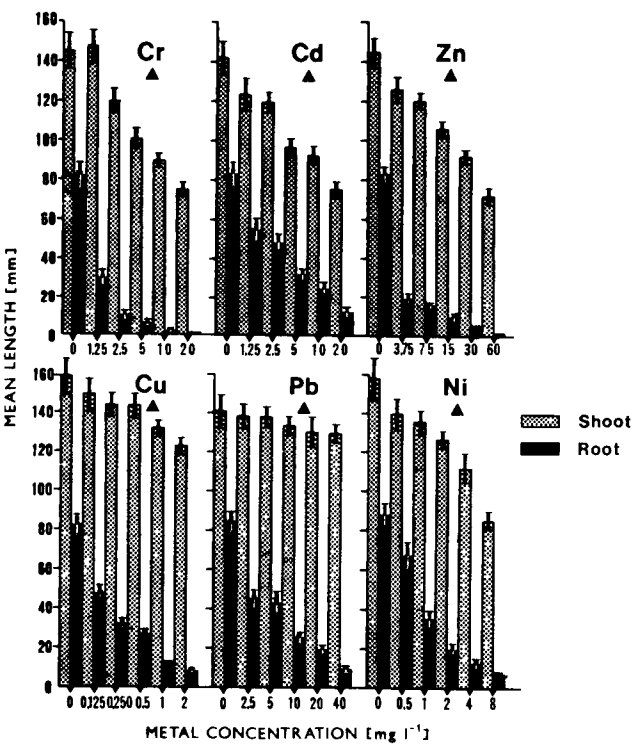


Fig. 1. Mean length of the shoot and of the longest root of *Triticum aestivum* cv. Vergina seedlings at each metal concentration used in the experiment. Each measurement is the mean value of 50 randomly sampled seedlings; vertical bars indicate standard deviation.

inhibition of root growth brought about by different metals, varied considerably, whereas at the higher metal concentrations employed root growth was almost completely inhibited. These different concentration effects produced by different metals, reflect their varying degrees of metal toxicity on plant growth (Karataglis 1987).

In some instances, apart from the shortening of the root, one could observe the root system becoming malformed with short, curved side roots. As regards the degree of metal toxicity, it appears that the most severe decrease of root length was caused by the 20 mgl-1 Cr treatment. In no case was shoot growth inhibited to the same extent as root growth (Fig. 1).

A general chlorosis of the younger leaves was observed, which according to Wainwright and Woolhouse (1975) is clearly a secondary characteristic since the plants are unable to utilize iron, thus resulting in an iron-deficiency chlorosis. According to Upadhyaya *et al.* (1985) chlorophyll loss is the end result of early senescence-linked events.

The growth reduction and chlorosis observed in *Triticum aestivum* cv. Vergina can be considered as consequences of the toxic effects of the heavy metals (Eleftheriou and Karataglis 1989).

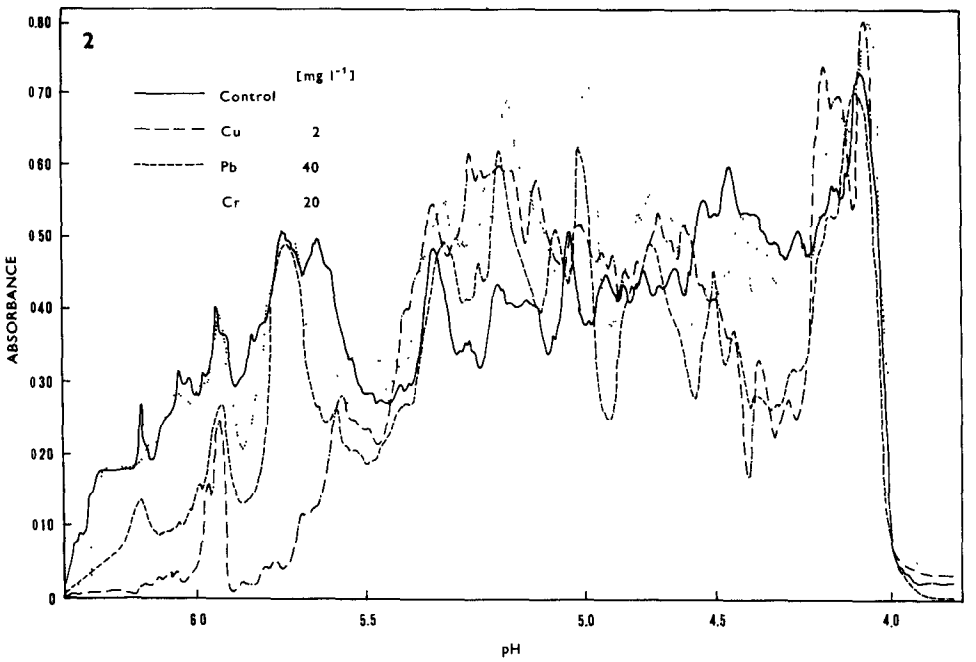
The heavy metal concentrations employed did not alter the number of peroxidase pI bands (Figs. 2,3) but almost in all cases enhanced the intensities of bands of pH 4.0–4.2 and 5.0–5.4, while they decreased the intensities of bands of pH 4.2–4.6 and 5.4–6.5. However, Maier (1978) demonstrated that the influence of lead on the multiple forms of peroxidases in *Zea mays* and *Medicago sativa* was negligible.

