

Review

The role of active oxygen species in plant signal transduction

Frank Van Breusegem, Eva Vranová, James F. Dat¹, Dirk Inzé*

Vakgroep Moleculaire Genetica, Departement Plantengenetica, Vlaams Interuniversitair Instituut voor Biotechnologie (VIB), Universiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium

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Abstract

Adequate responses to environmental changes are crucial for plant growth and survival. However, the molecular and biochemical mechanisms that orchestrate these responses are still poorly understood and the signaling networks involved remain elusive. A central role for active oxygen species (AOS) during biotic and abiotic stress responses is well-recognized, although under these situations AOS can either exacerbate damage or act as signal molecules that activate multiple defense responses. This duality can be obtained only when cellular levels of AOS are tightly controlled at both the production and consumption levels. This review focuses on the involvement of AOS in stress signal transduction in plants, guided by a summary of work performed in our laboratory on plants that are deficient in catalase activity. These plants not only reveal the importance of catalase in coping with environmental stresses, but also provide a powerful *in planta* model system to study the multiple roles of hydrogen peroxide during plant stress. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Active oxygen species; Catalase; Defense response; Oxidative stress; Signal transduction

1. Introduction

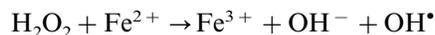
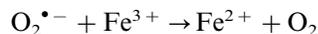
A wide range of environmental stresses (such as high and low temperatures, drought, salinity, UV or ozone stress and pathogen infections) is potentially harmful to plants. A common aspect of all these adverse conditions is the enhanced production of active oxygen species (AOS) within several subcellular compartments of the plant cell [1,2]. The reduction of oxygen to water provides the energy necessary for the impressive complexity of higher organisms, but its reduction is a mixed blessing. When AOS are incompletely reduced, they can be extremely reactive and oxidize biological molecules, such as DNA, proteins and lipids [3]. Molecular O₂ is reduced through four steps, thus generating several O₂ radical species (Eq. (1); [4]). The reaction chain requires initiation at the first step, whereas subsequent steps are exothermic and can occur spontaneously, either catalyzed or not.



The first step in O₂ reduction produces the relatively short-lived and not readily diffusible hydroperoxyl (HO₂^{•−}) and superoxide (O₂^{•−}) radicals. The half-life for O₂^{•−} is ≈ 2–4 μs [5,6]. These oxygen radicals are highly reactive, forming hydroperoxides with enes and dienes [7]. Furthermore, specific amino acids, such as histidine, methionine and tryptophan, can be oxidized by O₂^{•−} [6]. O₂^{•−} will also cause lipid peroxidation in a cellular environment, thereby weakening cell membranes [8].

Further reduction of O₂ generates hydrogen peroxide (H₂O₂), a relatively long-lived molecule (1 ms) that can diffuse some distance from its site of production [9,10]. The biological toxicity of H₂O₂ through oxidation of SH groups have long been known and is enhanced by the presence of metal catalysts through Haber–Weiss or Fenton-type reactions (Eq. (2)).

Fenton or Haber–Weiss reactions:



* Corresponding author. Tel.: +32-9-264-5170; fax +32-9-264-5349.

E-mail address: diinz@gengenp.rug.ac.be (D. Inzé).

¹ Present address: Département de Biologie et Ecophysiologie, Université de Franche-Comté, F-25030 Besançon Cedex, France.

The last species to be reduced in this reaction is the hydroxyl radical (OH^\bullet). It has a very strong potential and a half-life of $< 1 \mu\text{s}$. As a result, it has a very high affinity for biological molecules at its site of production, reacting at almost diffusion-controlled rates ($K > 10^9 \text{ m}^{-1} \text{ s}^{-1}$) [8].

AOS are mostly byproducts of the regular cellular metabolism, but they may be generated through the disruption of electron transport systems during stress conditions. The main sites of AOS production in the plant cell during abiotic stress are the organelles with highly oxidizing metabolic activities or with sustained electron flows: chloroplasts, mitochondria and microbodies (reviewed in Ref. [11]). In the chloroplasts, the primary source of H_2O_2 is thought to be the Mehler reaction. Photorespiration in the peroxisomes via glycolate oxidase is another source of H_2O_2 production. AOS production in plant mitochondria has received less attention in the past, but recent data suggest that they can be a source of AOS under specific stress conditions [12,13]. During plant–pathogen interactions, AOS formation is mechanistically similar to the oxidative burst in macrophages [14,15].

The reactive nature of AOS makes them potentially harmful to all cellular components. Fortunately, plants have the capacity to cope with these reactive oxygen species by eliminating them with an efficient AOS-scavenging system [16]. Because hydroxyl radicals are far too reactive to be controlled directly, aerobic organisms prefer to eliminate the less reactive precursor forms, superoxide and H_2O_2 . Superoxide dismutases (SOD) are considered key players within the antioxidant defense system, as they regulate the cellular concentration of $\text{O}_2^{\bullet-}$ and H_2O_2 ($\text{O}_2^{\bullet-} \xrightarrow{\text{SOD}} \text{H}_2\text{O}_2 + \text{O}_2$). Various SOD isozymes are active within the plant cell and are controlled developmentally and environmentally [17]. H_2O_2 is eliminated by catalases and peroxidases. Catalases remove the bulk of H_2O_2 , whereas ascorbate peroxidases (APX) can scavenge H_2O_2 that is inaccessible for catalase because of their higher affinity for H_2O_2 and their presence in different subcellular locations [18,19]. Other components of the plant AOS-scavenging system include all enzymes involved in the water–water cycle [20] and low-molecular weight antioxidant molecules, such as ascorbic acid, carotenoids and glutathione [21]. Under moderate stress conditions, the radicals are efficiently scavenged by this antioxidant defense system. However, during periods of more severe stress, the scavenging system may become saturated by the increased rate of radical production. Excessive levels of AOS result in damage to the photosynthetic apparatus (photoinhibition), ultimately leading to severe cellular damage and chlorosis of the leaves. The importance of the antioxidant defense system is demonstrated by the fact that overproduction of several AOS scavengers in different transgenic plants leads to a significant protec-

tion against oxidative stress [22]. Aside from their destructive nature, AOS can also be used in a beneficial way by the plant. AOS play an important role in inducing protection mechanisms during both biotic and abiotic stresses. The best known example is in the activation of resistance responses during incompatible plant–pathogen interactions. Upon infection, a plasma membrane NADPH oxidase is activated, producing superoxide radicals [23] that are converted into H_2O_2 via spontaneous dismutation or via SOD activity. The defensive properties of H_2O_2 are situated at several stages. (i) High levels of H_2O_2 are toxic for both pathogen and plant cells. Killing of the plant cells surrounding the infection site inhibits spreading of a (biotrophic) pathogen [24]. (ii) Hydrogen peroxide can serve as a substrate in peroxidative cross-linking reactions of lignin precursors and induce cross-linking of cell wall proteins. A reinforced plant cell wall slows down the spread of the pathogen and makes new infections more difficult. (iii) Because H_2O_2 is relatively stable and diffusible through membranes (in contrast with superoxide), it is a perfect candidate to act as a signal molecule during stress responses [2,25].

