

Effects of chromium on hypocotyl elongation, wall components, and peroxidase activity of *Phaseolus vulgaris* seedlings

SUMITRA V. CHANDA

NAYNA G. PARMAR

Department of Biosciences

Saurashtra University

Rajkot 360 005, India

email: sumitrachanda@yahoo.com

effect, specificity, and variation, and maximum activity occurred with caffeic acid. The role of peroxidase in the defense mechanism in response to Cr toxicity is discussed.

Keywords chromium toxicity; elongation; peroxidase activity; *Phaseolus*; wall components

Abstract In this investigation, the effect of the heavy metal chromium (Cr) on hypocotyl elongation of *Phaseolus vulgaris* seedlings in light was studied. The seedlings were subjected to two concentrations of Cr (0.5 and 1.0 mM) in the form of $K_2Cr_2O_7$. Hypocotyl elongation (growth) was measured in terms of length. Change in wall components (pectic polysaccharides and xyloglucan) and peroxidase activity (soluble and bound) were also studied. Both concentrations of Cr inhibited hypocotyl length and a clear concentration effect was observed. Maximum pectic polysaccharide content was observed at a high concentration of Cr and a minimum was observed in the distilled water control. A clear inverse correlation was observed, supporting the conclusion that pectic polysaccharides may be involved in cell wall loosening. Even xyloglucan (high and low) content showed a clear inverse correlation with hypocotyl length. It appears that Cr inhibits hypocotyl elongation and xyloglucan and pectic polysaccharide contents are not degraded to smaller oligosaccharides as they occur more in seedlings treated with Cr compared to seedlings treated with distilled water. Further soluble and bound peroxidase activity was assayed with four hydrogen donors—ferulic acid, caffeic acid, pyrocatechol, and pyrogallol during the initial phase of hypocotyl elongation. A clear inverse correlation between length and peroxidase activity was observed. All hydrogen donors showed a clear concentration

INTRODUCTION

Heavy metals are considered to be an important class of elements and cause of environmental pollution. The exposure of humans, animals, and plants to heavy metals is inevitable and remains a global environmental problem. Some heavy metals such as copper, iron, and zinc are essential nutritional elements whereas others such as mercury, lead, chromium (Cr), and cadmium are not essential for nutrition and are considered to be toxic metals. Plants are subjected to many stress factors in the natural environment as well as many man-made stresses. Metals are ubiquitous and they can be absorbed from the soil and the atmosphere, accumulate in the organs of plants and show their phytotoxic effects. Heavy metals are stable compounds that are not readily removed by oxidation, precipitation, or other processes. Heavy metals are now known to cause irreversible damage to a number of vital metabolic constituents and important biomolecules.

Different heavy metals have been found to affect the biosystem differently, resulting in a diversity of toxic symptoms. Moreover, the toxicity sequence varies not only among different plant groups but also among different plants of the same group. Growth inhibition, breakdown of enzymatic activity and cell wall elasticity, and inhibition of chlorophyll production are some of the important toxic effects of heavy metals. Inhibitory effects of heavy metals on plant growth and physiological processes have also been reported (Kalimuthu & Sivasubramaniam 1991). In most instances toxicity symptoms exhibited are attributed to a disruption in the metabolic pathway sensitive to that particular metal.

Chromium is used in dyeing, tanning, and as a refractory material. Pollution by Cr and its compounds occur primarily from these industrial processes and product use. Cr is present in soil as chromic (Cr III) or chromate (Cr VI) ions (Shewry & Peterson 1976). Cr III could be readily oxidised to Cr VI (Bartlett & James 1979) in soils, because of presence of oxidised manganese which serves as the electron acceptor in the reaction. Cr VI is more toxic to plants than Cr III; Cr VI is stable for several months and can be leached from the soil (Khasim et al. 1989).

Plant growth and development is a complex process that requires the participation of many biochemical circuits. Plant cell walls play an important role in mediating physiological events in plant development and are specifically involved in the mechanism of cell elongation. Plant cell walls are the dominant determinant of tissue mechanical properties and play major roles in the development of cellular structure during growth. The cell wall is a highly dynamic structure, not only important for growth and development but also for cell-to-cell communication and transport processes (Whitney et al. 1999).

The primary cell walls contain cellulose, xyloglucan, and pectin that are required for both inherent strength and the ability to respond to cell expansion. Plant cell enlargement occurs as a result of the force of water suction, which is derived from the osmotic pressure of cell solutes. Water suction occurs as a result of wall loosening. Many studies have demonstrated that an extensive turnover of cell wall polysaccharides occur during cell growth in higher plants. Auxin-induced elongation of plant organs is primarily triggered by cell wall loosening (Sakurai 1991) which appears as a result of biochemical modification of certain components of the cell wall.

Xyloglucans are the principal matrix polysaccharides of the primary cell walls. They possess a 1,4- β glucan backbone with 1,6 α -xylosyl residues attached to the 6-position of β -glucosyl residues. Species-specific differences occur according to the distribution of additional branching galactosyl or fucosyl-galactosyl residues. The important role of the breakdown of xyloglucans in the cell wall loosening induced by auxin has been confirmed with xyloglucan specific antibodies and lectins (Hoson et al. 1991). Nevertheless, the process of xyloglucan breakdown still remains unclear.

