Class III Peroxidases: Functions, Localization and Redox Regulation of Isoenzymes

Sonja Veljović Jovanović, Biljana Kukavica, Marija Vidović, Filis Morina and Ljiljana Menckhoff

Abstract Class III peroxidases (POXs; EC. 1.11.1.7), are secretory, multifunctional plant enzymes that catalyze the oxidation of a variety of substrates by hydrogen peroxide (H_2O_2). They show a remarkable diversity of isoenzymes, are encoded by a large number of paralogous genes, and are involved in a broad range of metabolic processes throughout plant growth and development. Peroxidases isoenzymes are located in the cell wall, apoplast and vacuole, and may be either soluble or ionically and covalently cell wall bound. They are involved in cell wall cross-linking and loosening, lignification and suberization, auxin catabolism and secondary metabolism. Due to their ability to control the levels of reactive oxygen species (ROS), POXs are efficient components of the antioxidative system induced in response to environmental stress, such as pathogen attack, metal excess, salinity, drought and high light intensity. In addition to the peroxidative function, POXs can catalyze H_2O_2 production in the oxidative cycle. Peroxidases are responsible either for cell elongation or cell wall stiffening, affecting carbon allocation, auxin level and redox homeostasis, which implicates their key role as being in the regulation of growth and defence under stress condition. This chapter will discuss novel insights into the functions of PODs with special emphasis on their localization, substrate specificity and the regulation of redox homeostasis.

Keywords Class III peroxidases · Vacuole · Apoplast · Cell wall
Isoenzymes · Phenolics

S. Veljović Jovanović (✉) · M. Vidović · F. Morina
Department of Life Sciences, Institute for Multidisciplinary Research,
University of Belgrade, Kneza Višeslava 1, 11030 Belgrade, Serbia
e-mail: sonjaveljov@gmail.com; sonjavel@imsi.rs

B. Kukavica
Faculty of Natural Sciences and Mathematics, University of Banja Luka,
Banja Luka, Bosnia and Herzegovina

L. Menckhoff
Biocenter Klein Flottbek and Botanical Garden, Biodiversity of Crop Plants,
University of Hamburg, Ohnhorststraße 18, D-22609 Hamburg, Germany

© Springer International Publishing AG 2018

D. K. Gupta et al. (eds.), *Antioxidants and Antioxidant Enzymes in Higher Plants*,
https://doi.org/10.1007/978-3-319-75088-0_13

1 Introduction

Under optimal physiological conditions, reactive oxygen species (ROS) accumulation and distribution are kept in the controlled range by a complex network of antioxidative enzymes and low molecular weight antioxidants. Intracellular accumulation of ROS is generally lower, while that of antioxidants is higher compared with the apoplast/cell wall compartment, which is, therefore, more oxidized. When accumulated above the certain threshold in the apoplast, H_2O_2 triggers signaling pathways and initiates a complex cascade of downstream events leading to the induction of the antioxidative defence response and transcriptional reprogramming.

The only H_2O_2-scavenging enzymes in the extracellular space are class III peroxidases (POXs; EC 1.11.1.7) classified by Welinder (1992). Peroxidases are glycoproteins synthesized in the endoplasmic reticulum and they are transported via the Golgi apparatus, both to the extracellular space and the vacuoles. Substrates of the endogenous POXs are electron donors for H_2O_2 and most of them belong to secondary metabolites such as phenolics, indoles and amines (Passardi et al. 2007), which are colocalized with POXs in the vacuoles and apoplastic compartment (Cheynier et al. 2013). Oxidation of phenolics by H_2O_2, catalyzed by POXs implies generation of phenoxyl radicals, which are in turn either polymerized or reduced by ascorbate (Asc) in the POXs/Phenolics/Asc (PPA) H_2O_2-scavenging system (Takahama 2004).

Class III plant peroxidases are a ubiquitous multigene family of enzymes with a number of genes identified in various species that are implicated in a broad range of physiological processes (Passardi et al. 2007). In addition to antioxidative defence, POXs are involved in all stages of plant development, from germination to aging, including formation of the secondary cell wall (Lopez-Serrano et al. 2004; Passardi et al. 2006), wound healing (Allison and Schultz 2004), seed germination (Scialabba et al. 2002), pollination (McInnis et al. 2006), fruit ripening (Pandey et al. 2012), senescence (Abarca et al. 2001), and auxin and anthocyanin catabolism (Gazaryan et al. 1996; Movahed et al. 2016). Such diversity of metabolic reactions catalyzed by POX isoforms, and a large number of genes with very divergent promoter sequences, implies a functional specialization of isoenzymes. However, low substrate specificity and a high redundancy of genes make it difficult to assign a specific function to the individual isoenzymes, which is still a considerable challenge in spite of the many attempts made so far (Shigeto and Tsutsumi 2016 and references therein). Molecular tools such as a transcriptome analysis and recombinant DNA in combination with biochemical and physiological approaches could be successful in addressing the specific function to particular POX gene and its protein, at least in *Arabidopsis thaliana* as a model plant. Recent work on the transcriptional regulation of a set of POXs in *A. thaliana* root by a transcription factor UPBEAT 1 (UPB1) suggested the important role of POXs in cell differentiation during root development (Tsukagoshi et al. 2010). Similarly, it has been shown that a transcription factor KUODA1 (KUA 1), which repressed the

expression of several POXs genes in the leaf of *A. thaliana* had a role in the redox homeostasis of leaf apoplast (Lu et al. 2014).

In this chapter, we give an overview of the antioxidative and pro-oxidative reactions of the multifunctional POX subfamily, with emphasis on the characterization of isoenzymes and responsible genes, the isoforms' specialization in stress defence, and role of POXs in the cross-talk between the primary and secondary antioxidants. Ascorbate and glutathione are the primary metabolites that, together with the antioxidative enzymes of ascorbate–glutathione (Asc–GSH) cycle, maintain redox homeostasis in plant cells (Foyer and Noctor 2011). On the other hand, flavonoids and other phenolic compounds, which are among numerous substrates of POXs, can constitute a 'secondary' antioxidant system that is activated upon severe stress conditions (Agati et al. 2012).

We incorporate recent findings on the subcellular distribution of POXs, phenolics, and Asc, as well as the intracellular transport of H_2O_2, which all together lead to the hypothesis that POX acts as a central H_2O_2 sink under severe stress and as a key player in the regulation of defence/secondary metabolism pathways.

Despite, POXs' numerous physiological functions and their abundance in all plant tissues and organs, their contribution in the antioxidative system has been surprisingly overlooked and disregarded in many excellent review papers on antioxidative defence and tolerance. We believe that the evidence from the literature reviewed here will incorporate POX into the complex cellular antioxidative network.

2 Reaction Mechanisms and Structure of POX Isoenzymes

2.1 Three Cycles of POXs

Class III peroxidases are oxidoreductases that catalyze the oxidation of a large number of different substrates (mainly phenolics) to corresponding radicals in the presence of H_2O_2 as an electron acceptor. In the reaction catalyzed by POX, phenoxyl radical ($PhO^{\cdot}$) is produced by the subtraction of one electron from the phenolic compound (PhOH) (reaction 1).

(1) $2PhOH + H_2O_2 \rightarrow 2PhO^{\cdot} + 2H_2O$
(2) $2PhO^{\cdot} \rightarrow$ cross-linking
(3) $PhO^{\cdot} + Asc \rightarrow PhOH + MDA^{\cdot}$
(4) $PhO^{\cdot} + MDA^{\cdot} \rightarrow PhOH + DHA$
(5) $2MDA^{\cdot} \rightarrow Asc + DHA$

In the absence of Asc, the generated $PhO^{\cdot}$ react with each other, resulting in the formation of cross-linked products (reaction 2); for example, lignin, suberin or quinones (Fry 1986; Bernards et al. 1999). One of the products is a quinhydrone-derived radical, which may be formed from caffeic and chlorogenic

acids and their quinones and hydroquinones (Takahama and Oniki 2000). The physiological function of quinhydrone as an electron-charged structure linked to the cell wall has been discussed elsewhere (Kukavica et al. 2008; Morina et al. 2010). Additionally, $PhO^{\cdot}$ (derived from monophenols, *p*-coumaric and ferulic acids) may react with Asc (reaction 3), yielding monodehydroascorbyl radical ($MDA^{\cdot}$), recovering the corresponding substrate in a non-radical way. Phenoxyl radicals can further react with $MDA^{\cdot}$ (reaction 4) to form dehydroascorbate (DHA), while Asc recovery is possible through dismutation of 2 $MDA^{\cdot}$ (reaction 5) (Takahama and Oniki 1992). As mentioned above, $PhO^{\cdot}$ can dismutate to quinones, such as *o*-dihydroxyphenolics, caffeic acid and 3,4-dihydroxyphenylalanine quinones (Takahama 1992, 1993).

Besides oxidizing various phenolic compounds during H_2O_2 reduction (peroxidatic cycle), POXs have the capacity to produce H_2O_2 via one-electron reduction of molecular oxygen (oxidative cycle), a mechanism proposed by Chen and Schopfer (1999). The oxidative cycle involves the transfer of one electron from a reducing substrate (NADH or phenolics) to O_2, with $O_2^{\cdot -}$ and H_2O_2 as intermediates. In addition, the oxidizing reaction of POX is determined by the trace amounts of dihydroxyphenols and Mn^{2+} (Halliwell 1978).

Horseradish peroxidase (HRP) is the most frequently used POX in in vitro mechanistic and kinetic studies. H_2O_2 coordinatively bonds to the iron atom and initiates the peroxidatic cycle and the oxidation of phenolic substrates to the corresponding radicals. The reduction of Compound I to Compound II, as well as Compound II's return to the resting state is carried out by reduction substrates (phenolics or aromatic amines).

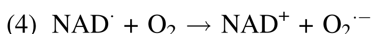
Superoxide radical converts the ferric form of the enzyme (Fe^{3+}-peroxidase) into the labile perferryl form ($Fe^{2+}-O_2{}^{-\cdot}Fe^{3+}-O_2{}^{\cdot -}$ POX), otherwise named Compound III. Compound III contains Fe^{2+} in the heme that can be converted to Fe^{3+} to act as a Fenton reagent and reduce H_2O_2 to $^{\cdot}OH$ in the hydroxylic cycle. Chen and Schopfer (1999) have demonstrated the ability of different types of POXs (e.g., unfractionated HRP mixture, acidic and alkaline HRP fractions, soybean POX) to catalyze the formation of $^{\cdot}OH$ in vitro in the presence of NADH (200 mM) as a reducing substrate. The largest capacity for generating $^{\cdot}OH$ was observed for the alkaline HRP fraction and *Arthromyces* POX, while myeloperoxidase showed the lowest capacity. The authors also showed that, beside NADH, HRP oxidizes NADPH and dihydroxyfumarate, while Asc was completely ineffective as a reducing substrate.

The proposed HRP catalyzed reactions in peroxidatic, oxidative and hydroxylic cycles in vitro with NAD(P)H acting as the exogenous reductant are presented below:

Peroxidatic cycle

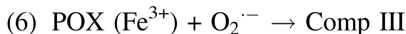
(1) POX (Fe^{3+}) + $H_2O_2 \rightarrow$ Comp I + $2H_2O$
(2) Comp I + PhOH $\rightarrow$ Comp II + $PhO^{\cdot}$
(3) Comp II + PhOH $\rightarrow$ POX (Fe^{3+}) + $PhO^{\cdot}$

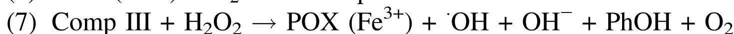
Oxidative cycle


(4) $NAD^{\cdot} + O_2 \rightarrow NAD^+ + O_2^{\cdot -}$

(5) $NADH + H^+ + O_2^{\cdot -} \rightarrow NAD^+ + H_2O_2$

Hydroxylic cycle


(6) $POX\ (Fe^{3+}) + O_2^{\cdot -} \rightarrow Comp\ III$


(7) $Comp\ III + H_2O_2 \rightarrow POX\ (Fe^{3+}) + {}^{\cdot}OH + OH^- + PhOH + O_2$

2.2 Structural Characterisation of POX Isoenzymes

Most of POXs are glycoproteins with one polypeptide chain of 300–350 amino acid residues, and their molecular weight (Mw) varies from 33–55 kDa. Though a majority of POXs are monomers, it has been shown that coconut POXs are homotetramers with a Mw of 55 kDa for each subunit (Mujer et al. 1983), POXs from *Leucaena leucocephala* are heterotrimers (consisting of two subunits of 66 kDa and one subunit of 58 kDa) (Pandey and Dwivedi 2011), while POXs from Brussels sprouts are homodimers (two subunits of 45 kDa) (Regalado et al. 1999).