2. Catalase-deficient plants: model system for *in planta* H_2O_2 research

The first focus on H_2O_2 as a potential signal in plant defense response came with the identification of catalase as a salicylic acid (SA)-binding protein. Catalase was proposed to be a receptor that becomes inactive after SA binding. Catalase inactivation would lead to H_2O_2 accumulation, which was shown to act as a secondary messenger to induce pathogenesis-related (PR) genes [26]. The specific induction of the genes coding for glutathione *S*-transferase (GST) and glutathione peroxidase (GPX) by 2 mM H_2O_2 in cell suspension cultures of soybean and the ability of H_2O_2 produced during an incompatible plant–pathogen interaction to induce the same genes after diffusion through a dialysis membrane confirmed the signaling properties of H_2O_2 in plants [9].

In an attempt to gather more evidence on the role of H_2O_2 in plant stress signaling, our laboratory used a transgenic approach to alter H_2O_2 homeostasis *in planta*. By using antisense and sense technology, transgenic *Nicotiana tabacum* lines (CAT1AS) were produced that retained only 10% of their residual catalase activity [27]. These transgenic plants were used to study the local and systemic signaling role of H_2O_2 in pathogen defense.

Catalases are tetrameric heme-containing enzymes that convert $2\text{H}_2\text{O}_2$ into $\text{O}_2 + 2\text{H}_2\text{O}$, primarily preventing the potential damaging effects caused by changes in H_2O_2 homeostasis. Plants, unlike animals, have multi-

ple isoforms of catalase, which are present in the peroxisomes and glyoxisomes. Catalases are the principal H_2O_2 -scavenging enzymes in plants and can directly dismutate H_2O_2 or oxidize substrates, such as methanol, ethanol, formaldehyde and formic acid. Our laboratory showed that plant catalases can be divided into three classes: class 1 catalases are most prominent in photosynthetic tissues and are involved in the removal of H_2O_2 produced during photorespiration; class 2 are highly produced in vascular tissues and may play a role in lignification, their exact biological role remaining unknown; and class 3 are highly abundant in seeds and young plants and their activity is linked with the removal of excessive H_2O_2 produced during fatty acid degradation in the glyoxylate cycle in glyoxisomes [28]. cDNAs and genes of plant catalases have been characterized in a wide range of species. In *Arabidopsis thaliana*, *Nicotiana plumbaginifolia*, rice and maize, cDNAs that code for the three different classes have been isolated [29,30]. *N. plumbaginifolia* contains three active catalase-encoding genes (*Cat1*, *Cat2*, *Cat3*), two of which are expressed in mature leaves [31]. *Cat1* represents $\approx 80\%$ of leaf catalase activity and is located in palisade parenchyma cells. *Cat2* accounts for $\approx 20\%$ of the total activity and is found mainly in the phloem. To characterize plant catalases functionally, transgenic tobacco plants deficient in *Cat1*, *Cat2* or both were generated in our laboratory. Transgenic tobacco lines with strongly reduced levels of both catalase isoforms were produced by introducing a catalase-overproducing cassette. mRNA levels of both endogenous and transgenic catalases were strongly reduced by a cosuppression process. Specific catalase isozyme activities were suppressed by using antisense cassettes of the *Cat1* and *Cat2* genes (CAT1AS, CAT2AS). Stress assessment of these plants showed that catalase functions as a sink for cellular H_2O_2 under adverse conditions. CAT1AS tobacco plants were more sensitive than wild-type plants to either the redox-cycling herbicide methyl viologen or to H_2O_2 . Enhanced sensitivity against ozone and salt stress of the CAT1AS plants demonstrated that H_2O_2 arising from photorespiration is an important mediator of cellular toxicity during environmental adversity and that catalase activity is crucial for the cellular defense against these stresses [32].

3. Launching the defense response

The production of these catalase-deficient transgenic tobacco plants provided a unique inducible and non-invasive system to assess the role of changes in H_2O_2 homeostasis in plant stress signal transduction. Under low light (LL) conditions ($< 100 \mu\text{mol}/\text{m}^2$ per second photosynthetic photon fluence rate), no obvious phenotypes are observed in CAT1AS plants. Yet, exposure to

moderate or high light intensities (HL; $300\text{--}1000 \mu\text{mol}/\text{m}^2$ per second photosynthetic photon fluence rate) produced photorespiration-dependent changes in H_2O_2 homeostasis. The changes in H_2O_2 homeostasis can be modulated depending on the intensity and duration of the HL exposure. Hence, the CAT1AS plants are an ideal system to study the signal function of H_2O_2 in *planta*. Perturbance of H_2O_2 homeostasis can be sustained in time and there is no need for invasive techniques (i.e. injections of AOS generators) to modulate the cellular redox. But, more importantly, the use of CAT1AS plants avoids a potential debate about the physiological relevance of the H_2O_2 concentrations used.

Whether pathogen defense-related responses could be induced in the CAT1AS plants was first assessed (Fig. 1). CAT1AS plants exposed to LL did not produce basic or acidic PR proteins constitutively. On the other hand, in leaf tissue exposed to HL intensities, PR protein accumulation was observed in the absence of any pathogenic stimulus. Induction of PR proteins was most prominent in necrotic leaves, but could be observed after 2 weeks in the upper leaves without any macroscopic damage [10]. This observation prompted us to investigate further whether localized H_2O_2 could be a signal capable of inducing local and/or systemic acquired resistance (SAR). Half parts of lower and entire upper leaves were wrapped in aluminum foil and exposed for 2 days to HL. Plants were subsequently returned to LL and covered leaf parts were unwrapped. After 2 more weeks, PR-1a production was analyzed in the covered leaf parts of CAT1AS and wild-type plants. Whereas wild-type plants did not produce PR-1a, CAT1AS plants accumulated PR-1a both in covered parts of HL-exposed leaves and in upper leaves that had been entirely covered [10]. This observation indicated that changes in H_2O_2 homeostasis function as a trigger for both local and systemic defense responses, similar to what is reported during pathogenesis [33,34]. The *in vivo* relevance of the induced PR response in CAT1AS plants, in which defense activation was uncoupled from damage by pre-exposing them for only a few hours to HL, was confirmed by an enhanced resistance against the bacterial pathogen *Pseudomonas syringae* pv. *syringae* [10]. Transgenic potato plants with constitutively elevated H_2O_2 levels due to the overproduction of a fungal glucose oxidase in the apoplast show increased acidic chitinase and anionic peroxidase levels. The induced defense gene expression correlated with enhanced resistance against soft rot disease and *Phytophthora infestans* [35,36].

