Reactive oxygen gene network of plants

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Reactive oxygen species (ROS) control many different processes in plants. However, being toxic molecules, they are also capable of injuring cells. How this conflict is resolved in plants is largely unknown. Nonetheless, it is clear that the steady-state level of ROS in cells needs to be tightly regulated. In Arabidopsis, a network of at least 152 genes is involved in managing the level of ROS. This network is highly dynamic and redundant, and encodes ROS-scavenging and ROS-producing proteins. Although recent studies have unraveled some of the key players in the network, many questions related to its mode of regulation, its protective roles and its modulation of signaling networks that control growth, development and stress response remain unanswered.

Ever since the introduction of molecular oxygen (O₂) into our atmosphere by O₂-evolving photosynthetic organisms ~2.7 billion years ago, reactive oxygen species (ROS) have been the unwelcome companions of aerobic life [1]. In contrast to O₂, these partially reduced or activated derivatives of oxygen (singlet oxygen (¹O₂), superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂) and hydroxyl radical (HO·)) are highly reactive and toxic, and can lead to the oxidative destruction of cells [2]. Consequently, the evolution of all aerobic organisms has been dependent on the development of efficient ROS-scavenging mechanisms. In recent years, a new role for ROS has been identified: the control and regulation of biological processes, such as growth, cell cycle, programmed cell death, hormone signaling, biotic and abiotic stress responses and development [3–13]. These studies extend our understanding of ROS and suggest a dual role for ROS in plant biology as both toxic byproducts of aerobic metabolism and key regulators of growth, development and defense pathways.

The use of ROS as signaling molecules by plant cells suggests that, during the course of evolution, plants were able to achieve a high degree of control over ROS toxicity and are now using ROS as signaling molecules. Controlling ROS toxicity while enabling ROS such as H₂O₂ or O₂⁻ to act as signaling molecules appears to require a large gene network composed of at least 152 genes in Arabidopsis. This ‘reactive oxygen gene network’ of plants is described below.

Modulation of ROS signaling by the reactive oxygen gene network of plants

Whereas Ca²⁺ signaling is predominantly controlled in plants by storage and release, ROS signaling is controlled by production and scavenging (Figure 1). Different developmental or environmental signals feed into the ROS signaling network and perturb ROS homeostasis in a compartment-specific or even cell-specific manner. Perturbed ROS levels are perceived by different proteins, enzymes or receptors and modulate different developmental, metabolic and defense pathways. ROS can be generated by various enzymatic activities, of which the best studied are NADPH oxidases, and removed by an array of ROS-scavenging enzymes (Table 1). The intensity, duration and localization of the different ROS signals are determined by interplay between the ROS-producing and ROS-scavenging pathways of the cell. This process requires a tight mode of regulation and might involve amplification and/or feedback inhibition loops. In addition to regulating the intensity and duration of the different ROS signals, the ROS-scavenging pathways are also responsible for maintaining a low steady-state baseline of ROS on which the different signals can be registered. The reactive oxygen gene network therefore modulates the steady-state level of ROS in the different cellular compartments for signaling purposes as well as for protection against oxidative damage.

It is possible that the use of ROS as versatile signaling molecules originated from their proposed use to sense stress. Most forms of biotic or abiotic stress disrupt the metabolic balance of cells, resulting in enhanced production of ROS. Simple organisms, such as bacteria or yeast, sense the enhanced production of ROS using redox-sensitive transcription factors and other molecular sensors, activate different ROS defense pathways, and regulate their metabolic pathways to lower the production rate of ROS [14,15]. This ‘basic cycle’ of ROS metabolism maintains a low steady-state level of ROS in cells. Variations on this pathway could have originated during evolution and contributed to the use of ROS as signaling molecules to control more specialized processes such as plant growth and defense, hormonal signaling, and development.
Production of ROS in plants

