CMLS, Cell. Mol. Life Sci. 57 (2000) 779–795
1420-682X/00/050779-17 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Dual action of the active oxygen species during plant stress responses

J. Dat, S. Vandenabeele, E. Vranová, M. Van Montagu, D. Inzé* and F. Van Breusegem

Vakgroep Moleculaire Genetica and Departement Plantengenetica, Vlaams Interuniversitair Instituut voor Biotechnologie (VIB), Universiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent (Belgium), Fax + 32 9 264 5349, e-mail: diinz@gengenp.rug.ac.be

Received 12 November 1999; accepted 15 January 2000

Abstract. Adaptation to environmental changes is crucial for plant growth and survival. However, the molecular and biochemical mechanisms of adaptation are still poorly understood and the signaling pathways involved remain elusive. Active oxygen species (AOS) have been proposed as a central component of plant adaptation to both biotic and abiotic stresses. Under such conditions, AOS may play two very different roles: exacerbating damage or signaling the activation of defense responses. Such a dual function was first described in pathogenesis but has also recently been demonstrated during several abiotic stress responses. To allow for these different roles, cellular levels of AOS must be tightly controlled. The numerous AOS sources and a complex system of oxidant scavengers provide the flexibility necessary for these functions. This review discusses the dual action of AOS during plant stress responses.

Key words. Abiotic stress; active oxygen species; biotic stress; catalase; oxidative stress.

Introduction

The last decade has seen many new and exciting findings paving the way to a better understanding of plant stress responses. Some of the major areas of focus include the role of antioxidants and active oxygen species (AOS) during biotic and abiotic stresses. Although both types of stress condition are associated with the regulation and potential signaling role of AOS, there has been little overlap between these two fields of research. Understanding the role of AOS during pathogenesis has progressed greatly since the early reports of their involvement in plant disease resistance. It is now clear that AOS play several crucial roles during pathogenesis. They are involved in the hypersensitive response typical of plant-pathogen incompatible interactions [1–7]. They can also limit the spread of pathogen infection by strengthening plant cell walls and/or by killing pathogens directly [8–12]. Finally, they also act as signaling molecules during local and systemic acquired resistance [5, 9, 13].

The role of AOS during pathogenesis has been extensively reviewed over the years and there are several excellent reviews on the topic [14–18]. As a result, this review will mainly focus on the role of AOS during abiotic stress responses.

The role of AOS during abiotic stress has become a subject of considerable interest given that AOS have been implicated in processes leading to plant stress acclimation [19–24]. High concentrations of AOS can lead to phytotoxicity whereas relatively low levels can be used for acclimatory signaling. This novel finding means that AOS are not simply toxic by-products of metabolism but also function as signaling molecules [24,

* Corresponding author.

25]. Therefore, the controlled modulation of AOS levels in planta is extremely important and may in part explain complex processes such as cross-tolerance.

The role of H_2O_2 during plant stress responses may be difficult to discern from that of other AOS. The reactive nature of these molecules makes it extremely difficult to distinguish effects linked to different species. Several methods are available for measuring AOS in planta but the technical difficulty associated with quantifying endogenous levels of these very reactive and short-lived species means that few studies have measured AOS during plant stress. Over the years, most of the evidence for increases in AOS has been provided through the study of antioxidants or other secondary processes such as lipid peroxidation.

Living with oxygen

Biochemical properties of AOS

Oxygen is necessary for plants. The reduction of O_2 to H_2O provides the energy that allows the impressive complexity of higher organisms but its reduction is a mixed blessing. When incompletely reduced, AOS can be extremely reactive and may oxidize biological molecules. All AOS can react with DNA, proteins, and lipids [26].

Reduction of molecular O_2 proceeds through four steps, thus generating several O_2 radical species [27]. The reaction chain requires initiation at the first step whereas subsequent steps are exothermic and can occur spontaneously, either catalyzed or not.

$$O_2 \rightarrow (H)O_2^{\cdot -} \rightarrow H_2O_2 \rightarrow OH^{\cdot} + H_2O \rightarrow 2\ H_2O \qquad (1)$$

The first step in O_2 reduction produces relatively short-lived AOS that are not readily diffusible: hydroperoxyl (HO_2^-) and superoxide ($O_2^{\cdot -}$). The half-life for $O_2^{\cdot -}$ is approximately 2–4 μs [28–30]. These oxygen radicals are highly reactive, forming hydroperoxides with enes and dienes [31]. Furthermore, specific amino acids, such as histidine, methionine, and tryptophan can be oxidized by $O_2^{\cdot -}$ [28]. In the cellular environment, $O_2^{\cdot -}$ will also cause lipid peroxidation, thereby weakening cell membranes.

The second O_2 reduction generates hydrogen peroxide (H_2O_2), a relatively long-lived molecule (1 ms) that can diffuse some distance from its site of production [5, 32]. The biological toxicity of H_2O_2 through oxidation of SH groups has long been known and it can be enhanced in the presence of metal catalysts through Haber-Weiss or Fenton-type reactions.

The Fenton or Haber-Weiss reactions are:

$$O_2^{\cdot -} + Fe^{3+} \rightarrow Fe^{2+} + O_2$$

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$$

Overall: $H_2O_2 + O_2^{\cdot -} \rightarrow OH^- + OH^{\cdot} + O_2$

The last species generated by this series of reductions is the hydroxyl radical ($OH^{\cdot}$). It has a very strong potential and a half-life of less than 1 μs. As a result, it has a very high affinity for biological molecules at its site of production, reacting at almost diffusion-controlled rates ($K > 10^9\ M^{-1}\ s^{-1}$).

As described above, several AOS can be generated by the reduction of molecular oxygen in plant systems, all with specific biochemical properties making them either extremely reactive or, in contrast, interesting candidates for cell signaling. This dual role for AOS is extremely important and will be discussed in more detail below.

Interactive effects of AOS

From the Haber-Weiss reaction, AOS can obviously react with one another leading to the generation of a third AOS species more toxic than the other two. Similarly, $O_2^{\cdot -}$ may react with other molecules such as lipid peroxides. It can also react with nitric oxide, leading to the formation of peroxynitrite, a species considered less reactive than peroxides [33, 34]. These reactions depend on concentration and on the preferential scavenging capacity of the cell. The plant may favor formation of one or the other species by preferentially scavenging H_2O_2 with antioxidants or, in contrast, accumulate H_2O_2 by activating superoxide dismutase (SOD). In addition, H_2O_2 accumulation may itself lead to higher AOS production. The H_2O_2 disruption of photosynthesis (see below), as well as the probable direct role of H_2O_2 in the activation of an NAD(P)H-dependent oxidase [35–37], suggests that H_2O_2 itself can stimulate AOS accumulation.

Sources of AOS in plants

There are several known sources of AOS in plants. Some of the most common include the leakage of electrons to O_2 from electron transport chains in the chloroplast and mitochondria. Other sources include photorespiration in the peroxisomes, and cell wall oxidases and peroxidases.

Photosynthesis

The chloroplasts are considered the most powerful source of AOS in plants [38]. Photosynthesis is a combination of successive redox reactions during which light energy is absorbed by the light-harvesting complexes and transferred to the reaction centers of the photosystems. Electrons are then passed on to a final electron acceptor, generally CO_2 (fig. 1). However, because very few plants have rates of CO_2 fixation high enough to

use more than 50% of the light energy absorbed [39], alternative acceptors are used, including oxygen. Conditions limiting CO_2 fixation will inevitably lead to an enhancement of AOS accumulation, as more O_2 molecules will be used as electron acceptors.

The photosynthetic electron transport chain includes a number of enzymes on the reducing (acceptor) side of photosystem I (PSI): Fe-S centers, reduced thioredoxin, and ferredoxin [40]. These electron transport components are auto-oxidizable and under conditions limiting the availability of $NADP^+$, $O_2^{\cdot -}$ can be formed [31, 41]. Superoxide can then initiate a chain reaction leading to the production of more toxic radicals. The sum of the Mehler peroxidase reaction is therefore the transfer of electrons from H_2O to O_2, resulting in production of O_2 at PSII, $O_2^{\cdot -}$ at PSI, and the trans-thylakoid proton gradient necessary to drive phosphorylation and photochemistry of PSII [40, 42, 43].

Although production of AOS during photosynthesis is often considered detrimental during stress conditions, under normal conditions, the ability of O_2 to accept excess electrons prevents overreduction of the electron transport chain, thereby reducing the risk that activated singlet oxygen is formed and minimizing energy exchange between excited chlorophyll and molecular oxygen [40].

Photorespiration

There is another route by which H_2O_2 can be produced during photosynthesis (fig. 2) [44]. During carbon assimilation, ribulose-1,5-bisphosphate carboxylase uses CO_2 to carboxylate ribulose-1,5-bisphosphate. However, ribulose-1,5-bisphosphate carboxylase can also use O_2 to oxygenate ribulose-1,5-bisphosphate [43]. Oxygenation yields two glycolates that are then transported from the chloroplasts to the peroxisomes. There, glycolate oxidation is catalyzed by glycolate oxidase yielding H_2O_2. In addition, the microbodies contain fatty acid β-oxidase and xanthine oxidase as H_2O_2- and $O_2^{\cdot -}$-producing enzymes, respectively [45].

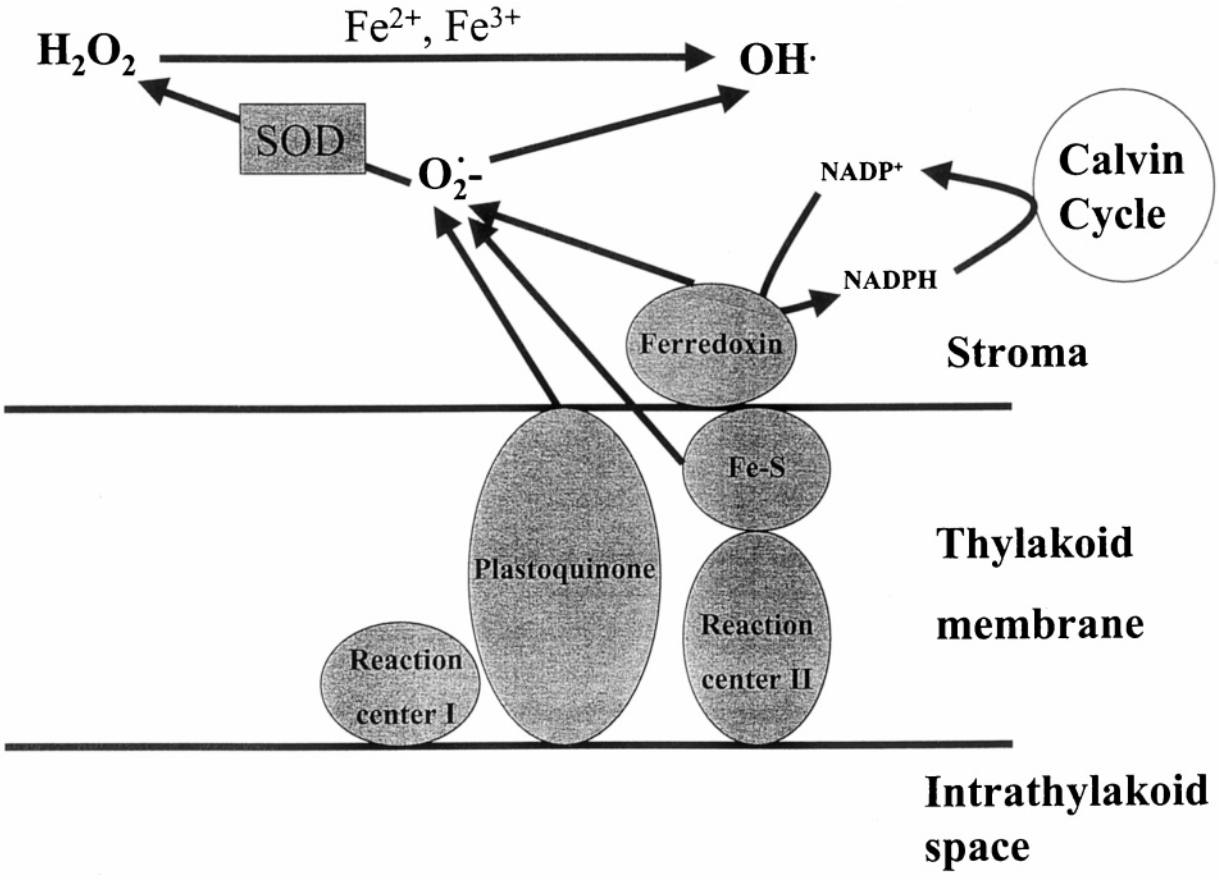

Figure 1. Schematic representation of the chloroplast showing the main sources of oxygen radicals. SOD, superoxide dismutase. Fe-S, iron sulfur cluster.