According to the crystallographic and modeling studies (Ros Barceló et al. 2007), the primary protein structure of POXs consists of 10–12 conserved α-helices in which the prosthetic group is embedded, two short β-strands, and four conserved disulphide bridges (Passardi et al. 2004). There are three highly conserved domains: one is a distal heme-binding domain, the other is unknown, and the third one is a proximal heme-binding domain (Cosio and Dunand 2008). Welinder (1992) showed that all POX proteins, encoded by 73 genes in *A. thaliana*, were expressed in all organs, and they had up to 98% amino acid sequence identity.

The variation in terms of Mw of POXs isolated from different species may be attributed to the carbohydrate component bound to the polypeptide chain, as it has been reported for POXs extracted from *Brasica napus* root (Duarte-Vázquez et al. 2000). Variations in the Mw of the same enzyme may be a result of the distinct techniques used for their purification and separation (Deepa and Arumughan 2002). The different Mw of secreted POXs of cell suspension cultures of *Cassia didymobotrya* were estimated using SDS PAGE (43 kDa) and gel filtration (50 kDa) (Vitali et al. 1998). The other extracellular POX isoenzymes were isolated from the cell suspension of *Vaccinium myrtillus* (Melo et al. 1995) and *Hevea brasiliensis* (Chanwun et al. 2013) with Mws of 34 and 38 kDa for *V. myrtillus* and 70 kDa for *H. brasiliensis*.

Cellular distribution of POXs (apoplastic or vacuolar) can be predicted by the absence or the presence of the extended C-terminal peptides, which are vacuolar sorting signals. However, when the C-terminal sequence was fused to the N-terminus of the protein, no vacuolar localization was obtained (Matsui et al. 2011). According to their N-terminal signal peptides, the majority of POXs are

targeted to the endoplasmic reticulum and are considered to be secreted extracellular proteins.

Profiling POX isoforms is an important tool for studying the species-specific isoenzymes and also for the identification of the specific function of individual isoforms of POXs induced by particular stress, despite their substrate redundancy (Bernards et al. 1999; Minibayeva et al. 2015). One of the most common classifications of POXs in the literature is based on their isoelectrophoretic (IEF) mobility, and they are classified as anionic, neutral and cationic isoforms, with quite a wide range of pI values (from 2 to 11.6) (Quiroga et al. 2000; Dicko et al. 2006). A commercial HRP that is widely used for studying POX kinetics and substrate affinity consists of 42 isoforms with a pI range of 2–10 (Hoyle 1977).

Basic isoenzymes were detected in the vacuoles of mesophyll cells, and in cell suspension cultures of tobacco (Schloss et al. 1987), in grape cell suspension cultures (García-Florenciano et al. 1992), and in *Catharanthus roseus* leaves (Sottomayor et al. 1996). On the other hand, the acidic and basic POX isoforms with different substrate specificities were determined in different plants: oat coleoptile (González et al. 1999), pea root (Kukavica et al. 2012), ginkgo and birch (Kukavica and Veljović-Jovanović 2004), *Ramonda serbica* (Veljović-Jovanović et al. 2006) and maize root (Šukalović et al. 2015).

Peroxidase isozyme pattern obtained by the separation on either native or semi-native IEF is also a useful indicator of the oxidizing capacity of specific POX isoenzymes (Bolwell et al. 1998; Daudi et al. 2012; Achary 2012). However, it is difficult to relate the isozyme to the corresponding gene, since there is no obvious quantitative relationship between the transcript expression level and the POX activity (Dunand et al. 2003).

Due to different post-translational modifications (PTMs), which are crucial mechanisms of regulation of enzymatic activities in vivo, often more than one protein form originates from a particular gene (Gabaldón et al. 2007; Laugesen et al. 2007). Moreover, it has been reported that particular isoenzymes were involved in two processes, such as, for example, lignification and the oxidative burst induced by a pathogen attack (Young et al. 1995; Morimoto et al. 1999). To our knowledge, there have been relatively few in-depth reports of post-translational regulation other than glycosylation of POXs. Class III peroxidases are mostly glycosylated, differing in the number of sugar moieties and the degree of branching, as well as their arrangement along the polypeptide chain (Kim and Kim 1996; Deepa and Arumughan 2002). It has been shown that an extensive glycosylation of peroxidase isolated from latex of *Ficus benghalensis* was related to N-glycosylation of seven asparagine residues (Palm et al. 2014). Despite the identification of more potential sites for various PTMs on the primary structure of ZePrxs in *Zinnia elegans*, only the formation of N-terminal pyroglutamate residues, disulphide bridges and N-glycosylation were documented (Gabaldón et al. 2007).

The role of glycans in the structure and function of POXs has been intensively examined, and it was reported that N-glycosylation affected protein folding, catalytic activity, subcellular localisation, Km value, thermostability, proteolytic sensitivity and trafficking within the cell (Hu and van Huystee ; Sánchez-Romero

et al. 1994; Lige et al. 2001; Zhang et al. 2004). For example, N-linked glycans attached to the vacuolar POX make up approximately 20% of its Mw (Welinder 1979), and the number of N-glycosylation signals is about four times higher in vacuolar than in extracellular POX (Matsui et al. 2011). One of the most stable and the most efficient POXs is the isoform of palm tree leaves that contains 21–29% of carbohydrates (Baker et al. 2016). In some cases, glycans did not interfere with substrate binding; for example, HRP retained full activity after deglycosylation (Tams and Welinder 1995). Moreover, the only two isolated plasma membrane-bound POX (PMPOX) isoforms are also glycosylated (Mika and Lüthje 2003). Furthermore, deglycosylation of ionically bound cell wall POXs isolated from pea affected the electrophoretic mobility without any effects on its activity (Kukavica et al. 2012).

The examination of the thermostability of different POX isoenzymes gives an alternative insight into their structural properties as well as to their structure-function ratio. Carbohydrate moiety of POXs generally increases thermostability, which has also been shown for anionic cotton ovule POXs (Mellon 1991), pepper fruit acidic POXs (Pomar et al. 1997), and cationic peanut POXs (Lige et al. 2001). In addition, the variation in thermostability between the individual isoenzymes can be a consequence of the different structural organization of enzymes—namely the folding pattern into the tertiary structure of the native protein—and therefore it may be used for the identification of different isoforms. Higher temperatures (<40 °C) inactivate an enzyme's activity due to the weakening of the bonds that hold the tertiary structure (e.g., hydrogen bonds), which may also result in the dissociation of the heme prosthetic group from apoprotein (Duarte-Vázquez et al. 2000). Thermostability studies are important for improving the biotechnological application of POXs in food and the pharmaceutical industry, and environmental protection (Bansal and Kanwar 2013; Gurung et al. 2013).

Determination of the thermostability of isoforms after IEF separation, instead of treating in solution, allows for the direct determination of the temperature effect on the individual isoforms. Kukavica and colleagues (2012) reported a quite difference in the thermostability of the ionic versus the covalently bound cell wall POX (CWPOX) in pea roots.

In addition to glycans, Ca^{2+} also plays an important role in the maintenance of the structural stability and activity of POX (Marañón and van Huystee 1994; Manu and Prasada Rao 2009; Fahmy et al. 2012).

3 Substrates

Peroxidases contain variable domains and substrate access channels, which may explain their great substrate diversity (Cosio and Dunand 2008). The reductant substrates of POXs include phenolics, amines, indoles, alkaloids and sulphonates (Sottomayor et al. 2004; Veitch 2004; Ferreres et al. 2011). The most common are phenolics and their glycosides: hydroxycinnamic acids (e.g., ferulic, chlorogenic, *p*-

coumaric), hydroxybenzoic acids, hydroxyl alcohols (e.g. coniferyl, synapyl), different flavonoids (e.g. anthocyanins, flavonols), coumarins and so on (Vidović et al. 2017 and references therein). Phenolics are involved in numerous processes in plants, from growth and development to plant interaction with the environment.

However, POX activity is often determined using an artificial electron donor, such as guaiacol, yielding colored products after oxidation with H_2O_2. Of course, considering that protein extract is a mixture of isoenzymes, this estimation can be taken only as a relative change in the total POX activity under certain conditions. Thus, the activity of isoforms with specific affinity for a different substrate might be masked by the average total POX activity (Bernards et al. 1999; Kukavica et al. 2012).

Class III peroxidases may oxidize phenolic glycosides too, but at much slower rates compared with aglycones (Hirota et al. 1998; Yamasaki et al. 1997). In addition, some specific isoforms of POXs have indole 3-acetic acid (IAA) oxidase activity but it has not yet been clearly established whether such activity could be exclusively ascribed to POX (Hoyle 1977). In their early studies, Hoyle (1977) showed that all HRP isozymes had the capacity to oxidize IAA. However, Van den Berg and colleagues (1983) showed that the peanut's cationic POX isoforms had a greater specific IAA oxidase activity compared with the anionic isoforms, while other authors demonstrated the activity of both anionic and cationic isoforms (Chibbar and van Huystee 1984). Kukavica and colleagues (2012) showed that three out of five purified ionic isoenzymes CWPOX showed IAA oxidase activity, while IAA oxidase activity was not detected for covalent isoforms. Mika and Lüthje (2003) showed that maize root PMPOXs also had the IAA activity.

Table 1 gives the Michaelis-Menten constant (Km) for each substrate, which is specific to a given isoenzyme–substrate complex; for example, the lower Km indicates a greater specificity. Km is a numerical value that enables the comparison of different enzymes, whereby the various values of Km may also suggest an isoenzyme's location within the plant since it is accepted that Km determines cellular substrate concentration. Based on the Km of POX isoenzymes, it can be concluded that specificity of POXs isolated from different plant species for the same substrates varies.

The final oxidation products of POX reactions with the same phenolic compound depend on the localization of isoforms. Among extracellular POX isoforms, those bound to the cell wall matrix were proposed to participate in lignification (Sato et al. 1993), while soluble POX isoforms function as scavengers of H_2O_2. Regardless of Asc in the apoplast, CWPOX exhibited highly specific activity in the formation of cell wall structure, since the oxidation products were continuously deposited even in the presence of Asc (Takahama 2004).

Table 1 Substrate specificity (Km) of various purified and partially purified POX isoforms (e.g. soluble, membrane-bound, ionic and covalent cell wall bound, apoplastic, vacuolar, anionic, cationic)

Origin of POXs		Substrate	Km (mM)	References
Korean radish (*Raphanus raphanistrum*) root	C1 isoform	H_2O_2 *o*-dianisidine	0.19 0.81	Kim and Lee (2005)
	C3 isoform	H_2O_2 *o*-dianisidine	0.77 1.20	
	C5 isoform	H_2O_2 *o*-dianisidine	1.27 1.18	
Oil palm (*Elaeis oleifera*) leaf	Soluble peroxidase	H_2O_2 Guaiacol H_2O_2 ABTS H_2O_2 Pyrogallol	1.3 3.96 0.0038 1.00 0.0056 0.84	Deepa and Arumughan (2002)
Turnip (*Brassica napus* var. esculenta D.C.) roots	Fraction C1	H_2O_2 ABTS	0.04 1.3	Duarte-Vázquez et al. (2000)
	Fraction C2	H_2O_2 ABTS	0.245 0.710	
	Fraction C3	H_2O_2 ABTS	0.850 0.470	
Lemon (*Citrus jambhiri*) peel	Isoform POII	H_2O_2 *o*-phenylenediamine Guaiacol *o*-dianisidine Pyrogallol Catehol	0.54 2.85 5 11 23 125	Mohamed et al. (2008)

(continued)

Table 1 (continued)

