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How to cite

PASSARDI, Filippo et al. Peroxidases have more functions than a Swiss army knife. In: Plant cell reports, 2005, vol. 24, n° 5, p. 255–265. doi: 10.1007/s00299-005-0972-6

This publication URL: https://archive-ouverte.unige.ch/unige:17916
Publication DOI: 10.1007/s00299-005-0972-6

Plant Cell Rep (2005) 24: 255–265
DOI 10.1007/s00299-005-0972-6

REVIEW

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Peroxidases have more functions than a Swiss army knife

Received: 15 December 2004 / Revised: 21 March 2005 / Accepted: 22 March 2005 / Published online: 22 April 2005

Abstract Plant peroxidases (class III peroxidases) are present in all land plants. They are members of a large multigenic family. Probably due to this high number of isoforms, and to a very heterogeneous regulation of their expression, plant peroxidases are involved in a broad range of physiological processes all along the plant life cycle. Due to two possible catalytic cycles, peroxidative and hydroxylic, peroxidases can generate reactive oxygen species (ROS) ($^{\bullet}OH$, $HOO^{\bullet}$), polymerise cell wall compounds, and regulate H_2O_2 levels. By modulating their activity and expression following internal and external stimuli, peroxidases are prevalent at every stage of plant growth, including the demands that the plant meets in stressful conditions. These multifunctional enzymes can build a rigid wall or produce ROS to make it more flexible; they can prevent biological and chemical attacks by raising physical barriers or by counterattacking with a large production of ROS; they can be involved in a more peaceful symbiosis. They are finally present from the first hours of a plant's life until its last moments. Although some functions look paradoxical, the whole process is probably regulated by a fine-tuning that has yet to be elucidated. This review will discuss the factors that can influence this delicate balance.

Keywords Evolution · ROS · (abiotic and biotic) stress · Cell wall loosening and cross-linking · Senescence · Fruit ripening · Symbiosis

Multigenic family, evolution and homology

Heme peroxidases specific to plants belong to a superfamily that contains three different classes of peroxidases (Welinder 1992b): the intracellular class I (EC 1.11.1.5/.6/.11), the class II released by fungi (EC 1.11.1.13/.14), and the secreted class III plant peroxidases (EC 1.11.1.7).

In their regular catalytic cycle, class III plant peroxidases (Welinder 1992a) catalyse the reduction of H_2O_2 by taking electrons to various donor molecules such as phenolic compounds, lignin precursors, auxin, or secondary metabolites (Hiraga et al. 2001). Their encoding genes form large multigenic families (Tognolli et al. 2002; Duroux and Welinder 2003; Passardi et al. 2004a) that are present in all land plants, but were not detected in the unicellular green algae (Passardi et al. 2004a). From the appearance of the first class III peroxidase, probably around the emergence of the terrestrial plants 450 MY ago, to the most evolved plants, the number of gene copies has largely increased. This increase seems to be correlated with the evolution of the plant architecture and complexity, as well as with the diversity of the biotopes and the pathogens.

The class III peroxidases gene structure, as well as key amino acid residues and protein size, are highly conserved between orthologs and paralogs. In spite of this conservation, their isoelectric points largely differ (anionic and cationic forms). Until now, no correlation has been established between their pI and their putative enzymatic function (Welinder 1992a).

The elevated number of paralogs found for example in *Arabidopsis thaliana* (Tognolli et al. 2002) and in *Oryza sativa* (Passardi et al. 2004a) can be related to the high duplication and conservation rate of the peroxidase genes. The conservation of duplicated genes can be explained by the acquisition of either a new expression profile (subfunctionalisation) or a novel function (neofunctionalisation). These two acquisitions can explain the rationale for the existence of multigenic families. Additionally, the diversity of the reactions catalysed by plant peroxidases accounts for the implication of these proteins in a broad range of physiological processes (Penel et al. 1992; Hiraga et al. 2001). Furthermore, the recent description of a separate hydroxylic cycle, which leads to the formation of various radical species, opens a new range of implications for

Communicated by P. Kumar

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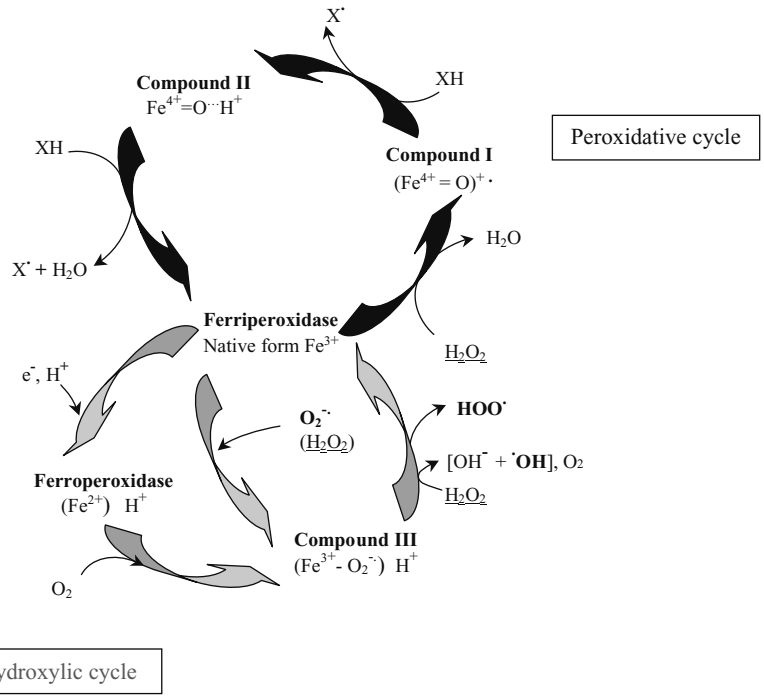

Fig. 1 Class III plant peroxidase cycles. The hydroxylic cycle (with grey arrows) can regulate the H_2O_2 level and release ROS (•OH, HOO•). The peroxidative cycle (with black arrows) can oxidise various substrates (XH) and release their oxidised form (X)

the peroxidases (Fig. 1) (Liszkay et al. 2003; Passardi et al. 2004b). Indeed, the reactive oxygen species (ROS) are considered as very reactive compounds, which can possess an intrinsic activity, or can act as part of signal transduction pathways.

Due to their implication in a broad range of physiological processes such as auxin metabolism, lignin and suberin formation, cross-linking of cell wall components, defence against pathogens or cell elongation, peroxidase activity can be easily detected in the whole lifespan of various plants: from the early stage of germination to the final step of senescence, through the control of cell elongation, defence mechanisms, and several other roles (Fig. 2).

Germination

Release of peroxidases and ROS during germination in the medium surrounding the seed has been reported in radish (*Raphanus sativus*) (Scialabba et al. 2002). To our knowledge, no in vivo direct proof has demonstrated the role of peroxidases in the generation of H_2O_2 during germination. Peroxidases have the capacity to generate H_2O_2 and subsequently •OH radicals via the hydroxylic cycle. Reactive oxygen species released during this cycle could play a defence role for the seed against pathogens, although they are also secreted in absence of pathogenic organisms (Scialabba et al. 2002). Class III peroxidases would then play a critical role in the very first days of life of the

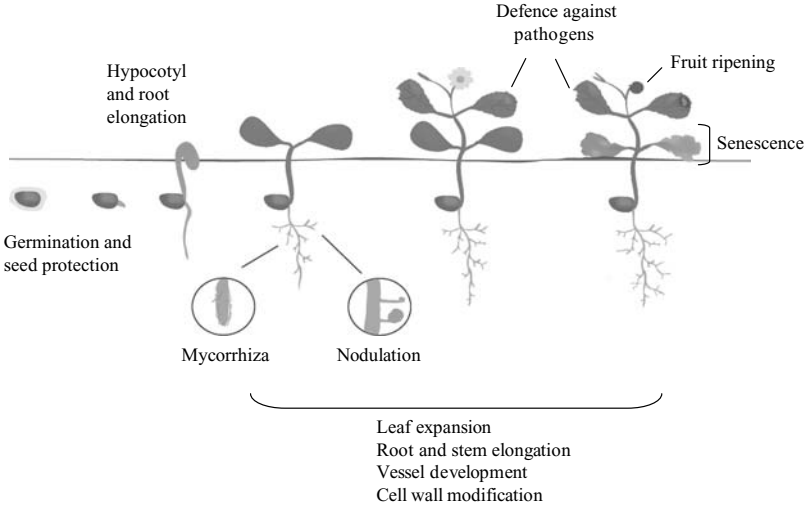

Fig. 2 From germination to senescence: implication of the Class III peroxidases through the whole plant lifespan

germinating seed by defending against pathogenic attacks and by cleaving the cell wall compounds around the radicle protrusion area. In agreement with this hypothesis, a study on tomato (*Lycopersicon esculentum*) seeds demonstrated that peroxidase genes start to be expressed as soon as the germination begins (Morohashi 2002). This event breaks down the endosperm, thus creating a wounded region vulnerable to pathogens: a burst of $^{\bullet}OH$ radicals at this precise time would certainly confer a great protective advantage and help the protrusion.