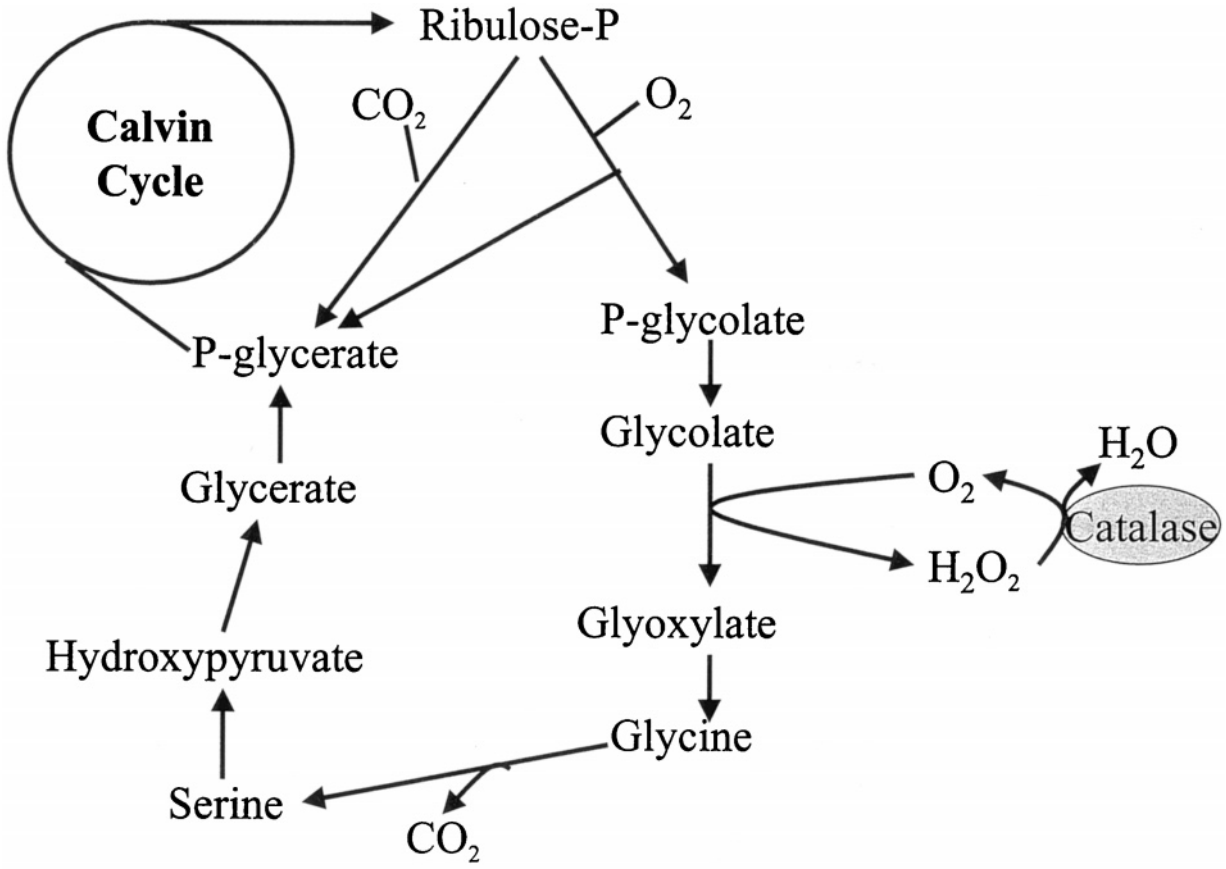

Figure 2. The photorespiratory cycle and the main source of H_2O_2 in the peroxisomes.

Respiration

Although AOS production in plant mitochondria has received little attention in the past, recent evidence suggests that mitochondria are an important source under specific conditions [45]. The mitochondria electron transport chain is made of several dehydrogenase complexes that reduce a common pool of ubiquinone (fig. 3) [46, 47]. The ubiquinone pool is then oxidized by either the cytochrome or the alternative pathway. In general, the main $O_2^{\cdot -}$ generators in the mitochondria are the ubiquinone radical and NADH dehydrogenases [26]. Superoxide is formed by the auto-oxidation of the reduced components of the respiratory chain. For example, H_2O_2 production in isolated pea stem mitochondria is mainly formed during substrate oxidation at the level of complex I [48].

Studies on the regulation of the alternative oxidase (AOX) pathway, a substitute for the cytochrome pathway, consisting of a single homodimeric protein, have provided additional evidence for mitochondrial AOS production during stress conditions [for a review, see ref. 49]. Transgenically cultured tobacco cells that are altered through antisense suppression of AOX accumulate higher level of AOS than wild-type cells [50]. In contrast, overproduction of AOX results in cells with lower AOS abundance. Similarly, use of inhibitors (salicyl hydroxamate and propyl gallate) to block the AOX leads to H_2O_2 accumulation in mitochondria of soybean and pea cotyledons [51]. The observation that AOX can be induced by various environmental stimuli makes mitochondrial AOS production an important component of stress responses [49, 52].

NAD(P)H-oxidase

In recent years, the source of AOS during the oxidative burst of plant-pathogen interactions has been the focus of numerous studies. Doke [53] demonstrated that an NADPH-dependent $O_2^{\cdot -}$ production played a part in the oxidative burst in both elicitor-treated potato protoplasts and microsomes infected with *Phytophthora infestans*. Pharmacological and immunological studies confirmed its presence in various plant species, suggesting its widespread role in plant defense [13, 54, 55].

Cross-reactivity of antibodies against components of the mammalian multiprotein complex that are homologous with proteins from various plant model systems helped identify $p22^{phox}$, $p47^{phox}$, $p67^{phox}$, $gp91^{phox}$, and $p21^{rac}$-like proteins [56–62]. Furthermore, several of the above plant homologues have been identified as expressed sequence tag cDNA sequences. However, questions remain regarding the precise role and importance of such a system [63]. In focusing on the NADPH-oxidase, other sources, such as pH-dependent peroxidases, may have been overlooked and their importance may become evident in the future. In addition, the potential role of NADPH-oxidase during other stress responses has been suggested [19, 37, 64], although further work is needed.

Other sources

Multiple AOS-producing systems are found in plants, some of which may be developmentally and/or environmentally regulated. However, many still await further characterization.

Cell wall peroxidases produce $O_2^{\cdot -}$ at the expense of NADH in a Mn^{2+}-dependent reaction [45]. The peroxidase-produced H_2O_2 is strongly pH dependent and believed to involve the reduction of the ferrous O_2 conjugate [16]. Most of the H_2O_2 generated during the oxidative burst can be produced by peroxidases [2, 10, 55].

Alternative sources for AOS include a germin-like oxalate oxidase, induced during infection of barley by *Erysiphe graminis* [65]. Oxalate oxidase gene expression is also induced by salt stress, salicylate, and methyl jasmonate [66].

Copper-containing amine oxidases can also catalyze the oxidation of various amines yielding NH_3 and H_2O_2 [16]. Flavin-containing oxidase and cell wall amine oxidase and peroxidase produce AOS in epidermal cells of tobacco following induction by cryptogein, a fungal elicitor [67].

An efficient system to regulate AOS

The antioxidant system

The omnipresence of O_2 in the environment and the various cellular locations where AOS are produced render oxidant scavengers necessary for plant growth and survival. The capacity for AOS to serve as signals adds to the importance of antioxidants to specifically regulate different AOS in various cellular locations. Plants have several antioxidant enzymes and metabolites located in different plant cell compartments, the main ones being SODs, a family of metalloenzymes catalyzing the dismutation of $O_2^{\cdot -}$ to H_2O_2 [for reviews, see refs 68–70], catalases (CATs), which are heme proteins that catalyze the removal of H_2O_2 [for reviews, see refs 12, 71], and the enzymes and metabolites of the ascorbate-glutathione cycle that are involved in the removal of H_2O_2 [for a review, see ref. 40].

The majority of enzymes of the ascorbate-glutathione cycle [ascorbate peroxidase (APX), glutathione reduc-

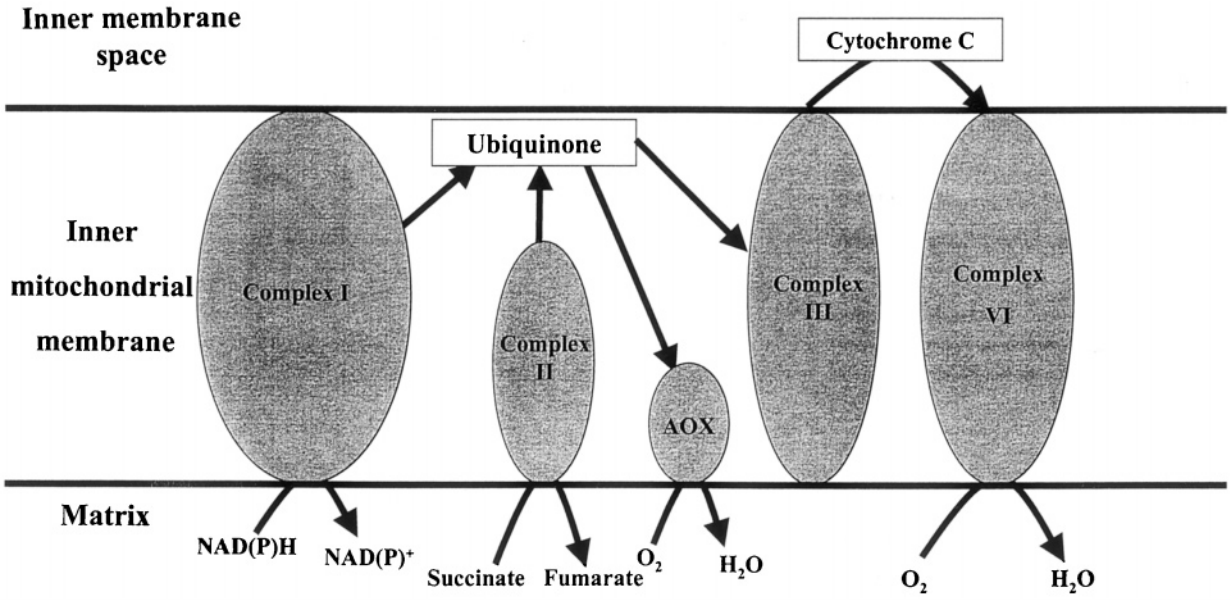

Figure 3. Schematic representation of the plant mitochondria complexes and the electron transport chain. AOX, alternative oxidase.

tase (GR), and dehydroascorbate reductase (DHAR)] have been found in chloroplasts, cytosol, mitochondria, and peroxisomes [40, 68, 72–75]. Catalase and the ascorbate-glutathione cycle are important in H_2O_2 scavenging. Although their properties and requirements are different, they function effectively in parallel. Catalase does not require reducing power and has a high reaction rate but a low affinity for H_2O_2, thereby only removing the bulk of H_2O_2 [32]. In contrast, APX requires a reductant (ascorbate) and has a higher affinity for H_2O_2, allowing for the scavenging of small amounts of H_2O_2 in more specific locations.

Antioxidant capacity is very much dependent on the severity of the stress as well as the species and its developmental stage [76–81]. The capacity of foliar antioxidants also varies with the physiological age of the leaf [82, 83] and its position in relation to irradiance interception [82].

Catalase

Catalases are tetrameric heme-containing enzymes that convert $2\ H_2O_2 \rightarrow O_2 + 2\ H_2O$, thus protecting the cell from the damaging effects of H_2O_2 accumulation [84]. Catalases have been mainly associated with the removal of H_2O_2 in microbodies [12] and can catalyze either the direct decomposition of H_2O_2 or the oxidation by H_2O_2 of substrates such as methanol, ethanol, formaldehyde, formate, nitrite, or elemental mercury [85]. Catalases are present in peroxisomes, glyoxysomes, and related organelles where H_2O_2-generating enzymes, such as glycolate oxidase, are found. There are three main isoforms: CAT1, CAT2, and CAT3 [32, 71, 85–88]. A basic nomenclature has been adopted to avoid confusion between the isozymes in the different species studied (table 1). The class I catalases include the SU2 of cotton [89], *Cat1* of *Nicotiana plumbaginifolia* [88, 90], *CAT2* of *Arabidopsis thaliana* [91], and *CAT-2* of *Zea mays* [71]. They are highly expressed in leaves, are light dependent, and involved in the removal of H_2O_2 during photorespiration. Class II catalases include the *Cat2* of *N. plumbaginifolia*, *CAT2* of castor bean [92], *CAT-3* of maize, a tomato catalase [93], *Cat2St* from potato [94], and *CAT1* from *A. thaliana* [91]. They are mainly found in vascular tissues. Finally, the class III catalases include *SU1* of cotton, *Cat3* of *N. plumbaginifolia*, *CAT1* from castor bean, *CAT-1* from maize, and *CAT3* from *A. thaliana* [91]. They are mainly involved in the removal of H_2O_2 from glyoxysomes and are highly abundant in seeds and young seedlings [12].

Table 1. Nomenclature of plant catalases [adapted from ref. 12].