Although H_2O_2 is a diffusible molecule, its half-life is only 1 ms, which essentially excludes it from being the mobile signal to induce defense responses in systemic tissues. This problem might be overcome by a relay mechanism. Such a model was proposed by Alvarez et

al. [34], who observed microscopic hypersensitive (HR) lesions in *A. thaliana* that appear throughout the plant upon infection with an avirulent bacterial pathogen or by injection of glucose/glucose oxidase (generating H_2O_2). These ‘microbursts’ correlated with the expression of defense genes (*GST*, *PR-2*) and the development of SAR. Microbursts were prevented by injecting diphenyleiodonium (an inhibitor of NADPH oxidase), suggesting a central role for NADPH oxidase in this reiterative signal network. Recently, a similar relay and/or amplification mechanism induced by short HL changes in H_2O_2 homeostasis was found sufficient to activate an oxidase-dependent burst, which is required for oxidative cell death in CAT1AS plants (J. Dat, unpublished results).

Although the field of pathogenesis certainly led the way in oxidative stress signaling in plants for many years, several studies in the field of plant stress acclimation have delivered additional evidence for a signaling role of H_2O_2 [25]. Maize seedlings injected with H_2O_2 and menadione, which is a superoxide-generating compound, became more tolerant against chilling stress. In the acclimated seedlings, chilling tolerance was partly due to an enhanced antioxidant system that prevents the accumulation of AOS during chilling stress [37,38]. Nodal potato explants subcultured from H_2O_2 -treated microplants were resistant to a 15-h heat shock at 42 °C (a normally lethal treatment) even after 4 weeks of treatment [39]. Thermotolerance in mustard seedlings induced by SA or heat acclimation was correlated with a transient peak in H_2O_2 [40]. Treatment of crowns of winter wheat with

various levels of H_2O_2 and a catalase inhibitor led to concentration-dependent synthesis of proteins that were also induced when the plants were exposed to low temperatures [41]. Small heat-shock proteins, including mitochondrial HSP22, accumulated in cell suspension cultures of tomato after 2 mM H_2O_2 were added, but not in response to superoxide-generating agents [42]. Partial exposure to excess light or injection of H_2O_2 in *Arabidopsis* leaves induced protection from a subsequent excess light-induced photobleaching [43]. This acclimation correlated with the H_2O_2 -dependent expression of at least one antioxidant gene, the cytosolic *APX*. The role of H_2O_2 as a signal for *APX* induction was further demonstrated in rice cell suspensions. Transcript levels of cytosolic *APX* were also significantly increased by H_2O_2 or paraquat treatment in cell suspension cultures of rice. Addition of diethyldithiocarbamate (a SOD inhibitor resulting in low H_2O_2 levels) reduced the induction of *APX*, whereas inhibition of catalase or *APX* (resulting in H_2O_2 accumulation) increased *APX* mRNA levels [44]. Thus, H_2O_2 is clearly part of the signaling cascade that induces cytosolic *APX* by providing some information on the signal transduction sequences and molecular mechanisms underlying the induction of defense genes. We have also shown that changes in H_2O_2 homeostasis in CAT1AS plants induces the production of antioxidant (*GPX* and *APX*) and heat-shock proteins (*HSP17.6*). The induction of these—other than *PR*—defense proteins was rapid (< 6 h) and clearly preceded the appearance of leaf damage (8 h), suggesting that induction is independent of injury (J. Dat, unpublished results). These

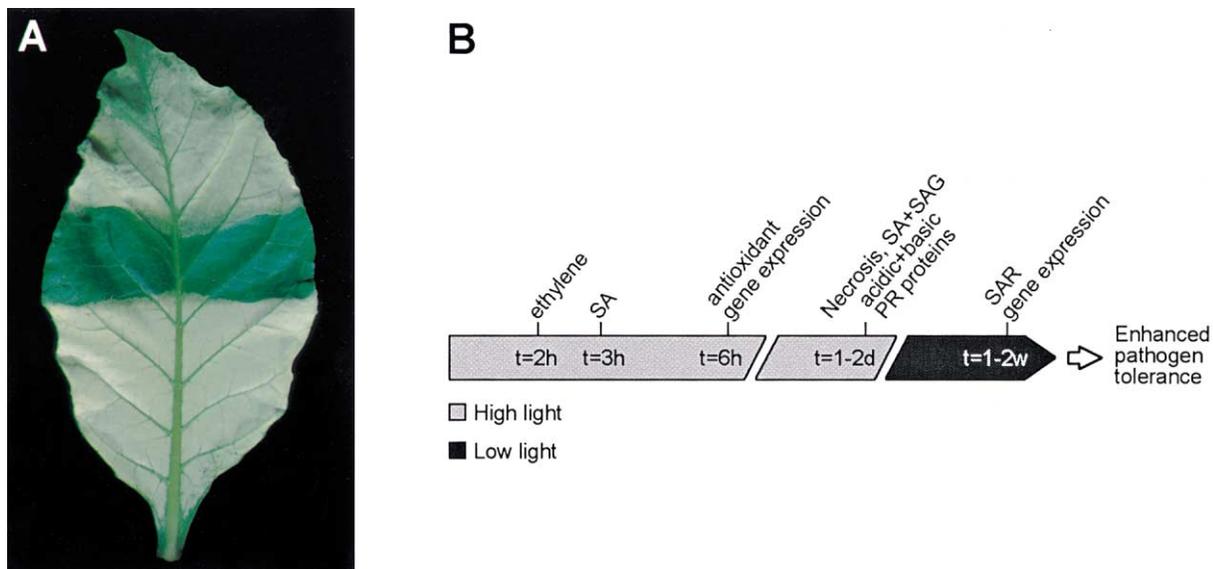


Fig. 1. Defense responses of CAT1AS plants. (A) Leaf of a CAT1AS plant exposed to high light (1000 $\mu\text{mol}/\text{m}^2$ per second photosynthetic photon fluence rate). The dark-green region was wrapped in foil during exposure. (B) Summary of responses induced during (grey) and after (black) HL exposure (courtesy of Dr Wim Van Camp).

results corroborate the role of H_2O_2 as a molecular signal for the induction of gene expression that is not limited to plant–pathogen interactions. We have recently obtained data showing that a short and controlled change in H_2O_2 homeostasis can be exploited to ‘immunize’ plants against a subsequent stress treatment (J. Dat, unpublished results).

As already indicated by the acclimatory effects of menadione in maize [37], the signaling properties of AOS are not exclusive to H_2O_2 . In bacteria and yeast, superoxide and H_2O_2 induce distinct sets of defense proteins, although the two responses overlap considerably [45,46]. However, increasing evidence points also toward differential signaling among AOS in plants. Digitonin or xanthine oxidase trigger the accumulation of a set of extensin transcripts in tomato cell suspensions [47]. One of these extensin transcripts is specifically induced by superoxide and not by H_2O_2 . Similarly, phytoalexin accumulation in parsley cell suspensions and lesion formation in lesion-stimulated disease resistance in *Arabidopsis* mutants are specifically induced by superoxide and not by H_2O_2 [48].