Cellular growth and cell wall loosening

Growth and elongation are related to cell extensibility, which occurs with a loosening of the cross-linking of cell wall compounds. Several enzymatic mechanisms are involved in cell wall loosening: the cleavage and the reassembly of xyloglucan polymers by xyloglucan endotransglycosylase or the suppression of hydrogen bonds between cellulose and xyloglucan by expansins (Cosgrove 2001). In addition, peroxidases, through their catalytic and hydroxylic cycles, regulate directly or indirectly the cell wall architecture.

The endogenous hydrogen peroxide (H_2O_2) level can be related to the elongation process. For example, in the Dicotyledons, such as soybean, the apoplastic H_2O_2 level is lower in the hypocotyl elongation zone (Schopfer 1994). In *A. thaliana*, the elongation during root curvature is also regulated by a variation in H_2O_2 concentration (Joo et al. 2001). On the contrary, in Monocotyledons such as onion, the location of H_2O_2 in the cell wall has been correlated with lignification and elongation during growth (Cordoba-Pedregosa et al. 2003). Peroxidases could participate in the control of the H_2O_2 level and therefore be directly related to the control of the elongation process. For example, transcripts of APRX, an anionic peroxidase from zucchini, accumulated strongly in the elongation zone of the hypocotyl and their accumulations were inversely correlated with lignin level (Dunand et al. 2003). Their specific localisations could be related to the high elongation rate observed in this region.

Besides H_2O_2, ascorbate has been shown to control elongation and expansion processes via the inhibition of enzymes involved in the cell wall stiffening. For example, the activity of apoplastic and cell wall isolated peroxidase involved in root elongation control was inhibited in presence of ascorbate (Cordoba-Pedregosa et al. 1996). Considering the peroxidase-driven cross-linking reaction, ascorbate treatment stimulates root elongation through the peroxidase inhibition. The same effect was observed during the mechanism of gravitropism in maize. Ascorbate can reduce the root curvature, which is directly related to the elongation process (differential elongation) (Schopfer 1994).

Other compounds such as hydroxyl radical ($^{\bullet}OH$) could be involved in the processes of elongation and expansion. This highly reactive radical produced by the Fenton reaction is capable of cleaving cell wall polysaccharides such as pectin and xyloglucan (Fry 1998). The production of $^{\bullet}OH$ near the cell wall could be related to non-enzymatic wall loosening mechanisms (Chen and Schopfer 1999). Hydroxyl radicals can be produced at the cell wall level from superoxide radical ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) by peroxidase (Schweikert et al. 2000; Schopfer et al. 2002; Liszkay et al. 2003). Auxin is also involved in this process, since it promotes the release of $O_2^{\cdot -}$ and production of $^{\bullet}OH$ when inducing elongation growth (Schopfer et al. 2002).

The analysis of these results suggests that peroxidases can regulate the growth elongation in different manners due to their two cycles and the various numbers of isoforms. They can be controlled by ascorbate and they can also induce the elongation process by generating $^{\bullet}OH$ (hydroxylic cycle) or by regulating the local concentration of H_2O_2 (owing to both cycles). However, peroxidases are not confined to this single elongation role: they can also physically inhibit the elongation process by creating cell wall cross-linking (peroxidative cycle).

Cell wall cross-linking

Peroxidases can create a physical barrier by catalysing cross-linking of cell wall compounds in response to different stimuli such as wounding, pathogen interactions, and climatic aggressions. This process also occurs simply as a normal cell wall evolution during the growth and senescence. The implication of the peroxidases in cross-linking is related to the oxidative capacity of the peroxidative cycle (Passardi et al. 2004b).

Lignification and suberisation

Cross-linking of the phenolic monomers in the formation of suberin and the oxidative coupling of lignin subunits has been associated with reduction of cell extensibility and growth. Peroxidases (Lewis and Yamamoto 1990) and laccases (EC 1.10.3.2) (Kiefer-Meyer et al. 1996) are both candidates for lignin units assembly by oxidative polymerisation. Using H_2O_2 as oxidant, peroxidases can generate monolignol phenoxy radicals that spontaneously form lignin polymers (Lewis and Yamamoto 1990). Down-regulation of the tobacco peroxidase TP60 and of the aspen anionic peroxidase prxA3a expressions lead to the reduction of lignin content (Blee et al. 2003; Li et al. 2003). Other peroxidases such as HRP A2 from horseradish, AtPrx21 (ATP2) from *A. thaliana*, various anionic peroxidases from poplar, and cationic peroxidases from tomato (TPX1) and *Zinnia* have also been shown to be involved in the lignification process (Ostergaard et al. 2000; Quiroga et al. 2000; Christensen et al. 2001; Nielsen et al. 2001; Lopez-Serrano et al. 2004).

The in vitro oxidation of lignin monomers by particular peroxidases and their specific localisation in lignifying plant tissues both suggest their role in the lignin biosynthetic pathways. Peroxidases are also involved in suberin formation through a similar process (Bernards et al. 1999; Keren-Keiserman et al. 2004).

Diferulic bonds and extensin network

The lignification step is not limited to the assembly of phenolic units. The cell wall network is further stiffened by association of monolignols to polysaccharides and by internal cross-linking of polysaccharides (mainly cellulose and pectin) through formation of diferulate bonds (Iiyama et al. 1994). The relationship between formation of diferulates and peroxidases is well-known in vitro. Different *Pinus elliotti* peroxidase fractions (soluble, ionically- and covalently-bound) were isolated from the cytoplasm and the wall of callus or cambial cells: only the cell wall fraction of cambium exhibited a ferulic acid oxidation activity (Whitmore 1976). Further studies confirmed this fact (Sanchez et al. 1996; MacAdam and Grabber 2002) and additionally showed that the proportion of different diferulic isomers varied according to the plant species (Ralph et al. 1994). Growth and cell wall extensibility are therefore inversely correlated with the increase in the content of diferulic acids in the cell wall (Sanchez et al. 1996; Kawamura et al. 2000; MacAdam and Grabber 2002).

Peroxidases are also major players in the building up of a dense extensin network destined to rigidify the cell wall. Peroxidases devoted to extensin cross-linking have been isolated from a large variety of plant species (Schnabelrauch et al. 1996; Magliano and Casal 1998; Jackson et al. 2001); they are known to act on the phenolic moiety of tyrosine, and maybe also on lysines, hence creating Tyr–Tyr or Tyr–Lys bonds (Schnabelrauch et al. 1996). Tyrosines and lysines are evenly spaced on extensins within conserved motifs, hence contributing to the formation of a very uniform mesh within the cell wall structure (Kieliszewski and Lamport 1994). Pectin is supposed to be involved in bringing together peroxidases and extensins, due to the affinity of some peroxidases to pectate–calcium complexes, and to a possible covalent bond between pectins and extensins (Qi et al. 1995; Dunand et al. 2002).