Peroxidases are heme-containing enzymes (EC 1.11.1.7) ubiquitous in the plant kingdom and

belong to a superfamily which comprises: Class I enzymes from mitochondria, chloroplasts, and bacteria; Class II from fungi; and Class III "classical" plant peroxidases (Boesewinkel & Bouman 1995). Plant peroxidases have been detected in vacuoles, tonoplast, and plasmalemma as well as inside cell walls. Plant peroxidases are glycoproteins characterised by the presence of oligosaccharide chains linked to the protein moiety and having effects on the stability of the enzyme (Egley et al. 1983).

Peroxidases have been implicated in numerous biochemical processes such as lignification (Lagrimini et al. 1987), suberisation (Espelie et al. 1986), cross linking of hydroxyproline-rich wall proteins and feruloylated polysaccharides (Fry 1986), both oxidation and polymerisation of soluble phenolics (Srivastava & van Huystee 1977), the formation of H₂O₂ (Mader et al. 1980), chlorophyll degradation and senescence (Yamauchi & Watada 1991), and auxin degradation (Jinnman & Lang 1965). One of its main functions is connected with its role as a part of the defense enzyme complex in the cells, ensuring the detoxification of the activated O₂ forms. This function is very important in the formation of the metabolic response of plants to different stress factors (Bakardjieva et al. 1996). Peroxidase induction is a general response of higher plants to uptake toxic amounts of heavy metals (Shaw 1995).

It is also well known that peroxidase activities can be fractionated into a large number of isozymes, the precise role of which is still uncertain. Assays for substrate specificity are often used to specify the respective function of the different isoperoxidases. The wall extensibility in plant cells is highly dependent on the nature and the number of cross links between matrix polymers. Peroxidases might decrease cell wall plasticity by cross-linking extensin molecules through the formation of intracovalent or intercovalent bonds between tyrosine residues to form isodityrosine. Further, covalent cross links may originate by the action of wall bound peroxidases on pectins or hemicelluloses via the formation of a diferulate bridge between ferulate residues (Johansson et al. 1992). Both the ability to modify cell wall structure and composition and to oxidise indole-3-acetic acid (IAA), explain why these enzymes have been considered to be implicated in plant growth and development.

Despite a large number of reports detailing plant responses to heavy metal toxicity, the mechanism(s) of plant tolerance to heavy metals are not properly

understood. Furthermore, the number of industries and occupations using and/or generating metal products are increasing the incidence of metal toxicity and will continue to make it critical to understand the effects and mechanism(s) by which these metals may act in modifying plant growth and development.

Considering the above, the effect of Cr was studied on two aspects of elongation growth of *Phaseolus* hypocotyls: (1) changes in cell wall polysaccharides; and (2) changes in the IAA oxidising system.

MATERIALS AND METHODS

Seeds of *P. vulgaris* were soaked in tap water for 3 h. They were then thoroughly washed with tap water followed by 3–4 rinses with distilled water. Then they were transferred to moistened filter paper for germination in the dark for 24 h. Uniformly germinated seeds were transferred to sieve culture dishes containing nutrient media (Doddemma & Telkamp 1979) and distilled water. Some dishes were kept as controls and the others were exposed to two different concentrations of Cr (0.5 mM (Treatment 1) and 1.0 mM (Treatment 2)) given in the form of $K_2Cr_2O_7$. Above this concentration of Cr, the seedlings did not survive. The sieve culture dishes were placed in a light room (c. 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with six fluorescent tubes at 0.5 m height. The time when sieve culture dishes were transferred to the light room was considered as 0 h. After 12 h, the hypocotyls were demarcated into two segments with India Ink. The segment near to the root was designated as the “lower” segment whereas the one near to the cotyledon was designated as the “upper” segment.

Growth analysis

At an interval of 8 h (initially) or 24 h (later on), 20 seedlings from each treatment were selected for growth analysis. The hypocotyls were cut into their respective segments, their lengths were measured to the nearest millimetre and fresh weights taken. In the present work, the lower segments did not show any growth in any treatment or hour in the presence or absence of Cr. Therefore, data for only the upper segment are presented; and “hypocotyl” refers to the upper segment only. Similar results were also reported in our earlier work (Bagatharia & Chanda 1998).

Biochemical analysis

For the estimation of cell wall polysaccharides, the required number of hypocotyls were killed by boiling in methanol for 5 min and then stored in methanol until the time of analysis. For estimation of peroxidase activity, the required number of hypocotyls were chilled and used for biochemical analysis.

Extraction of pectic polysaccharides

The hypocotyls (c. 500 mg) boiled in methanol were crushed in ice-cold water with a pinch of sterilised sand and centrifuged at 10 000g for 5 min. The supernatant, containing cytoplasmic enzymes, was discarded. The pellet was washed many times (nearly 12–15 times) with distilled water until it was free of cytoplasmic enzymes. After the pellet was washed, 10 ml of 1M NaCl was added for 1 h to remove wall-bound enzymes. This was done twice. After centrifugation, the supernatant was discarded and the pellet was washed successively with ice-cold water, acetone and chloroform-methanol mixture (1:1 v/v). This was also done twice before the pellet was air dried at room temperature. The dried pellet was treated with 15 ml of dimethyl sulfoxide for 12 h. This treatment dissolved starch; and it also resulted in more facile extraction of pectic substances (Wada & Ray 1978). After removing dimethyl sulfoxide by centrifugation, the pellet was extracted 3 times with 20 mM ammonium oxalate-oxalic acid buffer solution (pH 4) at 70°C for 1 h to remove pectic polysaccharides. The supernatant of all the three washes was collected together, mixed, and the volume was made up to 20 ml. This was the source of pectic polysaccharides (Nishitani & Masuda 1981).