Species	Class I	Class II	Class III
Gossypium hirsutum (cotton)	SU2		SU1
Nicotiana plumbaginifolia	Cat1	Cat2	Cat3
Ricinus communis L. (castor bean)	CAT2	CAT1	
Zea mays (maize)	CAT-2	CAT-3	CAT-1
Arabidopsis thaliana	CAT2	CAT1	CAT3
Lycopersicon esculentum (tomato)		TOMCAT1	
Solanum tuberosum (potato)		Cat2St	

Catalase and plant stress

The expression of catalase genes is not only developmentally regulated but it is also sensitive to various environmental signals [71, 90, 95–97]. The mRNA levels of *CAT2* and *CAT3* of *A. thaliana* [98], *Cat1* of *N. plumbaginifolia* [88], and *CAT-3* of *Z. mays* [99] are regulated by circadian rhythm, whereas others, such as *Cat2* and *Cat3* of *N. plumbaginifolia* and *CAT1* of *A. thaliana*, are not [91].

Catalase is photoinactivated in moderate light under conditions to which plants are adapted [100, 101]. Although they are involved in H_2O_2 scavenging, their turnover being continuous, their steady-state level can be rapidly lowered under any stress conditions in which translation is inhibited or degradation enhanced [100]. The various catalases are, however, differentially responsive to light. *CAT1* and *CAT2* mRNA abundance increases upon illumination, whereas *CAT3* shows a rapid and transient decline [102]. *Cat1* expression in tobacco leaves is unaffected by light [12, 32].

Differential sensitivity of catalases to stress is also observed during temperature extremes. In maize, CAT-3 is more sensitive to temperature than CAT-1 [85]. Generally, catalase activity is inhibited under low temperature [103], and heat shock [22, 23, 104, 105]. For example, catalase photoinactivation in rye seedlings was induced after 4 h at 40 °C [104]. *Cat1* and *Cat2* transcript abundance in *N. plumbaginifolia* decreased during a 5-h heat shock at 37 °C, but recovered rapidly after return at 22 °C. In contrast, *Cat3* expression was unchanged by these treatments [12, 88]. Catalases also exhibit differential responses to thermal extremes in maize and *Arabidopsis* [71, 102], and induction of *CAT-3* during cold acclimation in maize is correlated with chilling tolerance [106, 107].

Changes in catalase in response to other stress conditions have also been reported. For example, the three catalases of tobacco all responded to environmental challenges with UV-B, O_3, and SO_2 [88]. As a consequence of its sensitivity to environmental conditions, early loss of catalase was suggested as a signal for antioxidant defenses [100]. However, further studies are needed to confirm such a role.

The importance of catalase inhibition during pathogenesis has been the center of much debate. Catalase inhibition by salicylic acid (SA) was originally proposed as one of the mechanisms by which SA may mediate defense responses in tobacco [108, 109]. Identification and characterization of an SA-binding protein (SABP) with a cDNA sequence highly similar to catalase in tobacco [108, 110–112], cucumber, tomato, *Arabidopsis*, and rice [84, 113] was at the origin of this proposed model. Recently, an additional SABP (SABP2) has been identified and characterized in tobacco [114]. However, several studies have questioned the ability of SA to specifically inhibit catalase in vivo [115–118]. Because the SA concentrations that are required for in vitro inhibition of catalases are extremely high, this mechanism may only occur in restricted subcellular compartments [119]. Cat1- and Cat2-deficient transgenic tobacco plants are not altered in their pathogenesis-related (PR) response under normal growth conditions, which implies that catalase inhibition is not sufficient to generate a signal for activation of PR responses [120]. The Cat1-deficient tobacco plants are also more susceptible to infection [121].

The above discussion clearly demonstrates that the AOS-scavenging system in plants is an essential component of the protective mechanisms against biotic and abiotic stress. Corroborating the correlation between antioxidant potential and stress tolerance [40], free-radical scavenging capacity often increases with plant acclimation [64, 122, 123]. Acclimation to elevated AOS is generally associated with enhanced activity of antioxidant enzymes [124, 125]. This increase in stress tolerance has been exploited. Plants have been transformed to overproduce components of the antioxidant system [126–131]. However, results have not always been conclusive. SOD overproduction, though, shows more encouraging results [132–140]. Are these results related to the capacity of SOD to modulate endogenous levels of $O_2^{\cdot -}$ and H_2O_2, rather than to scavenge AOS like other antioxidants? Enhancement of the AOS-scavenging system may be sufficient to protect plants from some stress, but such transformation may also be detrimental. The fact that H_2O_2 and $O_2^{\cdot -}$ are involved in mediating cell defense responses is one possible explanation [4, 9, 22, 24, 25, 141]. Enhancing the antioxidant capacity may protect plants under specific conditions but it will also interfere with the signaling cascade involved in plant adaptation. Only recently, several research groups have realized that suppressing AOS accumulation in plants may be a futile approach. Instead, close control of AOS levels in various cellular compartments may offer greater possibilities, but the antioxidant mechanisms involved during stress responses and their complex interactions are only just being elucidated.

Generation of AOS under adverse conditions

Environmental stress is a major factor that limits plant productivity. Although AOS are formed in normal cell metabolism and their regulation is a common cellular event [24, 142], oxidative damage is often associated with plant stress. Most, if not all, abiotic stress conditions are exacerbated by the effect of AOS accumulation [24, 30].

Exposure to high light intensities

High-light exposure is one of the most common sources of oxidative stress in plants. High light-induced AOS generation arises principally when it is combined with additional stress factors in which CO_2 fixation is limiting [38]. Under normal photosynthesis, chloroplasts generate approximately 150–250 μmol of H_2O_2 per milligram of chlorophyll per hour [143]. Under excess light conditions, enzymatic processes for CO_2 fixation become rate limiting and, as a result, photosynthesis produces more NADPH and ATP than necessary. This accumulation of redox and energy equivalents will reduce the plastoquinone pool and/or inhibit the water-splitting complex, inevitably leading to PSII inactivation, so-called photoinhibition [144–147]. An increase in the rate of O_2 photoreduction with high irradiance has been reported for *Vinca major*, *Schefflera arboricola*, and *Mahonia repens* [125]. Exposure of *Arabidopsis* seedlings to excess light (>2000 μmol photons $m^{-2}\ s^{-1}$) for 1 h resulted in photoinhibition, accompanied by H_2O_2 accumulation [148]. When exposed to high light (>300 μmol photons $m^{-2}\ s^{-1}$), leaves of catalase-deficient (Cat1AS) tobacco plants become bleached as a result of H_2O_2 accumulation in the peroxisomes [32]. These results clearly demonstrate that a decline in catalase combined with high light results in oxidative stress.

Expression analysis of antioxidants has provided evidence for the accumulation of AOS during high-light exposure. Alteration in antioxidants after high-light treatment often infers enhancement of AOS accumulation. Tobacco plants exposed to high light showed a strong induction of chloroplastic FeSOD ($FeSOD_{chl}$) transcripts [149]. The $FeSOD_{chl}$ mRNA increase was not light dependent but directly responsive to oxidative stress, because 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea infiltration, which blocks re-oxidation of the primary electron-accepting quinone of PSII, abolished the induction of $FeSOD_{chl}$ mRNA. Activities of most antioxidant enzymes increased in pea seedlings transferred from low to high light [124].

Additional evidence for an involvement of AOS during high-light treatment is provided by the degradation of glutamine synthetase, phosphoglycolate phosphatase,

the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase, and an increased content of carbonyl groups in stromal proteins of pea [150]. All these processes result from oxidative damage.

Drought and salt stress

Drought-induced inhibition of photosynthesis leads to increased AOS production in the chloroplasts [30, 151, 152]. Numerous studies report increased oxidative stress during water deficit [153–158]. The accumulation of AOS during such conditions originates mainly from the decline in CO_2 fixation, leading to higher leakage of electrons to O_2. Accordingly, thylakoid membrane electron leakage to O_2 increased in sunflower [159] and in wheat [41] after drought.

Other AOS-dependent changes are often associated with water deficit. For example, lipid peroxidation, a commonly used indicator of oxidative stress, increases in various tissues during drought [81, 160–162]. Changes in antioxidants are also correlated with water deficit [161, 163, 164]. Chloroplastic and cytosolic Cu/ZnSOD activity increase in pea subjected to water deficit [81]. Activities of cytosolic and chloroplastic Cu/ZnSOD and cytosolic APX rise during drought of pea plants [78, 79], and osmotic stress enhances Mn-SOD transcript abundance in maize [165]. Drought-tolerant *Sorghum* bicolor has higher antioxidant capacity during water deficit than drought-susceptible varieties [166]. Drought stress can therefore alter the oxidative balance of the cell and acclimation to drought is generally correlated with keeping AOS levels relatively low through the antioxidant system.

Plant responses to salt stress often include drought-mediated symptons. As a result, it is not surprising that oxidative stress participates in salt damage. The generation of AOS during salt stress is probably similar to that during drought and is mainly attributed to increased leakage of electrons to O_2, ensuing from a decline in CO_2 fixation. Concentrations of H_2O_2 in shoot tissue of rice significantly increased upon salt stress [167]. Tobacco plants deficient in catalase (Cat1AS) were also more susceptible to salt stress [32].

Further evidence for the involvement of oxidative stress during salt stress comes from the study of antioxidants. Mitochondrial and chloroplastic SOD and APX isozymes increase during salt stress in pea [168, 169]. Various antioxidant enzymes are upregulated upon salt treatment of cotton cell calli [170] and in citrus cells [171]. NaCl treatment activates *Cat2* and *Cat3* genes in hydroponically grown tobacco [172]. Other salt-induced responses include AOS-mediated lipid peroxidation in citrus [173].

Low-temperature exposure

Low temperature is one of the most studied abiotic stresses in plants. Direct evidence for accumulation of AOS during chilling has been reported for several plant species. The chloroplast is a major source of AOS during chilling through inhibition of CO_2 fixation, although, recently, mitochondrial AOS accumulation during chilling has been demonstrated [174–176]. Early results by Okuda et al. [177] showed that cold treatment of winter wheat induces a transient but significant increase in endogenous H_2O_2. Similarly, chilled cucumber plants [103] and maize seedlings [20] accumulate H_2O_2. Exposure to 4°C of callus tissue of *A. thaliana* leads to conditions of oxidative stress [178]. Upon chilling, cellular H_2O_2 increases followed by lipid peroxidation. A correlation between H_2O_2 accumulation and chilling damage has been reported in *A. thaliana* callus tissue [178], cucumber, winter wheat [103, 177], rice [179], and maize [20, 180].

High-temperature exposure

Similar to low temperatures, high-temperature exposure involves AOS accumulation. The mechanisms by which AOS can accumulate under such conditions are diverse, but the prime site of production is probably the chloroplast, through leakage of electron following a decline in CO_2 fixation. Alternatively, an NAD(P)H-dependent oxidase might be activated upon heat treatment [19, 64]. Doke [181] showed that within 15 min after heat shock, potato leaf tissues produced an oxidative burst. More recently, exposure of whole tobacco seedlings to 40 °C for 1 h in the light induced a significant increase in H_2O_2 [24, 182]. A similar accumulation of H_2O_2 after a heat treatment was measured in mustard seedlings [23]. Although these latter studies show direct evidence of H_2O_2 accumulation in planta, other studies have reported changes in the antioxidants during high-temperature treatment.

SOD levels rise in tobacco under high-temperature treatment [149]. Heat protection of wheat is improved by keeping APX and GR activities high [183], and GR activity is significantly enhanced during heat-induced thermoprotection in mustard seedlings [64]. Furthermore, exposure of alfalfa to supra-optimal temperature leads to increases in APX and catalase [184].

Heavy metals

Although stress-related metal toxicity has received little attention in the past, it has become a major issue in reclamation of industrial sites. Copper, aluminum,

cadmium, zinc, and iron damage have all been linked to oxidative stress.

When present in excessive amounts, copper, as other heavy metals, causes uncontrolled redox reactions in the cell resulting in the formation of AOS and lipid peroxidation [185–188]. The differential activation of SODs in tobacco [189] and soybean [190] after Cu treatment suggests AOS accumulation during Cu stress. Other copper/AOS-mediated effects include the expression of PR10 protein in birch roots and leaves [191].

When present in high amounts, heavy metals, such as aluminum, cause AOS-mediated lipid peroxidation [192]. Induction of various AOS-related defense responses has also been reported. Aluminum induces glutathione-*S*-transferase (GST), peroxidase, β-1,3-glucanase, PR2, and phenylalanine ammonia lyase in *Arabidopsis* [193], and several antioxidant transcripts in soybean [194].

Zinc phytotoxicity is exacerbated by AOS. Zinc treatment of *Phaseolus vulgaris* increases the levels of H_2O_2 in roots and lipid peroxidation in primary leaves [195]. Interestingly, zinc deficiency symptoms also implicate AOS. Photo-oxidation of thylakoid constituents and impairment of detoxifying mechanisms are responsible for zinc deficiency symptoms, such as leaf chlorosis [196].