Origin of POXs		Substrate	Km (mM)	References
Buckwheat (*Fagopyrum esculentum*) seeds	POXI	Quercetin *o*-dianisidine ABTS Ascorbic acid Guaiacol	0.071 0.229 n.d. 0.043 0.288	Suzuki et al. (2006)
	POXII	Quercetin *o*-dianisidine ABTS Ascorbic acid Guaiacol	0.028 0.137 0.016 0.029 0.202	
Date palm (*Phoenix dactylifera*) leaves	Membrane bound peroxidases	H_2O_2 Guaiacol	0.045 0.77	Al-Senaidy and Ismael (2011)
Pencil tree (*Euphorbia tirucalli*) latex	Cationic peroxidases	H_2O_2 Guaiacol ABTS Aminoantipyren *o*-phenylenediamine	15 4.4 0.503 25 33.4	Fahmy et al. (2012)
Utazi, milkweed (*Gongronema latifolium*)		H_2O_2 *o*-dianisidine	1.80 0.12	Joy and Eze (2015)
Maize (*Zea mays*)	PMPOX1 PMPOX2	Guaiacol Guaiacol	12.2 14.3	Mika and Lüthje (2003)
Citrus reticulata var. Kinnowpeel		H_2O_2	1.428	Nouren et al. (2013)
		Guaiacol	0.66	
Turkish black radish (*Raphanus raphanistrum* var. niger)		H_2O_2 Guaiacol	0.0083 0.036	Sisecioglo et al. (2010)

(continued)

Table 1 (continued)

Origin of POXs		Substrate	Km (mM)	References
Tomato (*Lycopersicon esculentum*) roots	pI 9.6 pI 8.2 pI 7.5 pI 6.5 pI 3.6	Syringaldazine Syringaldazine Syringaldazine Syringaldazine Syringaldazine	11.4 14.7 8.0 46.3 26.0	Quiroga et al. (2000)
Sorghum (*Sorghum bicolor*) roots	Cationic peroxidase (PO-2)	H_2O_2 ABTS 2,7-diaminofluorene *o*-dianisidine	0.023 0.71 0.144 0.750	Dubrovskaya et al. (2017)
Soybean (*Glycine max*)		H_2O_2 Guaiacol	0.58 5.9	Sessa and Anderson (1981)
Papaya (*Carica papaya*) fruit		H_2O_2 Guaiacol *o*-dianisidine Ascorbic acid	0.25 0.8 0.125 5.2	Pandey et al. (2012)
White leadtree (*Leucaena leucocephala*)		H_2O_2 Guaiacol	5.6 2.9	Pandey and Dwivedi (2011)
Zucchini comparison with HRP *Km for HRP	Anionic peroxidase (ZOPA)	Hydroquinone 4-methylcatechol Catechol *p*-hydroxybenzoic acid	0.14/0.15* 0.23/0.29* 0.33/0.35* 0.29/0.36*	Casella et al. (1993)
Broccoli stems soluble POX	Acidic Basic Neutral	H_2O_2 Guaiacol H_2O_2 Guaiacol H_2O_2 Guaiacol	0.042 0.305 0.711 0.128 8.789 9.731	Thongsook and Barrett (2005)

(continued)

Table 1 (continued)

Origin of POXs		Substrate	Km (mM)	References
Garlic (*Allium sativum*) bulb	POX_2 isoform	H_2O_2	2.0	Marzouki et al. (2005)
		Guaiacol	9.5	
Brussels sprouts (*Brassica oleracea*)	pI 4.0	ABTS	0.2	Regalado et al. (1999)
		H_2O_2	0.0114	
	pI 4.7	ABTS	0.2	
		H_2O_2	0.0062	
Wheat grass (*Triticum aestivum*)	Crude extract	*o*-phenylenediamine	2.9	Lai et al. (2006)
		Catechol	18.2	
		Pyrogallol	2.5	
		Guaiacol	3.8	
Pea (*Pisum sativum*) root	Apoplastic	H_2O_2	1.4	Kukavica et al. (2012)
		Pyrogallol	2.6	
		Chlorogenic acid	0.6	
		Caffeic acid	2.7	
		Ferulic acid	4.0	
	Ionically-bound cell wall	H_2O_2	1.3	
		Pyrogallol	2.5	
		Chlorogenic acid	1.0	
		Caffeic acid	2.4	
		Ferulic acid	1.2	
	Covalently-bound cell wall	H_2O_2	2.4	
		Pyrogallol	2.7	
		Chlorogenic acid	0.7	
		Caffeic acid	2.1	
		Ferulic acid	0.6	
	HRP	H_2O_2	1.4	
		Pyrogallol	1.1	
		Chlorogenic acid	1.6	

(continued)

Table 1 (continued)

Origin of POXs		Substrate	Km (mM)	References
		Caffeic acid Ferulic acid	0.9 1.1	
Maize (*Zea mays*) root cell wall fractions	isPOX	H_2O_2 Ferulic acid *p*-coumaric acid	0.182 0.176 0.0086	Šukalović et al. (2015)
	cEsPOX	H_2O_2 Ferulic acid *p*-coumaric acid	0 164 0.184 0.10	
	cAsPOX	H_2O_2 Ferulic acid *p*-coumaric acid	0.289 0.112 0.095	
	caPOX	H_2O_2 Ferulic acid *p*-coumaric acid	0.058 0.218 0.053	
Rosy periwinkle (*Catharanthus roseus*) leaf	Vacuoles CrPrxl basic isoform pI 8.68	Quercetin-3-*O*-arabinoside Ferulic acid Quercetin Caffeic acid Kaempferol Coniferyl aldehyde 5-*O*-caffeoylquinic acid	1.589 1.606 0.045 0.058 0.018 1.337 0.025	Ferreres et al. (2011)

4 Antioxidative Function

In addition to numerous physiological functions, the antioxidative role of POXs is based on the scavenging of H_2O_2, which accumulates in the apoplast and vacuoles (Tognolli et al. 2002; Welinder et al. 2002). Cellular trafficking of H_2O_2 is enabled and facilitated by specific members of the aquaporin family, the Tonoplast Intrinsic Proteins (TIPs) (Bienert et al. 2006; Maurel et al. 2009). Under stress condition that promote enhanced H_2O_2 accumulation in the chloroplast, which in turn overcomes the scavenging capacity of the Asc–GSH cycle, H_2O_2 may escape to the vacuole where it is scavenged (Karuppanapandian et al. 2011; Ferreres et al. 2011; Bienert and Chaumont 2014; Koffler et al. 2014). Ferreres et al. (2011) estimated that specific activity of the leaf vacuolar POX isoform from *C. roseus* represents a very efficient H_2O_2 sink of up to 9 mM s^{-1}, utilizing endogenous phenolic substrates in the vacuole. The concept of delocalized H_2O_2 scavenging (scavenging in a compartment different from its source) was proposed for the first time by Takahama (2004) based on the experiments with paraquat (Pq)-induced oxidative stress in *Vicia faba* leaves (Takahama and Oniki 1992, 1997). The same authors reported that Pq-induced accumulation of H_2O_2 in the chloroplasts of *V. faba* diffused into the vacuoles where it was utilized in the POX-dependent oxidation of 3,4-dihydroxyphenylalanine (DOPA) to melanin-like compounds (Takahama and Oniki 2000). The importance of a vacuolar PPA system as a general H_2O_2 sink for plant cells under oxidative stress has been reviewed and evaluated by Ferreres et al. (2011) and Zipor et al. (2015).

4.1 Redox Regulation of Peroxidatic Cycle and Subcellular Compartmentation

While the Asc–GSH cycle and 2-Cys peroxiredoxins (PRXs) are the major scavengers of H_2O_2 in chloroplasts, cytosol, and mitochondria, as well as catalase in peroxisomes, none of these enzymes have been found in the apoplast or the vacuole (Noctor and Foyer 2016). The only antioxidative enzymes found to be located in apoplast/cell wall compartment and vacuoles are superoxide dismutase (SOD) and POXs (Takahama 2004; Ferreres et al. 2011).

It has been reported that POXs make up 6.4% of 500 analyzed cell wall proteins (Jamet et al. 2008). About 10% of all POXs are vacuolar, but in some plants, like in *C. roseus*, this ratio may even reach 90% (Ferreres et al. 2011). The analysis of subcellular localization of 10 POXs genes from poplar labelled with a C-terminal green fluorescence protein (GFP) and transiently expressed in *Nicotiana benthamiana* showed that eight of them were targeted to vacuoles, while two were transported to the cell wall (Ren et al. 2014). In addition, proteomic studies showed that 32 POXs isoforms in *A. thaliana* were in the extracellular fractions, with 17

embedded in the plasma membrane, while seven were in the vacuole-enriched fractions (Francoz et al. 2015).

Vacuolar POXs account for more than 90% of soluble guaiacol peroxidase activity of bulk leaf extract, while the rest is attributed to ionically and covalently bound fractions originating from the cell wall. According to the concept of subcellular compartmentation of the antioxidative system, POXs' endogenous substrates predominately accumulate in the vacuoles and apoplast (reviewed in Vidović et al. 2017). Indeed, upon biosynthesis, phenolic compounds are transported to the vacuole or apoplast, either conjugated with GSH (by glutathione-S-transferase: GST), esterified with malonate, or glycosylated (Kitamura 2006; Zhao 2015). Depending on the conjugation type, flavonoids and anthocyanins are transferred into the vacuole through specific transporters on the tonoplast, such as, ATP-binding cassette (ABC) transporters, multidrug resistance-associated proteins (MRPs; preferentially glutathione-flavonoid complexes), and multidrug and toxic compound extrusion proteins (MATE, preferentially glycosides) (Petrussa et al. 2013; Vidović et al. 2017 and references therein). However, this compartmentation of POXs' endogenous substrate is specific. Thus, kaempferol, quercetin, and the indole alkaloids were found in the vacuole (Sottomayor et al. 1998; Harborne and Williams 2000; Marinova et al. 2007), while ferulic acid and coniferyl aldehyde were targeted to the apoplastic cell wall compartment (Ros Barceló et al. 2004).

Although Asc transport across plasma membranes has been described previously (Horemans et al. 2000), the specific transporters that carry Asc have not been identified at the molecular level in plants so far (Maurino et al. 2006). It has been proposed that DHA was exclusively transferred through the plasma membrane via different transporters than the glucose transporters (Fernie and Tóth 2015). It has also been suggested that Asc uptake in some plant cells is an active process that requires the presence of a proton-electrochemical gradient across the plasma membrane. During the last decade, the involvement of the nucleobase-Asc transporter (NAT) family proteins in Asc transport through plasma membrane was proposed and studied (Girke et al. 2014; Pick and Weber 2014). To date, 12 members of the NAT family have been identified and molecularly characterized in *A. thaliana*, rice and tomato (Maurino et al. 2006; Cai et al. 2014; Niopek-Witz et al. 2014). Trans-membrane Asc-driven electron transport is catalyzed by cytochromes (Cyts) b561. The Asc reducible Cyt b561 is present in the plasma membrane (Horemans et al. 1994; Asard et al. 2001) and in tonoplasts (Griesen et al. 2004).

The Asc localization in the apoplast and vacuoles, concomitantly with the absence of GSH in apoplast and very low concentration of highly oxidized glutathione pool in the vacuole (<0.03 mM; <10%) (Noctor and Foyer 2016 and references therein), justifies Asc function as the main reductant involved in the PPA system (Takahama 2004). Besides biochemical approaches, Asc in the vacuoles was visualized using transmission electron microscopy after immunocytochemical labeling (reviewed in Zechmann 2017), and concentration around 2 mM was revealed. Compared with vacuolar Asc, where it is mostly in the reduced form (Ferreres et al. 2011), Asc in the apoplast is mostly present in its oxidized form,

DHA. In the apoplast, Asc plays a crucial role in plant growth, development and defence against pathogens, drought and high ozone levels (Veljovic Jovanovic et al. 2018). Apoplastic Asc is also involved in the redox regulation of antioxidant enzymes, modifications in plant growth through changes in the hormone synthesis and MAPK activity, as well as changes in calcium signaling (Liso et al. 2004; Karpinska et al. 2017).