Extraction of xyloglucan

The hemicellulosic xyloglucans were fractionated into low and high molecular weight xyloglucans. The pectin-free wall pellet was extracted twice with 4% KOH solution (2 h each time) to obtain low molecular weight xyloglucans. The residue was then extracted with 24% KOH solution (for 24 h) to obtain high molecular weight xyloglucans. Each alkali extract was acidified (pH 5) with 5% and 33% acetic acid respectively. The acidification caused no precipitation of hemicellulosic polysaccharides.

Determination of total polysaccharides and xyloglucan content

The total polysaccharide contents in the pectic fraction was determined by the phenol sulfuric acid

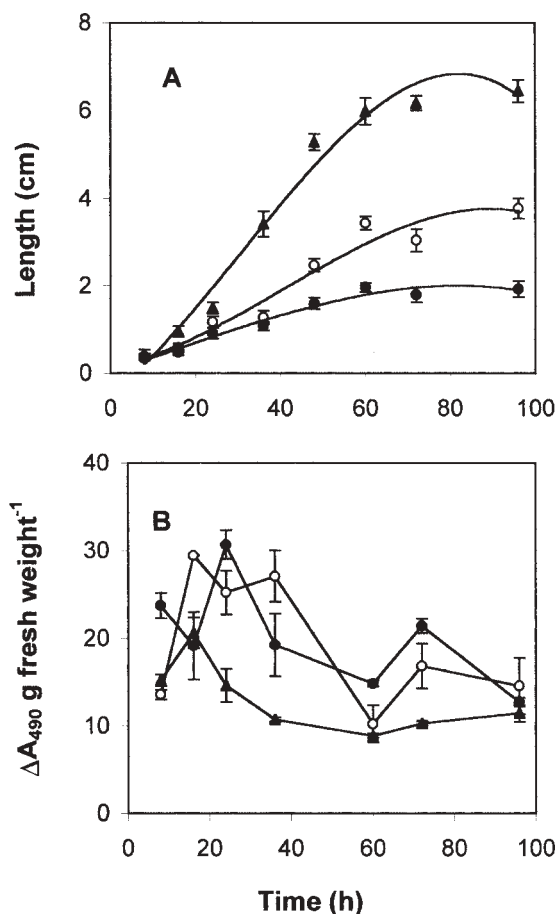


Fig. 1 Effect of different concentrations of chromium on: **A**, hypocotyl length; and **B**, pectic polysaccharide content in *Phaseolus vulgaris* seedlings. (▲, distilled water control; ○, Treatment 1—0.5 mM and ●, Treatment 2—1.0 mM. Vertical bars represent \pm standard deviation.)

method (Dubois et al. 1956) and expressed as ΔA_{490} g fresh weight⁻¹. 0.5 ml of the extract was mixed with 0.5 ml of 5% phenol and 2.5 ml of 98% sulfuric acid with constant stirring. After 10 min, the tubes were placed on a water bath at 30°C for 20 min. The yellow-orange colour developed was read at 490 nm. The xyloglucan contents were determined by iodine staining by the modified method of Kooiman (1960). 1.0 ml of acidified extract was mixed with 0.25 ml of I₂KI solution (0.5% I₂ and 1% KI). The reaction mixture was then kept in the dark for 1 h at 4°C. The bluish-green colour developed was read at 640 nm.

Extraction of soluble and bound enzyme

The hypocotyls were homogenised in a pre-chilled mortar and pestle with a pinch of sand in K-phosphate buffer (0.02 M, pH 6.4). The mixture was centrifuged at 10 000g for 10 min. The supernatant was used for estimating soluble peroxidase activity. The residual wall material, after the extraction of soluble cytoplasmic enzymes, was thoroughly washed with distilled water and centrifuged until the washings were free of the peroxidase reaction with guaiacol. The wall fraction was then kept with 10 ml of 1M NaCl for 1 h at room temperature with regular shaking to release bound (ionically) enzymes. After centrifugation at 10 000g for 10 min, the supernatant served as a source of bound enzyme.

Biochemical peroxidase assay

Peroxidase activity was measured by recording changes in absorbance at 400 nm (ΔA_{400}). The various hydrogen donors used were ferulic acid, caffeic acid, pyrocatechol, and pyrogallol. The brown colour that developed as a result of the oxidation of different hydrogen donors in the presence of H₂O₂ was spectrophotometrically measured. The assay mixture consisted of 12 mM K-phosphate buffer (pH 6.4), 4 mM hydrogen donors, enzyme, and 1 mM H₂O₂. The activity is expressed as ΔA_{400} g fresh weight⁻¹ min⁻¹.

RESULTS AND DISCUSSION

Addition of heavy metal salts to the plant growth medium or assay medium caused a continuous and concentration-dependent decrease in growth and biochemical activities. However, the decrease was greater when the heavy metal salts were added to the growth medium. Hence, in the present work, the heavy metal salt (Cr) was added to the growth medium. It is now well recognised that, when the metal concentration surpasses a characteristic value for any species, the organism will enter into a toxic situation that produces a wide range of effects and responses at all levels.

Changes in hypocotyl length under the influence of the presence or absence of Cr are shown in Fig. 1A. A cubic polynomial was the best fit and hence in all the three treatments a cubic polynomial was fitted ($R^2 > 0.96$). In distilled water, the hypocotyls slowly started increasing in length until 24 h and then sharply increased until 60 h, after which time the length almost stabilised. A similar effect was

observed in both the Cr treatments. A clear concentration effect was observed at all hours of growth. At all times Treatment 2 had minimum length and maximum inhibition was at 96 h. In Treatment 1, the length showed a slight increasing trend up to 60 h after which it stabilised whereas in Treatment 2, it increased very slightly up to 60 h and then stabilised.