Iron excess can also result in oxidative stress [186]. Iron toxicity is in part due to its potential for reacting with H_2O_2 and $O_2^{\cdot -}$ to generate the more toxic radical $OH^{\cdot}$. Increased activity of catalase and APX in tobacco seedlings after iron excess supports this model [197]. Iron-induced oxidative stress in maize was proposed as a possible trigger for ferritin mRNA and protein accumulation [198].

UV radiation

By exciting electrons by electronic transition in appropriate photosensitizers, UV radiation induces production of $O_2^{\cdot -}$ [199]. SOD can then dismutate the produced $O_2^{\cdot -}$ to H_2O_2. Accordingly, H_2O_2 accumulation peaks 60–90 min after UV-C irradiation of *Rosa damascena* cell suspensions [199]. AOS are also implicated in the regulation of transcript levels of photosynthetic and acidic PR proteins in *Arabidopsis* exposed to UV-B [200].

Exposure of *N. plumbaginifolia* to UV-B strongly increases Cat2 and glutathione peroxidase (GPX) mRNA [90]. In contrast, APX and SOD transcript levels remain relatively unaltered. Similarly, SOD transcript levels of *Pisum sativum* decrease after UV-B exposure, whereas those of GR are several–fold higher [201].

Air pollutants

Ozone enters plant tissues through the open stomata, where it reacts with components of the cell wall and plasma membranes to form various AOS [202]. Mehlhorn et al. [203] clearly showed formation of AOS during ozone exposure by using electron spin resonance. Bluegrass and ryegrass [204] and *Arabidopsis* [37] accumulated $O_2^{\cdot -}$ and H_2O_2, respectively, upon ozone treatment. Recently, O_3 was shown to trigger an oxidative burst by activating an NADPH-dependent oxidase in *Arabidopsis* [37]. Analysis of antioxidants also clearly demonstrates a mediating role for AOS during ozone stress.

Expression of the cytosolic *APX1* gene of *Arabidopsis* increases after low-level exposure to ozone [205]. Similarly, cytosolic APX, Cu/ZnSOD, and GST mRNA increase with the onset of visible injury in tobacco, *Arabidopsis*, and bean [90, 206–208]. In contrast, $FeSOD_{chl}$ and GR decrease in tobacco and *Arabidopsis* during ozone treatment [90, 208]. Additional evidence for an involvement of AOS was provided by the overlap in AOS-dependent signaling induced by ozone exposure and pathogen infection [209–214].

Ozone is not the only air pollutant linked to AOS formation and damage. Once it enters the leaf, sulfur dioxide (SO_2) is hydrated to form HSO_3^- and SO_3^{2-} which can affect carbon metabolism [215]. The presence of sulfite and bisulfite leads to a free radical chain reaction initiated by the interaction of sulfite with $O_2^{\cdot -}$ from the Mehler reaction. Exposure to SO_2 may also enhance the level of AOS in plant tissues. In tobacco exposed to SO_2, Cat2 and GPX transcript levels rise whereas other antioxidants are either repressed or unaltered by the treatment [90]. Increased SOD activity correlates with SO_2 tolerance in bean [215, 216] and poplar [217]. In *Arabidopsis*, APX and guaiacol peroxidase activities are enhanced [205], whereas in *Cassia siamea*, SOD and guaiacol peroxidase activities increase [218].

Mechanical and physical stress

Several pathways are involved in physical and mechanical wounding responses. One of these clearly involves AOS accumulation. The source of AOS during such conditions is believed to be NAD(P)H dependent, because wound-induced AOS accumulation in various species is inhibited by diphenyleneiodonium, an NADPH-oxidase inhibitor [219]. Wounding stimulates H_2O_2 generation systemically in tomato leaves [219]. AOS accumulate after slicing tissues of various species [220], mechanical wounding of mesocarp tissue of winter squash [221] or potato tissues [19, 181], and mechanical stress in red macroalgae [222].

Pathogen attack

Several excellent reviews on the role of AOS during plant-pathogen interactions have been published recently [63, 223–225]. A very brief overview is given of the most relevant findings that implicate AOS.

Generation of AOS in plants can be detected within minutes of elicitor or microbial treatment [14, 226–228]. The production of AOS occurs in two distinct phases: an initial, non-specific phase follows within minutes of pathogen addition, and a secondary phase, dependent on recognition of incompatible pathogens by the host begins 1–3 h after the initial burst [1, 5, 228–230]. The exact role of these two AOS bursts is still unclear, but potentiation effects of SA, defense gene activation, and programmed cell death probably require different H_2O_2 kinetics for induction. The initial burst probably originates from an NADPH-dependent oxidase, although as noted above, other candidate sources exist. The produced $O_2^{\cdot -}$ is dismutated by SOD to H_2O_2 and will activate benzoic acid 2-hydroxylase [231] leading to SA accumulation. This rise in SA, in combination with H_2O_2, is necessary for potentiating defense gene activation and plant cell death [232]. The second, longer-lasting burst is involved in activating defense responses, albeit thought to be NAD(P)H dependent. Its role is supported by results obtained through direct injection of either H_2O_2 or pathogen elicitors. The artificial oxidative burst obtained induces the expression of PR genes [110, 233], phytoalexins [226, 234, 235], cross-linking of the hydroxyproline-rich cell wall glycoproteins [11, 236], and benzoic acid and SA accumulation [115, 231, 237].

A large body of evidence substantiates the damaging effects of AOS during biotic and abiotic stress. Many stress-related symptoms are exacerbated, if not directly dependent on AOS. However, evidence also suggests that AOS may play another role during stress responses, related to their capacity to serve as secondary messengers.

AOS: friends or foes?

Although plant signaling responses and possible cross-talk between different signaling cascades are increasingly well-understood, the mode of action of H_2O_2 remains unknown [63]. It may function as a secondary messenger at low concentrations but still be phytotoxic at higher concentrations [238]. Early evidence in the field of pathogenesis clearly supported a signaling role for AOS during plant-pathogen interactions. In recent years, specific induction of defense responses has been obtained with H_2O_2 treatments and corroborates the idea that H_2O_2 directly regulates defense responses. The specific induction of GPX and GST by 2 mM H_2O_2 in cell suspension cultures of soybean and the cross-membrane trafficking of H_2O_2 were the first demonstration of H_2O_2-inducible gene expression in plants [5]. Use of transgenic technology has helped to characterize the H_2O_2 signaling role. Transgenic potato overexpressing a fungal glucose oxidase gene targeted into the apoplast contains constitutively elevated H_2O_2 levels, accumulates SA, and increases production of acidic chitinase and anionic peroxidase transcript levels [8, 239]. Similarly, tobacco deficient in catalase (Cat1AS), which accumulates H_2O_2 under high light, was used to investigate the role of H_2O_2 during systemic and locally acquired resistance. Acidic PR proteins accumulate in both local and distal leaves protected from high light-imposed H_2O_2 stress, clearly demonstrating that enhanced H_2O_2 levels in the exposed part can activate the expression of defense proteins [9]. In both the potato and tobacco system, disease resistance was enhanced.

Although the field of pathogenesis led the way in oxidative stress signaling in plants for many years, several recent studies have implicated H_2O_2 in a more general signaling role. Transcript levels of cytosolic APX are significantly increased by H_2O_2 or paraquat treatment in cell suspension cultures of rice [240]. Addition of diethyldithiocarbamate (a SOD inhibitor resulting in lower H_2O_2) reduces the induction of APX, whereas inhibition of catalase or APX (resulting in H_2O_2 accumulation) increases APX mRNA levels [240]. These results suggest that H_2O_2 is part of the signaling cascade leading to cytosolic APX induction. This study was the first thorough analysis of APX induction by H_2O_2, although the regulation of APX by paraquat [78, 80, 241] or H_2O_2 [241, 242] had already been reported.

Additional evidence for a signaling role for AOS was also provided by recent findings in the field of plant stress acclimation. Nodal potato explants subcultured from H_2O_2-treated microplants are resistant to a 15-h heat shock at 42 °C [22, 24]. Small heat shock proteins (HSPs), including mitochondrial HSP22, accumulate in cell suspension cultures of tomato after addition of 2 mM H_2O_2, but not in response to $O_2^{\cdot -}$-generating agents [243]. Increased levels of H_2O_2 in Cat1AS plants exposed to high light lead to HSP17.6 accumulation [9]. Injection of H_2O_2 in *Arabidopsis* leaves induces protection from high light-induced photobleaching [21]. Maize coleoptiles treated with H_2O_2 are protected from subsequent chilling stress [20, 106]. Treatment of crowns of winter wheat with various levels of H_2O_2 and a catalase inhibitor lead to concentration-dependent synthesis of various polypeptides, similar to those found when the plants are exposed to low temperature [244]. Additionally, chilling or H_2O_2 treatment of tobacco induces a similar transient burst of calcium [245, 246]. AOS, and more particularly H_2O_2, can clearly function as a signaling molecule during abiotic stress responses.

For some time, signaling properties of AOS were considered exclusive to H_2O_2. However, increasing evidence points to differential signaling amongst AOS. Tomato cells treated with digitonin or xanthine oxidase are able to trigger the accumulation of a set of extensin transcripts [247]. One of these is specifically induced by $O_2^{\cdot -}$ and not H_2O_2 or H_2O_2-generating compounds, such as glucose oxidase. Similarly, phytoalexin accumulation in cell suspension cultures of parsley and lesion formation in lesion-stimulated disease resistance mutants of *Arabidopsis* are induced by $O_2^{\cdot -}$ rather than H_2O_2 [4, 141]. A cDNA sequence with high homology to 1-aminocyclopropane-1-carboxylate synthase (WSACS2) is preferentially expressed after $O_2^{\cdot -}$ treatment [221].

In addition to the direct evidence of AOS signaling during plant stress adaptation, tolerance to a specific environmental stress can sometimes improve the ability to withstand one or more other stresses. Cross-tolerance is often attributed to the fact that various stresses produce a similar effect at the cellular level, including oxidative stress [68]. Ozone or UV exposure of *A. thaliana* and *N. tabacum* induces resistance against virulent *Pseudomonas syringae* strains and tobacco mosaic virus, respectively [210, 248]. Pre-exposure of mung bean (*Vigna radiata* L.) and pea (*P. sativum* L.) to ethylene makes them more resistant against further exposure to ozone [249], and cotton plants exposed to water deficit are more resistant than irrigated plants against paraquat treatment [250]. Finally, drought treatment induces chilling acclimation in various plant species [251, 252]. The capacity for H_2O_2-induced protection and reports of cross-tolerance suggest that AOS play a common mediator role under these conditions. AOS, and more particularly H_2O_2 and $O_2^{\cdot -}$, may therefore be involved in a general stress resistance signal transduction pathway. Although the stress conditions described above involve the accumulation of individual AOS, AOS levels were not monitored in these studies. The role of AOS in cross-tolerance is therefore still not clearly demonstrated.

Conclusion

This review has summarized the wealth of data on the importance of oxidative stress during many biotic and abiotic stress responses. Besides exacerbating cellular damage, AOS are capable of inducing defense gene expression. H_2O_2 and/or $O_2^{\cdot -}$ can induce different genes, in combination or separately, thereby giving more flexibility to the AOS signaling function. In addition, the evidence emerging from abiotic stress responses clearly indicates that AOS may also play a more general signaling role during plant stress adaptation.

Acknowledgments. J. D. was financially supported by a European Science Foundation postdoctoral fellowship.