4. AOS induce plant cell death

Cell death is an essential process in a plant’s life cycle. Two main modes of action have been described in plants: programmed cell death (PCD) and necrosis. PCD is controlled genetically and shares some characteristic features with apoptotic cell death in animal cells, such as cell shrinkage, cytoplasmic condensation, chromatin condensation and DNA fragmentation. Necrosis results from severe and persistent trauma and is not considered to be orchestrated genetically [49,50].

Plant cell death has been best studied during the HR, which is typical of an incompatible plant–pathogen interaction. During the HR, an oxidative burst coincides with the induction of cell death at the site of pathogen attack. This localized cell death limits the spread of the invading pathogen. The source of the oxidative burst is considered to be partly a NADPH oxidase complex and pH-dependent cell wall peroxidases [2,51]. However, a decrease in activity of antioxidant enzymes probably generates AOS during the HR of several plant–pathogen interactions as well. In tobacco cells that undergo a HR upon infiltration with a fungal elicitor, accumulation of H_2O_2 is correlated with a decrease in *CAT1* and *CAT2* transcript and protein levels together with a decrease in total catalase activity [52]. Similarly, viral- and pathogen-induced HR-like cell death is accompanied by a post-transcriptional suppression of cytosolic APX production [53]. Suppression of H_2O_2 scavenging activity probably contributes to the accumulation of threshold levels of H_2O_2 or changes in H_2O_2 homeostasis, which are necessary for the activation of an active cell death program.

The first direct evidence that AOS induce plant cell death by initiating a transduction pathway rather than by direct killing due to phytotoxic levels was provided by experiments in soybean cell cultures. A short pulse of H_2O_2 was sufficient to activate a hypersensitive cell death program [9]. Five millimolar H_2O_2 initiated an active cell death pathway, requiring RNA and protein synthesis in *Arabidopsis* cell suspensions. To initiate an irreversible cell death process, H_2O_2 had to be present for 60 min [54]. We have recently demonstrated that *in planta*-generated changes in H_2O_2 homeostasis can induce an active cell death pathway. A transient change in H_2O_2 homeostasis in CAT1AS plants was sufficient to activate a PCD program similar to that observed during the HR. In CAT1AS tobacco plants, the cell death program that is induced by an increase in H_2O_2 can be impeded by inhibiting *de novo* protein synthesis, blocking Ca^{2+} fluxes, kinase/phosphatase activities and an oxidase-dependent burst (J. Dat, unpublished results). Thus, changes in H_2O_2 levels are not *per se* the cell death executioners, but they trigger a signal transduction cascade that ultimately leads to an active cell death program. In the ‘lesion-simulating disease resistance response’ mutant (*lsd1*) of *Arabidopsis*, superoxide, and not H_2O_2 , initiates a runaway cell death phenotype, providing genetic evidence for a role of $O_2^{\bullet-}$ in plant cell death [48]. The *lsd1* mutant grown under long days forms spontaneously necrotic lesions on leaves and cannot stop the spreading of cell death. $O_2^{\bullet-}$ drastically accumulates in front of the spreading zone of cell death. Hence, $O_2^{\bullet-}$ seems to be the critical signal in the cell death process, which is monitored via the ‘rheostat’ LSD1. In the ozone-sensitive *rcd1* mutant of *Arabidopsis*, $O_2^{\bullet-}$ is both necessary and sufficient to initiate an ethylene-dependent cell death signaling pathway, hereby propagating cell death [55]. Thus, H_2O_2 and $O_2^{\bullet-}$ are indisputably involved in genetically controlled cell death programs in plants.

5. Decision makers during stress situations

Under environmental stress conditions, plant growth is reduced or even stopped. Upon several stress conditions, a decrease in cell numbers, mitotic activity or cell division rates is observed in either leaves or roots [56,57]. The reduction of division under unfavorable conditions allows the conservation of energy, thereby launching the appropriate defense response and also reducing the risk of heritable damage [58,59]. Although little is known about how various stress conditions affect the cell cycle, dehydration of wheat leaves reduced the activity of the key cell cycle regulator, the cyclin-dependent kinase (CDK), probably as a result of an inhibitory phosphorylation [60]. Oxidative stress can arrest cell division and, in animal and yeast cells,

checkpoints have been identified that lead to cell cycle arrest upon oxidative stress [61]. AOS have been shown also to be good candidates to link stress responses and cell cycle progression in plants. Menadione impaired the G1-to-S transition, slowed down DNA replication and delayed the entry into mitosis in tobacco cell suspensions [62]. Cell cycle arrest was associated with an inhibition of CDK activity, cell cycle gene expression and a concomitant activation of stress genes. Similar effects were observed on tobacco plants [62]. Accordingly, exogenous application of micromolar concentrations of glutathione (GSH) increases the number of meristematic root cells undergoing mitosis, whereas depletion of GSH has the opposite effect [63]. A GSH-dependent developmental pathway is essential to initiate and maintain cell division during post-embryonic root development [58]. Whereas cell cycle progression is under negative control of AOS, somatic embryogenesis is stimulated by H_2O_2 [64] and is essential for root gravitropism [65]. The first molecular link that was demonstrated between oxidative stress and growth responses is a mitogen-activated protein kinase, ANP1. H_2O_2 can specifically activate ANP1 that triggers a phosphorylation cascade, resulting in the induction of specific stress genes, but blocks simultaneously the mitogenic action of auxin [66]. Although the exact role of AOS during plant growth and development is still poorly understood, the above examples demonstrate that under environmental stress conditions, AOS can play a decisive role at specific checkpoints during the plant cell cycle, leading to an adequate defense response.

6. Part of a team: interaction between AOS and other signaling components

It would be difficult to imagine that H_2O_2 is the only signal responsible for the orchestration of the diverse responses described above. A close interaction with other signaling molecules and pathways is a more realistic scenario. Ethylene, SA, jasmonic acid (JA) and nitric oxide (NO) are other well-known players in the induction of plant defense responses against many biotic and abiotic stresses [67–70].

The relationship between AOS and SA is well-documented. SA is mainly associated with the establishment of SAR [68]. Upon pathogen infection, levels of SA increase in both challenged and non-challenged leaves and plants become more resistant against a subsequent infection. In transgenic plants that do not accumulate SA because of the overexpression of the bacterial SA-dehydrogenase gene (*nahG*), SAR is compromised [71]. Originally, H_2O_2 was thought to be the downstream signal of SA in SAR. SA and its active analogues were proposed to bind and inactivate catalase. However,