Numerous reports show that all three constituents of PPA, phenolics, POXs and Asc, were targeted to vacuoles under high light conditions (Neill and Gould 2003; Pollastri and Tattini 2011; Ferreres et al. 2011; Heyneke et al. 2013; Zipor and Oren-Shamir 2013) as well as during early response to drought (Koffler et al. 2014). Although it is lower than in other compartments (chloroplasts, peroxisomes, mitochondria) (Zechmann et al. 2011), a strong enhancement of vacuolar Asc content has been observed in *A. thaliana* exposed to high light, drought and after inoculation with *Pseudomonas syringae* (Großkinsky et al. 2012; Heyneke et al. 2013; Koffler et al. 2014). In addition, the redox state of apoplastic Asc has a significant role in tolerance to high light stress (Karpinska et al. 2017). Recycling of Asc from $MDA^{\cdot}$ and DHA takes place on the plasma membrane and tonoplast through the cytoplasmic Asc–GSH cycle (Fig. 1). As previously mentioned, $MDA^{\cdot}$ generated in the vicinity of plasma membrane may accept electrons from membrane-bound Cyt b561 (Asard et al. 2001) and form Asc and DHA independently, or accept electron by the action of membrane-bound MDAR (Drazkiewicz et al. 2003). The DHA is further transported (see the previous section) to the cytoplasm where it is reduced to Asc in Asc–GSH cycle (Horemans et al. 2000).

Taking into the account the light-induced accumulation of all three constituents of the PPA system in vacuoles and apoplast, Km values for endogenous POX substrates, and the relative volume of these compartments (50–55% of the total mesophyll cell volume; Vidović et al. 2016), the PPA system may represent an important sink for excess H_2O_2 under high light stress.

5 Pro-oxidative Functions

5.1 H_2O_2-Producing System (Oxidative Cycle)

In response to various unfavourable conditions, including high levels of ozone, wounding, and infection by pathogens, apoplastic POXs can act as a source of ROS, contributing to the oxidative burst (Doke 1983; Bolwell et al. 1998; Gill and Tuteja 2010).

The involvement of CWPOXs (besides plasma membrane NADPH oxidase) was confirmed using the heme inhibition studies with salicylhydroxamic acid (SHAM), NaN_3, and KCN, and inhibitors of NAD(P)H oxidase: diphenyleneiodonium chloride (DPI) and imidazole (IMZ). In addition, treatments with elicitors and pathogen inoculation, besides H_2O_2, trigger the $^{\cdot}OH$ and $O_2^{\cdot-}$ production in

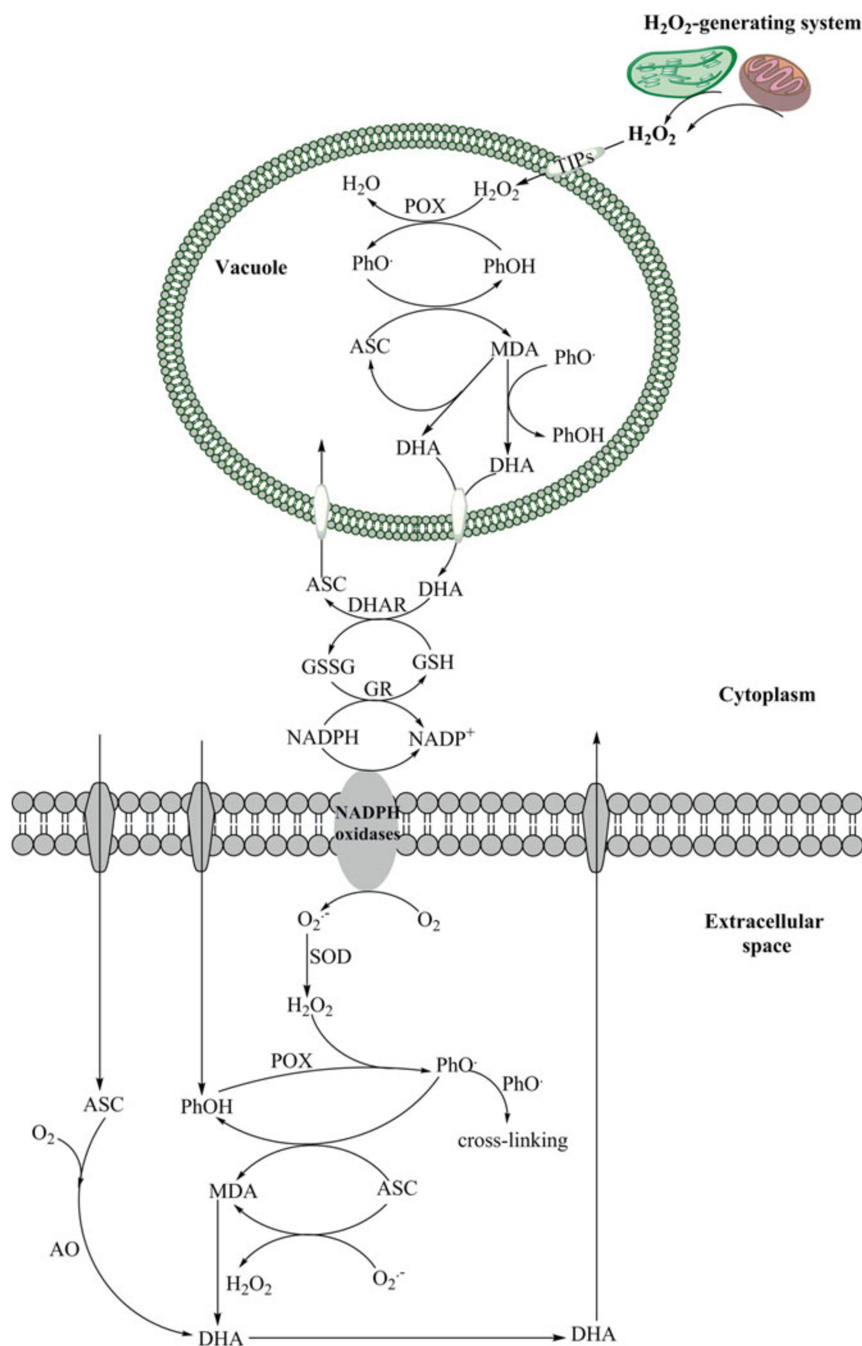


Fig. 1 Schematic overview of the vacuolar and apoplastic H_2O_2 scavenging mechanism by the POX/PhOH/Asc system. Explanation in the text. POX class III peroxidases, DHAR dehydroascorbate reductase, GR glutathione reductase, SOD superoxide dismutase, AO ascorbate oxidase

cultured plant cells, protoplasts and tissues (Bestwick et al. 1998; Choi et al. 2007). The oxidative cycle of POX is considered to be an $O_2^{\cdot -}$-generating system and a source of H_2O_2 required for cell wall stiffening, and for the formation of the secondary cell wall by lignification (Halliwell 1978). According to the proposed reaction mechanism, in the absence of H_2O_2 and the presence of a suitable reductant, the intermediate catalytically inactive form of the POX (Compound III, Fig. 2) may oxidize NADH. In this reaction, PhOH returns Compound III to the ground state, making the enzyme functional again, while Mn^{2+}, as a second cofactor, catalyzes the non-enzymatic reaction between $O_2^{\cdot -}$ and NADH to produce H_2O_2, directing the reaction to peroxidatic cycle (Halliwell 1978).

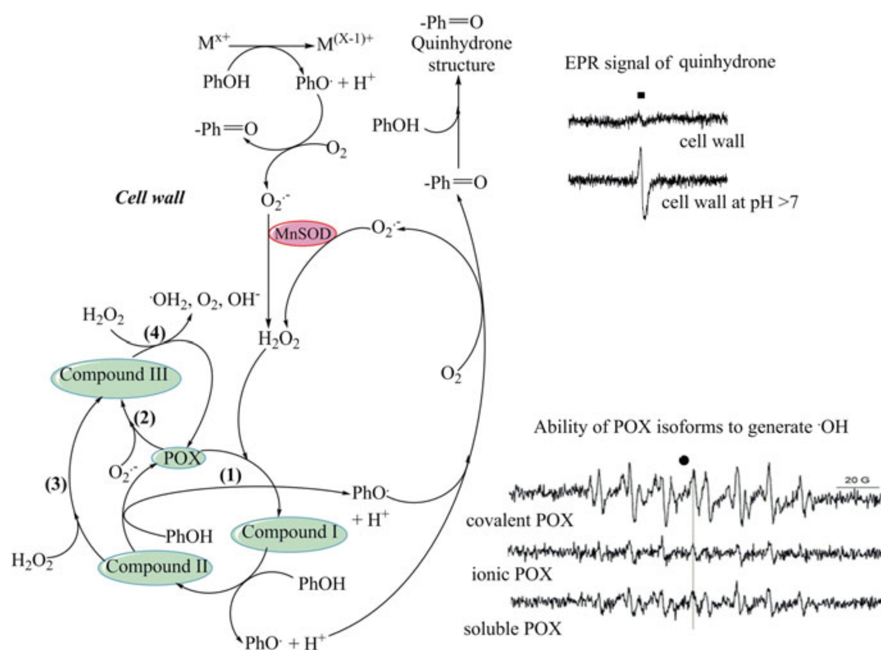


Fig. 2 Proposed model of $^{\cdot}$OH generation in the pea root cell wall (Adopted from Kukavica et al. 2008). In the presence of metal ions PhOH and MnSOD, cell wall-bound POX catalyze the formation of $^{\cdot}$OH through the following reactions: (1) $POX\,(Fe^{3+}) + H_2O_2 \rightarrow Comp\,I\,(Fe^{4+}{=}O)^{+} + H_2O_2$; (2) $POX\,(Fe^{3+}) + O_2^{\cdot -} \rightarrow Comp\,III\,(Fe^{2+}{-}O_2)$; (3) $Comp\,II\,(Fe^{4+}{=}O) + H_2O_2 \rightarrow Comp\,III\,(Fe^{2+}{-}O_2) + H_2O$; (4) $Comp\,III\,(Fe^{2+}{-}O_2) + H_2O_2 \rightarrow POX\,(Fe^{3+}) + {}^{\cdot}OH + O_2 + OH^{-}$. M^{x+} and $M^{(x-1)+}$ oxidized and reduced metal ions, PhOH phenolics compounds, PhO$^{\cdot}$ phenoxyl radical, –Ph=O quinhydrone. The upper panel represents the electron paramagnetic resonance (EPR) spectra of the quinhydrone structure detected without the presence of spin trap in the pea root cell wall. Basic pH increased the signal intensity of quinhydrone. The bottom panel represents the EPR spectra of the DEMPO/OH adduct produced by POX covalently bound to the cell wall, the ionic POX and soluble intracellular POX. Adopted from Kukavica et al. 2012.

The exclusive involvement of the cell wall in the $O_2^{\cdot-}$-production system has been reported for the pea and cowpea during plant-microbe interactions (Kiba et al. 1997) and upon metal toxicity, such as Al excess in the onion root (Achary et al. 2012) or Mn excess in cowpea leaf (Fecht-Christoffers et al. 2006). Kiba and colleagues (1997) also showed that the $O_2^{\cdot-}$-production system of the same plant species induced by the elicitor, was inhibited by POX inhibitor, SHAM, and not by NADPH oxidase inhibitors (i.e., imidazole and quinacrine).

5.2 Hydroxyl Radical-Generating System (Hydroxylic Cycle)

The capacity of POXs to generate $^{\cdot}OH$ has been documented by a number of in vitro and in vivo studies. Several enzymatic and non-enzymatic mechanisms can be found in the literature explaining the cell wall loosening required for extension and cell growth (Fry 1998; Chen and Schopfer 1999; Liszkay et al. 2003; Kukavica et al. 2007, 2008). Cell wall loosening is important under osmotic, drought or salt stress in order to ensure the possibility of cells and organs to expand (Tenhaken 2014). Furthermore, it has been reported that POX can produce $^{\cdot}OH$ in the presence of NADH (Liszkay et al. 2003; Schopfer et al. 2001).