The two principal material requirements for primary walls are a high intrinsic strength and the ability to accommodate cellular expansion during active growth (McQueen-Mason 1997). However, despite a large number of studies describing metal cytotoxicity, the molecular mechanisms involved are still poorly understood. It is now evident that heavy metals can interact with enzyme functional groups and proteins involved in various biochemical functions. Impairment of the ability of cells to adequately respond to the stimulation by hormones and growth factors may result in the loss of important cell functions.

The plant cell wall is a strong fibrillar network that provides each cell with its stable shape. To enlarge, cells selectively loosen this network, enabling it to yield to the expansive forces generated by cell turgor pressure. Primary plant cell walls are composed of a number of structurally complex polysaccharides, including hemicelluloses (xyloglucan) and pectin (rhamnogalacturonans I and II). Pectin is suggested to play a major role in cell wall architecture and mechanical properties, cell wall porosity, cation acquisition, etc (Cosgrove 2001).

Changes in pectic polysaccharides are shown in Fig. 1B. Maximum pectic polysaccharide content was found in Treatment 2 followed by Treatment 1 and a minimum was found in the distilled water control hypocotyls. Initially, in all the three treatments, the pectic polysaccharide content was high and steadily decreased with the advancement in the age; minimum content being at 96 h. Here also a clear inverse correlation was observed with hypocotyl elongation. Seedlings treated with distilled water had maximum length and minimum pectic polysaccharide content whereas Treatment 2 seedlings showed minimum length and hence maximum content. In Treatment 1 seedlings, length and content both were in between the other two treatments.

The physiological responses of plants for survival in the stressed environment are based on their ability to express the pre-existing defense programme and/or adaptation, in which the plants adjust to stress. Under stress conditions, the adaptive responses are elicited by plants mainly through changes in

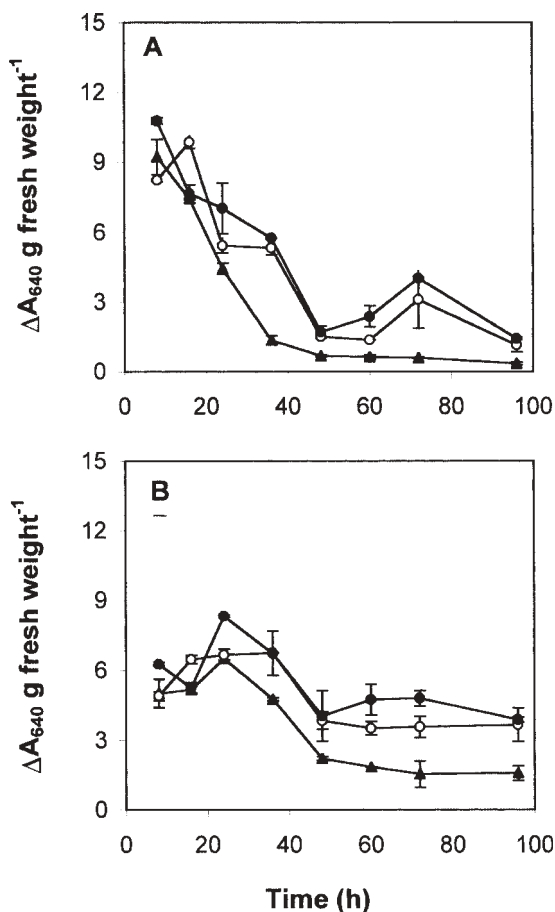


Fig. 2 Effect of different concentrations of chromium on: **A**, low molecular weight xyloglucan content; and **B**, high molecular weight xyloglucan content in *Phaseolus vulgaris* seedlings. (▲, distilled water control; ○, Treatment 1—0.5 mM and ●, Treatment 2—1.0 mM. Vertical bars represent \pm standard deviation.)

endogenous levels as well as balances of phytohormones. The re-establishment of hormonal equilibrium under the new environment probably plays a central role in the survival of plants under stress conditions (Amzallag & Lerner 1995). However, under severe stress conditions, plants occasionally fail to express their pre-adaptation and adaptation ability probably because of high catabolism. In the present work, the pectic polysaccharide content also showed an inverse correlation with hypocotyl length, both in distilled water and Cr treatments. Under the Cr treatment, growth was arrested and hence had more pectic polysaccharide content than the distilled water

control. Inverse correlation between pectic polysaccharide content and growth has already been reported in *Phaseolus* (Chanda et al. 1995; Bagatharia & Chanda 1998) and this further supports the notion that pectic polysaccharides are involved in cell wall loosening.

The cell elongation of the pea stem is promoted by a high concentration of xyloglucan oligosaccharides. The promotion results in a decrease in cell wall viscoelastic properties. Xyloglucan is degraded into oligosaccharides in the growing plant cell wall and the oligosaccharides provide either positive or negative feedback control during cell elongation. It has been reported that an antibody against xyloglucan prevents not only an increase in xyloglucan degradation but also cell elongation. Potential enzymes involved in xyloglucan solubilisation may be expansin, xyloglucanase, xyloglucan endotrans glycosylase, and cellulase (Baydoun & Fry 1989). Changes in low molecular weight xyloglucan (Fig. 2A) and high molecular weight xyloglucan (Fig. 2B) are shown in Fig. 2. The low molecular weight xyloglucan content was slightly higher than that of high molecular weight xyloglucan content. Both low and high xyloglucan content showed a clear inverse correlation but an absolute clear trend was seen in low molecular weight xyloglucan only. Maximum low molecular weight xyloglucan content in all the three treatments was initially at 8 h. The content steadily sharply decreased until 40 h and then low levels were maintained until 96 h. Here also minimum levels were at 96 h and amongst the three treatments, minimum being in the control. In high molecular weight xyloglucan content, the differences between the control and treatments were more after 40 h and the difference was maintained until 96 h.