Organelles with a highly oxidizing metabolic activity or with an intense rate of electron flow, such as chloroplasts, mitochondria and microbodies, are a major source of ROS production in plant cells. Together with an extensive battery of oxidases, the plant cell is well armed for bountiful yet flexible ROS production. In chloroplasts, the primary sources of ROS production are the Mehler reaction and the antenna pigments [2]. Production of ROS by these sources is enhanced in plants by conditions limiting CO₂ fixation, such as drought, salt and temperature stress, as well as by the combination of these conditions with high-light stress. In C3 plants, limiting CO₂ conditions can also activate the photorespiratory pathway [16]. As part of this pathway, H₂O₂ is generated in peroxisomes by the enzymatic activity of glycolate oxidase. Production of H₂O₂ in microbodies can also occur during lipid catabolism as a side-product of fatty acid oxidation. In mitochondria, over-reduction of the electron transport chain is the main source of O₂⁻ production under specific stress conditions [17]. Additional sources of ROS in plant cells include the detoxifying reactions catalyzed by cytochromes in both the cytoplasm and the endoplasmic reticulum [18].

Plasma membrane NADPH-dependent oxidases, similar to the mammalian calcium-regulated NADPH oxidase (NOX5), have been the subject of intense investigation [19–23]. They are thought to play a key role in ROS signaling, and contain a multimeric flavocytochrome that forms an electron transport chain capable of reducing O₂ to O₂⁻. Chemical inhibitors of mammalian NADPH oxidase (such as diphenylene iodium) have been shown to block or impair ROS production during biotic or abiotic stress in plants [24–27]. Moreover, genes homologous to the mammalian subunit gp91phox have been identified in different plant genomes [28–31] (Table 1). In addition to NADPH oxidases, pH-dependent cell wall peroxidases, germin-like oxalate oxidases and amine oxidases have been proposed to generate ROS at the apoplast [32–34]. Although much attention has been given to NADPH oxidases and their possible role in cell signaling, other ROS-producing mechanisms in the mitochondria, apoplast and peroxisome are likely to play a role in ROS signaling in response to different stimuli or developmental signals.

Enzymatic components of the ROS-scavenging pathways of plants

Major ROS-scavenging enzymes of plants include superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX) and peroxiredoxin (PrxR) (Table 1). Together with the antioxidants ascorbic acid and glutathione [35], these enzymes provide...
Table 1. Gene annotation and expression of the reactive oxygen species scavenging network of *Arabidopsis*<sup>a</sup>

<table>
<thead>
<tr>
<th>Enzyme and reaction</th>
<th>Gene name</th>
<th>AGI code</th>
<th>Localization</th>
<th>Expression (mutant to wild-type)</th>
<th>Expression (stress to control)</th>
</tr>
</thead>
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<tr>
<td><strong>Superoxide dismutase (SOD)</strong></td>
<td>FeSOD (FSD2)</td>
<td>At5g51100.1</td>
<td>Chi</td>
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<td>*</td>
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<tr>
<td></td>
<td>FeSOD (FSD3)</td>
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<tr>
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<td>CuSOD (CSD1)</td>
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<td>CuZnSOD (CSD2)</td>
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<td>MnSOD-like</td>
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<td>*</td>
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<td>APX2</td>
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<td>Cyt</td>
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<td>APX3</td>
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<td>APX6</td>
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<td>Cyt, chi, mit</td>
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<td>*</td>
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<td></td>
<td>APX7</td>
<td>At1g33680.1</td>
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<td>GR2</td>
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<tr>
<td><strong>Dehydroascorbate reductase (DHAR)</strong></td>
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<td>Phospholipid GPX8</td>
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<td>Chi</td>
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<tr>
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<td>Ferritin 2</td>
<td>At3g56080.1</td>
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<td>Ferritin 3</td>
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<td>Chi, mit</td>
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<td>Ferritin 4</td>
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<td><strong>NADPH oxidase</strong></td>
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<td>NADPH oxidase (RbohB)</td>
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<td><strong>Aox</strong></td>
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<tr>
<td><strong>Peroxiredoxin (Prx)</strong></td>
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<td>Nuc</td>
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<td>2-cys PrxR B</td>
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<td>Cyt</td>
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<td></td>
<td>2-cys PrxR F</td>
<td>Atg30050.1</td>
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<td>PrxR G</td>
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<td>Mem, chi</td>
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<td>Type 2 PrxR D</td>
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<td>Cyt</td>
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<tr>
<td><strong>Thioredoxins (Trx)</strong></td>
<td>See supplementary material (31 genes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Glutaredoxin (GR)</strong></td>
<td>DHA + 2GSH → Asc + NADPH</td>
<td></td>
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</tr>
</tbody>
</table>