treatment of tobacco plants with SA increased H_2O_2 levels and H_2O_2 induced the production of PR-1, which is associated with SAR [26,72]. Later studies revealed that exogenously applied H_2O_2 could not induce PR-1 in *nahG* plants [73,74] and that the induction of PR proteins in CAT1AS plants followed an SA-dependent pathway. PR induction was investigated in crosses between CAT1AS and *nahG* plants. These plants were impaired for PR induction after exposure to HL. In addition, a biphasic (after 6 and 33 h) increase in free SA as well as a monophasic increase in SA glucoside was found in HL-treated CAT1AS plants [10]. These data caused a debate on the location of H_2O_2 versus SA in the plant defense signaling pathway, because other reports indicate that SA can enhance H_2O_2 accumulation [75,76]. A model was suggested by Van Camp et al. [77] that seems to have put a hold on this controversy. In this model, H_2O_2 and SA work in unison as a self-amplifying system. In support of such a model, H_2O_2 induces SA accumulation [10,76] and SA enhances H_2O_2 accumulation [26,78]. This self-amplification loop, involving H_2O_2 and SA, may generate microbursts that intensify and spread the H_2O_2 signal required for oxidative cell death and establishment of acquired resistance against pathogens [34,77]. Evidence is accumulating that H_2O_2 and SA are also complementary secondary messengers in acclimation to abiotic stress; for instance, SA and H_2O_2 levels are enhanced during acclimation to heat stress [40,79] and plants become acclimated to heat and chilling stress by spraying with H_2O_2 or SA [39,80,81]. Thus, similar mechanisms that utilize both SA and H_2O_2 may operate to establish immunity against environmental cues in plants.

Ethylene is another well-established signaling molecule that has long been recognized in plant stress responses. It is linked to stress responses following wounding, chilling and pathogen attack [69,82]. By using the CAT1AS tobacco plants, an interplay with AOS was demonstrated. Enhanced production of bPR-2 in CAT1AS plants had already suggested that H_2O_2 induces biosynthesis of stress ethylene, which was confirmed by a dramatic, but transient, increase in ethylene production in CAT1AS plants, 2–3 h after exposure to HL. This result is consistent with ethylene kinetics during pathogen infection. The ethylene peak followed accumulation of H_2O_2 , but preceded that of SA, thereby implying that H_2O_2 can work as an intermediate signal upstream of both ethylene and SA during plant stress responses [10]. Accordingly, exogenous application of H_2O_2 activates ethylene production in pine needles [83]. Furthermore, ozone, which is believed to form AOS in the apoplast, induces accumulation of ethylene in tobacco plants within 1 h of the start of the treatment. This early induction positively correlates with ozone sensitivity. Such an early ethylene burst

does not occur in the ozone-tolerant tobacco cultivar BelB [84–86]. The ethylene burst seems to originate from *de novo* ethylene synthesis, because this peak in ethylene emission corresponds with elevated levels of its precursor, 1-aminocyclopropane-1-carboxylic acid (ACC). The ethylene burst can be blocked by inhibitors of enzymes involved in ethylene biosynthesis enzymes, such as ACC synthase and ACC oxidase [83,87]. Recently, the specific activation of ethylene biosynthesis and functional ethylene signaling was shown to be required for $O_2^{\bullet-}$ accumulation and cell death [55].

Jasmonic acid (JA) is a stress hormone primarily involved in wound stress responses. In comparison with ethylene, JA has opposite effects on cell death. The JA-insensitive and -biosynthetic *Arabidopsis* mutants *jar1* and *fad3/7/8* show an increased magnitude of an ozone-induced oxidative burst, SA accumulation and HR-like cell death [88]. Accordingly, pretreatment of ozone-sensitive *A. thaliana* ecotype Cvi-0 with methyl jasmonate abolishes ozone-induced cell death [55]. This observation is in agreement with the potentiating effect of JA on GSH synthesis, therewith limiting AOS accumulation and consequent cell death [89].

Nitric oxide (NO^-) is used by mammals to regulate various biological process of the immune, nervous and vascular systems [4]. It is now becoming evident that NO is also a ubiquitous signal in plants. NO promotes leaf expansion, seed germination and de-etiolation [90,91], but it also inhibits hypocotyl and internode elongation, induces defense genes and phytoalexin production and potentiates the induction of hypersensitive cell death [91–93]. Hence, NO can provoke beneficial or harmful effects in plant cells. This dual role probably depends on a concentration-dependent threshold window and on direct interactions with AOS species [94]. Because of an unpaired electron, NO readily interacts with $O_2^{\bullet-}$ to form peroxynitrite ($ONOO^-$) that oxidizes DNA, lipids, protein thiols and iron clusters, resulting in impaired enzyme activities and cellular damage. However, interaction of NO with lipid alcoxyl or lipid peroxy radicals breaks the self-perpetuating chain reaction during lipid peroxidation [94]. The inhibition of some enzymes by NO may be beneficial. NO-mediated inhibition of aconitase activity may reduce the electron flow through the mitochondrial electron transport chain, thereby decreasing mitochondrial oxidative stress. Moreover, NO converts cytosolic aconitase into an Fe-regulatory protein that controls iron homeostasis [95]. Because Fe catalyzes the Fenton reaction that produces harmful hydroxyl radicals, a limited availability of iron may prevent oxidative damage. Thus, NO could affect oxidative metabolism at multiple levels either by exacerbating or counteracting AOS effects.

7. Conclusions

We have tried to summarize the wealth of data on the role and importance of AOS during biotic and abiotic stress responses. The level and kind of AOS are determining factors for the type of response. H_2O_2 and $O_2^{\bullet-}$ can induce different genes, in combination or separately, thereby giving more flexibility to the AOS signaling function. At low concentrations, AOS induce defense genes and adaptive responses. Sublethal levels of AOS can acclimate plants to biotic and abiotic stress conditions and reduce plant growth, probably as part of an adaptational response. Although substantial genomic responses and enzyme activities are affected by AOS, the molecular mechanisms of adaptation are still poorly understood and the signaling pathways involved remain elusive. At higher concentrations, AOS trigger a genetically controlled cell death program. AOS also communicate with other signal molecules and pathways forming a network that can orchestrate downstream responses. The recently established role of AOS during growth and morphogenesis suggests that AOS are not only stress signal molecules, but that they are an intrinsic signal in plant growth and development. Genetic analysis and further physiological studies will help position AOS signals in transduction cascades and improve our understanding on how AOS are perceived and transduced into specific downstream responses.

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References

- [1] C.H. Foyer, P.M. Mullineaux, Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants, CRC Press, Boca Raton, FL, 1994.
- [2] C. Lamb, R.A. Dixon, The oxidative burst in plant disease resistance, *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 48 (1997) 251–275.
- [3] C. Richter, M. Schweizer, Oxidative stress in mitochondria, in: J.G. Scandalios (Ed.), *Oxidative Stress and the Molecular Biology of Antioxidant Defenses*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1997, pp. 169–200, Monograph Series, vol. 34.