It has been proved that auxin-induced cell enlargement accompanies xyloglucan degradation and solubilisation. An inverse correlation between hypocotyl elongation and xyloglucan has been proposed (Fry 1989; Bagatharia & Chanda 1998). In the present work also, a distinct inverse correlation between hypocotyl elongation and xyloglucan content is observed. In the presence of Cr, growth is inhibited and consequently xyloglucan content is higher in seedlings treated with Cr compared to seedlings treated with distilled water. The difference in high molecular weight xyloglucan content between seedlings treated with distilled water and seedlings treated with Cr was more than that of the low molecular weight xyloglucan content and distilled water.

Numerous studies indicate that multiple forms of peroxidase exist in higher plants including horseradish, turnip, Japanese radish, and tobacco. Stitch & Ebermann (1988) showed that most peroxidase isoforms from sapwood were able to oxidise a wide range of substrates. Peroxidases can use various organic and inorganic substrates which act as hydrogen donors, *in vitro*, in the presence of H_2O_2 . Changes in soluble peroxidase activity with four hydrogen donors—ferulic acid, caffeic acid, pyrocatechol, and pyrogallol are shown in Fig. 3. In all the four hydrogen donors studied, the peroxidase activity was at a maximum at 24 h. It decreased with increase in age, minimum activity being at 96 h. A clear inverse correlation between length and peroxidase activity was observed. With the exception of pyrocatechol, all the other three hydrogen donors showed a clear concentration effect of Cr toxicity. Treatment 2 had a higher concentration of Cr and correspondingly, maximum peroxidase activity was observed. Incidentally, this treatment had minimum length. Furthermore, a very high peroxidase activity was found when hydrogen donors were either caffeic or pyrogallol. However, when ferulic or pyrocatechol were used as hydrogen donors, a much lower peroxidase activity was found. Studies done by van Huystee's group suggested an inhibitory role played by peroxidase activity on cell growth (van Huystee & Esnault 1995).

Changes in bound peroxidase activity are shown in Fig. 4. Initially, in all the four hydrogen donors studied, the activity was less and subsequently it increased immensely in all except in pyrocatechol. In all the four hydrogen donors, Treatment 2 had distinct maximum activity at all stages of growth; maximum difference and activity being at 96 h. Amongst all the four hydrogen donors and distilled water, the distilled water seedlings had minimum activity. Here also, hydrogen donor specificity was seen; maximum activity was with caffeic acid.

All kinds of environmental stresses induced the formation of H_2O_2 , which is a very harmful molecule for the cell. One of the main functions of peroxidase is its role as a part of the defense enzyme complex, ensuring the detoxification of the activated O_2 forms. Peroxidase induction is a general response of higher plants to uptake of toxic amounts of metals (Stroinski 1994). The expression of distinct peroxidase isozymes is specific to particular organs, tissues, or cell types. The profile of isozymes and the total amount of activity in the leaf may be vastly different from that in stem or flower of the same plant. This differential expression of peroxidase isozymes is