The proposed mechanism for the oxidative cycle of cell wall-bound POXs is based on the oxidation of NADH only in the presence of *p*-coumaric acid and Mn^{2+}, while, in the absence of these two cofactors, oxidation of NADH is negligible. However, there is no evidence for the presence of NAD(P)H in the apoplastic compartment, suggesting that $^{\cdot}OH$ generation using NADH is unlikely under natural conditions. Instead, plasma membrane-bound NAD(P)H oxidase is regarded as a crucial enzyme responsible for the generation of $O_2^{\cdot-}$ in the apoplast (Murphy and Auh 1996; Van Gestelen et al. 1997). Moreover, various cellular components are capable of generating $O_2^{\cdot-}$ in the vicinity of plasma membranes (Mojović et al. 2004). An extreme reactivity of $O_2^{\cdot-}$ (half-life in water is 0.2 and 20 ms at 10 and 1 μM, respectively, Bielski et al. 1985) is even more accelerated in the presence of SOD. Therefore, it is doubtful that the membrane-bound NAD(P)H oxidase is a source of $^{\cdot}OH$ for cell wall reactions. Kukavica et al. (2008) demonstrated that isolated cell wall free from plasma membrane had the capacity for NADH-independent generation of $^{\cdot}OH$, and suggested a similar mechanism in situ. In isolated cell walls of pea roots, $^{\cdot}OH$ was detected using a spin-trapping reagent DEPMPO in the absence of any exogenous compounds (Fig. 2). The alternative mechanism for $O_2^{\cdot-}$ formation in the pea cell wall isolates involved the oxidation of hydroxycinnamic acids by redox active metals, Fe^{3+} and Cu^{2+}, to phenoxyl radicals, which can reduce O_2 to $O_2^{\cdot-}$. Both, caffeic and chlorogenic acid are found in apoplastic fluid and cell wall isolates, and are easily auto-oxidizable yielding either a charge-transfer complex, quinhydrone, or polymers that are also auto-oxidizable. Once generated, $O_2^{\cdot-}$ may induce the formation of CWPOX Compound III, which can enter the hydroxylic cycle yielding $^{\cdot}OH$ and H_2O_2. The cell wall-bound MnSOD can regulate the concentration of $O_2^{\cdot-}$ and $^{\cdot}OH$, while apoplastic H_2O_2 can

catalyze the formation of $^{\cdot}OH$ via the Fenton reaction (Yim et al. 1993). An overview of the whole mechanism proposed for cell wall generation of $^{\cdot}OH$ is given in Fig. 2.

The authors demonstrated an exclusive role of covalently CWPOX in the DEPMPO/OH adduct formation (Fig. 2). During the generation of $^{\cdot}OH$, the concentration of quinhydrone structures (as detected by EPR spectroscopy) increased, suggesting that H_2O_2 required for the formation of $^{\cdot}OH$ in isolated cell walls was produced during the oxidation of hydroxycinnamic acids, which form a quinhydrone dimer.

6 Effects of Environmental Stresses on POXs

Induction of overall peroxidase activity is widely accepted as an indicator of abiotic and biotic stress response in plants. In addition to the measurements of guaiacol peroxidase activity of the whole tissue extract or cell wall isolates, numerous studies have revealed differences in POXs isoform profiles. These variations may correspond either to a stimulation or attenuation of some pre-existing isoforms, or to the appearance of the new isoforms (Kukavica et al. 2012; Liu et al. 2013). Such modulations of POXs profile patterns prove their role in the defence mechanism. The differences in POXs profile patterns are also related to the plant species, the type, intensity and the duration of stress. Sometimes a contrasting response of POX isoforms to stress was demonstrated, such as in the case of ionic and covalently cell wall-bound isoforms in pea roots induced by elicitor chitosan (Kukavica et al. 2012).

The expression of POX genes is regulated in response to biotic and abiotic stresses and the underlying molecular mechanism is related to the nature of the 5′ flanking regions with stress-responsive *cis*-elements (Sasaki et al. 2007; Kim et al. 2012). However, POX gene expression patterns show great variations, and the stress-induced upregulation depends on the developmental stage and organs. According to Cosio and Dunand (2008), at least 19 *AtPrx*s genes were involved in the specific abiotic stress mechanisms. However, the stimulating effect on POX activity was not always obvious.

Since it has been shown that POXs are quite sensitive to atmospheric pollution and heavy metals, the measurements of their activities have been widely used for the phytomonitoring of industrial and urban areas (Cho and Park 2000; Klumpp et al. 2000; Wu and von Tiedemann 2002; Geebelen et al. 2002). It has been shown that induction of POX activity was higher in metal-sensitive species/populations compared with tolerant ones (Tamás et al. 2002; Morina et al. 2016). Cadmium treatment increased the accumulation of lignin and apoplastic guaiacol peroxidase activities to a higher extent in the sensitive compared with the resistant cultivars of *V. faba*. In addition, ten *ZmPrxs* genes were altered (seven of them were down and three were upregulated) in the stress response to Cd (Yue et al. 2017). Tao and colleagues (2013) suggested a synergistic action between salicylic acid and POXs

in the *snc1* (suppressor of non-expressor of pathogenesis-related gene 1 (*npr1-1*) constitutive 1) plant leading to its sensitive phenotype under metal excess. Native PAGE of cell wall proteins of *Cassia tora* exposed to Al stress showed a strong induction of POXs (Xue et al. 2008). The expression of *AtPrx64* enhanced root growth and decreased the accumulation of Al and ROS in the roots of transgenic plants in comparison with WT plants (Wu et al. 2017).

The influence of temperature variation and water status on POX activity has been also demonstrated. Exposure to cold stress gradually increased POX activities in the leaves of several *Medicago* accessions at the beginning of stress; however, POX activity decreased with prolonged stress duration (Nourredine et al. 2015). Nourredine and coauthors (2015) also showed that the decrease in POD activity was less in tolerant than in sensitive plants. Similar results were obtained for naked oat plants (Liu et al. 2013). The decrease of POX activity under long-term exposure to cold stress indicated that low temperatures might affect RNA transcription, and consequently translation, reducing the POX synthesis (Liu et al. 2013). It was also shown that two POX isoforms (TaPrx04 and TaPrxo3) from an apical root segment were responsible for drought tolerance in two wheat cultivars (Csiszár et al. 2012). In addition, seven isoforms of POXs from *Tamarix hispida* were either upregulated or downregulated upon NaCl, polyethylene glycol (PEG), $NaHCO_3$ and Cd stress (Gao et al. 2010). Five *ZmPrxs* genes (Wang et al. 2015), and six *POXs* from sweet potato (*Ipomoea batatas*) responded to different abiotic stresses (H_2O_2, SA, NaCl and PEG treatment) (Kim et al. 2007). The POX isoform, MsPrx16, from alfalfa (*Medicago sativa*) showed decreased expression under cold stress as a part of a general strategy of root cell walls to maintain flexibility under temperature stress (Behr et al. 2015). In addition, the dominant PMPOX from maize, ZmPrx66, was downregulated and two others were upregulated by H_2O_2 stress (Mika et al. 2009). After a short time of flooding (4 h), the neutral form of soluble POX (pI 7.0) of maize was downregulated while long-time flooding (28 or 52 h) resulted in the upregulation of alkaline POXs (pI 9.2, 8 and 7.8) (Meisrimler et al. 2014).

In contrast to abiotic stress, a complex role of POXs in biotic stress is based on peroxidatic and pro-oxidative catalytic action (Sects. 4.1 and 5) and possible generation and detoxification of ROS, though a reductant for the formation of the 'oxidative burst' has not yet been identified. They are induced in host plant tissues by pathogen infection and belong to the pathogen-related protein 9 subfamily (van Loon et al. 2006). POXs are crucial for the establishment of structural barriers to limit pathogen invasion or the generation of extremely toxic ROS and RNS (Passardi et al. 2005). It has been shown that POX activity or POX gene expression in higher plants was induced by fungi, bacteria, viruses and viroids (references in Almagro et al. 2009). The activity of NADH-peroxidases was involved in the generation of $O_2^{\cdot -}$ and H_2O_2, and $^{\cdot}OH$ in response to biotic stress as was shown in many plants: in pea and cowpea in response to a pathogen (Kiba et al. 1997); in cultured cells of rose and French bean in response to an elicitor derived from the cell walls of *Colletotrichum lindemuthianum* (Bolwell et al. 1998); in cotton cotyledons in response to a hypersensitive reaction to *Xanthomonas campestris* (Martinez et al. 1998); and in lettuce leaves during the non-host hypersensitive

reaction induced by *P. syringae* (Bestwick et al. 1998). *Lycopersicum esculentum* POX (Prxs06) was induced at infection site by accumulating H_2O_2 (Coego et al. 2005). The extracellular wheat isoforms were upregulated by wounding stress (Minibayeva et al. 2015).

As previously mentioned, assigning a function to a particular POX isoform is a rather complex task and requires the integration of physiological, biochemical and genetic studies, especially regarding the possible use for generation of useful transgenic plants (Sasaki et al. 2007).

7 Genetic Manipulation of POX Isoenzymes Related to Plant Defence Against Environmental Stress Conditions

A number of studies on transgenic plants with altered expression of genes of POXs were carried out with the aim to understand the specific role of individual isoenzymes under unfavourable environmental conditions. Overexpression of sweet potato *swpa4* POX gene in tobacco plants (*Nicotiana tabacum*) (Kim et al. 2008) and overexpression of *AtPrx3* in *A. thaliana* significantly increased salinity and drought tolerance (Llorente et al. 2002). Moreover, it has been shown that the overexpression of *AtPrx22*, *AtPrx39*, and *AtPrx69* increased cold tolerance in the brassinosteroid-insensitive mutants (Kim et al. 2012). The same results—increased germination rate under salt and dehydration, and decreased sensitivity to cold stress—were shown in the experiments with heterologous expression of two POX genes from *C. roseus* in *N. tabacum* (Kumar et al. 2012). The knockdown of *AtPrx33* and *AtPrx34* transcripts by transduction of antisense *FBP1* from *Phaseolus vulgaris* into *A. thaliana* resulted in a failed oxidative burst and an increased sensitivity to fungal and bacterial pathogens (Daudi et al. 2012). In addition, transgenic expression of POX2 from *Capsicum annuum* (*CaPrx2*) in *A. thaliana* (Choi et al. 2007) enhanced a broad spectrum resistance (pathogenesis-related gene induction) and H_2O_2 accumulation, as well as tolerance to drought and salt. However, there are no data about changing the phenotype by knockout of simple vacuolar *POXs* genes.

The overexpression of *AtPrx* in transgenic tobacco plants enhanced root growth under Al excess and decreased the accumulation of Al in the roots (Wu et al. 2017). The role of specific POX isoforms in growth inhibition was revealed in a genetic study in which AtPRX71 expression was suppressed and the phenotype had a bigger rosette and biomass. On the other hand, a retarded growth of the *35S:AnPGII* plants was accompanied by a high activity level of POXs (Raggi et al. 2015). Transgenic plants with antisense suppression of *Pry60* in tobacco had a significant reduction in lignin content (Blee et al. 2003). Similarly, overexpression of *AtPRX37* caused dwarfism, probably by affecting cell expansion and not cell division (Pedreira et al. 2011). The underlying inhibitory mechanism of these isoforms on cell expansion is proposed to be the promotion of H_2O_2 generation. However, the

qua2-1 atprx71-1 double mutant showed decreased ROS accumulation that can be explained by the involvement of the 71 and 53 genes in the cross-linking of cell wall in the hydroxylic POX cycle (Raggi et al. 2015).

8 Conclusion

Class III peroxidases are a ubiquitous, multigene family of secretory enzymes, localized in all plant organs, which is in accordance with their well-established role in plant growth and development, as well as in plant–environment interactions. Their role in stress-related processes (e.g., oxidative burst, signaling, cell wall re-arrangement and antioxidative defence) is determined by (i) the cellular distribution of the specific substrates and isoenzymes; (ii) PTMs (especially the glycosylation pattern) of isoenzymes; (iii) the microenvironment such as pH, Ca^{2+}, Mn^{2+} concentration, Asc redox state; and (iv) POXs' bifunctionality—namely, scavenging/generating H_2O_2 activities. The mechanisms underlying these processes have not yet been fully established and remain a challenge for future research. In the light of recent findings related to H_2O_2 intracellular trafficking and the subcellular localization of the constituents of Takahama's H_2O_2 scavenging system (POX/Phe/Asc), we re-established the antioxidative role of POXs incorporating it in a perfectly orchestrated complex cellular antioxidative system (Fig. 2). We propose that, in this way, vacuolar POXs are the important sink for H_2O_2 in the plant cell, taking into account its size and the presence of millimolar concentrations of phenolics and Asc, which emphasize the key role of the vacuole in the cellular antioxidant network. Taking all of this into account, POXs can be regarded as a crossover point in metabolism involved in growth and defence regulation by redirecting phenolics to the cell wall and, finally, in adaptation to stress. Due to the more oxidized state and the low redox buffering in the apoplast, POXs located in apoplast/cell wall compartment may have different properties compared with those in the vacuole, such as contribution to cell wall loosening and elongation or, on the other hand, to an oxidative burst.