1 Baker C. J. and Orlandi E. W. (1995) Active oxygen in plant pathogenesis. Annu. Rev. Phytopathol. **33:** 299–321
2 Bestwick C. S., Brown I. R., Bennett M. H. R. and Mansfield J. W. (1997) Localization of hydrogen peroxide accumulation during the hypersensitive reaction of lettuce cells to *Pseudomonas syringae* pv *phaseolicola*. Plant Cell **9:** 209–221
3 Doke N. and Ohashi Y. (1988) Involvement of a superoxide anion generating system in the induction of necrotic lesions on tobacco leaves infected with tobacco mosaic virus. Physiol. Mol. Plant Pathol. **32:** 163–175
4 Jabs T., Dietrich R. A. and Dangl J. L. (1996) Initiation of runaway cell death in an *Arabidopsis* mutant by extracellular superoxide. Science **273:** 1853–1856
5 Levine A., Tenhaken R., Dixon R. and Lamb C. (1994) H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell **79:** 583–593
6 Marrè M. T., Amicucci E., Zingarelli L., Albergoni F. and Marrè E. (1998) The respiratory burst and electrolyte leakage induced by sulfhydryl blockers in *Egeria densa* leaves are associated with H_2O_2 production and are dependent on Ca^{2+} influx. Plant Physiol. **118:** 1379–1387
7 Mittler R., Shulaev V., Seskar M. and Lam E. (1996) Inhibition of programmed cell death in tobacco plants during a pathogen-induced hypersensitive response at low oxygen pressure. Plant Cell **8:** 1991–2001
8 Wu G., Shortt B. J., Lawrence E. B., León J., Fitzsimmons K. C., Levine E. B. et al. (1997) Activation of host defense mechanisms by elevated production of H_2O_2 in transgenic plants. Plant Physiol. **115:** 427–435
9 Chamnongpol S., Willekens H., Moeder W., Langebartels C., Sandermann H. Jr, Van Montagu M. et al. (1998) Defense activation and enhanced pathogen tolerance induced by H_2O_2 in transgenic plants. Proc. Natl. Acad. Sci. USA **95:** 5818–5823
10 Peng M. and Kuc J. (1992) Peroxidase-generated hydrogen peroxide as a source of antifungal activity in vitro and on tobacco leaf disks. Phytopathology **82:** 696–699
11 Bradley D. J., Kjellbom P. and Lamb C. J. (1992) Elicitor- and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: a novel, rapid defense response. Cell **70:** 21–30
12 Willekens H., Inzé D., Van Montagu M. and Van Camp W. (1995) Catalases in plants. Mol. Breed. **1:** 207–228
13 Alvarez M. E., Pennell R. I., Meijer P.-J., Ishikawa A., Dixon R. A. and Lamb C. (1998) Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. Cell **92:** 773–784
14 Low P. S. and Merida J. R. (1996) The oxidative burst in plant defense: function and signal transduction. Physiol. Plant. **96:** 533–542
15 Medhy M. C. (1994) Active oxygen species in plant defense against pathogens. Plant Physiol. **105:** 467–472
16 Bolwell G. P. and Wojtaszek P. (1997) Mechanisms for the generation of reactive oxygen species in plant defence – broad perspective. Physiol. Mol. Plant Pathol. **51:** 347–366
17 Greenberg J. T. (1997) Programmed cell death in plant-pathogen interactions. Annu. Rev. Plant Physiol. Plant Mol. Biol. **48:** 525–545
18 Lamb C. and Dixon R. A. (1997) The oxidative burst in plant disease resistance. Annu. Rev. Plant Physiol. Plant Mol. Biol. **48:** 251–275
19 Doke N., Miura Y., Sanchez L. M. and Kawakita K. (1994) Involvement of superoxide in signal transduction: responses to attack by pathogens, physical and chemical shocks, and UV irradiation. In: Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants, pp. 177–197, Foyer C. H. and Mullineaux P. M. (eds), CRC, Boca Raton

20 Prasad T. K., Anderson M. D., Martin B. A. and Stewart C. R. (1994) Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. Plant Cell **6:** 65–74

21 Karpinski S., Reynolds H., Karpinska B., Wingsle G., Creissen G. and Mullineaux P. (1999) Systemic signaling and acclimation in response to excess excitation energy in *Arabidopsis*. Science **284:** 654–657

22 Lopez-Delgado H., Dat J. F., Foyer C. H. and Scott I. M. (1998) Induction of thermotolerance in potato microplants by acetylsalicylic acid and H_2O_2. J. Exp. Bot. **49:** 713–720

23 Dat J. F., Lopez-Delgado H., Foyer C. H. and Scott I. M. (1998) Parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. Plant Physiol. **116:** 1351–1357

24 Foyer C. H., Lopez-Delgado H., Dat J. F. and Scott I. M. (1997) Hydrogen peroxide- and glutathione-associated mechanisms of acclimatory stress tolerance and signalling. Physiol. Plant. **100:** 241–254

25 Van Camp W., Van Montagu M. and Inzé D. (1998) H_2O_2 and NO: redox signals in disease resistance. Trends Plant Sci. **3:** 330–334

26 Richter C. and Schweizer M. (1997) Oxidative stress in mitochondria. In: Oxidative Stress and the Molecular Biology of Antioxidant Defenses, pp. 169–200, Scandalios J. G. (ed.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

27 Hippeli S., Heiser I. and Elstner E. F. (1999) Activated oxygen and free oxygen radicals in pathology: new insights and analogies between animals and plants. Plant Physiol. Biochem. **37:** 167–178

28 Knox J. P. and Dodge A. D. (1985) Singlet oxygen and plants. Phytochemistry **24:** 889–896

29 Sutherland M. W. (1991) The generation of oxygen radicals during host plant responses to infection. Physiol. Mol. Plant Pathol. **39:** 79–93

30 Smirnoff N. (1993) The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol. **125:** 27–58

31 Salin M. L. (1987) Toxic oxygen species and protective systems of the chloroplast. Physiol. Plant. **72:** 681–689

32 Willekens H., Chamnongpol S., Davey M., Schraudner M., Langebartels C., Van Montagu M. et al. (1997) Catalase is a sink for H_2O_2 and is indispensable for stress defence in C_3 plants. EMBO J. **16:** 4806–4816

33 Beligni M. V. and Lamattina L. (1999) Nitric oxide counteracts cytotoxic processes mediated by reactive oxygen species in plants. Planta **208:** 337–344

34 Beligni M. V. and Lamattina L. (1999) Is nitric oxide toxic or protective? Trends Plant Sci. **4:** 299–300

35 Park H.-J., Miura Y., Kawakita K., Yoshioka H. and Doke N. (1998) Physiological mechanisms of a sub-systemic oxidative burst triggered by elicitor-induced local oxidative burst in potato tuber slices. Plant Cell Physiol. **39:** 1218–1225

36 Park H.-J., Doke N., Miura Y., Kawakita K., Noritake T. and Komatsubara H. (1998) Induction of a sub-systemic oxidative burst by elicitor-stimulated local oxidative burst in potato plant tissues: a possible systemic signaling in systemic acquired resistance. Plant Sci. **138:** 197–208

37 Rao M. V. and Davis K. R. (1999) Ozone-induced cell death occurs via two distinct mechanisms in *Arabidopsis*: the role of salicylic acid. Plant J. **17:** 603–614

38 Foyer C. H., Descourvières P. and Kunert K. J. (1994) Protection against oxygen radicals: an important defence mechanism studied in transgenic plants. Plant Cell Environ. **17:** 507–523

39 Baker N. R. (1991) A possible role for photosystem II in environmental perturbations of photosynthesis. Physiol. Plant. **81:** 563–570

40 Noctor G. and Foyer C. H. (1998) Ascorbate and glutathione: keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Biol. **49:** 249–279

41 Biehler K. and Fock H. (1996) Evidence for the contribution of the Mehler-peroxidase reaction in dissipating excess electrons in drought-stressed wheat. Plant Physiol. **112:** 265–272

42 Polle A. (1996) Mehler reaction: friend of foe in photosynthesis? Bot. Acta **109:** 84–89

43 Foyer C. H. (1996) Oxygen processing in photosynthesis. Biochem. Soc. Trans. **24:** 427–433

44 Elstner E. F. (1982) Oxygen activation and oxygen toxicity. Annu. Rev. Plant Physiol. **33:** 73–96

45 Elstner E. F. and Osswald W. (1994) Mechanisms of oxygen activation during plant stress. Proc. R. Soc. Edinb. B **102:** 131–154

46 Møller I. M. (1997) The oxidation of cytosolic NAD(P)H by external NAD(P)H dehydrogenases in the respiratory chain of plant mitochondria. Physiol. Plant. **100:** 85–90

47 Elthon T. E., Nickels R. L. and McIntosh L. (1989) Monoclonal antibodies to the alternative oxidase of higher plant mitochondria. Plant Physiol. **89:** 1311–1317

48 Braidot E., Petrussa E., Vianello A. and Macri F. (1999) Hydrogen peroxide generation by higher plant mitochondria oxidizing complex I or complex II substrates. FEBS Lett. **451:** 347–350

49 Vanlerberghe G. C. and McIntosh L. (1997) Alternative oxidase: from gene to function. Annu. Rev. Plant Physiol. Plant Mol. Biol. **48:** 703–734

50 Maxwell D. P., Wang Y. and McIntosh L. (1999) The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. Proc. Natl. Acad. Sci. USA **96:** 8271–8276

51 Popov V. N., Simonian R. A., Skulachev V. P. and Starkov A. A. (1997) Inhibition of the alternative oxidase stimulates H_2O_2 production in plant mitochondria. FEBS Lett. **415:** 87–90

52 Purvis A. C. (1997) Role of the alternative oxidase in limiting superoxide production by plant mitochondria. Physiol. Plant. **100:** 165–170

53 Doke N. (1985) NADPH-dependent O_2^- generation in membrane fractions isolated from wounded potato tubers inoculated with *Phytophthora infestans*. Physiol. Plant Pathol. **27:** 311–322

54 Auh C.-K. and Murphy T. M. (1995) Plasma membrane redox enzyme is involved in the synthesis of O_2^- and H_2O_2 by *Phytophthora* elicitor-stimulated rose cells. Plant Physiol. **107:** 1241–1247

55 Vera-Estrella R., Blumwald E. and Higgins V. J. (1992) Effect of specific elicitors of *Cladosporium fulvum* on tomato suspension cells: evidence for the involvement of active oxygen species. Plant Physiol. **99:** 1208–1215

56 Tenhaken R., Levine A., Brisson L. F., Dixon R. A. and Lamb C. (1995) Function of the oxidative burst in hypersensitive disease resistance. Proc. Natl. Acad. Sci. USA **92:** 4158–4163

57 Desikan R., Hancock J. T., Coffey M. J. and Neill N. J. (1996) Generation of active oxygen in elicited cells of *Arabidopsis thaliana* is mediated by a NADPH oxidase-like enzyme. FEBS Lett. **382:** 213–217

58 Xing T., Higgins V. J. and Blumwald E. (1997) Race-specific elicitors of *Cladosporium fulvum* promote translocation of cytosolic components of NADPH oxidase to the plasma membrane of tomato cells. Plant Cell **9:** 249–259

59 Kieffer F., Simon-Plas F., Maume B. F. and Blein J.-P. (1997) Tobacco cells contain a protein, immunologically related to the neutrophil small G protein Rac2 and involved in elicitor-induced oxidative burst. FEBS Lett. **403:** 149–153

60 Groom Q. J., Torres M. A., Fordham-Skelton A. P., Hammond-Kosack K. E., Robinson N. J. and Jones J. D. G. (1996) *rbohA*, a rice homologue of the mammalian *gp91phox* respiratory burst oxidase gene. Plant J. **10:** 515–522

61 Keller T., Damude H. G., Werner D., Doerner P., Dixon R. A. and Lamb C. (1998) A plant homolog of the neutrophil NADPH oxidase $gp91^{phox}$ subunit gene encodes a plasma membrane protein with Ca^{2+} binding motifs. Plant Cell **10:** 255–266

62 Torres M. A., Onouchi H., Hamada S., Machida C., Hammond-Kosack K. E. and Jones J. D. G. (1998) Six *Arabidopsis thaliana* homologues of the human respiratory burst oxidase (*gp91^{phox}*). Plant J. **14:** 365–370

63 Bolwell G. P. (1999) Role of active oxygen species and NO in plant defence responses. Curr. Opin. Plant Biol. **2:** 287–294

64 Dat J. F., Foyer C. H. and Scott I. M. (1998) Changes in salicylic acid and antioxidants during induced thermotolerance in mustard seedlings. Plant Physiol. **118:** 1455–1461

65 Dumas B., Freyssinet G. and Pallett K. E. (1995) Tissue-specific expression of germin-like oxalate oxidase during development and fungal infection of barley seedlings. Plant Physiol. **107:** 1091–1096

66 Hurkman W. J. and Tanaka C. K. (1996) Effect of salt stress on germin gene expression in barley roots. Plant Physiol. **110:** 971–977

67 Allan A. C. and Fluhr R. (1997) Two distinct sources of elicited reactive oxygen species in tobacco epidermal cells. Plant Cell **9:** 1559–1572

68 Bowler C., Van Montagu M. and Inzé D. (1992) Superoxide dismutase and stress tolerance. Annu. Rev. Plant Physiol. Plant Mol. Biol. **43:** 83–116

69 Bowler C., Van Camp W., Van Montagu M. and Inzé D. (1994) Superoxide dismutase in plants. CRC Crit. Rev. Plant Sci. **13:** 199–218

70 Van Camp W., Van Montagu M. and Inzé D. (1994) Superoxide dismutases. In: Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants, pp. 317–341, Foyer C. H. and Mullineaux P. M. (eds), CRC, Boca Raton

71 Scandalios J. G., Guan L. and Polidoros A. N. (1997) Catalases in plants: gene structure, properties, regulation, and expression. In: Oxidative Stress and the Molecular Biology of Antioxidant Defenses, pp. 343–406, Scandalios J. G. (ed.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

72 Biemelt S., Keetman U. and Albrecht G. (1998) Re-aeration following hypoxia or anoxia leads to activation of the antioxidative defense system in roots of wheat seedlings. Plant Physiol. **116:** 651–658

73 Pastori G. M. and Río L. A. del (1997) Natural senescence of pea leaves: an activated oxygen-mediated function for peroxisomes. Plant Physiol. **113:** 411–418