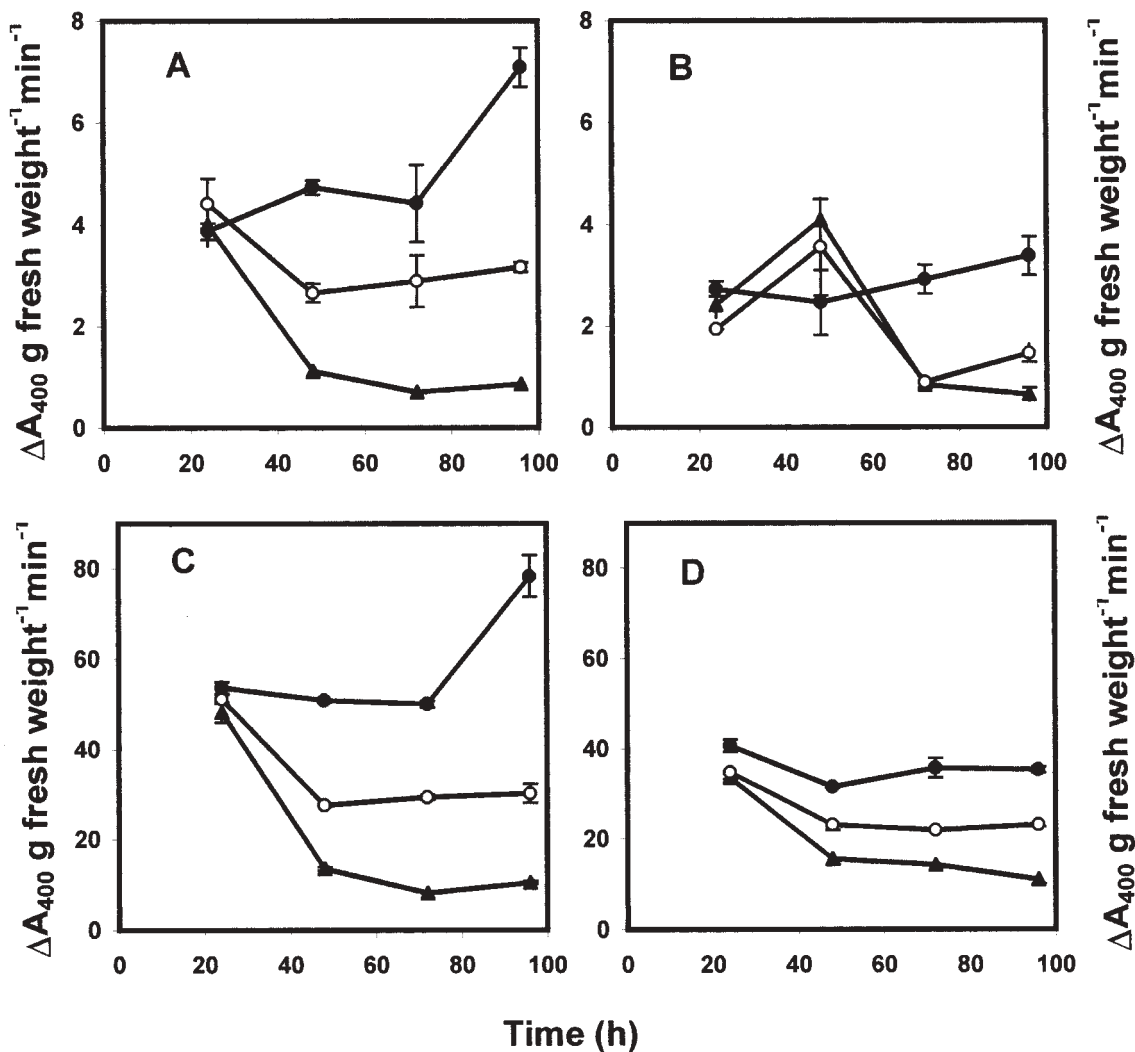


Fig. 3 Changes in soluble peroxidase activity with four hydrogen donors: A, ferulic acid; B, pyrocatechol; C, caffeic acid; and D, pyrogallol in *Phaseolus vulgaris* seedlings. (▲, distilled water control; ○, Treatment 1—0.5 mM and ●, Treatment 2—1.0 mM. Vertical bars represent \pm standard deviation.)

possible because these enzymes are typically represented by large gene families in most plant species (Welinder et al. 1996). The presence of several active sites would explain the great molecular diversity of the compounds which can be oxidised by isoperoxidases as well as the lack of specificity of the various isozymes, at least, *in vitro* (Stitch & Ebermann 1988). Bakardjieva et al. (1996) also suggested a polyfunctionality of peroxidase. Usually, peroxidases are separated into two distinct groups, cationic and anionic, according to the electrophoretic data. An inhibitory effect of anionic peroxidase was

observed with stress mediating compounds, suggesting a direct involvement of the anionic peroxidase in host plant defense reactions. A role in defense processes has also been suggested for a cationic isozyme from horseradish plants. However in the present work in *P. vulgaris*, the response seems to be a result from the co-ordinated expression of both anionic and cathodic isoperoxidases. Similar results were reported in other crop plants.

It appears from this study that peroxidases have other functions besides lignification and cell wall biosynthesis. In this stress condition caused by a