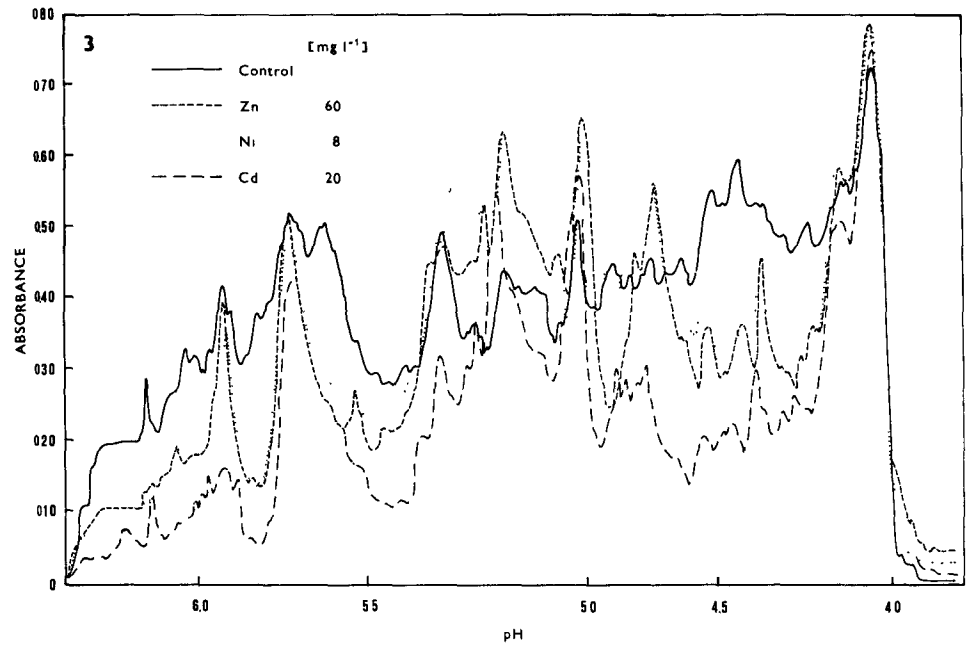
Our results suggest that the different heavy metals employed have caused similar effects on the isoperoxidases of *Triticum aestivum* cv. Vergina shoots, while Van Assche *et al.* (1986) reported that in plants exposed to toxic levels of several heavy metals the isoperoxidase pattern changes as a function of the heavy metal applied.

According to Van Assche and Clijsters (1987) only part of the heavy metals taken up by the plant is phytotoxic, namely the fraction which interferes with cellular metabolism. Consequently the metal bound to cell walls or accumulated in vacuoles has no physiological effect. Chemical analysis cannot distinguish between these two fractions. Increase of enzyme activity is considered to be due to the phytotoxic fraction, which also induces growth inhibition. This explains the close relationship between plant growth and enzyme activity (Van Assche and Clijsters 1987).

Mukherji and Daş Gupta (1972) showed that excess copper resulted in an increase in peroxidase activity which can be attributed to an accelerated synthesis of protein. As it is generally believed, the stimulation of enzymes by metal ions is assumed to be an indirect effect. However, this stimulation is not known to be caused by a metal-induced *de novo* synthesis of the enzyme protein (Weigel and Jäger 1980).

Braber (1980) reports that the peroxidase activity level and the number of isoenzymes increases during leaf senescence. Senescence in plants involves a sequ-





Figs. 2 and 3. Isozyme spectra of the isoelectric focusing gel showing the effects of different metals on shoot isoperoxidases of *Triticum aestivum* cv. Vergina as compared with the control.

ence of specific, genetically controlled events which result in chlorophyll loss, and in nucleic acid and protein breakdown (Thomas and Stoddart 1980).

The increased intensities of peroxidase bands of pH 4.0–4.2 and 5.0–5.4 of *Triticum aestivum* cv. Vergina shoots can be considered as an indication of enhanced senescence caused by the heavy metal treatments, while the similar effects of the different heavy metals on the isoperoxidases of *Triticum aestivum* cv. Vergina shoots, support the statement of Wong and Bradshaw (1982) that "most metals produce a similar kind of metabolic disturbance".

Generally, our results suggest that the heavy metals employed have caused complex changes on the multiple forms of peroxidases.

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Ahuja, M. R. (ed.): Somatic Cell Genetics of Woody Plants. – Kluwer Acad. Publ., Dordrecht–Boston–London, 1988. 225 pp., US $ 59.00, UK £ 31.50.

The thirtieth volume of the renowned Kluwer Forestry Sciences edition is an outcome of the first Workshop of the International Union of Forestry Research Organization (IUFRO) Working Party "Somatic Cell Genetics of Woody Plants" held at Grosshansdorf, F. R. G., 10–13 August, 1987.

The volume contains 28 papers presented by 59 authors from ten countries, mainly from the U.S.A. and F.R.G. The articles are arranged in four thematic parts. Part 1 is devoted to somatic embryogenesis (7 articles); part 2 to genetic transformation by *Agrobacterium*, by direct gene transfer or injection of DNA (5 articles). The largest third part is dedicated to genetic control of morphogenesis (9 articles). Two remaining Workshop sessions comprising the last part deal with *in vitro* screening, somaclonal selection, testing and deployment (7 articles). The majority of papers presents original experimental results; the review articles including up-to date information are written with profound knowledge of the subject and they are supplemented by a list of references the most recent latest.

The specialized Working Party set up at the IUFRO World Congress in Ljubljana in 1986 and the subsequent publication of proceedings of the first Working Party Workshop bespeaks the increasing importance of somatic cell genetics in research and breeding of woody plants. This volume summarizing the Workshop activities is a comprehensive, up-to-date treatise on somatic cell differentiation, genetics and biotechnology of woody plants. Besides, it reflects the continuous and promising progress which has been made in introducing *in vitro* techniques to the culture of tissues, organs, cells and protoplasts of tree species considered recalcitrant a decade ago.

The book will undoubtedly be an excellent source of information not only for researcher engaged in genetics and breeding of woody plants but for researchers in plant physiology, genetics, and related branches, in general.

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