Abiotic and biotic stresses

Plants exposed to acute stress are known to up-regulate their overall peroxidase activity. This reaction happens equally with various abiotic and biotic stresses such as chemical (heavy metal, industrial, or agriculture pollution), biological (pathogens), or physical (wounding) assaults. Peroxidase expression results in plant defence either passively (building up of stronger walls) or actively (production of ROS against attacking organisms). When the stress factor manages to overcome the plant barriers and penetrates inside the plant, peroxidases may play a major role by isolating or eliminating the foreign body.

Chemical stresses

Toxicity of heavy metals in plants is probably due to their ability to promote damaging oxidative reactions (Schützendübel and Polle 2002). Cu and Fe ions can directly generate ROS from oxygenated molecules through the Haber–Weiss and Fenton reactions. However, the mechanisms of ROS production by metals such as Pb, Cd, and Zn ions is less clear, and may be mediated, for instance, by activation of lipoxygenase or binding to membrane proteins, thus triggering electron leakages responsible for formation of ROS (Seregin and Ivanov 2001). A possible function of peroxidases in treatment of heavy metals is their contribution in accumulating plants. For example, the waterlily *Nymphaea* probably uses peroxidases to produce phenolic polymers that trap Cd and isolate it in the form of Ca–Cd crystals in specific glands situated on the aquatic side of its leaves (Lavid et al. 2001b). When another aquatic plant, *Nymphoides peltata*, which contains a lower basal level of phenols, was subjected to the same growth condition and Cd exposure, it showed severe damages, thus supporting the implication of phenolic polymers and peroxidase in phytoaccumulation of metals (Lavid et al. 2001a).

Besides their participation to the entrapment of heavy metals, peroxidases can also degrade toxic molecules. Hairy root cultures of turnip (*Brassica napus*), are very efficient in degrading the toxic pesticide 2,4-dichlorophenol (2,4-DCP) (Agostini et al. 2003). The resulting products have not yet been precisely identified both in terms of chemical nature and toxicity, but they are probably a mixture of polymers created by the action of peroxidases. These enzymes can indeed attack phenolic moieties and form radicals that will then non-enzymatically polymerise. A further proof of the importance of peroxidases in the process was the appearance of an isozyme on an IEF gel upon repeated exposure to 2,4-DCP.

Although peroxidases are clearly induced by heavy metals and other toxic chemicals, it is still difficult to assign them a precise role in phytoremediation. Despite the poor knowledge about their function, the peroxidase up-regulation as a response of the plant to pollutants can already be used for the phytomonitoring of industrial or densely urbanised areas. Peroxidases have been shown to be quite sensitive to atmospheric pollution, with a response that can be stronger than the one of other classical biomarkers, such as superoxide dismutase, glutathione reductase levels (Wu and von Tiedemann 2002) or ascorbate concentration (Moraes et al. 2002). Similar observations have been made with heavy metals present in soil (Cho and Park 2000; Geebelen et al. 2002), although the response may be lower than that observed with atmospheric stress (Klumpp et al. 2000). The major drawback of this method is that it does not allow to distinguish between pollutants. However, it still remains one of the most sensitive methods to biologically evaluate the impact of pollution.

Biological stresses

Peroxidases are known to be activated in response to pathogen attacks and several roles have been attributed to plant peroxidases in host–pathogen interaction. They can

have a cell wall cross-linking activity (formation of lignin, extensin cross-links, dityrosine bonds) and create a highly toxic environment by massively producing ROS (oxidative burst), which results in adverse growth conditions for microorganisms.

In the defence of cotton against bacterial blight (*Xanthomonas campestris* pv. *malvacearum*), the resistance phenotype is characterised by a rapid localised tissue collapse resulting in necrotisation and immobilisation of the intruding pathogen at the sites of attack. Production and accumulation of superoxide anions and hydrogen peroxide in cell wall has been related to the presence of pathogens (Martinez et al. 1998). Total peroxidase activity is highly increased in the infected region 12 h after treatment and mainly localised in the apoplast and close to the bacterial infection site (Martinez et al. 1998; Delannoy et al. 2003). Similarly, rice leaves infected by rice blight (*Xanthomonas oryzae* pv. *oryzae*) strongly upregulated a single peroxidase isoform in xylem parenchyma. This peroxidase was then secreted to the xylem vessels, resulting in secondary wall thickening and reducing access of the pathogen to the pit membrane (the pathogen's contact point with living cells) (Hilaire et al. 2001). Lignin is not the only phenolic subunit that is used by peroxidase in defensive polymerisation reactions. Several other phenolics are involved and result in different morphological "barriers" against pathogens. In onion, for instance, granular deposits formed upon *Botrytis allii* infection contained tyramine derivatives, thought to strengthen the cell wall against fungal intrusion. These phenolics were probably polymerised by peroxidases (McLusky et al. 1999).

During germination, the aleurone layer of radish seeds functions as a secretory tissue. The seeds release ROS and peroxidases in the apoplastic space, respectively, between 6 to 12 h and between 24 and to 36 h after sowing to prevent pathogenic attack (Schopfer 2001). Reactive oxygen species and peroxidase act probably as a constitutive defence reaction against possible infections. A strong increase in H_2O_2 has also been reported following bacterial inoculation of lettuce (*Lactuca sativa* L.), together with a rise in apoplast peroxidasic activity. Localisation of the protective response was confined to the site of pathogenic intrusion (Bestwick et al. 1998).

Besides their functions during symbiosis, peroxidases from legumes have been associated with the plant's defence mechanisms. For example, FBP1, a French bean peroxidase, is responsible for the apoplastic oxidative burst and can generate hydrogen peroxide using cysteine (Blee et al. 2001). LEP1 from lupine is highly efficient in the cross-linking of extensin and could therefore form a physical barrier against invading organisms (Price et al. 2003). Other examples are reported, although no specific role has been assigned to the peroxidase: GMIPER1, from soybean, is induced in response to infection with *Phytophthora sojae* and other external stresses (Yi and Hwang 1998); Msprx1B, and the similar 1A and 1C peroxidases from *Medicago sativa*, were identified in alfalfa leaves after infection with *Pseudomonas syringae* (el-Turk et al. 1996).

Plant parasitism, the special case of Orobanche

Orobanche sp. (broomrape) are obligate root holoparasitic plants (Musselman 1980). They are one of the most important agricultural pests in several major cropping systems worldwide (Goldwasser and Yoder 2001). Several control strategies have been proposed and employed, but none have provided complete protection (Rubiales et al. 2003).

Several reports reveal that typical plant defence responses against pathogenic microorganisms are also induced upon parasitic plant infection. These include an increase of peroxidase activity, lignification, and cell-wall phenolic deposition (Goldwasser et al. 1999; Vieira Dos Santos et al. 2003a). For example, among the genes induced after infection of *A. thaliana* by *Orobanche ramose*, a gene coding for a class III peroxidase was upregulated (Vieira Dos Santos et al. 2003a). The expression of this gene was transient, early induced, and the transcript accumulated during the first 24 h after infection. A second induction was further detected at 7 days when the first parasite attachment was observed (Vieira Dos Santos et al. 2003b). A time-dependent increase of peroxidase activity was also reported in *O. aegyptiaca*-infected vetch (*Vicia sativa* cv. Yovel) plants (Goldwasser et al. 1999). However, *O. aegyptiaca* inoculation resulted in a relatively small increase in the enzyme activity in roots of susceptible vetch plants, whereas in roots of resistant plants (*Vicia atropurpurea* Desf. cv. Popany) the activity was four-fold increased compared to the non-inoculated treatments (Goldwasser et al. 1999). In addition, a sharp increase in free and bound phenolics was reported as well as an increase in lignin for resistant vetch, whereas only low increase in lignin and phenolics in response to infection were found for susceptible vetch (Goldwasser et al. 1999). Interestingly, another study revealed that most of the *Orobanche*-pea (*Pisum* spp.) resistant genotypes showed a higher constitutive peroxidase activity than susceptible ones (Castillejo et al. 2004). It can, therefore, be suggested that peroxidase proteins play a crucial role in plants' resistance against *Orobanche* by increasing the cell wall cross-linking or the generation of ROS.