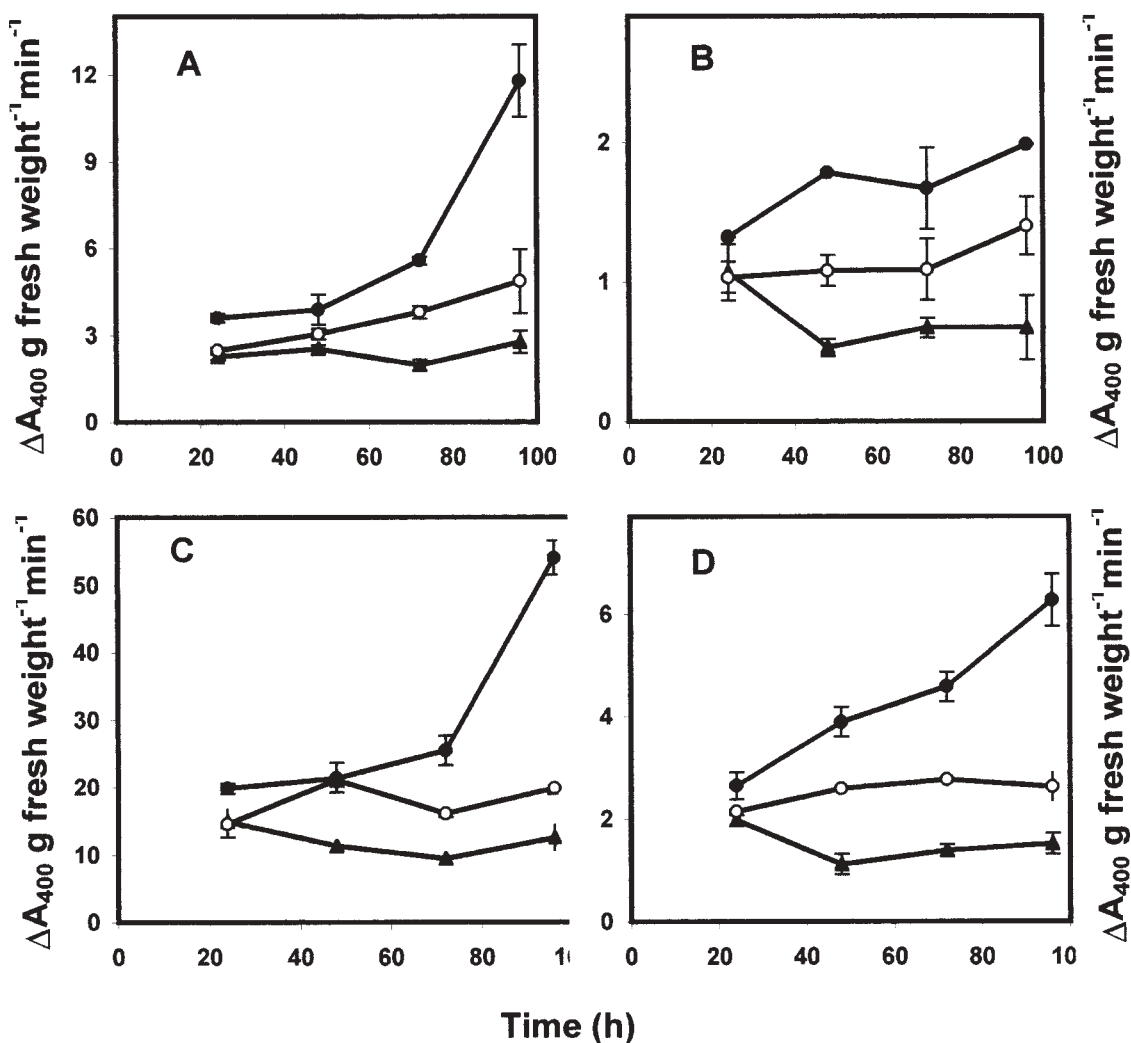


Fig. 4 Changes in bound peroxidase activity with four hydrogen donors: A, ferulic acid; B, pyrocatechol; C, caffeic acid; and D, pyrogallol in *Phaseolus vulgaris* seedlings. (▲, distilled water control; ○, Treatment 1—0.5 mM and ●, Treatment 2—1.0 mM. Vertical bars represent \pm standard deviation.)

heavy metal, the enzyme may provide a degree of selective advantage in defense or disease resistance and play a role in regulating seed germination or modify the micro-environment of the developing seed. It appears that peroxidase activity provides resistance to the plants against the formation of H_2O_2 in response to heavy metal Cr toxicity. They also protect cells against harmful concentration of hydroperoxides. Similar results are reported in coniferous trees (Scalet et al. 1995). Peroxidases also protect the cell membrane against active oxidants and enable

plants to be resistant to heavy metal stress factors as reported to other stress factors (Edreva et al. 1993).

Chromium affected hypocotyl elongation of *Phaseolus* seedlings. A higher concentration of Cr caused maximum inhibition and hence had more pectic polysaccharide and xyloglucan contents. Once again, this proves the earlier conclusion that degradation of wall components is involved in cell wall loosening which in turn is responsible for hypocotyl elongation. Further, Cr toxicity induced peroxidase activity, may be as a defense mechanism.