74 Jiménez A., Hernández J. A., Río L. A. del and Sevilla F. (1997) Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. Plant Physiol. **114:** 275–284

75 Jiménez A., Hernández J. A., Pastori G., Río L. A. del and Sevilla F. (1998) Role of the ascorbate-glutathione cycle of mitochondria and peroxisomes in the senescence of pea leaves. Plant Physiol. **118:** 1327–1335

76 Smirnoff N. and Colombé S. V. (1988) Drought influences the activity of enzymes of the chloroplast hydrogen peroxide scavenging system. J. Exp. Bot. **39:** 1097–1108

77 Perl-Treves R. and Galun E. (1991) The tomato Cu,Zn superoxide dismutase genes are developmentally regulated and respond to light and stress. Plant Mol. Biol. **17:** 745–760

78 Mittler R. and Zilinskas B. A. (1992) Molecular cloning and characterization of a gene encoding pea cytosolic ascorbate peroxidase. J. Biol. Chem. **267:** 21802–21807

79 Mittler R. and Zilinskas B. A. (1994) Regulation of pea cytosolic ascorbate peroxidase and other antioxidant enzymes during the progression of drought stress and following recovery from drought. Plant J. **5:** 397–405

80 Donahue J. L., Okpodu C. M., Cramer C. L., Grabau E. A. and Alscher R. G. (1997) Responses of antioxidants to paraquat in pea leaves. Plant Physiol. **113:** 249–257

81 Iturbe-Ormaetxe I., Escuredo P. R., Arrese-Igor C. and Becana M. (1998) Oxidative damage in pea plants exposed to water deficit or paraquat. Plant Physiol. **116:** 173–181

82 Foyer C. (1993) Ascorbic acid. In: Antioxidants in Higher Plants, pp. 31–58, Alscher R. G. and Hess J. L. (eds), CRC, Boca Raton

83 Pastori G. M. and Río L. A. del (1994) An activated-oxygen-mediated role for peroxisomes in the mechanism of senescence of *Pisum sativum* L. leaves. Planta **193:** 385–391

84 Sánchez-Casas P. and Klessig D. F. (1994) A salicylic acid-binding activity and a salicylic acid-inhibitable catalase activity are present in a variety of plant species. Plant Physiol. **106:** 1675–1779

85 Havir E. A. and McHale N. A. (1989) Enhanced-peroxidatic activity in specific catalase isozymes of tobacco, barley, and maize. Plant Physiol. **91:** 812–815

86 Ni W. and Trelease R. N. (1991) Post-transcriptional regulation of catalase isozyme expression in cotton seeds. Plant Cell **3:** 737–744

87 Havir E. A. (1992) The in vivo and in vitro inhibition of catalase from leaves of *Nicotiana sylvestris* by 3-amino-1,2,4-triazole. Plant Physiol. **99:** 533–537

88 Willekens H., Villarroel R., Van Montagu M., Inzé D. and Van Camp W. (1994) Molecular identification of catalases from *Nicotiana plumbaginifolia* (L.). FEBS Lett. **352:** 79–83

89 Ni W., Turley R. B. and Trelease R. N. (1990) Characterization of a cDNA encoding cottonseed catalase. Biochim. Biophys. Acta **1049:** 219–222

90 Willekens H., Van Camp W., Van Montagu M., Inzé D., Sandermann H. Jr and Langebartels C. (1994) Ozone, sulfur dioxide, and ultraviolet B have similar effects on mRNA accumulation of antioxidant genes in *Nicotiana plumbaginifolia* (L.). Plant Physiol. **106:** 1007–1014

91 Frugoli J. A., Zhong H. H., Nuccio M. L., McCourt P., McPeek M. A., Thomas T. L. et al. (1996) Catalase is encoded by a multigene family in *Arabidopsis thaliana* (L.) Heynh. Plant Physiol. **112:** 327–336

92 Suzuki M., Miyamoto R., Hattori T., Nakamura K. and Asahi T. (1995) Differential regulation of the expression in transgenic tobacco of the gene for β-glucuronidase under the control of the 5′-upstream regions of two catalase genes from castor bean. Plant Cell Physiol. **36:** 273–279

93 Drory A. and Woodson W. R. (1992) Molecular cloning and nucleotide sequence of a cDNA encoding catalase from tomato. Plant Physiol. **100:** 1605–1606

94 Niebel A., Heungens K., Barthels N., Inzé D., Van Montagu M. and Gheysen G. (1995) Characterization of a pathogen-induced potato catalase and its systemic expression upon nematode and bacterial infection. Mol. Plant-Microbe Interact. **8:** 371–378

95 Matters G. L. and Scandalios J. G. (1986) Effect of the free radical-generating herbicide paraquat on the expression of the superoxide dismutase (*Sod*) genes in maize. Biochem. Biophys. Acta **882:** 29–38

96 Matters G. L. and Scandalios J. G. (1986) Effect of elevated temperature on catalase and superoxide dismutase during maize development. Differentiation **30:** 190–196

97 Scandalios J. G. (1994) Regulation and properties of plant catalases. In: Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants, pp. 275–315, Foyer C. H. and Mullineaux P. M. (eds), CRC, Boca Raton

98 Zhong H. H. and McClung C. R. (1996) The circadian clock gates expression of two *Arabidopsis* catalase genes to distinct and opposite circadian phases. Mol. Gen. Genet. **251:** 196–203

99 Boldt R. and Scandalios J. G. (1995) Circadian regulation of the Cat3 catalase gene in maize (*Zea mays* L.): entrainment of the circadian rhythm of Cat3 by different light treatments. Plant J. **7:** 989–999

100 Feierabend J., Streb P., Schmidt M., Dehne S. and Shang W. (1996) Expression of catalase and its relation to light stress and stress tolerance. In: Physical Stresses in Plants: Genes and Their Products for Tolerance, pp. 223–234, Grillo S. and Leone A. (eds), Springer, Berlin

101 Streb P. and Feierabend J. (1996) Oxidative stress responses accompanying photoinactivation of catalase in NaCl-treated rye leaves. Bot. Acta **109:** 125–132

102 McClung C. R. (1997) Regulation of catalases in *Arabidopsis*. Free Rad. Biol. Med. **23:** 489–496

103 Omran R. G. (1980) Peroxide levels and the activities of catalase, peroxidase, and indoleacetic acid oxidase during and after chilling cucumber seedlings. Plant Physiol. **65:** 407–408

104 Feierabend J., Schaan C. and Hertwig B. (1992) Photoinactivation of catalase occurs under both high- and low-temperature stress conditions and accompanies photoinhibition of photosystem II. Plant Physiol. **100:** 1554–1561

105 Hertwig B., Streb P. and Feierabend J. (1992) Light dependence of catalase synthesis and degradation in leaves and the influence of interfering stress conditions. Plant Physiol. **100:** 1547–1553

106 Prasad T. K., Anderson M. D. and Stewart C. R. (1994) Acclimation, hydrogen peroxide, and abscisic acid protect mitochondria against irreversible chilling injury in maize seedlings. Plant Physiol. **105:** 619–627

107 Anderson M. D., Prasad T. K., Martin B. A. and Stewart C. R. (1994) Differential gene expression in chilling-acclimated maize seedlings and evidence for the involvement of abscisic acid in chilling tolerance. Plant Physiol. **105:** 331–339

108 Chen Z. and Klessig D. F. (1991) Identification of a soluble salicylic acid-binding protein that may function in signal transduction in the plant disease-resistance response. Proc. Natl. Acad. Sci. USA **88:** 8179–8183

109 Chen Z., Ricigliano J. W. and Klessig D. F. (1993) Purification and characterization of a soluble salicylic acid-binding protein from tobacco. Proc. Natl. Acad. Sci. USA **90:** 9533–9537

110 Chen Z., Silva H. and Klessig D. F. (1993) Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science **262:** 1883–1886

111 Conrath U., Chen Z., Ricigliano J. R. and Klessig D. F. (1995) Two inducers of plant defense responses, 2,6-dichloroisonicotinic acid and salicylic acid, inhibit catalase activity in tobacco. Proc. Natl. Acad. Sci. USA **92:** 7143–7147

112 Wendehenne D., Durner J., Chen Z. and Klessig D. F. (1998) Benzothiadiazole, an inducer of plant defenses, inhibits catalase and ascorbate peroxidase. Phytochemistry **47:** 651–657

113 Chen Z., Iyer S., Caplan A., Klessig D. F. and Fan B. (1997) Differential accumulation of salicylic acid and salicylic acid-sensitive catalase in different rice tissues. Plant Physiol. **114:** 193–201

114 Du H. and Klessig D. F. (1997) Identification of a soluble, high-affinity salicylic acid-binding protein in tobacco. Plant Physiol. **113:** 1319–1327

115 Bi Y.-M., Kenton P., Mur L., Darby R. and Draper J. (1995) Hydrogen peroxide does not function downstream of salicylic acid in the induction of PR protein expression. Plant J. **8:** 235–245

116 Rüffer M., Steipe B. and Zenk M. H. (1995) Evidence against specific binding of salicylic acid to plant catalase. FEBS Lett. **377:** 175–180

117 Summermatter K., Sticher L. and Métraux J.-P. (1995) Systemic responses in *Arabidopsis thaliana* infected and challenged with *Pseudomonas syringae* pv *syringae*. Plant Physiol. **108:** 1379–1385

118 Tenhaken R. and Rübel C. (1997) Salicylic acid is needed in hypersensitive cell death in soybean but does not act as a catalase inhibitor. Plant Physiol. **115:** 291–298

119 Ryals J. A., Neuenschwander U. H., Willits M. G., Molina A., Steiner H.-Y. and Hunt M. D. (1996) Systemic acquired resistance. Plant Cell **8:** 1809–1819

120 Chamnongpol S., Willekens H., Langebartels C., Van Montagu M., Inzé D. and Van Camp W. (1996) Transgenic tobacco with a reduced catalase activity develops necrotic lesions and induces pathogenesis-related expression under high light. Plant J. **10:** 491–503

121 Mittler R., Herr E. H., Orvar B. L., Van Camp W., Willekens H., Inzé D. et al. (1999) Enhanced pathogen sensitivity in transgenic tobacco plants with reduced capability to detoxify reactive oxygen intermediates. Proc. Natl. Acad. Sci. USA **96:** 14165–14170

122 Kendall E. J. and McKersie B. D. (1989) Free radical and freezing injury to cell membranes of winter wheat. Physiol. Plant. **76:** 86–94

123 Bridger G. M., Yang W., Falk D. E. and McKersie B. D. (1994) Cold acclimation increases tolerance of activated oxygen in winter cereals. J. Plant Physiol. **144:** 235–240

124 Mishra N. P., Fatma T. and Singhal G. S. (1995) Development of antioxidative defense system of wheat seedlings in response to high light. Physiol. Plant. **95:** 77–82

125 Grace S. C. and Logan B. A. (1996) Acclimation of foliar antioxidant systems to growth irradiance in three broad-leaved evergreen species. Plant Physiol. **112:** 1631–1640

126 Torsethaugen G., Pitcher L. H., Zilinskas B. A. and Pell E. J. (1997) Overproduction of ascorbate peroxidase in the tobacco chloroplast does not provide protection against ozone. Plant Physiol. **114:** 529–537

127 Aono M., Kubo A., Saji H., Natori T., Tanaka K. and Kondo N. (1991) Resistance to active oxygen toxicity of transgenic *Nicotiana tabacum* that expresses the gene for glutathione reductase from *Escherichia coli*. Plant Cell Physiol. **32:** 691–697

128 Foyer C. H., Souriau N., Perret S., Lelandais M., Kunert K.-J., Pruvost C. et al. (1995) Overexpression of glutathione reductase but not glutathione synthetase leads to increases in antioxidant capacity and resistance to photoinhibition in poplar trees. Plant Physiol. **109:** 1047–1057

129 Van Camp W., Willekens H., Bowler C., Van Montagu M., Inzé D., Reupold-Popp P. et al. (1994) Elevated levels of superoxide dismutase protect transgenic plants against ozone damage. Bio/technology **12:** 165–168

130 Pitcher L. H. and Zilinskas B. A. (1996) Overexpression of copper/zinc superoxide dismutase in the cytosol of transgenic tobacco confers partial resistance to ozone-induced foliar necrosis. Plant Physiol. **110:** 583–588

131 Pitcher L. H., Brennan E., Hurley A., Dunsmuir P., Tepperman J. M. and Zilinskas B. A. (1991) Overproduction of petunia chloroplastic copper/zinc superoxide dismutase does not confer ozone tolerance in transgenic tobacco. Plant Physiol. **97:** 452–455

132 Sen Gupta A., Heinen J. L., Holaday A. S., Burke J. J. and Allen R. D. (1993) Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase. Proc. Natl. Acad. Sci. USA **90:** 1629–1633