Physical stresses

During a pathogenic attack, plant tissues can be damaged. Alternatively, wounding can occur through meteorological adversities or larger animals. Peroxidase expression during wounding is probably triggered in order to repair the damaged tissue, but also as a preventive defence mechanism against foreign attacks.

A study in sweet potato showed that the acidic peroxidase gene *swpa4* is not expressed in any tissue of the plant during normal growth, but that it is strongly up-regulated upon wounding of leaves, as well as incubation with high concentrations of H_2O_2 or NaCl (Park et al. 2003). This would mean either that the stress factors wounding and some chemicals follow the same pathway leading to peroxidase activation or that *swpa4* promoter is involved in

several response pathways. The latter hypothesis would not be surprising considering the high number of different regulatory sequences generally present in promoter sequences of peroxidase genes. Similarly, *ZmPox2* gene from *Zea mays* was both induced by wounding and ethylene, but not by methyl-jasmonate, another well-known wounding marker (Reymond et al. 2000; de Obeso et al. 2003). In striking contrast, two isozymes from northern red oak tree (*Quercus rubra* L.) were specifically induced by wounding and jasmonate, whereas another isozyme was induced by wounding, but not by jasmonate (Allison and Schultz 2004). This discrepancy may reflect the existence of different pathways in wounding signalling, that could also be due to the nature of the wounding. Indeed, a more detailed analysis performed on rice showed that the nature of the wound induces different peroxidase isoforms: rubbing or cutting leaves or tips did not induce the same peroxidases (Hiraga et al. 2000).

Symbiosis

During pathogenic interaction (fungi or bacteria), plants have the capability to respond with a set of defence reactions that include several peroxidases (as described above). In the case of symbiosis, the question arises whether symbionts influence peroxidase activities and isozyme patterns in a similar or different manner than pathogen attacks. Indeed, in symbiotic interactions, defence mechanisms have to be controlled and limited to allow the association of two different organisms.

Nodulation

After inoculation with compatible rhizobia, certain leguminous plants form nodules on their roots, providing conditions necessary for bacterial conversion of atmospheric dinitrogen to ammonia. Rhizobia soil bacteria colonise host cells and tissues through infection threads, which are tubular in-growths of the plant cell wall (Rathbun et al. 2002). The initiation of infection threads is apparently preceded by localised cell wall degradation (van Spronsen et al. 1994) and may be accompanied by modifications in cell wall peroxidase activities (Salzwedel and Dazzo 1993; Cook et al. 1995). Prior to infection, flavonoids or other plant metabolites contained in root exudates induce the expression of a set of bacterial genes (*nod* genes) (Cook et al. 1995; Mathesius 2001). The *nod* genes are required and are essential for the synthesis of rhizobial signal molecules called "Nod factors" and then for nodulation.

A *Rhizobium*-induced peroxidase, *rip1*, is rapidly and transiently expressed in the very early interactions of *Medicago truncatula*, with compatible *R. meliloti* (Cook et al. 1995). The *R. meliloti* Nod factor is both necessary and sufficient for the *rip1* induction (Peng et al. 1996) and for ROS production (Ramu et al. 2002). The *rip1* transcript and the ROS accumulations are both localised in the root epidermal cells and the level of *rip1* is maximal by 3 h post-inoculation and decreases by 48 h (Peng et al. 1996; Ramu et al. 2002). In contrast, maximal induction of other early genes by Nod factors is associated with rhizobial infection and early nodule morphogenesis, and these genes continue to be expressed in mature nodules (Cook et al. 1995). The *rip1* gene is also activated by exogenous H_2O_2 treatment in absence of Nod factors (Ramu et al. 2002), with a dose response similar to that of other characterised ROS-responsive genes (Chen et al. 1996). The RIP1 peroxidase could, therefore, mediate cell wall alteration in the early nodule development and at sites of infection thread formation (Salzwedel and Dazzo 1993). The increasing level of RIP1 and ROS could contribute to cell wall loosening facilitating early infection events. In addition, other peroxidases could be involved in cell wall cross-linking rapidly after rhizobia infection.

Several investigators highlighted the involvement of auxin in nodulation by demonstrating that the external application of auxin transport inhibitors lead to the formation of nodule-like structures (Allen et al. 1953; Hirsch et al. 1989). Moreover, auxin accumulated at the site of nodule initiation and subsequently diminished during nodule primordium formation (Mathesius et al. 1998; Penmetsa et al. 2003). The increase of peroxidases during nodulation could be related to the control of auxin level in roots. Indeed, some class III peroxidases are known to oxidise auxin and could then indirectly control nodule formation through the auxin catabolism (Savitsky et al. 1999; Mathesius 2001).

Mycorrhization

Ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) fungi are ubiquitous symbiotic microorganisms associated with plants of most families of Angiosperms and Gymnosperms (Koide and Schreiner 1992). They play an essential role in plant nutrition and stress tolerance and may be related to expression of various oxidative enzymes. Mycorrhiza are known to modify root enzymes (activity, isoforms, etc.), including peroxidase activity. As during nodulation, the mycorrhizal symbiotic interaction is also probably associated with a modification of the cell wall structure of the infected cells.

Arbuscular mycorrhizal fungal inoculation of leeks (*Allium porrum*) resulted in a transiently higher level of peroxidase activity compared to uninoculated controls (Spanu and Bonfante-Fasolo 1988). Ultrastructural localisation of peroxidase activity further revealed an accumulation in the middle lamella of cortical cells penetrated by intercellular hyphae, whereas no activity was found in the walls of cortical cells already containing arbuscular intracellular structures (Spanu and Bonfante-Fasolo 1988). Similarly, peroxidase activity in the fine roots of *Pinus sylvestris* seedlings planted in forest humus was highest at the beginning of the experiment and decreased during growing season (Tarvainen et al. 2004). This decrease of peroxidase can be associated with the increase of the number of ECM morphotypes as well as root biomass. These and several other results (Albrecht et al. 1994) suggest that the mycorrhizal

fungus generally induces peroxidase expression during the initial stage of symbiotic interaction (Günther et al. 1998; Salzer et al. 1999) and that this response is later either controlled by the plant or circumvented in mature mycorrhiza (Münzenberger et al. 1997). Nevertheless, some contradictory results have also been reported. In roots of *Medicago sativa* L. colonised by a mature AM fungi (*Glomus mossae*), increased peroxidase levels were observed (Criquet et al. 2000). A similar amount of peroxidases was found in roots of Scots pine (*Pinus sylvestris*) colonised and not colonised by different ECM fungi (Timonen and Sen 1998). However, the differences reported between the different mycorrhizal fungi are likely to be due to potential species-specific enzyme activities. Eucalyptus roots inoculated with different ECM strain of *Pisolithus* spp. showed a transient increase of peroxidase activity during the first days of inoculation in relation to the colonisation efficiency of the strain. Peroxidase activity of "poor" root colonisers remained relatively low, whereas activity in roots inoculated with "good" root colonisers increased sharply (Albrecht et al. 1994). Besides acting on the total peroxidase activity, mycorrhyza interaction can also regulate specific isoforms. Changes in root peroxidase profile following mycorrhiza colonisation were observed indicating a specific differential isozyme expression in roots (Münzenberger et al. 1997; Timonen and Sen 1998).

It has been suggested that auxins produced by the ECM fungi reduce peroxidase-catalysed cross-linking of cell-wall constituents (Salzer and Hager 1993; Charvet-Candela et al. 2002). This mechanism may make the cell walls in roots less rigid and allow colonisation of the intercellular space by symbiotic fungi. However, other investigators have shown that IAA levels were inversely related to peroxidase activity (Mitchell et al. 1986), suggesting that in mycorrhiza like in nodules, peroxidases could be involved in auxin catabolism. The role of auxin and peroxidases in mycorrhization is still difficult to define, because of the lack of information about the relative importance of these two mechanisms.