REFERENCES

- Amzallag, G. N.; Lerner H. R. 1995: Physiological adaptation of plants to environmental stresses. In: Pessarkali, M. ed. Handbook of plant and crop physiology. New York, Marcel Decker. Pp. 557–576.
- Bagatharia, S. B.; Chanda S. V. 1998: Modification of cell wall polysaccharides during cell elongation in *Phaseolus vulgaris* hypocotyls. *Acta Physiologia Plantarum* 20: 15–18.
- Bakardjieva, N. T.; Christova, N. V.; Christov, K. 1996: Reaction of peroxidase from different plant species to increased temperatures and the effect of calcium and zinc ions. In: Obinger, C.; Burner, U.; Ebermann, R. ed. Plant peroxidases: biochemistry and physiology. University of Geneva. Pp. 345–351.
- Bartlett, R. J.; James B. 1979: Behaviour of chromium in soils III. Oxidation. *Journal of Environment Quality* 8: 31–35.
- Baydoun, E. A. H.; Fry, S. C. 1989: *In vivo* degradation and extracellular polymer-binding of xyloglucan, nonsaccharide, a naturally occurring anti-auxin. *Journal of Plant Physiology* 134: 453–459.
- Boesewinke, F. D.; Bouman, F. 1995: The seed: structure and function. In: Seed development and germination. New York, Marcel Dekker Inc. Pp.1–24.
- Chanda, S. V.; Bapodara, C.; Singh, Y. D. 1995: Degradation of xyloglucan and pectic polysaccharides during cell elongation in *Phaseolus vulgaris* hypocotyls. *Acta Physiologia Plantarum* 17: 349–356.
- Cosgrove, D. J. 2001: Wall structure and wall loosening. A look backwards and forwards. *Plant Physiology* 125: 131–134.
- Doddemma, H.; Telkamp, G. P. 1979: Uptake of nitrate by mutants of *Arabidopsis thaliana*, disturbed in uptake of reduction of nitrate. II. Kinetics. *Physiologia Plantarum* 45: 332–338.
- Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. 1956: Colometric method for determination of sugars and related substances. *Analytical Chemistry* 28: 350–356.
- Edreva, A.; Salcheva, G.; Georgieva, D. 1993: Stress damage is related to peroxidase induction in wheat plants. In: Welinder, K. G.; Rasmussen, S. K.; Penel, C.; Greppin, H. Plant peroxidases: biochemistry and physiology. University of Geneva. Pp. 401–404.
- Egley, G. H.; Paul, R. N.; Vaughn, K.C.; Duke, S. O. 1983: Role of peroxidase in the development of water impermeable seed coats in *Sida spinosa* L. *Planta* 157: 224–232.
- Espelie, K. E.; Franeschi, V. R.; Kolattukudy, P. E. 1986: Immunocytochemical localization and time course of appearance of an anionic peroxidase associated with suberization in wound healing potato tuber tissue. *Plant Physiology* 81: 487–492.
- Fry, S. C. 1986: Cross-linking of matrix polymers in the growing cell wall of angiosperms. *Annual Review of Plant Physiology* 37: 165–186.
- Fry, S. C. 1989: The structure and functions of xyloglucan. *Journal of Experimental Botany* 40: 1–11.
- Hoson, T.; Masuda, Y.; Sone, Y.; Misaki, A. 1991: Xyloglucan antibodies inhibit auxin-induced elongation and cell wall loosening of azuki bean epicotyls but not of oat coleoptiles. *Plant Physiology* 96: 551–557.
- Jinnman, R. L.; Lang, J. 1965: Peroxidase catalyzed oxidation of indole-3-acetic acid. *Biochemistry* 4: 144–158.
- Johansson, A.; Rasmussen, S. K.; Harthill, J. E.; Welinder, K. G. 1992: cDNA, amino acid and carbohydrate sequence of barley seed-specific peroxidase BP1. *Plant Molecular Biology* 18: 1151–1161.
- Kalimuthu, K.; Sivasubramaniam, R. 1991: Physiological effects of heavy metals on *Zea mays* (maize) seedlings. *Indian Journal of Plant Physiology* 33: 242–244.
- Khasim, D. I.; Nanda, N.V.; Hussain, R. C. 1989: Environmental contamination of chromium in agricultural and animal products near chromate industry. *Bulletin Environmental Contamination Toxicology* 43: 745–746.
- Kooiman, P. 1960: A method for the determination of amyloid in plant seeds. *Recueil des Travaux Chimiques du Pays Bas et de la Belgique* 79: 675–678.
- Lagrimini, L. M.; Burkhart, W.; Moyer, M.; Rothstein, S. 1987: Molecular cloning of complementary DNA encoding the lignin forming peroxidase from tobacco: molecular analysis and tissue specific expression. *Proceedings of National Academy of Science, USA*, 84: 7542–7546.
- Mader, M.; Ungemach, J.; Schloss, P. 1980: The role of peroxidase isozyme groups of *Nicotina tabacum* in hydrogen peroxide formation. *Planta* 147: 467–470.
- Mcqueen-Mason, S. 1997: Plant cell walls and the control of growth. *Biochem Society Transactions* 25: 204–214.
- Nishitani, K.; Masuda, Y. 1981: Auxin induced changes in the cell wall structure: change in the sugar composition intrinsic viscosity and molecular weight distribution of matrix polysaccharides of the epicotyl cell wall of *Vigna angularis*. *Physiologia Plantarum* 52: 482–494.

- Sakurai, N. 1991: Cell wall functions in growth and development: a physical and chemical point of view. *Botanical Magazine* 104: 235–251.
- Scalet, M.; Federico, R.; Guido, M. C.; Manes, F. 1995: Peroxidase activity and polyamine changes in response to ozone and simulated acid rain in Aleppo pine needles. *Environmental Experimental Botany* 35: 417–425.
- Shaw, B. P. 1995: Effects of mercury and cadmium on the activities of antioxidative enzymes in the seedlings of *Phaseolus aureus*. *Biologia Plantarum* 37: 587–596.
- Shewry, P. R.; Peterson, P. J. 1976: Distribution of chromium and nickel in plants and soil from serpentine and other sites. *Journal of Ecology* 64: 195–212.
- Srivastava, O. P.; van Huystee, R. B. 1977: An interrelationship among peroxidase, IAA oxidase and polyphenoloxidase from peanut cells. *Canadian Journal of Botany* 55: 2630–2635.
- Stitch, K.; Ebermann, R. 1988: Investigation of the substrate specificity of peroxidase isozymes occurring in wood of different species. *Holzforschung* 42: 221–224.
- Stroinski, A. 1994: Cadmium signaling in plant cell. *Biologia Plantarum* 36: 299.
- van Huystee, R. B.; Esnault, R. 1995: Reflections on peanut peroxidase regulation of growth. *Plant Peroxidase Newsletter* 6: 8–10.
- Wada, S.; Ray, P. M. 1978: Matrix polysaccharides of oat coleoptile cell walls. *Phytochemistry* 17: 923–931.
- Welinder, K. G.; Je, H. M.; Kjaersgard, I. V. H.; Ostergaard, L.; Abeleskov, A. K.; Hansen, L. N.; Rasmussen, S. K. 1996: What can we learn from Arabidopsis peroxidases? In: Obinger, C.; Burner, U.; Ebermann, R.; Penel, C.; Greppin, H. ed. Plant peroxidases: biochemistry and physiology, IV. IV International Symposium, University of Geneva. Pp. 173–178.
- Whitney, S. E. C.; Gothard, M. G. E.; Mitchell, J. T.; Gidley, M. J. 1999: Roles of cellulose and xyloglucan in determining the mechanical properties of primary plant cell walls. *Plant Physiology* 121: 657–664.
- Yamauchi, N.; Watada, A. E. 1991: Regulated chlorophyll degradation in spinach leaves during storage. *Journal of American Society Horticultural Science* 116: 58–62.