133 Sen Gupta A., Webb R. P., Holaday A. S. and Allen R. D. (1993) Overexpression of superoxide dismutase protects plants from oxidative stress. Plant Physiol. **103:** 1067–1073

134 Van Breusegem F., Kushnir S., Slooten L., Bauw G., Botterman J., Van Montagu M. et al. (1998) Processing of a chimeric protein in chloroplasts is different in transgenic maize and tobacco plants. Plant Mol. Biol. **38:** 491–496

135 Van Breusegem F., Slooten L., Stassart J.-M., Botterman J., Moens T., Van Montagu M. et al. (1999) Effects of overexpression of tobacco MnSOD in maize chloroplasts on foliar tolerance to cold and oxidative stress. J. Exp. Bot. **50:** 71–78

136 Van Breusegem F., Slooten L., Stassart J.-M., Moens T., Botterman J., Van Montagu M. et al. (1999) Overproduction of *Arabidopsis thaliana* FeSOD confers oxidative stress tolerance to transgenic maize. Plant Cell Physiol. **40:** 515–523

137 Van Camp W., Capiau K., Van Montagu M., Inzé D. and Slooten L. (1996) Enhancement of oxidative stress tolerance in transgenic tobacco plants overexpressing Fe-superoxide dismutase in chloroplasts. Plant Physiol. **112:** 1703–1714

138 McKersie B. D., Bowley S. R., Harjanto E. and Leprince O. (1996) Water-deficit tolerance and field performance of transgenic alfalfa overexpressing superoxide dismutase. Plant Physiol. **111:** 1177–1181

139 Aono M., Saji H., Sakamoto A., Tanaka K., Kondo N. and Tanaka K. (1995) Paraquat tolerance of transgenic *Nicotiana tabacum* with enhanced activities of glutathione reductase and superoxide dismutase. Plant Cell Physiol. **36:** 1687–1691

140 Slooten L., Capiau K., Van Camp W., Van Montagu M., Sybesma C. and Inzé D. (1995) Factors affecting the en-

hancement of oxidative stress tolerance in transgenic tobacco overexpressing manganese superoxide dismutase in the chloroplasts. Plant Physiol. **107:** 737–750
141 Jabs T., Tschöpe M., Colling C., Hahlbrock K. and Scheel D. (1997) Elicitor-stimulated ion fluxes and O_2^- from the oxidative burst are essential components in triggering defense gene activation and phytoalexin synthesis in parsley. Proc. Natl. Acad. Sci. USA **94:** 4800–4805
142 Asada K. (1992) Ascorbate peroxidase – a hydrogen peroxide-scavenging enzyme in plants. Physiol. Plant. **85:** 235–241
143 Asada K. (1994) Molecular properties of ascorbate peroxidase – a hydrogen peroxide-scavenging enzyme in plants. In: Frontiers of Reactive Oxygen Species in Biology and Medicine, pp. 103–106, Asada K. and Yoshikawa T. (eds), Elsevier, Amsterdam
144 Krause G. H. (1988) Photoinhibition of photosynthesis: an evaluation of damaging and protective mechanisms. Physiol. Plant. **74:** 566–574
145 Giardi M. T., Masojídek J. and Godde D. (1997) Effects of abiotic stresses on the turnover of the D_1 reaction centre II protein. Physiol. Plant. **101:** 635–642
146 Anderson J. M., Park Y.-I. and Chow W. S. (1997) Photoinactivation and photoprotection of photosystem II in nature. Physiol. Plant. **100:** 214–223
147 Powles S. B. (1984) Photoinhibition of photosynthesis induced by visible light. Annu. Rev. Plant Physiol. **35:** 15–44
148 Karpinski S., Escobar C., Karpinska B., Creissen G. and Mullineaux P. M. (1997) Photosynthetic electron transport regulates the expression of cytosolic ascorbate peroxidase genes in *Arabidopsis* during excess light stress. Plant Cell **9:** 627–640
149 Tsang E. W. T., Bowler C., Hérouart D., Van Camp W., Villarroel R., Genetello C. et al. (1991) Differential regulation of superoxide dismutases in plants exposed to environmental stress. Plant Cell. **3:** 783–792
150 Stieger P. A. and Feller U. (1997) Degradation of stromal proteins in pea (*Pisum sativum* L.) chloroplasts under oxidising conditions. J. Plant Physiol. **151:** 556–562
151 Smirnoff N. (1998) Plant resistance to environmental stress. Curr. Opin. Biotechnol. **9:** 214–219
152 Leprince O., Hendry G. A. F. and Atherton N. M. (1994) Free radical processes induced by desiccation in germinating maize: the relationship with respiration and loss of desiccation tolerance. Proc. R. Soc. Edinb. B **102:** 211–218
153 Sgherri C. L. M., Pinzino C. and Navari-Izzo F. (1993) Chemical changes and O_2^- production in thylakoid membranes under water stress. Physiol. Plant. **87:** 211–216
154 Sgherri C. L. M., Loggini B., Puliga S. and Navari-Izzo F. (1994) Antioxidant system in *Sporobolus stapfianus*: changes in response to desiccation and rehydration. Phytochemistry **35:** 561–565
155 Sgherri C. L. M. and Navari-Izzo F. (1995) Sunflower seedlings subjected to increasing water deficit stress: oxidative stress and defence mechanisms. Physiol. Plant. **93:** 25–30
156 Wellburn F. A. M., Lau K.-K., Milling P. M. K. and Wellburn A. R. (1996) Drought and air pollution affect nitrogen cycling and free radical scavenging in *Pinus halepensis* (Mill.). J. Exp. Bot. **47:** 1361–1367
157 Loggini B., Scartazza A., Brugnoli E. and Navari-Izzo F. (1999) Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. Plant Physiol. **119:** 1091–1099
158 Boo Y. C. and Jung J. (1999) Water deficit-induced oxidative stress and antioxidative defenses in rice plants. J. Plant Physiol. **155:** 255–261
159 Sgherri C. L. M., Pinzino C. and Navari-Izzo F. (1996) Sunflower seedlings subjected to increasing stress by water deficit: changes in O_2^- production related to the composition of thylakoid membranes. Physiol. Plant. **96:** 446–452
160 Moran J. F., Becana M., Iturbe-Ormaetxe I., Frechilla S., Klucas R. V. and Aparicio-Tejo P. (1994) Drought induces oxidative stress in pea plants. Planta **194:** 346–352
161 Gogorcena Y., Iturbe-Ormaetxe I., Escuredo P. R. and Becana M. (1995) Antioxidant defenses against activated oxygen in pea nodules subjected to water stress. Plant Physiol. **108:** 753–759
162 Aziz A. and Larher F. (1998) Osmotic stress induced changes in lipid composition and peroxidation in leaf discs of *Brassica napus* L. J. Plant Physiol. **153:** 754–762
163 Olsson M. (1995) Alterations in lipid composition, lipid peroxidation and anti-oxidative protection during senescence in drought stressed plants and non-drought stressed plants in *Pisum sativum*. Plant Physiol. Biochem. **33:** 547–553
164 Schwanz P., Picon C., Vivin P., Dreyer E., Guehl J.-M. and Polle A. (1996) Responses of antioxidative systems to drought stress in pendunculate oak and maritime pine as modulated by elevated CO_2. Plant Physiol. **110:** 393–402
165 Zhu D. and Scandalios J. G. (1994) Differential accumulation of manganese-superoxide dismutase transcripts in maize in response to abscisic acid and high osmoticum. Plant Physiol. **106:** 173–178
166 Jagtap V. and Bhargava S. (1995) Variation in the antioxidant metabolism of drought tolerant and drought susceptible varieties of *Sorghum bicolor* (L.) Moench. exposed to high light, low water and high temperature stress. J. Plant Physiol. **145:** 195–197
167 Fadzillah N. M., Finch R. P. and Burdon R. H. (1997) Salinity, oxidative stress and antioxidant responses in shoot cultures of rice. J. Exp. Bot. **48:** 325–331
168 Hernández J. A., Corpas F. J., Gómez M., Río L. A. del and Sevilla F. (1993) Salt-induced oxidative stress mediated by activated oxygen species in pea leaf mitochondria. Physiol. Plant. **89:** 103–110
169 Hernández J. A., Olmos E., Corpas F. J., Sevilla F. and Río L. A. del (1995) Salt-induced oxidative stress in chloroplasts of pea plants. Plant Sci. **105:** 151–167
170 Gossett D. R., Banks S. W., Millhollon E. P. and Lucas M. C. (1996) Antioxidant response to NaCl stress in a control and an NaCl-tolerant cotton cell line grown in the presence of paraquat, buthionine sulfoximine, and exogenous glutathione. Plant Physiol. **112:** 803–809
171 Piqueras A., Hernández J. A., Olmos E., Hellín E. and Sevilla F. (1996) Changes in antioxidant enzymes and organic solutes associated with adaptation of citrus cells to salt stress. Plant Cell Tissue Organ. Cult. **45:** 53–60
172 Savouré A., Thorin D., Davey M., Hua X.-J., Mauro S., Van Montagu M. et al. (1999) NaCl and $CuSO_4$ treatments trigger distinct oxidative defence mechanisms in *Nicotiana plumbaginifolia* L. Plant Cell Environ. **22:** 387–396
173 Gueta-Dahan Y., Yaniv Z., Zilinskas B. A. and Ben-Hayyim G. (1997) Salt and oxidative stress: similar and specific responses and their relation to salt tolerance in citrus. Planta **203:** 460–469
174 Gonzàlez-Meler M. A., Ribas-Carbo M., Giles L. and Siedow J. N. (1999) The effect of growth and measurement temperature on the activity of the alternative respiratory pathway. Plant Physiol. **120:** 765–772
175 De Santis A., Landi P. and Genchi G. (1999) Changes in mitochondrial properties in maize seedlings associated with selection for germination at low temperature: fatty acid composition, cytochrome *c* oxidase, and adenine nucleotide translocase activities. Plant Physiol. **119:** 743–754
176 Prasad T. K., Anderson M. D. and Stewart C. R. (1995) Localization and characterization of peroxidases in the mitochondria of chilling-acclimated maize seedlings. Plant Physiol. **108:** 1597–1605
177 Okuda T., Matsuda Y., Yamanaka A. and Sagisaka S. (1991) Abrupt increase in the level of hydrogen peroxide in leaves of winter wheat is caused by cold treatment. Plant Physiol. **97:** 1265–1267
178 O'Kane D., Gill V., Boyd P. and Burdon R. (1996) Chilling, oxidative stress and antioxidant responses in *Arabidopsis thaliana* callus. Planta **198:** 371–377
179 Fadzillah N. M., Gill V., Finch R. P. and Burdon R. H. (1996) Chilling, oxidative stress and antioxidant responses in shoot cultures of rice. Planta **199:** 552–556