From these results it appears that symbionts, mycorrhizal fungi, as well as bacteria, may not trigger the full host defence response particularly where peroxidase activity is concerned. In successful infections, these defence responses are either controlled by the plant or overcome by the successful symbiont. The contradictory reports concerning the interactions of peroxidase with auxins afford only a limited first insight into mutual interactions of the symbiotic partners and need further exploration to be clarified. Expression of peroxidases in host roots might nevertheless limit and regulate the growth of the symbiont. During the early infection, peroxidase production can generate ROS implicated in the cell wall loosening to facilitate the symbiont entrance. During the time of colonisation and development of symbiosis, it is also likely that defence-related peroxidases are expressed to limit the intracellular spreading of the fungus but also to protect against soil microbial pathogens, through the establishment of a highly toxic environment by massively producing ROS (oxidative burst).

Senescence

It is generally accepted that cell death, senescence, ripening, necrosis, and lesions are correlated and depended on overlapping mechanisms. The first three events can be considered as normal evolution for a plant, the last ones being related to foreign actions. One difference could be the notion of reversibility: until a specific threshold, cell death, senescence, and ripening can be considered as reversible processes.

Plant senescence

Leaf senescence is the last stage before complete cell death. A number of cellular changes have been associated with this mechanism. For example, increase of ethylene synthesis and peroxidase activity have been reported (Jimenez et al. 1998). A change in gene expression has also been observed during senescence: a decrease for the photosynthesis-associated genes and an increase for the senescence-associated genes (Abarca et al. 2001). Reactive oxygen species such as $O_2^{\cdot -}$ have been shown to be involved in the induction and development of the senescence stage. The superoxide radical $O_2^{\cdot -}$ could be generated by extracellular peroxidase activity following salicylic acid treatment (Kawano et al. 1998) and could act on the senescence induction pathway. On the other hand, total peroxidase activity increased during *A. thaliana* leaf expansion as well as in the senescent tissues, with a stronger increase at the end of the leaf expansion (Abarca et al. 2001). It seems difficult to dissociate the leaf senescence from the cell wall stiffening and cross-linking. In *A. thaliana* leaf, the peroxidase activity is mainly associated with cell extension between the 1st and the 4th week of growth, the 4th to 5th weeks of growth correspond to a transition phase, and after the 5th week of growth peroxidase activity is associated in majority with the cross-linking of cell-wall compounds (Abarca et al. 2001). In agreement with this observation, the increase of syringaldazine-peroxidase activity in plants exposed to ozone may be related to the induction of early senescence through the activation or acceleration of lignification process (Ranieri et al. 2000).

Fruit growth and ripening

Fruit growth occurs by cell expansion rather than division. During tomato fruit development as in other cell elongation processes described previously, cell expansion is dependent on cell wall loosening (Andrews et al. 2000). Hence the implication of peroxidases in such a process is related to the fine balance between cell wall loosening and stiffening.

However, fruit ripening syndrome, which involves certain hormonal and enzyme classes, is commonly promoted by ethylene in climacteric plant (Alexander and Grierson 2002). The final fruit evolution is correlated with an increased peroxidase activity acting on cell-wall

modification. The cessation of tomato fruit growth follows a decrease of wall extensibility at the fruit skin level (Andrews et al. 2000). Interestingly, peroxidases associated to the cell wall of the epidermis are induced during fruit maturity. These peroxidases are probably not directly related to the fruit ripening but could control the growth arrest by changing the mechanical properties of cell wall and consequently produce a protective barrier in the epidermis.

Conclusions

In plant cells, class III peroxidases are present in soluble, ionically- and covalently-cell wall bound forms. They are involved in many functions as a consequence of their two cycles (Fig. 1): ROS generation and regulation, H_2O_2 level regulation, and oxidation of various substrates (which leads to the catabolism or the polymerisation of substrates) (Fig. 3). Using these three mechanisms, plants have at their disposal a "Swiss army knife-like" multifunctional tool that they can use in every tissue at any time throughout their life cycle. The high number of isoforms further allows a fine balance between antagonistic peroxidase functions such as cell wall cross-linking and loosening. The variation of the number of isoforms between species could explain the numerous discrepancies reported on the regulation of the different peroxidases related to physiological processes.

The comparison of the different situations described in this review shows that peroxidases up-regulation is generally transient. Peroxidases are often strongly induced at the beginning of an event, and then slowly decrease with time. This type of response has indeed been shown in chemical stress, where peroxidase expression was only significantly increased in acute stress (Klumpp et al. 2000). Plants respond to pathogens and symbionts similarly, with a rapid oxidative burst for the former (Blee et al. 2001), and the increase in peroxidase activity being mainly expressed in the first moments of colonisation in the latter (Cook et al. 1995; Tarvainen et al. 2004). This transient induction is, however, not common to all peroxidases. There is always a basal level of peroxidase activity in plants, probably to perform "housekeeping" functions, such as growth by elongation and lignification. It is possible that the appearance of the first peroxidase during evolution has allowed plants to build up a cell-wall structure that is able to stand out of the water and hence adapted to land colonisation. Later on, with the advent of insects, climatic changes, human impact, and other stresses, a quickly inducible peroxidase was advantageous to respond to these novel factors, and, therefore, was naturally selected and further duplicated. However, these assumptions remain purely speculative as long as the in vivo functions of single peroxidases have not been determined. To our knowledge, no studies have reported such information on peroxidases yet. When identified, this crucial point will certainly help us to better understand the evolution, the roles, and the regulations of this multifunctional enzyme.

Acknowledgements We thank William Deakin for his critical reading, his constructive suggestions, and for his original title. We thank the University of Geneva and the Swiss National Science Foundation (grant 31-068003.02) for research support to C.P. and C.D. The authors F. Passardi and C. Cosio have both contributed equally to this work

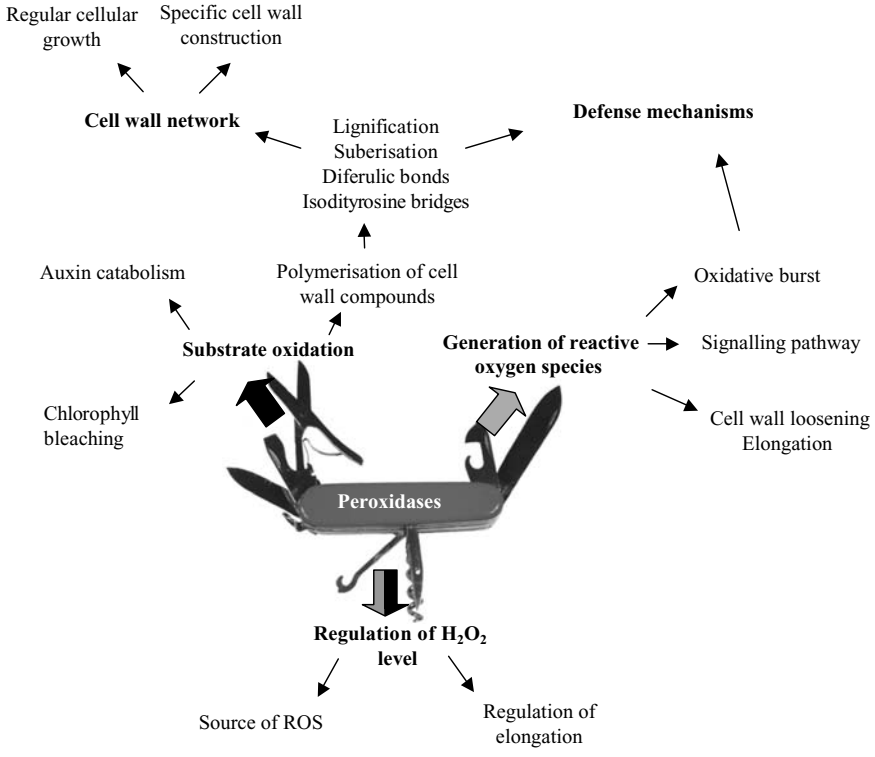

Fig. 3 Overview of the putative multiple functions of the class III peroxidases through both of their cycles. Peroxidases oxidise various subtrates through the peroxidative cycle (black arrow). Reactive oxygen species are generated through the hydroxylic cycle (grey arrow). H_2O_2 level can be regulated through both catalytic cycles (grey and black arrows)