Further studies including molecular approaches, such as a transcriptome analysis and recombinant DNA, should be conducted in order to reveal the specific functions of particular POX isoforms and to assign a specific function to a particular POX gene and its protein. Comprehensive knowledge of the link between genes, primary structure and PTMs will provide new solutions for developing and engineering plants with improved vigor and stress tolerance. A range of biotechnological approaches are being employed for the manipulation of lignin content for increased stress tolerance, as well as for optimal utilization of plant biomass in different branches of industry.

Acknowledgements This work was supported by the Ministry of Education, Science, and Technological Development of the Republic of Serbia (Project No. III 43010).

References

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

Achary VMM, Parinandi NL, Panda BB (2012) Aluminum induces oxidative burst, cell wall NADH peroxidase activity, and DNA damage in root cells of *Allium cepa* L. Environ Mol Mutagen 53:550–560

Agati G, Azzarello E, Pollastri S, Tattini M (2012) Flavonoids as antioxidants in plants: location and functional significance. Plant Sci 196:67–76

Al-Senaidy AM, Ismael MA (2011) Purification and characterization of membrane-bound peroxidase from date palm leaves (*Phoenix dactylifera* L.). Saudi J Biol Sci 18:293–298

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

Almagro L, Gómez Ros LV, Belchi-Navarro S, Bru R, Ros Barceló A, Pedreno MA (2009) Class III peroxidases in plant defence reactions. J Exp Bot 60:377–390

Asard H, Kapila J, Verelst W, Bérczi A (2001) Higher-plant plasma membrane cytochrome b561: a protein in search of a function. Protoplasma 217:77–93

Baker MR, Tabb DL, Ching T, Zimmerman LJ, Sakharov IY, Li QX (2016) Site-specific N-glycosylation characterization of windmill palm tree peroxidase using novel tools for analysis of plant glycopeptide mass spectrometry data. J Prot Res 15:2026–2038

Bansal N, Kanwar SS (2013) Peroxidase (s) in environment protection. Sci World J Article ID: 714639

Barceló AR, Ros LG, Gabaldón C, López-Serrano M, Pomar F, Carrión JS, Pedreño MA (2004) Basic peroxidases: the gateway for lignin evolution? Phytochem Rev 3:61–78

Behr M, Legay S, Hausman JF, Guerriero G (2015) Analysis of cell wall-related genes in organs of *Medicago sativa* L. under different abiotic stresses. Int J Mol Sci 16:16104–16124

Bernards MA, Fleming WD, Llewellyn DB, Priefer R, Yang X, Sabatino A, Plourude GL (1999) Biochemical characterization of suberization-associated anionic peroxidase of potato. Plant Physiol 121:135–145

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

Bielski BHJ, Cabelli DE, Arudi RL (1985) Reactivity of HO_2/O_2^- radicals in aqueous solution. J Phys Chem Ref D 14:1041–1100

Bienert GP, Chaumont F (2014) Aquaporin-facilitated transmembrane diffusion of hydrogen peroxide. Biochim Biophys Acta-Gen Subj 1840:1596–1604

Bienert GP, Schjoerring JK, Jahn TP (2006) Membrane transport of hydrogen peroxide. Biochim Biophys Acta Biomembr 1758:994–1003

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

Bolwell GP, Davies DR, Gerrish C, Auh CK, Murphy TM (1998) Comparative biochemistry of the oxidative burst produced by rose and French bean cells reveals two distinct mechanisms. Plant Physiol 116:1379–1385

Cai X, Ye J, Hu T, Zhang Y, Ye Z, Li H (2014) Genome-wide classification and expression analysis of nucleobase–ascorbate transporter (NAT) gene family in tomato. Plant Growth Reg 73:19–30

Casella L, Gullotti M, Poli S, Ferrari RP, Laurenti E, Marchesini A (1993) Purification, characterization and catalytic activity of anionic zucchini peroxidase. Biometals 6:213–222

Chanwun T, Muhamad N, Chirapongsatonkul N, Churngchow N (2013) *Hevea brasiliensis* cell suspension peroxidase: purification, characterization and application for dye decolorization. AMB Express 3:14

Chen S, Schopfer P (1999) Hydroxyl-radical production in physiological reactions. A novel function of peroxidase. Eur J Biochem 260:726–773

Cheynier V, Comte G, Davies KM, Lattanzio V, Martens S (2013) Plant phenolics: recent advances on their biosynthesis, genetics, and ecophysiology. Plant Physiol Biochem 72:1–20

Chibbar RN, van Huystee RB (1984) Characterization of peroxidase in plant cells. Plant Physiol 75:956–958

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

Choi HW, Kim YJ, Lee SC, Hong JK, Hwang BK (2007) Hydrogen Peroxide generation by the pepper extracellular Peroxidase $CaPO_2$ activates local and systemic cell death and defense response to bacterial pathogens. Plant Physiol 145:890–904

Coego A, Ramirez V, Ellul P, Mayda E, Vera P (2005) The H_2O_2-regulated Ep5C gene encodes a peroxidase required for bacterial speck susceptibility in tomato. Plant J 42:283–293

Cosio C, Dunand C (2008) Specific functions of individual class III peroxidase genes. J Exp Bot 60:391–408

Csiszár J, Gallé A, Horváth E, Dancsó P, Gombos M, Váry Z, Erdei L, Györgyey J, Tari I (2012) Different peroxidase activities and expression of abiotic stress-related peroxidases in apical root segments of wheat genotypes with different drought stress tolerance under osmotic stress. Plant Physiol Biochem 52:119–129

Daudi A, Cheng Z, O'Brien JA, Mammarella N, Khan S, Ausubel FM, Bolwell GP (2012) The apoplastic oxidative burst peroxidase in Arabidopsis is a major component of pattern-triggered immunity. Plant Cell 24:275–287

Deepa SS, Arumughan C (2002) Purification and characterization of soluble peroxidase from oil palm (*Elaeis guineensis* Jacq.) leaf. Phytochemistry 61:503–511

Dicko MH, Gruppen H, Hilhorst R, Voragen AG, van Berkel WJ (2006) Biochemical characterization of the major sorghum grain peroxidase. FEBS J 273:2293–2307

Doke N (1983) Involvement of superoxide anion generation in the hypersensitive response of potato tuber tissues to infection with an incompatible race of *Phytophthora infestans* and to the hyphal wall components. Physiol Plant Pathol 23:345–357

Drazkiewicz M, Skórzyńska-Polit E, Krupa Z (2003) Response of the ascorbate–glutathione cycle to excess copper in *Arabidopsis thaliana* (L.). Plant Sci 164:195–202

Duarte-Vázquez MA, García-Almendárez B, Regalado C, Whitaker JR (2000) Purification and partial characterization of three turnip (*Brassica napus* L. var. *esculenta* DC) Peroxidases. J Agric Food Chem 48:1574–1579

Dubrovskaya E, Pozdnyakova N, Golubev S, Muratova A, Grinev V, Bondarenkova A, Turkovskaya O (2017) Peroxidases from root exudates of *Medicago sativa* and *Sorghum bicolor*: catalytic properties and involvement in PAH degradation. Chemosphere 169:224–232

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

Fahmy AS, Salem AM, Abd MMS (2012) Role of calcium in enhancing the activity and thermal stability of a new cationic peroxidase purified from *Euphorbia tirucalli latex*. Egypt J Biochem Mol Biol 30:245–268

Fecht-Christoffers MM, Führs H, Braun HP, Horst WJ (2006) The role of hydrogen peroxide-producing and hydrogen peroxide-consuming peroxidases in the leaf apoplast of cowpea in manganese tolerance. Plant Physiol 140:1451–1463

Fernie AR, Tóth SZ (2015) Identification of the elusive chloroplast ascorbate transporter extends the substrate specificity of the PHT Family. Mol Plant 8:674–676

Ferreres F, Figueiredo R, Bettencourt S, Carqueijeiro I, Oliveira J, Gil-Izquierdo A, Sottomayor M (2011) Identification of phenolic compounds in isolated vacuoles of the medicinal plant *Catharanthus roseus* and their interaction with vacuolar class III peroxidase: an H_2O_2 affair? J Exp Bot 62:2841–2854

Foyer CH, Noctor G (2011) Ascorbate and glutathione: the heart of the redox hub. Plant Physiol 155:2–18

Francoz E, Ranocha P, Nguyen-Kim H, Jamet E, Burlat V, Dunand C (2015) Roles of cell wall peroxidases in plant development. Phytochemistry 112:15–21

Fry SC (1986) Cross-linking of matrix polymers in the growing cell walls of angiosperms. Ann Rev Plant Physiol 37:165–186

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

Gabaldón C, Gómez-Ros LV, Núñez-Flores MJL, Esteban-Carrasco A, Ros Barceló AR (2007) Post-translational modifications of the basic peroxidase isoenzyme from *Zinnia elegans*. Plant Mol Biol 65:43–61

Gao C, Wang Y, Liu G, Wang C, Jiang J, Yang C (2010) Cloning of Ten Peroxidase (POD) Genes from *Tamarix hispida* and characterization of their responses to abiotic stress. Plant Mol Biol Rep 28:77–89

García-Florenciano E, Calderón AA, Muñoz R, Ros A (1992) The decarboxylative pathway of indole-3-acetic acid catabolism is not functional in grapevine protoplasts. J Exp Bot 43:715–721

Gazaryan IG, Lagrimini LM, Ashby GA, Thorneley RN (1996) Mechanism of indole-3-acetic acid oxidation by plant peroxidases: anaerobic stopped-flow spectrophotometric studies on horseradish and tobacco peroxidases. Biochem J 313:841–847

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

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930

Girke C, Daumann M, Niopek-Witz S, Möhlmann T (2014) Nucleobase and nucleoside transport and integration into plant metabolism. Front Plant Sci 5:443

González LF, Perez F, Rojas MC (1999) Indole-3-acetic acid Control on acidic oat cell wall peroxidases. J Plant Growth Regul 18:25–31

Griesen D, Su D, Bérczi A, Asard H (2004) Localization of an ascorbate-reducible cytochrome b561 in the plant tonoplast. Plant Physiol 134:726–734

Großkinsky DK, Koffler BE, Roitsch T, Maier R, Zechmann B (2012) Compartment-specific antioxidative defense in Arabidopsis against virulent and avirulent *Pseudomonas syringae*. Phytopathology 102:662–673

Gurung N, Ray S, Bose S, Rai V (2013) A broader view: microbial enzymes and their relevance in industries, medicine, and beyond. BioMed Res Int 2013:1–18

Halliwell B (1978) Lignin synthesis: the generation of hydrogen peroxidase and superoxide by horseradish peroxidase and its stimulation by manganese (II) and phenols. Planta 140:81–88

Harborne JB, Williams CA (2000) Advances in flavonoid research since 1992. Phytochemistry 55:481–504

Heyneke E, Luschin-Ebengreuth N, Krajcer I, Wolkinger V, Müller M, Zechmann B (2013) Dynamic compartment specific changes in glutathione and ascorbate levels in Arabidopsis plants exposed to different light intensities. BMC Plant Biol 13:104

Hirota S, Shimoda T, Takahama U (1998) Tissue and spatial distribution of flavonol and peroxidase in onion bulbs and stability of flavonol glucosides during boiling of the scales. J Agric Food Chem 46:3497–3502

Horemans N, Asard H, Caubergs RJ (1994) The role of ascorbate free-radical as an electron acceptor to cytochrome b-mediated trans-plasma membrane electron transport in higher plants. Plant Physiol 104:1455–1458

Horemans N, Foyer CH, Asard H (2000) Transport and action of ascorbate at the plant plasma membrane. Trend Plant Sci 5:263–267

Hoyle MC (1977) High resolution of peroxidase-indoleacetic acid oxidase isoenzymes from horseradish by isoelectric focusing. Plant Physiol 60:787–793

Jamet E, Albenne C, Boudart G, Irshad M, Canut H, Pont-Lezica R (2008) Recent advances in plant cell wall proteomics. Proteomics 8:893–908

Joy O, Eze SOO (2015) Partial purification and characterization of peroxidase extracted from *Gongronema latifolium*. Am Euras J Sci Res 10:221–227

Karpinska B, Zhang K, Rasool B, Pastok D, Morris J, Verrall SR, Foyer CH (2017) The redox state of the apoplast influences the acclimation of photosynthesis and leaf metabolism to changing irradiance. Plant, Cell Environ. https://doi.org/10.1111/pce.12960