<sup>a</sup>Main reactive oxygen species (ROS) genes and their corresponding enzymatic activities are listed (a complete list can be viewed as supplementary material in the online version). With the exception of NADPH oxidase (a ROS producer), all genes included in the table encode ROS-scavenging enzymes. Arabidopsis genome initiative (AGI) codes were obtained from the TIGR annotation database. Predicted subcellular localization was determined with Ipsort, Predotar and Targetp. When in bold, localization was based on published data and on recent studies on the chloroplast and mitochondrial proteomes [78,79]. Relative expression data (mutant to wild-type or stress to control; right columns) visualized with Genesis software [80] was obtained from DNA chip analyses (Arabidopsis ATH1 Affymetrix chips). Comparative analysis of samples was performed using the Affymetrix MAS5.0 software and the Silicon Genetics GeneSpring version 5.1. Each column represents the different mutants or stress conditions (AsCAT, antisense Cat2; KoAPX, knockout APX1; KoSOD, knockout SOD2). Red indicates an increase, green indicates a decrease. Positive and negative values indicate upregulation or downregulation compared with the control line or unstressed condition, respectively. Cells containing asterisks have one absent call (i.e. no detectable expression) in either control, mutant or stressed situation. Gray cells indicate absent calls in both samples. White cells indicate that the genes are not represented on the ATH1 GeneChip.

Abbreviations: chi, chloroplast; cyt, cytosol; ER, endoplasmic reticulum; HL, high light; mem, membrane; mit, mitochondria; nuc, nuclei; per, peroxisomes.
cells with highly efficient machinery for detoxifying $\text{O}_2$ and $\text{H}_2\text{O}_2$. The balance between SODs and the different $\text{H}_2\text{O}_2$-scavenging enzymes in cells is considered to be crucial in determining the steady-state level of $\text{O}_2$ and $\text{H}_2\text{O}_2$. This balance, together with the sequestration of metal ions by ferritin and other metal-binding proteins, prevents the formation of the highly toxic HO· radical via the metal-dependent Haber–Weiss reaction or the Fenton reaction [2,36]. The cellular pools of the antioxidants ascorbic acid and glutathione are maintained in their reduced state by a set of enzymes capable of using NAD(P)H to regenerate oxidized glutathione or ascorbic acid (e.g. monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase; Table 1). Although dehydroascorbate reductases and glutaredoxins are indicated in Table 1 as capable of reducing dehydroascorbic acid, many other enzymes in plants can catalyze this reaction with different efficiencies [37]. In addition, monodehydroascorbate radicals can be reduced back into ascorbic acid via ferredoxin using electrons diverted from the photosynthetic apparatus in the water–water cycle in chloroplasts [38] (Figure 2). Scavenging of $\text{H}_2\text{O}_2$ can also be mediated in plants by ‘classical’ plant peroxidases (class III) using a range of reductants (not included in Table 1). These enzymes are encoded by a large gene family of at least 73 genes in Arabidopsis and are found in the cytosol, vacuole, apoplast or cell wall [39,40]. Although transcriptome analysis of knockout and antisense plants deficient in Apx1 or Cat2 indicated that the steady-state level of transcripts that encode certain classical plant peroxidases is elevated in these plants, the significance of these findings is unknown at present. Additional research is needed to determine whether specific class III plant peroxidases contribute to the ROS-scavenging capacity of cells and should be included in the ROS gene network of plants.

Substrate affinity, reaction rate and enzyme concentration are important parameters when assessing the relative contribution of the different enzymes shown in Table 1 to ROS detoxification [6]. Under optimal conditions, a combination of these parameters allows enzymes such as the CuZnSOD to have a diffusion-limited reaction rate ($2 \times 10^8 \text{M}^{-1} \text{s}^{-1}$) [2]. Membranes are highly susceptible to oxidative stress. In plant cells, they are protected by the activity of specific phospholipid glutathione peroxidases and by $\alpha$-tocopherol (vitamin E), which is kept in its reduced state by the pool of reduced ascorbic acid [41–43]. Protection of cells against $^5\text{O}_2$ is generally believed to be mediated by carotenoids. Although it would be interesting to explore how these pathways, as well as those involved in the biosynthesis of ascorbate and glutathione, and those responsible for the repair of oxidative damage in cells, are linked to the genes described in Table 1, these mechanisms are not described here [20,35,44,45].