References

Abarca D, Martin M, Sabater B (2001) Differential leaf stress responses in young and senescent plants. Physiol Plant 113:409–415

Agostini E, Coniglio MS, Milrad SR, Tigier HA, Giulietti AM (2003) Phytoremediation of 2,4-dichlorophenol by *Brassica napus* hairy root cultures. Biotechnol Appl Biochem 37:139–144

Albrecht C, Burgess T, Dell B, Lapeyrie F (1994) Chitinase and peroxidase activities are induced in eucalyptus roots according to aggressiveness of Australian ectomycorrhizal strains of *Pisolithus* sp. New Phytol 127:217–222

Alexander L, Grierson D (2002) Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. J Exp Bot 53:2039–2055

Allen EK, Allen ON, Newman AS (1953) Pseudonodulation of leguminous plants induced by 2-bromo-3,5-dichlorobenzoic acid. Am J Bot 40:429–435

Allison SD, Schultz JC (2004) Differential activity of peroxidase isozymes in response to wounding, gypsy moth, and plant hormones in northern red oak (*Quercus rubra* L.). J Chem Ecol 30:1363–1379

Andrews J, Malone M, Thompson DS, Ho LC, Burton KS (2000) Peroxidase isozyme patterns in the skin of maturing tomato fruit. Plant Cell Environ 23:415–422

Bernards MA, Fleming WD, Llewellyn DB, Priefer R, Yang X, Sabatino A, Plourde GL (1999) Biochemical characterization of the suberization-associated anionic peroxidase of potato. Plant Physiol 121:135–146

Bestwick CS, Brown IR, Mansfield JW (1998) Localized changes in peroxidase activity accompany hydrogen peroxide generation during the development of a nonhost hypersensitive reaction in lettuce. Plant Physiol 118:1067–1078

Blee KA, Jupe SC, Richard G, Zimmerlin A, Davies DR, Bolwell GP (2001) Molecular identification and expression of the peroxidase responsible for the oxidative burst in French bean (*Phaseolus vulgaris* L.) and related members of the gene family. Plant Mol Biol 47:607–620

Blee KA, Choi JW, O'Connell AP, Schuch W, Lewis NG, Bolwell GP (2003) A lignin-specific peroxidase in tobacco whose antisense suppression leads to vascular tissue modification. Phytochemistry 64:163–176

Castillejo MA, Amiour N, Dumas-Gaudot E, Rubiales D, Jorrin JV (2004) A proteomic approach to studying plant response to crenate broomrape (*Orobanche crenate*) in pea (*Pisum sativum*). Phytochemistry 65:1817–1828

Charvet-Candela V, Hitchin S, Reddy MS, Cournoyer B, Marmeisse R, Gay G (2002) Characterization of a *Pinus pinaster* cDNA encoding an auxin up-regulated putative peroxidase in roots. Tree physiol 22:231–238

Chen SX, Schopfer P (1999) Hydroxyl-radical production in physiological reactions. A novel function of peroxidase. Eur J Biochem 260:726–735

Chen W, Chao G, Singh KB (1996) The promoter of a H_2O_2-inducible, *Arabidopsis* glutathione S-transferase gene contains closely linked OBF- and OBP1-binding sites. Plant J 10:955–966

Cho U, Park J (2000) Mercury-induced oxidative stress in tomato seedlings. Plant Sci 156:1–9

Christensen JH, Overney S, Rohde A, Diaz WA, Bauw G, Simon P, Van Montagu M, Boerjan W (2001) The syringaldazine-oxidizing peroxidase PXP 3-4 from poplar xylem: cDNA isolation, characterization and expression. Plant Mol Biol 47:581–593

Cook D, Dreyer D, Bonnet D, Howell M, Nony E, Van den Bosch K (1995) Transient induction of a peroxidase gene in *Medicago truncatula* precedes infection by *Rhizobium meliloti*. Plant Cell 7:43–55

Cordoba-Pedregosa M, Gonzalez-Reyes JA, Canadillas M, Navas P, Cordoba F (1996) Role of apoplastic and cell-wall peroxidases on the stimulation of root elongation by ascorbate. Plant Physiol 112:1119–1125

Cordoba-Pedregosa M, Cordoba F, Villalba JM, Gonzalez-Reyes JA (2003) Zonal changes in ascorbate and hydrogen peroxide contents, peroxidase, and ascorbate-related enzyme activities in onion roots. Plant Physiol 131:697–706

Cosgrove DJ (2001) Wall structure and wall loosening. A look backwards and forwards. Plant Physiol 125:131–134

Criquet S, Joner E, Leglize P, Leyval C (2000) Anthracene and mycorrhiza affect the activity of oxidoreductase in the roots and rhizosphere of lucerne (*Medicago sativa* L.). Biotechnol Lett 22:1733–1737

Delannoy E, Jalloul A, Assigbetse K, Marmey P, Geiger JP, Lherminier J, Daniel JF, Martinez C, Nicole M (2003) Activity of class III peroxidases in the defense of cotton to bacterial blight. Mol Plant Microbe Interact 16:1030–1038

de Obeso M, Caparros-Ruiz D, Vignols F, Puigdomenech P, Rigau J (2003) Characterisation of maize peroxidases having differential patterns of mRNA accumulation in relation to lignifying tissues. Gene 309:23–33

Dunand C, Tognolli M, Overney S, von Tobel L, De Meyer M, Simon P, Penel C (2002) Identification and characterisation of Ca2+-pectate binding peroxidases in *Arabidopsis thaliana*. J Plant Physiol 159:1165–1171

Dunand C, de Meyer M, Crévecoeur M, Penel C (2003) Expression of a peroxidase gene in zucchini in relation with hypocotyl growth. Plant Physiol Biochem 41:805–811

Duroux L, Welinder KG (2003) The peroxidase gene family in plants: a phylogenetic overview. J Mol Evol 57:397–407

el-Turk J, Asemota O, Leymarie J, Sallaud C, Mesnage S, Breda C, Buffard D, Kondorosi A, Esnault R (1996) Nucleotide sequences of four pathogen-induced alfalfa peroxidase-encoding cDNAs. Gene 170:213–216

Fry SC (1998) Oxidative scission of plant cell wall polysaccharides by ascorbate-induced hydroxyl radicals. Biochem J 332:507–515

Geebelen W, Vangronsveld J, Adriano DC, Van Poucke LC, Clijsters H (2002) Effects of Pb-EDTA and EDTA on oxidative stress reactions and mineral uptake in *Phaseolus vulgaris*. Physiol Plant 115:377–384

Goldwasser Y, Yoder JI (2001) Differential induction of *Orobanche* seed germination by *Arabidopsis thaliana*. Plant Sci 160:951–959

Goldwasser Y, Hershenhorn J, Plakhine D, Kleifeld Y, Rubin B (1999) Biochemical factors involved in vetch resistance to *Orobanche aegyptiaca*. Physiol Mol Plant Pathol 54: 87–96

Günther T, Perner B, Gramss G (1998) Activities of phenol oxidizing enzymes of ectomycorrhizal fungi in axenic culture and in symbiosis with Scots pine (*Pinus sylvestris* L.). J Basic Microbiol 38:197–206

Hilaire E, Young SA, Willard LH, McGee JD, Sweat T, Chittoor JM, Guikema JA, Leach JE (2001) Vascular defense responses in rice: peroxidase accumulation in xylem parenchyma cells and xylem wall thickening. Mol Plant Microbe Interact 14:1411–1419