Karuppanapandian T, Moon JC, Kim C, Manoharan K, Kim W (2011) Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. Aust J Crop Sci 5:709–725

Kiba A, Miyake C, Toyoda K, Ichinose Y, Yamada T, Shiraishi T (1997) Superoxide generation in extracts from isolated plant cell walls is regulated by fungal signal molecules. Phytopathology 87:846–852

Kim SH, Kim SS (1996) Carbohydrate moieties of three radish peroxidases. Phytochemistry 42:287–290

Kim BH, Kim SY, Nam KH (2012) Genes encoding plant-specific Class III Peroxidases are responsible for increased cold tolerance of the brassinosteroid-insensitive 1 mutant. Mol Cells 34:539–548

Kim YH, Kim CY, Song WK, Kwak SS (2008) Overexpression of sweet potato swpa4 peroxidase results in increased hydrogen peroxide production and enhances stress tolerance in tobacco. Planta 22:867–881

Kim SS, Lee DJ (2005) Purification and characterization of a cationic peroxidase Cs in *Raphanus sativus*. J Plant Physiol 162:609–617

Kim YH, Lim S, Han SH, Lee JC, Song WK, Kwak SS (2007) Differential expression of 10 sweetpotato peroxidases in response to sulfur dioxide, ozone, and ultraviolet radiation. Plant Physiol Biochem 45:908–914

Kitamura S (2006) Transport of flavonoids. From cytosolic synthesis to vacuolar accumulation. In: Grotewald E (ed) The science of flavonoids. Springer, New York, pp 123–146

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 Cubatão, Brazil. Sci Total Environ 246:79–91

Koffler BE, Luschin-Ebengreuth N, Stabentheiner E, Müller M, Zechmann B (2014) Compartment specific response of antioxidants to drought stress in Arabidopsis. Plant Sci 227:133–144

Kukavica B, Mitrović A, Mojović M, Veljović-Jovanović S (2007) Effect of indole-3-acetic acid on pea root growth, peroxidase profiles and hydroxyl radical formation. Arch Bio Sci 59:319–326

Kukavica B, Mojović M, Vučinić Ž, Maksimović V, Takahama U, Veljović-Jovanović S (2008) Generation of hydroxyl radical in isolated pea root cell wall, and the role of cell wall-bound peroxidase, Mn-SOD and phenolics in their production. Plant Cell Physiol 50:304–317

Kukavica B, Veljović-Jovanovic S (2004) Senescence-related changes in the antioxidant status of ginkgo and birch leaves during autumn yellowing. Physiol Plant 22:321–327

Kukavica B, Veljović-Jovanović S, Menckhoff L, Lüthje S (2012) Cell wall-bound cationic and anionic class III isoperoxidases of pea root: biochemical characterization and function in root growth. J Exp Bot 63:4631–4645

Kumar S, Jaggi M, Sinha AK (2012) Ectopic overexpression of vacuolar and apoplastic *Catharanthus roseus* peroxidases confers differential tolerance to salt and dehydration stress in transgenic tobacco. Protoplasma 249:423–432

Lai LS, Wang DJ, Chang CT, Wang CH (2006) Catalytic characteristics of peroxidase from wheat grass. J Agric Food Chem 54:8611–8616

Laugesen S, Bak-Jensen KS, Hägglund P, Henriksen A, Finnie C, Svensson B, Roepstorff P (2007) Barley peroxidase isozymes: expression and post-translational modification in mature seeds as identified by two-dimensional gel electrophoresis and mass spectrometry. Int J Mass Spectrom 268:244–253

Lige B, Ma S, van Huystee RB (2001) The effects of the site-directed removal of N-glycosylation from cationic peanut peroxidase on its function. Arch Biochem Biophys 386:17–24

Liso R, De Tullio MC, Ciraci S, Balestrini R, La Rocca N, Bruno L, Arrigoni O (2004) Localization of ascorbic acid, ascorbic acid oxidase, and glutathione in roots of *Cucurbita maxima* L. J Exp Bot 55:2589–2597

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

Liu W, Yu K, He T, Li F, Zhang D, Liu J (2013) The low temperature induced physiological responses of *Avena nuda* L., a cold-tolerant plant species. Sci World J Article ID: 658793

Llorente F, Lopez-Cobollo RM, Catála R, Martinez-Zapater JM, Salinas J (2002) A novel cold-inducible gene from Arabidopsis, RCI3, encodes a peroxidase that constitutes a component for stress tolerance. Plant J 32:13–24

López-Serrano M, Fernández MD, Pomar F, Pedreño MA, Ros Barceló A (2004) *Zinnia elegans* uses the same peroxidase isoenzyme complement for cell wall lignification in both singe-cell treachery elements and xylem vessels. J Exp Bot 55:423–431

Lu D, Wang T, Persson S, Mueller-Roeber B, Schippers JH (2014) Transcriptional control of ROS homeostasis by KUODA1 regulates cell expansion during leaf development. Nat Commun Article No 3767

Manu BT, Prasada Rao UJS (2009) Calcium modulated activity enhancement and thermal stability study of a cationic peroxidase purified from wheat bran. Food Chem 114:66–71

Marañón MJR, van Huystee RB (1994) Plant peroxidases: interaction between their prosthetic groups. Phytochemistry 37:1217–1225

Marinova K, Pourcel L, Weder B, Schwarz M, Barron D, Routaboul JM, Klein M (2007) The Arabidopsis MATE transporter TT12 acts as a vacuolar flavonoid/H^+-antiporter active in proanthocyanidin-accumulating cells of the seed coat. Plant Cell 19:2023–2038

Martinez C, Montillet JL, Bresson E, Agnel JP, Dai GH, Daniel JF, Geiger JP, 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

Marzouki SM, Limam F, Smaali MI, Ulber R, Marzouki MN (2005) A new thermostable peroxidase from garlic *Allium sativum*. Appl Biochem Biotechnol 127:201–214

Matsui T, Tabayashi A, Iwano M, Shinmyo A, Kato K, Nakayama H (2011) Activity of the C-terminal-dependent vacuolar sorting signal of horseradish peroxidase C1a is enhanced by its secondary structure. Plant Cell Physiol 52:413–420

Maurel C, Santoni V, Luu DT, Wudick MM, Verdoucq L (2009) The cellular dynamics of plant aquaporin expression and functions. Curr Opin Plant Biol 12:690–698

Maurino VG, Grube E, Zielinsk J, Schild A, Fischer K, Flugge UI (2006) Identification and expression analysisof twelve members of the nucleobase-ascorbate transporter (NAT) gene family in *Arabidopsis thaliana*. Plant CellPhysiol 47:1381–1393

McInnis SM, Desikan R, Hancock JT, Hiscock SJ (2006) Production of reactive oxygen species and reactive nitrogen species by angiosperm stigmas and pollen: potential signalling crosstalk? New Phytol 172:221–228

Meisrimler CN, Buck F, Lüthje S (2014) Alterations in soluble class III Peroxidases of maize shoots by flooding stress. Proteomes 2:303–322

Mellon JE (1991) Purification and characterization of isoperoxidases elicited by *Aspergillus flavus* in cotton ovule cultures. Plant Physiol 95:14–20

Melo NS, Cabral JMS, Fevereiro MP (1995) Extracellular peroxidases from cell suspension cultures of *Vaccinium myrtillus*. Purification and characterization of two cationic enzymes. Plant Sci 106:177–184

Mika A, Lüthje S (2003) Properties of guaiacol peroxidase activities isolated from corn root plasma membranes. Plant Physiol 132:1489–1498

Mika A, Boenisch MJ, Hopff D, Lüthje S (2009) Membrane-bound guaiacol peroxidases from maize (*Zea mays* L.) roots are regulated by methyl jasmonate, salicylic acid, and pathogen elicitors. J Exp Bot 61:831–841

Minibayeva F, Beckett RP, Kranner I (2015) Roles of apoplastic peroxidases in plant response to wounding. Phytochemistry 112:122–129

Mohamed SA, El-Badry MO, Drees EA, Fahmy AS (2008) Properties of a cationic peroxidase from *Citrus jambhiri* cv. Adalia. Appl Biochem Biotechnol 150:127–137

Mojović M, Vuletić M, Bačić G, Vučinić Ž (2004) Oxygen radicals produced by plant plasma membranes: an EPR spin-trap study. J Exp Bot 55:2523–2531

Morimoto S, Tateish N, Inuyama M, Taura F, Tanaka H, Shoyama Y (1999) Identification and molecular characterization of novel peroxidase with structural protein-like properties. J Biol Chem 274:26192–26198

Morina F, Jovanović Lj, Mojović M, Vidović M, Panković D, Veljović-Jovanović S (2010) Zinc-induced oxidative stress in *Verbascum thapsus* is caused by an accumulation of reactive oxygen species and quinhydrone in the cell wall. Physiol Plant 140:209–224

Morina F, Takahama U, Mojović M, Popović-Bijelić A, Veljović-Jovanović S (2016) Formation of stable radicals in catechin/nitrous acid systems: participation of dinitrosocatechin. Food Chem 194:1116–1122

Movahed N, Pastore C, Cellini A, Allegro G, Valentini G, Zenoni S, Filippetti I (2016) The grapevine VviPrx31 peroxidase as a candidate gene involved in anthocyanin degradation in ripening berries under high temperature. J Plant Res 129:513–526

Mujer CV, Mendoza EMT, Ramirez DA (1983) Coconut peroxidase isoenzymes: isolation, partial purification and physicochemical properties. Phytochemistry 22:1335–1340

Murphy TM, Auh CK (1996) The superoxide synthases of plasma membrane preparations from cultured rose cells. Plant Physiol 110:621–629

Neill SO, Gould KS (2003) Anthocyanins in leaves: light attenuators or antioxidants? Funct Plant Biol 30:865–873

Niopek-Witz S, Deppe J, Lemieux MJ, Möhlmann T (2014) Biochemical characterization and structure-function relationship of two plant NCS2 proteins, the nucleobase transporters NAT3 and NAT12 from *Arabidopsis thaliana*. Biochim Biophys Acta 1838:3025–3035

Noctor G, Foyer CH (2016) Intracellular redox compartmentation and ROS-related communication in regulation and signaling. Plant Physiol 171:1581–1592

Nouren S, Bhatti HN, Bhatti IA, Asgher M (2013) Kinetic and thermal characterization of peroxidase from peels of *Citrus reticulata* var. *Kinnow*. J Anim Plant Sci 23:430–435

Nourredine Y, Naima A, Dalila H, Habib S, Karim S (2015) Changes of peroxidase activities under cold stress in annuals populations of Medicago. Mol Plant Breed 6:1–9

Palm GJ, Sharma A, Kumari M, Panjikar S, Albrecht D, Jagannadham MV, Hinrichs W (2014) Post-translational modification and extended glycosylation pattern of a plant latex peroxidase of native source characterized by X-ray crystallography. FEBS J 281:4319–4333

Pandey VP, Dwivedi UN (2011) Purification and characterization of peroxidase from *Leucaena leucocephala*, a tree legume. J Mol Catal B Enzym 68:168–173

Pandey VP, Singh S, Singh R, Dwivedi UN (2012) Purification and characterization of peroxidase from papaya (*Carica papaya*) fruit. Appl Biochem Biotech 167:367–376

Passardi F, Cosio C, Penel C, Dunand C (2005) Peroxidases have more functions than a Swiss army knife. Plant Cell Rep 24:255–265

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

Passardi F, Theiler G, Zamocky M, Cosio C, Rouhier N, Teixera F, Margis-Pinheiro M, Dunand C (2007) PeroxiBase: the peroxidase database. Phytochemistry 68:1605–1611

Passardi F, Tognolli M, De Meyer M, Penel C, Dunand C (2006) Two cell wall associated peroxidases from *Arabidopsis* influence root elongation. Planta 223:965–974

Pedreira J, Herrera MT, Zarra I, Revilla G (2011) The overexpression of AtPrx37, an apoplastic peroxidase, reduces growth in Arabidopsis. Physiol Plant 141:177–187

Petrussa E, Braidot E, Zancani M, Peresson C, Bertolini A, Patui S, Vianello A (2013) Plant flavonoids—biosynthesis, transport and involvement in stress responses. Int J Mol Sci 14:14950–14973

Pick TR, Weber APM (2014) Unknown components of the plastidial permeome. Front Plant Sci 5:410