- [4] S. Hippeli, I. Heiser, E.F. Elstner, Activated oxygen and free oxygen radicals in pathology: new insights and analogies between animals and plants, *Plant Physiol. Biochem.* 37 (1999) 167–178.
- [5] M.W. Sutherland, The generation of oxygen radicals during host plant responses to infection, *Physiol. Mol. Plant Pathol.* 39 (1991) 79–93.
- [6] J.P. Knox, A.D. Dodge, Singlet oxygen and plants, *Phytochemistry* 24 (1985) 889–896.
- [7] M.L. Salin, Toxic oxygen species and protective systems of the chloroplast, *Physiol. Plant.* 72 (1987) 681–689.
- [8] B. Halliwell, J.M.C. Gutteridge, *Free Radicals in Biology and Medicine*, Clarendon Press, Oxford, 1989.
- [9] A. Levine, R. Tenhaken, R. Dixon, C. Lamb, H₂O₂ from the oxidative burst orchestrates the plant hypersensitive disease resistance response, *Cell* 79 (1994) 583–593.
- [10] S. Chamnongpol, H. Willekens, W. Moeder, C. Langebartels, H. Sandermann Jr, M. Van Montagu, D. Inzé, W. Van Camp, Defense activation and enhanced pathogen tolerance induced by H₂O₂ in transgenic plants, *Proc. Natl. Acad. Sci. USA* 95 (1998) 5818–5823.
- [11] J. Dat, S. Vandenabeele, E. Vranová, M. Van Montagu, D. Inzé, F. Van Breusegem, Dual action of the active oxygen species during plant stress responses, *Cell. Mol. Life Sci.* 57 (2000) 779–795.
- [12] R. Pellinen, T. Palva, J. Kangasjärvi, Subcellular localization of ozone-induced hydrogen peroxide production in birch (*Betula pendula*) leaf cells, *Plant J.* 20 (1999) 349–356.
- [13] D.P. Maxwell, Y. Wang, L. McIntosh, The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells, *Proc. Natl. Acad. Sci. USA* 96 (1999) 8271–8276.
- [14] T. Keller, H.G. Damude, D. Werner, P. Doerner, R.A. Dixon, C. Lamb, A plant homolog of the neutrophil NADPH oxidase gp91^{phox} subunit gene encodes a plasma membrane protein with Ca²⁺ binding motifs, *Plant Cell* 10 (1998) 255–266.
- [15] T. Xing, V.J. Higgins, E. Blumwald, Race-specific elicitors of *Cladosporium fulvum* promote translocation of cytosolic components of NADPH oxidase to the plasma membrane of tomato cells, *Plant Cell* 9 (1997) 249–259.
- [16] L. Slooten, M. Van Montagu, D. Inzé, Manipulation of oxidative stress tolerance in transgenic plants, in: K. Lindsey (Ed.), *Transgenic Plant Research*, Harwood, Amsterdam, 1998, pp. 241–262.
- [17] W. Van Camp, D. Inzé, M. Van Montagu, The regulation and function of tobacco superoxide dismutases, *Free Rad. Biol. Med.* 23 (1997) 515–520.
- [18] J.G. Scandalios, Regulation and properties of plant catalases, in: C.H. Foyer, P.M. Mullineaux (Eds.), *Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants*, CRC Press, Boca Raton, 1994, pp. 275–315.
- [19] G.P. Creissen, E.A. Edwards, P.M. Mullineaux, Glutathione reductase and ascorbate peroxidase, in: C.H. Foyer, P.M. Mullineaux (Eds.), *Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants*, CRC Press, Boca Raton, FL, 1994, pp. 343–364.
- [20] K. Asada, The water–water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons, *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 50 (1999) 601–639.
- [21] R.G. Alscher, J.L. Hess, *Antioxidants in Higher Plants*, CRC Press, Boca Raton, FL, 1993.
- [22] F. Van Breusegem, M. Van Montagu, D. Inzé, Engineering stress tolerance in maize, *Outlook Agric.* 27 (1998) 115–124.
- [23] R. Desikan, J.T. Hancock, M.J. Coffey, N.J. Neill, Generation of active oxygen in elicited cells of *Arabidopsis thaliana* is mediated by a NADPH oxidase-like enzyme, *FEBS Lett.* 382 (1996) 213–217.
- [24] A. Levine, R.I. Pennell, M.E. Alvarez, R. Palmer, C. Lamb, Calcium-mediated apoptosis in a plant hypersensitive disease resistance response, *Curr. Biol.* 6 (1996) 427–437.
- [25] C.H. Foyer, H. Lopez-Delgado, J.F. Dat, I.M. Scott, Hydrogen peroxide- and glutathione-associated mechanisms of acclimatory stress tolerance and signalling, *Physiol. Plant.* 100 (1997) 241–254.
- [26] Z. Chen, H. Silva, D.F. Klessig, Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid, *Science* 262 (1993) 1883–1886.
- [27] S. Chamnongpol, H. Willekens, C. Langebartels, M. Van Montagu, D. Inzé, W. Van Camp, Transgenic tobacco with a reduced catalase activity develops necrotic lesions and induces pathogenesis-related expression under high light, *Plant J.* 10 (1996) 491–503.
- [28] H. Willekens, R. Villarroel, M. Van Montagu, D. Inzé, W. Van Camp, Molecular identification of catalases from *Nicotiana plumbaginifolia* (L.), *FEBS Lett.* 352 (1994) 79–83.
- [29] J.A. Frugoli, M.A. McPeck, T.L. Thomas, C.R. McClung, Intron loss and gain during evolution of the catalase gene family in angiosperms, *Genetics* 149 (1998) 355–365.
- [30] C.R. McClung, Regulation of catalases in *Arabidopsis*, *Free Rad. Biol. Med.* 23 (1997) 489–496.
- [31] H. Willekens, C. Langebartels, C. Tiré, M. Van Montagu, D. Inzé, W. Van Camp, Differential expression of catalase genes in *Nicotiana plumbaginifolia* (L.), *Proc. Natl. Acad. Sci. USA* 91 (1994) 10450–10454.
- [32] H. Willekens, S. Chamnongpol, M. Davey, M. Schraudner, C. Langebartels, M. Van Montagu, D. Inzé, W. Van Camp, Catalase is a sink for H₂O₂ and is indispensable for stress defence in C₃ plants, *EMBO J.* 16 (1997) 4806–4816.
- [33] S. Dorey, F. Baillieul, M.-A. Pierrel, P. Saindrenan, B. Fritig, S. Kauffmann, Spatial and temporal induction of cell death, defense genes, and accumulation of salicylic acid in tobacco leaves reacting hypersensitively to a fungal glycoprotein elicitor, *Mol. Plant-Microbe Interact.* 10 (1997) 646–655.
- [34] M.E. Alvarez, R.I. Pennell, P.-J. Meijer, A. Ishikawa, R.A. Dixon, C. Lamb, Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity, *Cell* 92 (1998) 773–784.
- [35] G. Wu, B.J. Shortt, E.B. Lawrence, J. León, K.C. Fitzsimmons, E.B. Levine, I. Raskin, D.M. Shah, Activation of host defense mechanisms by elevated production of H₂O₂ in transgenic plants, *Plant Physiol.* 115 (1997) 427–435.
- [36] G. Wu, B.J. Shortt, E.B. Lawrence, E.B. Levine, K.C. Fitzsimmons, D.M. Shah, Disease resistance conferred by expression of a gene encoding H₂O₂-generating glucose oxidase in transgenic potato plants, *Plant Cell* 7 (1995) 1357–1368.
- [37] T.K. Prasad, M.D. Anderson, B.A. Martin, C.R. Stewart, Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide, *Plant Cell* 6 (1994) 65–74.
- [38] T.K. Prasad, Mechanisms of chilling-induced oxidative stress injury and tolerance in developing maize seedlings: changes in antioxidant system, oxidation of proteins and lipids, and protease activities, *Plant J.* 10 (1996) 1017–1026.
- [39] H. Lopez-Delgado, J.F. Dat, C.H. Foyer, I.M. Scott, Induction of thermotolerance in potato microplants by acetylsalicylic acid and H₂O₂, *J. Exp. Bot.* 49 (1998) 713–720.
- [40] J.F. Dat, C.H. Foyer, I.M. Scott, Changes in salicylic acid and antioxidants during induced thermotolerance in mustard seedlings, *Plant Physiol.* 118 (1998) 1455–1461.
- [41] Y. Matsuda, T. Okuda, S. Sagisaka, Differences in activities related to cessation of synthesis of inducible proteins as an early response to cold between hardy and less hardy cultivars of winter wheat, *Biosci. Biotech. Biochem.* 58 (1994) 32–37.