Hiraga S, Yamamoto K, Ito H, Sasaki K, Matsui H, Honma M, Nagamura Y, Sasaki T, Ohashi Y (2000) Diverse expression profiles of 21 rice peroxidase genes. FEBS Lett 471: 245–250

Hiraga S, Sasaki K, Ito H, Ohashi Y, Matsui H (2001) A large family of class III plant peroxidases. Plant Cell Physiol 42: 462–468

Hirsch AM, Bhuvaneswari TV, Torrey JG, Bisseling T (1989) Early nodulin genes are induced in alfalfa root outgrowths elicited by auxin transport inhibitor. Proc Natl Acad Sci USA 86:1244–1248

Iiyama K, Lam T, Stone BA (1994) Covalent cross-links in the cell wall. Plant Physiol 104:315–320

Jackson PA, Galinha CI, Pereira CS, Fortunato A, Soares NC, Amancio SB, Pinto Ricardo CP (2001) Rapid deposition of extensin during the elicitation of grapevine callus cultures is specifically catalyzed by a 40-kilodalton peroxidase. Plant Physiol 127:1065–1076

Jimenez A, Hernandez JA, Pastori G, del Rio LA, Sevilla F (1998) Role of the ascorbate-glutathione cycle of mitochondria and peroxisomes in the senescence of pea leaves. Plant Physiol 118:1327–1335

Joo JH, Bae YS, Lee JS (2001) Role of auxin-induced reactive oxygen species in root gravitropism. Plant Physiol 126:1055–1060

Kawamura Y, Wakabayashi K, Hoson T, Yamamoto R, Kamisaka S (2000) Stress-relaxation analysis of submerged and air-grown rice coleoptiles: correlation with cell wall biosynthesis and growth. J Plant Physiol 156:689–694

Kawano T, Sahashi N, Takahashi K, Uozumi N, Muto S (1998) Salicylic acid induces extracellular superoxide generation followed by an increased in cytosolic calcium ion in tobacco suspension culture: the earliest events in salicylic acid signal transduction. Plant Cell Physiol 39:721–730

Keren-Keiserman A, Tanami Z, Shoseyov O, Ginzberg I (2004) Peroxidase activity associated with suberization processes of the muskmelon (*Cucumis melo*) rind. Physiol Plant 121:141–148

Kiefer-Meyer MC, Gomord V, O'Connell A, Halpin C, Faye L (1996) Cloning and sequence analysis of laccase-encoding cDNA clones from tobacco. Gene 178:205–207

Kieliszewski MJ, Lamport DT (1994) Extensin: repetitive motifs, functional sites, post-translational codes, and phylogeny. Plant J 5:157–172

Klumpp G, Furlan CM, Domingos M, Klumpp A (2000) Response of stress indicators and growth parameters of *Tibouchina pulchra* Cogn. exposed to air and soil pollution near the industrial complex of Cubatao, Brazil. Sci Total Environ 246:79–91

Koide RT, Schreiner RP (1992) Regulation of the vesicular-arbuscuar mycorrhizal symbiosis. Annu Rev Plant Physiol Plant Mol Biol 43:557–581

Lavid N, Schwartz A, Lewinsohn E, Tel-Or E (2001a) Phenols and phenol oxidases are involved in cadmium accumulation in the water plants Nymphoides peltata (*Menyanthaceae*) and Nymphaeae (*Nymphaeaceae*). Planta 214:189–195

Lavid N, Schwartz A, Yarden O, Tel-Or E (2001b) The involvement of polyphenols and peroxidase activities in heavy-metal accumulation by epidermal glands of the waterlily (*Nymphaeaceae*). Planta 212:323–331

Lewis NG, Yamamoto E (1990) Lignin: occurrence, biogenesis and biodegradation. Annu Rev Plant Physiol Plant Mol Biol 41:455–496

Li Y, Kajita S, Kawai S, Katayama Y, Morohoshi N (2003) Down-regulation of an anionic peroxidase in transgenic aspen and its effect on lignin characteristics. J Plant Res 116:175–182

Liszkay A, Kenk B, Schopfer P (2003) Evidence for the involvement of cell wall peroxidase in the generation of hydroxyl radicals mediating extension growth. Planta 217:658–667

Lopez-Serrano M, Fernandez MD, Pomar F, Pedreno MA, Ros Barcelo A (2004) *Zinnia elegans* uses the same peroxidase isoenzyme complement for cell wall lignification in both single-cell tracheary elements and xylem vessels. J Exp Bot 55:423–431

MacAdam JW, Grabber JH (2002) Relationship of growth cessation with the formation of diferulate cross-links and p-coumaroylated lignins in tall fescue leaf blades. Planta 215:785–793

Magliano TMA, Casal JJ (1998) In vitro cross-linking of extensin precursors by mustard extracellular isoforms of peroxidase that respond either to phytochrome or to wounding. J Exp Bot 49:1491–1499

Martinez C, Montillet J, Bresson E, Agnel J, Dai G, Daniel J, Geiger J, Nicole M (1998) Apoplastic peroxidase generates superoxide anions in cells of cotton cotyledons undergoing the hypersensitive reaction to *Xanthomonas campestris* pv. *malvacearum* race 18. Mol Plant Microbe Interact 11:1038–1047

Mathesius U (2001) Flavonoids induced in cells undergoing nodule organogenesis in white clover are regulators of auxin breakdown by peroxidase. J Exp Bot 52:419–426

Mathesius U, Schlaman HRM, Spaink HP, Sautter C, Rolfe BG, Djorhjevic MA (1998) Auxin transport inhibition precedes root nodule formation in white clover roots and is regulated by flavonoids and derivatives of chitin oligosaccharides. Plant J 14:23–34

McLusky SR, Bennett MH, Beale MH, Lewis MJ, Gaskin P, Mansfield JW (1999) Cell wall alterations and localized accumulation of feruloyl-3′-methoxytyramine in onion epidermis at sites of attempted penetration by *Botrytis allii* are associated with actin polarisation, peroxidase activity and suppression of flavonoid biosynthesis. Plant J 17:523–534

Mitchell RJ, Garrett HE, Cox GS, Atalay A (1986) Boron and ectomycorrhizal influences on indole-3-acetic acid levels and indole-3-acetic acid oxidase and peroxidase activities of *Pinus echinata* Mill. roots. Tree Physiol 1:1–8

Moraes RM, Klumpp A, Furlan CM, Klumpp G, Domingos M, Rinaldi MC, Modesto IF (2002) Tropical fruit trees as bioindicators of industrial air pollution in southeast Brazil. Environ Int 28:367–374

Morohashi Y (2002) Peroxidase activity develops in the micropylar endosperm of tomato seeds prior to radicle protrusion. J Exp Bot 53:1643–1650

Münzenberger B, Otter T, Wüstrich D, Polle A (1997) Peroxidase and laccase activities in mycorrhizal and non-mycorrhizal fine roots of Norway spruce (*Picea abies*) and larch (*Larix decidua*). Can J Bot 75:932–938

Musselman LJ (1980) The biology of *Striga*, *Orobanche*, and other root-parasitic weeds. Ann Rev Plant Physiol 18:463–489

Nielsen KL, Indiani C, Henriksen A, Feis A, Becucci M, Gajhede M, Smulevich G, Welinder KG (2001) Differential activity and structure of highly similar peroxidases. Spectroscopic, crystallographic, and enzymatic analyses of lignifying *Arabidopsis thaliana* peroxidase A2 and horseradish peroxidase A2. Biochemistry 40:11013–11021

Ostergaard L, Teilum K, Mirza O, Mattsson O, Petersen M, Welinder KG, Mundy J, Gajhede M, Henriksen A (2000) *Arabidopsis* ATP A2 peroxidase. Expression and high-resolution structure of a plant peroxidase with implications for lignification. Plant Mol Biol 44:231–243

Park SY, Ryu SH, Kwon SY, Lee HS, Kim JG, Kwak SS (2003) Differential expression of six novel peroxidase cDNAs from cell cultures of sweet potato in response to stress. Mol Genet Genomics 269:542–552