Pollastri S, Tattini M (2011) Flavonols: old compounds for old roles. Ann Bot 108:1225–1233

Pomar F, Bernal MA, Díaz J, Merino F (1997) Purification, characterization and kinetic properties of pepper fruit acidic peroxidase. Phytochemistry 46:1313–1317

Quiroga M, Guerrero C, Botella MA, Barceló A, Amaya I, Medina MI, Valpuesta V (2000) A tomato peroxidase involved in the synthesis of lignin and suberin. Plant Physiol 122:1119–1128

Raggi S, Ferrarini A, Delledonne M, Dunand C, Ranocha P, De Lorenzo G, Cervone F, Ferrari S (2015) The Arabidopsis Class III Peroxidase AtPRX71 negatively regulates growth under physiological conditions and in response to cell wall damage. Plant Physiol 169:2513–2525

Regalado G, Perez-Arvizu O, Garcia-Almendarez B, Whitaker JR (1999) Purification and properties of two acid peroxidases from Brussels sprouts. J Food Biochem 23:435–450

Ren LL, Liu YJ, Liu HJ, Qian TT, Qi LW, Wang XR, Zenga QY (2014) Subcellular relocalization and positive selection play key roles in the retention of duplicate genes of Populus Class III Peroxidase family. Plant Cell 26:2404–2419

Ros Barceló A, Gómez-Ros LV, Carrasco AE (2007) Looking for syringyl peroxidases. Trend Plant Sci 12:486–491

Sánchez-Romero C, García-Gómez ML, Pliego-Alfaro F, Heredia A (1994) Effect of partial deglycosylation on catalytic characteristics and stability of an avocado peroxidase. Physiol Plant 92:97–101

Sasaki K, Yuichi O, Hiraga S, Gotoh Y, Seo S, Mitsuhara I, Ito H, Matusi H, Ohashi Y (2007) Characterization of two rice peroxidase promoters that respond to blast fungus-infection. Mol Genet Genomics 278:709–722

Sato Y, Sugiyama M, Górecki RJ, Fukuda H, Komamine A (1993) Interrelationship between lignin deposition and the activities of peroxidase isoenzymes in differentiating tracheary elements of *Zinnia*. Planta 189:584–589

Schloss P, Walter C, Mäder M (1987) Basic peroxidases in isolated vacuoles of *Nicotiana tabacum* L. Planta 170:225–229

Schopfer P, Plachy C, Frahry G (2001) Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin and abscisic acid. Plant Physiol 125:1591–1602

Scialabba N, Grandi C, Henatsch C (2002) Organic agriculture and genetic resources for food and agriculture. In: Biodiversity and the ecosystem approach in agriculture, forestry, and fisheries: satellite event on the occasion of the ninth regular session of the Commission on Genetic Resources for Food and Agriculture, pp 74–98

Sessa DJ, Anderson RL (1981) Soybean peroxidases: purification and some properties. J Agric Food Chem 29:960–965

Shigeto J, Tsutsumi Y (2016) Diverse functions and reactions of class III peroxidases. New Phytol 209:1395–1402

Sisecioglo M, Gülçin M, Çankaya A, Atasever MH, Sehitoglu H, Kaya B, Özdemir H (2010) Purification and characterization of peroxidase from Turkish black radish (*Raphanus sativus* L.) J Med Plants Res 4:1187–1196

Sottomayor M, Cardoso IL, Pereira LG, Ros Barceló AR (2004) Peroxidase and the biosynthesis of terpenoid indole alkaloids in the medicinal plant *Catharanthus roseus* (L.) G. Don. Phytochem Rev 3:159–171

Sottomayor M, Lopez-Serrano M, DiCosmo F, Ros Barceló A (1998) Purification and characterization of α-3′, 4′-anhydrovinblastine synthase (peroxidase-like) from *Catharanthus roseus* (L.) G. Don. FEBS Lett 428:299–303

Sottomayor M, Pinto MD, Salema R, DiCosmo F, Pedreoo MA, Ros Barcelo A (1996) The vacuolar localization of a basic peroxidase isoenzyme responsible for the synthesis of α-31, 41-anhydrovinblastine in *Catharanthus roseus* (L.) G. Don Leaves. Plant, Cell Environ 19:761–767

Šukalović VH-T, Vuletić M, Marković K, Antić TC, Vučinić Ž (2015) Comparative biochemical characterization of peroxidases (class III) tightly bound to the maize root cell walls and modulation of the enzyme properties as a result of covalent binding. Protoplasma 252:335–343

Suzuki T, Honda Y, Mukasa Y, Kim SJ (2006) Characterization of peroxidase in buckwheat seed. Phytochemistry 67:219–224

Tao Y, Lin Y, Huang Z, Ren J, Qu X (2013) Incorporating graphene oxide and gold nanoclusters: A synergistic catalyst with surprisingly high peroxidase-like activity over a broad pH range and its application for cancer cell detection. Adv Mater 25:2594–2599

Takahama U (1992) Hydrogen peroxide scavenging systems in vacuoles of mesophyll cells of *Vicia faba*. Phytochemistry 31:1127–1133

Takahama U (1993) Regulation of peroxidase-dependent oxidation of phenolics by ascorbic acid: different effects of ascorbic acid on the oxidation of coniferyl alcohol by the apoplastic soluble and cell wall-bound peroxidases from epicotyls of *Vigna angularis*. Plant Cell Physiol 34:809–817

Takahama U (2004) Oxidation of vacuolar and apoplastic phenolic substrates by peroxidase: physiological significance of the oxidation reactions. Phytochem Rev 3:207–219

Takahama U, Oniki T (1992) Regulation of peroxidase-dependent oxidation of phenolics in the apoplast of spinach leaves by ascorbate. Plant Cell Physiol 33:379–387

Takahama U, Oniki T (1997) A peroxidase/phenolics/ascorbate system can scavenge hydrogen peroxide in plant cells. Physiol Plant 101:845–852

Takahama U, Oniki T (2000) Flavonoids and some other phenolics as substrates of peroxidase: physiological significance of the redox reactions. J Plant Res 113:301–309

Tams JW, Welinder KG (1995) Mild chemical deglycosylation of horseradish peroxidase yields a fully active, homogeneous enzyme. Anal Biochem 228:48–55

Tamás L, Huttová J, Mistrík I (2002) Effect of aluminium on peroxidase activity in roots of Al-sensitive and Al-resistant barley cultivars. Rostlinná Výroba 48:76–79

Tenhaken R (2014) Cell wall remodeling under abiotic stress. Front Plant Sci 5:771

Thongsook T, Barrett DM (2005) Purification and partial characterization of broccoli (*Brassica oleracea* Var. *italica*) peroxidases. J Agric Food Chem 53:3206–3214

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

Tsukagoshi H, Busch W, Benfey PN (2010) Transcriptional regulation of ROS controls transition from proliferation to differentiation in the root. Cell 143:606–616

Van den Berg BM, Chibbar RN, van Huystee RB (1983) A comparative study of a cationic peroxidase from peanut and an anionic peroxidase from petunia. Plant Cell Rep 2:304–307

Van Gestelen P, Asard H, Caubergs RJ (1997) Solubilization and separation of a plant plasma membrane NADPH-O_2-synthase from other NAD(P)H oxidoreductases. Plant Physiol 115:543–550

van Loon LC, Rep M, Pieterse CM (2006) Significance of inducible defense-related proteins in infected plants. Annu Rev Phytopathol 44:135–162

Veitch NC (2004) Horseradish peroxidase: a modern view of a classic enzyme. Phytochemistry 65:249–259

Veljovic-Jovanovic S, Vidovic M, Morina F (2018) Ascorbate as a key player in plant abiotic stress response and tolerance. In: Hossain MA, Munné-Bosch S, Burritt DJ, Vivancos PD, Fujita M, Lorence A (eds) Ascorbic acid in plant growth, development and stress tolerance. Springer International Publishing.

Veljović-Jovanović S, Kukavica B, Stevanović B, Navari-Izzo F (2006) Senescence-and drought-related changes in peroxidase and superoxide dismutase isoforms in leaves of *Ramonda serbica*. J Exp Bot 57:1759–1768

Vidović M, Morina F, Milić-Komić S, Vuleta A, Zechmann B, Prokić L, Veljović-Jovanović S (2016) Characterisation of antioxidants in photosynthetic and non-photosynthetic leaf tissues of variegated *Pelargonium zonale* plants. Plant Biol 18:669–680

Vidović M, Morina F, Veljović-Jovanović S (2017) Stimulation of various phenolics in plants under ambient UV-B radiation. In: Singh VP, Singh S, Prasad SM, Parihar P (eds) UV-B Radiation: from environmental stressor to regulator of plant growth. Wiley-Blackwell, Chichester, West Sussex, UK, pp 9–56

Vitali A, Botta B, Delle Monache G, Zappitelli S, Ricciardi P, Melino S, Giardina B (1998) Purification and partial characterization of a peroxidase from plant cell cultures of *Cassia didymobotrya* and biotransformation studies. Biochem J 331:513–519

Wang CS, Pan H, Weerasekare GM, Stewart RJ, (2015) Peroxidase-catalysed interfacial adhesion of aquatic caddisworm silk. J R Soc Interface 12:20150710
Welinder KG (1979) Amino acid sequence studies of horseradish peroxidase. Amino and carboxyl termini, cyanogen bromide and tryptic fragments, the complete sequence, and some structural characteristics of horseradish peroxidase. Eur J Biochem 96:483–502
Welinder KG (1992) Superfamily of plant, fungal and bacterial peroxidases. Curr Opin Struct Biol 2:388–393
Welinder KG, Justesen AF, Kjaersgård IV, Jensen RB, Rasmussen SK, Jespersen HM, Duroux L, (2002) Structural diversity and transcription of class III peroxidases from *Arabidopsis thaliana*. Eur J Biochem 269:6063–6081
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 Pollut 116:37–47
Wu Y, Yang Z, How J, Xu H, Chen L, Li K (2017) Overexpression of a peroxidase gene (AtPrx64) of *Arabidopsis thaliana* in tobacco improves plant's tolerance to aluminium stress. Plant Mol Biol 95:157–168
Xue YJ, Tao L, Yang ZM (2008) Aluminium-induced cell wall peroxidase activity and lignin synthesis are differentially regulated by jasmonate and nitric oxide. J Agric Food Chem 56:9676–9684
Yamasaki H, Sakihama Y, Ikehara N (1997) Flavonoid-peroxidase reaction as a detoxification mechanism of plant cells against H_2O_2. Plant Physiol 115:1405–1412
Yim MB, Chock PB, Stadtman ER (1993) Enzyme function of copper, zinc superoxide dismutase as a free radical generator. J Bio Chem 268:4099–4105
Young SA, Guo A, Guikema JA, White FF, Leach JE (1995) Rice cationic peroxidase accumulates in xylem vessels during incompatible interactions with *Xanthomonas oryzae* pv. *oryzae*. Plant Physiol 107:1333–1341
Yue R, Lu C, Qi J, Han X, Yan S, Guo S, Liu L, Fu X, Chen N, Yin H, Chi H, Tie S (2017) Transcriptome analysis of cadmium-treated roots in maize (*Zea mays* L.). Front Plant Sci 7:1298
Zechmann B (2017) Compartment-specific importance of Ascorbate during environmental stress in plants. Antioxid Redox Signal. https://doi.org/10.1089/ars.2017.7232
Zechmann B, Stumpe M, Mauch F (2011) Immunocytochemical determination of the subcellular distribution of ascorbate in plants. Planta 233:1–12
Zhang C, Doherty-Kirby A, van Huystee R, Lajoie G (2004) Investigation of cationic peanut peroxidase glycans by electrospray ionization mass spectrometry. Phytochemistry 65:1575–1588
Zhao J (2015) Flavonoid transport mechanisms: how to go, and with whom. Trend Plant Sci 20:576–585
Zipor G, Oren-Shamir M (2013) Do vacuolar peroxidases act as plant caretakers? Plant sci 199:41–47
Zipor G, Duarte P, Carqueijeiro I, Shahar L, Ovadia R, Teper-Bamnolker P, Eshel D, Levin Y, Doron-Faigenboim A, Sottomayor M, Oren-Shamir M (2015) In planta anthocyanin degradation by a vacuolarclass III peroxidase in Brunfelsia calycina flowers. New Phytol 205:653–665