180 Kingston-Smith A. H., Harbinson J. and Foyer C. H. (1999) Acclimation of photosynthesis, H_2O_2 content and antioxidants in maize (*Zea mays*) grown at sub-optimal temperatures. Plant Cell Environ. **22:** 1071–1083
181 Doke N. (1997) The oxidative burst: roles in signal transduction and plant stress. In: Oxidative Stress and the Molecular Biology of Antioxidant Defenses, pp. 785–813, Scandalios J. G. (ed.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
182 Dat J. F., Lopez-Delgado H., Foyer C. H. and Scott I. M. (2000) Effects of salicylic acid on oxidative stress and thermotolerance in tobacco. J. Plant Physiol. (in press)
183 Kraus T. E. and Fletcher R. A. (1994) Paclobutrazol protects wheat seedlings from heat and paraquat injury: is detoxification of active oxygen involved? Plant Cell Physiol. **35:** 45–52
184 Badiani M., Paolacci A. R., Fusari A., D'Ovidio R., Scandalios J. G. et al. (1997) Non-optimal growth temperatures and antioxidants in the leaves of Sorghum bicolor (L.) Moench. II. Short-term acclimation. J. Plant Physiol. **151:** 409–421
185 Sandmann G. and Böger P. (1980) Copper-mediated lipid peroxidation processes in photosynthetic membranes. Plant Physiol. **66:** 797–800
186 Halliwell B. and Gutteridge J. M. C. (1989) Free Radicals in Biology and Medicine. Clarendon, Oxford
187 De Vos C. H. R., Vonk M. J., Vooijs R. and Schat H. (1992) Glutathione depletion due to copper-induced phytochelatin synthesis causes oxidative stress in *Silene cucubalus*. Plant Physiol. **98:** 853–858
188 Weckx J. E. J. and Clijsters H. M. M. (1996) Oxidative damage and defense mechanisms in primary leaves of *Phaseolus vulgaris* as a result of root assimilation of toxic amounts of copper. Physiol. Plant. **96:** 506–512
189 Kurepa J., Hérouart D., Van Montagu M. and Inzé D. (1997) Differential expression of CuZn- and Fe-superoxide dismutase genes of tobacco during development, oxidative stress and hormonal treatments. Plant Cell Physiol. **38:** 463–470
190 Chongpraditnun P., Mori S. and Chino M. (1992) Excess copper induces a cytosolic Cu, Zn-superoxide dismutase in soybean root. Plant Cell Physiol. **33:** 239–244
191 Utriainen M., Kokko H., Auriola S., Sarrazin O. and Kärenlampi S. (1998) PR-10 protein is induced by copper stress in roots and leaves of a Cu/Zn tolerant clone of birch, *Betula pendula*. Plant Cell Environ. **21:** 821–828
192 Kochian L. V. (1995) Cellular mechanisms of aluminum toxicity and resistance in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **46:** 237–260
193 Richards K. D., Schott E. J., Sharma Y. K., Davis K. R. and Gardner R. C. (1998) Aluminum induces oxidative stress genes in *Arabidopsis thaliana*. Plant Physiol. **116:** 409–418
194 Cakmak I. and Horst W. J. (1991) Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). Physiol. Plant. **83:** 463–468
195 Weckx J. E. J. and Clijsters H. M. M. (1997) Zn phytotoxicity induces oxidative stress in primary leaves of *Phaseolus vulgaris*. Plant Physiol. Biochem. **35:** 405–410
196 Cakmack I. and Marschner H. (1993) Effect of zinc nutritional status on activities of superoxide radical and hydrogen peroxide scavenging enzymes in bean leaves. Plant Soil **155/156:** 127–130
197 Kampfenkel K., Van Montagu M. and Inzé D. (1995) Effects of iron excess on *Nicotiana plumbaginifolia* plants: implications to oxidative stress. Plant Physiol. **107:** 725–735
198 Lobréaux S., Thoiron S. and Briat J.-F. (1995) Induction of ferritin synthesis in maize leaves by an iron-mediated oxidative stress. Plant J. **8:** 443–449
199 Murphy T. M. and Huerta A. J. (1990) Hydrogen peroxide formation in cultured rose cells in response to UV-C radiation. Physiol. Plant. **78:** 247–253
200 Surplus S. L., Jordan B. R., Murphy A. M., Carr J. P., Thomas B. and Mackerness S. A.-H. (1998) Ultraviolet-B-induced responses in *Arabidopsis thaliana*: role of salicylic acid and reactive oxygen species in the regulation of transcripts encoding photosynthetic and acidic pathogenesis-related proteins. Plant Cell Environ. **21:** 685–694
201 Strid Å. (1993) Alteration in expression of defence genes in *Pisum sativum* after exposure to supplementary ultraviolet-B radiation. Plant Cell Physiol. **34:** 949–953
202 Kangasjärvi J., Talvinen J., Utriainen M. and Karjalainen R. (1994) Plant defence systems induced by ozone. Plant Cell Environ. **17:** 783–794
203 Mehlhorn H., Tabner B. J. and Wellburn A. R. (1990) Electron spin resonance evidence for the formation of free radicals in plants exposed to ozone. Physiol. Plant. **79:** 377–383
204 Runeckles V. C. and Vaartnou M. (1997) EPR evidence for superoxide anion formation in leaves during exposure to low levels of ozone. Plant Cell Environ. **20:** 306–314
205 Kubo A., Saji H., Tanaka K. and Kondo N. (1995) Expression of *Arabidopsis* cytosolic ascorbate peroxidase gene in response to ozone or sulfur dioxide. Plant Mol. Biol. **29:** 479–489
206 Pitcher L. H., Brennan E. and Zilinskas B. A. (1992) The antiozonant ethylenediurea does not act via superoxide dismutase induction in bean. Plant Physiol. **99:** 1388–1392
207 Hérouart D., Bowler C., Willekens H., Van Camp W., Slooten L., Van Montagu M. et al. (1993) Genetic engineering of oxidative stress resistance in higher plants. Phil. Trans. R. Soc. Lond. B **342:** 235–240
208 Conklin P. L. and Last R. L. (1995) Differential accumulation of antioxidant mRNAs in *Arabidopsis thaliana* exposed to ozone. Plant Physiol. **109:** 203–212
209 Sharma Y. and Davis K. R. (1997) The effects of ozone on antioxidant responses in plants. Free Rad. Biol. Med. **23:** 480–488
210 Sharma Y., León J., Raskin I. and Davis K. R. (1996) Ozone-induced responses in *Arabidopsis thaliana*: the role of salicylic acid in the accumulation of defense-related transcripts and induced resistance. Proc. Natl. Acad. Sci. USA **93:** 5099–5104
211 Sandermann H. Jr, Ernst D., Heller W. and Langebartels C. (1998) Ozone: an abiotic elicitor of plant defence reactions. Trends Plant Sci. **3:** 47–50
212 Schraudner M., Langebartels C. and Sandermann H. (1997) Changes in the biochemical status of plant cells induced by the environmental pollutant ozone. Physiol. Plant. **100:** 274–280
213 Schraudner M., Moeder W., Wiese C., Van Camp W., Inzé D., Langebartels C. et al. (1998) Ozone-induced oxidative burst in the ozone biomonitor plant, tobacco Bel W3. Plant J. **16:** 235–245
214 Pell E. J., Schlagnhaufer C. D. and Arteca R. N. (1997) Ozone-induced oxidative stress: mechanisms of action and reaction. Physiol. Plant. **100:** 264–273
215 Madamanchi N. R. and Alscher R. G. (1991) Metabolic bases for differences in sensitivity of two pea cultivars to sulfur dioxide. Plant Physiol. **97:** 88–93
216 Madamanchi N. R., Donahue J. L., Cramer C. L., Alscher R. G. and Pedersen K. (1994) Differential response of Cu,Zn superoxide dismutases in two pea cultivars during a short-term exposure to sulfur dioxide. Plant Mol. Biol. **26:** 95–103
217 Tanaka K. and Sugahara K. (1980) Role of superoxide dismutase in defense against SO_2 toxicity and an increase in superoxide dismutase activity with SO_2 fumigation. Plant Cell Physiol. **21:** 601–611
218 Rao M. V. (1992) Cellular detoxifying mechanisms determine the age dependent injury in tropical trees exposed to SO_2. J. Plant Physiol. **140:** 733–740
219 Orozco-Cardenas M. and Ryan C. A. (1999) Hydrogen peroxide is generated systemically in plant leaves by wounding and systemin via the octadecanoid pathway. Proc. Natl. Acad. Sci. USA **96:** 6553–6557

220 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
221 Watanabe T. and Sakai S. (1998) Effects of active oxygen species and methyl jasmonate on expression of the gene for a wound-inducible 1-aminocyclopropane-1-carboxylate synthase in winter squash (*Cucurbita maxima*). Planta **206:** 570–576
222 Collén J. and Pedersén M. (1994) A stress-induced oxidative burst in *Eucheuma platycladum* (Rhodophyta). Physiol. Plant. **92:** 417–422
223 Grant M. and Mansfield J. (1999) Early events in host-pathogen interactions. Curr. Opin. Plant Biol. **2:** 312–319
224 Dong X. (1998) SA, JA, ethylene, and disease resistance in plants. Curr. Opin. Plant Biol. **1:** 316–323
225 Hammond-Kosack K. E. and Jones J. D. G. (1996) Resistance gene-dependent plant defense responses. Plant Cell **8:** 1773–1791
226 Apostol I., Heinstein P. F. and Low P. S. (1989) Rapid stimulation of an oxidative burst during elicitation of cultured plant cells: role in defense and signal transduction. Plant Physiol. **90:** 109–116
227 Low P. S. and Heinstein P. F. (1986) Elicitor stimulation of the defense response in cultured plant cells monitored by fluorescent dyes. Arch. Biochem. Biophys. **249:** 472–479
228 Draper J. (1997) Salicylate, superoxide synthesis and cell suicide in plant defence. Trends Plant Sci. **2:** 162–165
229 Baker C. J., Orlandi E. W. and Mock N. M. (1993) Harpin, an elicitor of the hypersensitive response in tobacco caused by *Erwinia amylovora*, elicits active oxygen production in suspension cells. Plant Physiol. **102:** 1341–1344
230 Shirasu K., Nakajima H., Rajasekhar V. K., Dixon R. A. and Lamb C. (1997) Salicylic acid potentiates an agonist-dependent gain control that amplifies pathogen signals in the activation of defense mechanisms. Plant Cell **9:** 261–270
231 León J., Lawton M. A. and Raskin I. (1995) Hydrogen peroxide stimulates salicylic acid biosynthesis in tobacco. Plant Physiol. **108:** 1673–1678
232 Dempsey D. A., Shah J. and Klessig D. F. (1999) Salicylic acid and disease resistance in plants. Crit. Rev. Plant Sci. **18:** 547–575
233 Klessig D. F. and Malamy J. (1994) The salicylic acid signal in plants. Plant Mol. Biol. **26:** 1439–1458
234 Davis D., Merida J., Legendre L., Low P. S. and Heinstein P. (1993) Independent elicitation of the oxidative burst and phytoalexin formation in cultured plant cells. Phytochemistry **32:** 607–611
235 Degousée N., Triantaphylidès C. and Montillet J.-L. (1994) Involvement of oxidative processes in the signaling mechanisms leading to the activation of glyceollin synthesis in soybean (*Glycine max*). Plant Physiol. **104:** 945–952
236 Brisson L. F., Tenhaken R. and Lamb C. (1994) Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. Plant Cell **6:** 1703–1712
237 Neuenschwander U., Vernooij B., Friedrich L., Uknes S., Kessmann H. and Ryals J. (1995) Is hydrogen peroxide a second messenger of salicylic acid in systemic acquired resistance? Plant J. **8:** 227–233
238 Greenberg J. T., Monach P., Chou J. H., Josephy P. D. and Demple B. (1990) Positive control of a global antioxidant defense regulon activated by superoxide-generating agents in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87:** 6181–6185
239 Wu G., Shortt B. J., Lawrence E. B., Levine E. B., Fitzsimmons K. C. and Shah D. M. (1995) Disease resistance conferred by expression of a gene encoding H_2O_2-generating glucose oxidase in transgenic potato plants. Plant Cell **7:** 1357–1368
240 Morita S., Kaminaka H., Masumura T. and Tanaka K. (1999) Induction of rice cytosolic ascorbate peroxidase mRNA by oxidative stress: the involvement of hydrogen peroxide in oxidative stress signalling. Plant Cell Physiol. **40:** 417–422
241 Pastori G. M. and Trippi V. S. (1992) Oxidative stress induces high rate of glutathione reductase synthesis in a drought-resistant maize strain. Plant Cell Physiol. **33:** 957–961
242 Lappartient A. G. and Touraine B. (1997) Glutathione-mediated regulation of ATP sulfurylase activity, SO_4^{2-} uptake, and oxidative stress response in intact canola roots. Plant Physiol. **114:** 177–183
243 Banzet N., Richaud C., Deveaux Y., Kazmaizer M., Gagnon J. and Triantaphylidès C. (1998) Accumulation of small heat shock proteins, including mitochondrial HSP22, induced by oxidative stress and adaptive response in tomato cells. Plant J. **13:** 519–527
244 Matsuda Y., Okuda T. and Sagisaka S. (1994) Regulation of protein synthesis by hydrogen peroxide in crowns of winter wheat. Biosci. Biotech. Biochem. **58:** 906–909
245 Price A. H., Taylor A., Ripley S. J., Griffiths A., Trewavas A. J. and Knight M. R. (1994) Oxidative signals in tobacco increase cytosolic calcium. Plant Cell **6:** 1301–1310
246 Knight H., Trewavas A. J. and Knight M. R. (1996) Cold calcium signaling in Arabidopsis involves two cellular pools and a change in calcium signature after acclimation. Plant Cell **8:** 489–503
247 Wisniewski J.-P., Cornille P., Agnel J.-P. and Montillet J.-L. (1999) The extensin multigene family responds differentially to superoxide or hydrogen peroxide in tomato cell cultures. FEBS Lett. **447:** 264–268
248 Yalpani N., Enyedi A. J., León J. and Raskin I. (1994) Ultraviolet light and ozone stimulate accumulation of salicylic acid, pathogenesis-related proteins and virus resistance in tobacco. Planta **193:** 372–376
249 Mehlhorn H. (1990) Ethylene-promoted ascorbate peroxidase activity protects plants against hydrogen peroxide, ozone and paraquat. Plant Cell Environ. **13:** 971–976
250 Burke J. J., Gamble P. E., Hatfield J. L. and Quisenberry J. E. (1985) Plant morphological and biochemical responses to field water deficits. I. Responses of glutathione reductase activity and paraquat sensitivity. Plant Physiol. **79:** 415–419
251 Brüggemann W., Kooij T. A. W. van der and Hasselt P. R. van (1992) Long-term chilling of young tomato plants under low light and subsequent recovery. I. Growth, development and photosynthesis. Planta **186:** 172–178
252 Irigoyen J. J., Perez de Juan J. and Sanchez-Diaz M. (1996) Drought enhances chilling tolerance in a chilling-sensitive maize (*Zea mays*) variety. New Phytol. **134:** 53–59