Passardi F, Longet D, Penel C, Dunand C (2004a) The plant peroxidase multigenic family in rice and its evolution in green plants. Phytochemistry 65:1879–1893

Passardi F, Penel C, Dunand C (2004b) Performing the paradoxical: how plant peroxidases modify the cell wall. Trends Plant Sci 9:534–540

Penel C, Gaspar T, Greppin H (1992) Plant peroxidases. University of Geneva, Geneva, Switzerland

Peng HM, Dreyer DA, VandenBosch KA, Cook D (1996) Gene structure and differential regulation of the Rhizobium-induced peroxidase gene rip1. Plant Physiol 112:1437–1446

Penmetsa RV, Frugoli JA, Smith LS, Long SR, Cook D (2003) Dual genetic pathways controlling nodule number in *Medicago truncatula*. Plant Physiol 131:998–1008

Price NJ, Pinheiro C, Soares CM, Ashford DA, Ricardo CP, Jackson PA (2003) A biochemical and molecular characterization of LEP1, an extensin peroxidase from Lupin. J Biol Chem 278:41389–41399

Qi X, Behrens BX, West PR, Mort AJ (1995) Solubilization and partial characterization of extensin fragments from cell walls of cotton suspension cultures. Evidence for a covalent cross-link between extensin and pectin. Plant Physiol 108: 1691–1701

Quiroga M, Guerrero C, Botella MA, Barcelo A, Amaya I, Medina MI, Alonso FJ, de Forchetti SM, Tigier H, Valpuesta V (2000) A tomato peroxidase involved in the synthesis of lignin and suberin. Plant Physiol 122:1119–1127

Ralph J, Quideau S, Grabber JH, Hatfield RD (1994) Identification and synthesis of new ferulic dehydrodimers present in grass cell walls. J Chem Soc Perkin Trans 1:3485–3498

Ramu SK, Peng HM, Cook DR (2002) Nod factor induction of reactive oxygen species production is correlated with expression of the early nodulin gene rip1 in *Medicago truncatula*. Mol Plant Microbe Interact 15:522–528

Ranieri A, Petacco F, Castagna A, Soldatini GF (2000) Redox state and peroxidase system in sunflower plants exposed to ozone. Plant Sci 159:159–167

Rathbun EA, Naldrett MJ, Brewin NJ (2002) Identification of a family of extensin-like gltcoproteins in the lumen of *Rhizobium*-induced infection threads in pea root nodules. Mol Plant Microbe Interact 15:350–359

Reymond P, Weber H, Damond M, Farmer EE (2000) Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. Plant Cell 12:707–720

Rubiales D, Pérez-de-Luque A, Joel DM, Alcantara C (2003) Characterization of resistance in chickpea to crenate broomrape (*Orobanche crenata*). Weed Sci 51:702–707

Salzer P, Hager A (1993) Effects of auxins and ectomycorrhizal elicitors on wall-bond proteins and enzymes of spruce (*Picea abies* (L.) Karst.) cells. Trees 8:49–55

Salzer P, Corbière H, Boller T (1999) Hydrogen peroxide accumulation in *Medicago truncatula* roots colonized by the arbuscular mycorrhiza-forming fungus *Glomus intaradices*. Planta 208:319–325

Salzwedel JL, Dazzo FB (1993) pSym nod gene influence on elicitation of peroxidase activity from white clover and pea roots by rhizobia and their cell-free supernatants. Mol Plant Microbe Interact 6:127–134

Sanchez M, Pena MJ, Revilla G, Zarra I (1996) Changes in dehydrodiferulic acids and peroxidase activity against ferulic acid associated with cell walls during growth of *Pinus pinaster* hypocotyl. Plant Physiol 111:941–946

Savitsky PA, Gazaryan IG, Tishkov VI, Lagrimini LM, Ruzgas T, Gorton L (1999) Oxidation of indole-3-acetic acid by dioxygen catalysed by plant peroxidases: specificity for the enzyme structure. Biochem J 340:579–583

Schnabelrauch LS, Kieliszewski M, Upham BL, Alizedeh H, Lamport DT (1996) Isolation of pl 4.6 extensin peroxidase from tomato cell suspension cultures and identification of Val-Tyr-Lys as putative intermolecular cross-link site. Plant J 9:477–489

Schopfer P (1994) Histochemical demonstration and localization of H_2O_2 in organs of higher plants by tissue printing on nitrocellulose paper. Plant Physiol 104:1269–1275

Schopfer P (2001) Hydroxyl radical-induced cell-wall loosening in vitro and in vivo: implications for the control of elongation growth. Plant J 28:679–688

Schopfer P, Liszkay A, Bechtold M, Frahry G, Wagner A (2002) Evidence that hydroxyl radicals mediate auxin-induced extension growth. Planta 214:821–828

Schützendübel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by micorrhization. J Exp Bot 53:1351–1365

Schweikert C, Liszkay A, Schopfer P (2000) Scission of polysaccharides by peroxidase-generated hydroxyl radicals. Phytochemistry 53:565–570

Scialabba A, Bellani LM, Dell'Aquila A (2002) Effects of ageing on peroxidase activity and localization in radish (*Raphanus sativus* L.) seeds. Eur J Histochem 46:351–358

Seregin IV, Ivanov VB (2001) Physiological aspects of cadmium and lead toxic effects on higher plants. Russ J Plant Physiol 48:523–544

Spanu P, Bonfante-Fasolo P (1988) Cell-wall-bound peroxidase activity in roots of mycorrhizal *Allium porrum*. New Phytol 109:119–124

Tarvainen O, Markkola AM, Ahonen-Jonnarth U, Jumpponen A, Strömmer R (2004) Changes in ectomycorrhizal colonization and root peroxidase activity in *Pinus sylvestris* nursery seedlings planted in forest humus. Scan J For Res 19:400–408

Timonen S, Sen R (1998) Heterogeneity of fungal and plant enzyme expression in intact Scots pine- *Suillus bovinus* and *-Paxillus involutus* mycorrhizospheres developed in natural forest humus. New Phytol 138:355–366

Tognolli M, Penel C, Greppin H, Simon P (2002) Analysis and expression of the class III peroxidase large gene family in *Arabidopsis thaliana*. Gene 288:129–138

van Spronsen PC, Bakhuizen R, van Brussel AAN, Kijne JW (1994) Cell wall degradation during infection thread formation by the root nodule bacterium *Rhizobium leguminosarum* is a two-step process. Eur J Cell Biol 64:88–94

Vieira Dos Santos C, Delavault P, Letousey P, Thalouarn P (2003a) Identification by suppression subtractive hybridization and expression analysis of *Arabidopsis thaliana* putative defence genes during *Orobanche ramosa* infection. Physiol Mol Plant Pathol 62:297–303

Vieira Dos Santos C, Letousey P, Delavault P, Thalouarn P (2003b) Defense gene expression analysis of *Arabidopsis thaliana* parasited by *Orobanche ramosa*. Phytopathology 93:415–457

Welinder KG (1992a) Plant peroxidases: structure-function relationships. In: Penel C, Gaspar T, Greppin H (eds) Plant peroxidases. University of Geneva, Geneva, Switzerland, pp 1–24

Welinder KG (1992b) Superfamily of plant, fungal and bacterial peroxidases. Curr Opin Struct Biol 2:388–393

Whitmore FW (1976) Binding of ferulic acid to cell-walls by peroxidases of *Pinus-elliottii*. Phytochemistry 15:375–378

Wu YX, von Tiedemann A (2002) Impact of fungicides on active oxygen species and antioxidant enzymes in spring barley (*Hordeum vulgare* L.) exposed to ozone. Environ Poll 116:37–47

Yi SY, Hwang BK (1998) Molecular cloning and characterization of a new basic peroxidase cDNA from soybean hypocotyls infected with *Phytophthora sojae* f.sp. glycines. Mol Cell 8:556–564