- [42] N. Banzet, C. Richaud, Y. Deveaux, M. Kazmaizer, J. Gagnon, C. Triantaphylidès, Accumulation of small heat shock proteins, including mitochondrial HSP22, induced by oxidative stress and adaptive response in tomato cells, *Plant J.* 13 (1998) 519–527.
- [43] S. Karpinski, H. Reynolds, B. Karpinska, G. Wingsle, G. Creissen, P. Mullineaux, Systemic signaling and acclimation in response to excess excitation energy in *Arabidopsis*, *Science* 284 (1999) 654–657.
- [44] S. Morita, H. Kaminaka, T. Masumura, K. Tanaka, Induction of rice cytosolic ascorbate peroxidase mRNA by oxidative stress; the involvement of hydrogen peroxide in oxidative stress signalling, *Plant Cell Physiol.* 40 (1999) 417–422.
- [45] B. Demple, C.F. Amábile-Cuevas, Redox redux: the control of oxidative stress responses, *Cell* 67 (1991) 837–839.
- [46] D.J. Jamieson, Oxidative stress responses of the yeast *Saccharomyces cerevisiae*, *Yeast* 14 (1998) 1511–1527.
- [47] J.-P. Wisniewski, P. Cornille, J.-P. Agnel, J.-L. Montillet, The extensin multigene family responds differentially to superoxide or hydrogen peroxide in tomato cell cultures, *FEBS Lett.* 447 (1999) 264–268.
- [48] T. Jabs, R.A. Dietrich, J.L. Dangl, Initiation of runaway cell death in an *Arabidopsis* mutant by extracellular superoxide, *Science* 273 (1996) 1853–1856.
- [49] D.G. Gilchrist, Programmed cell death in plant disease: The purpose and promise of cellular suicide, *Ann. Rev. Phytopathol.* 36 (1998) 393–414.
- [50] R.I. Pennell, C. Lamb, Programmed cell death in plants, *Plant Cell* 9 (1997) 1157–1168.
- [51] G.P. Bolwell, P. Wojtaszek, Mechanisms for the generation of reactive oxygen species in plant defence—broad perspective, *Physiol. Mol. Plant Pathol.* 51 (1997) 347–366.
- [52] S. Dorey, F. Baillieul, P. Saindrenan, B. Fritig, S. Kauffmann, Tobacco class I and II catalases are differentially expressed during elicitor-induced hypersensitive cell death and localized acquired resistance, *Mol. Plant-Microbe Interact.* 11 (1998) 1102–1109.
- [53] R. Mittler, X. Feng, M. Cohen, Post-transcriptional suppression of cytosolic ascorbate peroxidase expression during pathogen-induced programmed cell death in tobacco, *Plant Cell* 10 (1998) 461–473.
- [54] R. Desikan, A. Reynolds, J.T. Hancock, S.J. Neill, Harpin and hydrogen peroxide both initiate programmed cell death but have differential effects on defence gene expression in *Arabidopsis* suspension cultures, *Biochem. J.* 330 (1998) 115–120.
- [55] K. Overmyer, H. Tuominen, R. Kettunen, C. Betz, C. Langebartels, H. Sandermann Jr, J. Kangasjärvi, Ozone-sensitive *Arabidopsis rcd1* mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death, *Plant Cell* 12 (2000) 1849–1862.
- [56] S. Burssens, K. Himanen, B. van de Cotte, T. Beeckman, M. Van Montagu, D. Inzé, N. Verbruggen, Expression of cell cycle regulatory genes and morphological alterations in response to salt stress in *Arabidopsis thaliana*, *Planta* 211 (2000) 632–640.
- [57] C. Granier, D. Inzé, F. Tardieu, Spatial distribution of cell division rate can be deduced from that of p34^{cdc2} kinase activity in maize leaves grown at contrasting temperatures and soil water conditions, *Plant Physiol.* 124 (2000) 1393–1402.
- [58] T. Vernoux, R.C. Wilson, K.A. Seeley, J.-P. Reichheld, S. Muroy, S. Brown, S.C. Maughan, C.S. Cobbett, M. Van Montagu, D. Inzé, M.J. May, Z.R. Sung, The root meristemless1/cadmium sensitive2 gene defines a glutathione-dependent pathway involved in initiation and maintenance of cell division during postembryonic root development, *Plant Cell* 12 (2000) 97–110.
- [59] M.J. May, T. Vernoux, C. Leaver, M. Van Montagu, D. Inzé, Glutathione homeostasis in plants: implications for environmental sensing and plant development, *J. Exp. Bot.* 49 (1998) 649–667.
- [60] U. Schuppler, P.-H. He, P.C.L. John, R. Munns, Effect of water stress on cell division and cell-division-cycle 2-like cell-cycle kinase activity in wheat leaves, *Plant Physiol.* 117 (1998) 667–678.
- [61] A.G. Paulovich, D.P. Toczyski, L.H. Hartwell, When checkpoints fail, *Cell* 88 (1997) 315–321.
- [62] J.-P. Reichheld, T. Vernoux, F. Lardon, M. Van Montagu, D. Inzé, Specific checkpoints regulate plant cell cycle progression in response to oxidative stress, *Plant J.* 17 (1999) 647–656.
- [63] R. Sánchez-Fernández, M. Fricker, L.B. Corben, N.S. White, N. Sheard, C.J. Leaver, M. Van Montagu, D. Inzé, M.J. May, Cell proliferation and hair tip growth in the *Arabidopsis* root are under mechanistically different forms of redox control, *Proc. Natl. Acad. Sci. USA* 94 (1997) 2745–2750.
- [64] K. Cui, G. Xing, X. Liu, G. Xing, Y. Wang, Effect of hydrogen peroxide on somatic embryogenesis of *Lycium barbarum* L., *Plant Sci.* 146 (1999) 9–16.
- [65] J. Joo, Y. Bae, J. Lee, Generation of reactive oxygen species is essential for gravitropism in primary root of maize. Abstract presented at the Plant Biology 2000 Meeting, San Diego, CA, July 15–19, 2000 (No. 22004).
- [66] Y. Kovtun, W.-L. Chiu, G. Tena, J. Sheen, Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants, *Proc. Natl. Acad. Sci. USA* 97 (2000) 2940–2945.
- [67] D.F. Klessig, J. Durner, R. Noad, D.A. Navarre, D. Wendehenne, D. Kumar, J.M. Zhou, J. Shah, S. Zhang, P. Kachroo, Y. Trifa, D. Pontier, E. Lam, H. Silva, Nitric oxide and salicylic acid signaling in plant defense, *Proc. Natl. Acad. Sci. USA* 97 (2000) 8849–8855.
- [68] J. Durner, J. Shah, D.F. Klessig, Salicylic acid and disease resistance in plants, *Trends Plant Sci.* 2 (1997) 266–274.
- [69] X. Dong, SA, JA, ethylene, and disease resistance in plants, *Curr. Opin. Plant Biol.* 1 (1998) 316–323.
- [70] I.M. Scott, J.F. Dat, H. Lopez-Delgado, C.H. Foyer, Salicylic acid and hydrogen peroxide in abiotic stress signaling in plants, *Phyton* 39 (1999) 13–17.
- [71] T.P. Delaney, L. Friedrich, J.A. Ryals, *Arabidopsis* signal transduction mutant defective in chemically and biologically induced disease resistance, *Proc. Natl. Acad. Sci. USA* 92 (1995) 6602–6606.
- [72] Z. Chen, J.W. Ricigliano, D.F. Klessig, Purification and characterization of a soluble salicylic acid-binding protein from tobacco, *Proc. Natl. Acad. Sci. USA* 90 (1993) 9533–9537.
- [73] Y.-M. Bi, P. Kenton, L. Mur, R. Darby, J. Draper, Hydrogen peroxide does not function downstream of salicylic acid in the induction of PR protein expression, *Plant J.* 8 (1995) 235–245.
- [74] U. Neuenschwander, B. Vernooij, L. Friedrich, S. Uknes, H. Kessmann, J. Ryals, Is hydrogen peroxide a second messenger of salicylic acid in systemic acquired resistance?, *Plant J.* 8 (1995) 227–233.
- [75] H. Kauss, W. Jeblick, Pretreatment of parsley suspension cultures with salicylic acid enhances spontaneous and elicited production of H₂O₂, *Plant Physiol.* 108 (1995) 1171–1178.
- [76] J. León, M.A. Lawton, I. Raskin, Hydrogen peroxide stimulates salicylic acid biosynthesis in tobacco, *Plant Physiol.* 108 (1995) 1673–1678.
- [77] W. Van Camp, M. Van Montagu, D. Inzé, H₂O₂ and NO: redox signals in disease resistance, *Trends Plant Sci.* 3 (1998) 330–334.
- [78] M.V. Rao, G. Paliyath, D.P. Ormrod, D.P. Murr, C.B. Watkins, Influence of salicylic acid on H₂O₂ production, oxidative stress, and H₂O₂-metabolizing enzymes, *Plant Physiol.* 115 (1997) 137–149.
- [79] J.F. Dat, H. Lopez-Delgado, C.H. Foyer, I.M. Scott, Parallel changes in H₂O₂ and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings, *Plant Physiol.* 116 (1998) 1351–1357.

- [80] T. Senaratna, D. Touchell, E. Bunn, K. Dixon, Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants, *Plant Growth Reg.* 30 (2000) 157–161.
- [81] T.K. Prasad, M.D. Anderson, C.R. Stewart, Acclimation, hydrogen peroxide, and abscisic acid protect mitochondria against irreversible chilling injury in maize seedlings, *Plant Physiol.* 105 (1994) 619–627.
- [82] R. Solano, J.R. Ecker, Ethylene gas: perception, signaling and response, *Curr. Opin. Plant Biol.* 1 (1998) 393–398.
- [83] G. Ievinsh, E. Tillberg, Stress-induced ethylene biosynthesis in pine needles: a search for the putative 1-aminocyclopropane-1-carboxylic-independent pathway, *J. Plant Physiol.* 145 (1995) 308–314.
- [84] M. Schraudner, W. Moeder, C. Wiese, W. Van Camp, D. Inzé, C. Langebartels, H. Sandermann Jr, Ozone-induced oxidative burst in the ozone biomonitor plant, tobacco Bel W3, *Plant J.* 16 (1998) 235–245.
- [85] M. Schraudner, C. Langebartels, H. Sandermann Jr, Plant defence systems and ozone, *Biochem. Soc. Trans.* 24 (1996) 456–461.
- [86] M. Schraudner, C. Langebartels, H. Sandermann, Changes in the biochemical status of plant cells induced by the environmental pollutant ozone, *Physiol. Plant.* 100 (1997) 274–280.
- [87] J. Tuomainen, C. Betz, J. Kangasjärvi, D. Ernst, Z.-H. Yin, C. Langebartels, H. Sandermann Jr, Ozone induction of ethylene emission in tomato plants: regulation by differential accumulation of transcripts for the biosynthetic enzymes, *Plant J.* 12 (1997) 1151–1162.
- [88] M.V. Rao, H.-i. Lee, R.A. Creelman, J.E. Mullet, K.R. Davis, Jasmonic acid signaling modulates ozone-induced hypersensitive cell death, *Plant Cell* 12 (2000) 1633–1646.
- [89] C. Xiang, D.J. Oliver, Glutathione metabolic genes coordinately respond to heavy metals and jasmonic acid in Arabidopsis, *Plant Cell* 10 (1998) 1539–1550.
- [90] Y.Y. Leshem, E. Haramaty, The characterization and contrasting effects of the nitric oxide free radical in vegetative stress and senescence of *Pisum sativum* Linn. foliage, *J. Plant Physiol.* 148 (1996) 258–263.
- [91] M.V. Beligni, L. Lamattina, Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants, *Planta* 210 (2000) 215–221.
- [92] M. Delledonne, Y. Xia, R.A. Dixon, C. Lamb, Nitric oxide functions as a signal in plant disease resistance, *Nature (Lond.)* 394 (1998) 585–588.
- [93] J. Durner, D. Wendehenne, D.F. Klessig, Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose, *Proc. Natl. Acad. Sci. USA* 95 (1998) 10328–10333.
- [94] M.V. Beligni, L. Lamattina, Is nitric oxide toxic or protective?, *Trends Plant Sci.* 4 (1999) 299–300.
- [95] D.A. Navarre, D. Wendehenne, J. Durner, R. Noad, D.F. Klessig, Nitric oxide modulates the activity of tobacco aconitase, *Plant Physiol.* 122 (2000) 573–582.