**Cellular localization and coordination of the ROS-scavenging pathways of plants**

The various scavenging enzymes encoded by the ROS network can be found in almost every subcellular compartment (Figure 2). In addition, usually more than one enzymatic scavenging activity per a particular ROS can be found in each of the different compartments (e.g. GPXs, PrxRs and APXs in the cytosol and chloroplast, and APXs and CATs in peroxisomes; Figure 2). When the relative function of the different enzymes in the different cellular compartments is considered, it is important to remember that ROS such as $\text{H}_2\text{O}_2$ can diffuse between different compartments [46]. Furthermore, transporters for the antioxidants ascorbic acid and glutathione are likely to be central in determining the specific concentrations of these compounds and the redox potential in the different cellular compartments [35,47]. An anonymous player in the ROS signaling network is the vacuole. Its ROS-scavenging and ROS-producing potentials are unknown. It is possible that this organelle, because of its relatively large cellular volume, plays an unanticipated essential role in the control of ROS metabolism in plants. Likewise, the antioxidant capacity and signaling role of the apoplast and peroxisomes have only recently begun to gain recognition [27,47,48]. However, the specific enzymes and genes involved in controlling ROS metabolism in these compartments are only partially known (Table 1; Figure 2).

Recent studies in Arabidopsis have suggested that the mode of coordination between different components of the ROS removal network of plants is complex [49,50]. For example, the application of light stress to Arabidopsis results in the induction of cytosolic and not chloroplastic defense enzymes [49,51,52], even though most ROS produced during light stress are thought to be generated in chloroplasts or peroxisomes [38]. In addition, at least three different enzymes of the ROS-scavenging pathways of plants have been found to be targeted to both the chloroplast and mitochondria, suggesting a high degree of coordination in defense responses between these different cellular compartments [37,53,54]. Future studies with knockout lines for the different ROS-scavenging or ROS-producing enzymes would reveal how the different branches of the ROS network co-operate during biotic and abiotic stresses.

**Gene annotation and expression of the ROS network in Arabidopsis**

Table 1 and the table in the supplementary material (available in the on-line version) summarize all known ROS-scavenging genes and NADPH oxidases in Arabidopsis. Expression data for the different genes in three different knockout or antisense lines (Apx1, CSD2 and Cat2) and in plants subjected to different abiotic stresses (e.g. drought, salt, cold or high light) are also included. Although data were assembled from different experiments and should only be considered from a qualitative point of view, Table 1 highlights two fundamental principles of a genetic network: redundancy and flexibility. Redundancy is evident from the transcript expression in the different knockouts. Thus, deficiency in Apx1 (KoAPX; Table 1) results in the enhanced expression of a type 2 PrxR (E) and a ferritin; deficiency in Cat2 (AsCAT; Table 1) results in the enhanced expression of a copper-binding protein, glutaredoxin and thioredoxin; and deficiency in CSD2 (KoSOD; Table 1) results in the enhanced expression of FeSODs, ferritin and CATs. Flexibility is evident from transcript expression in response to different stresses, as
the ROS network responds in a highly specific manner to each of the different stresses included in Table 1.

Several studies have shown that biotic and abiotic stresses are accompanied by an oxidative burst mediated by NADPH oxidases [26,55]. Changes in the expression of NADPH oxidases provoked through perturbed ROS homeostasis by internal (knockouts) or external stresses suggest that transcriptional activation of certain NADPH oxidases is an essential intermediate step in the activation or amplification of defense responses [27,50,56,57].

When using changes in transcript expression as a measure for how important a specific gene is in protecting cells against ROS or other stresses, it is important to remember that, although many genes can play a key role in cell protection, their expression might not respond to stress. For example, transcript expression for CSD2 or
chlAOX (Immutans) shows no response to the different stresses (Table 1). However, analysis of knockouts for these genes revealed that they play a pivotal role in chloroplast protection in the presence or absence of abiotic stress [50,58].

**Key components of the reactive oxygen gene network identified by reverse genetics**

Recent studies of knockout and antisense lines for Cat2, Apx1, chlAOX, mitAOX, CSD2, 2-cysteine PrxR and various NADPH oxidases have revealed a strong link between ROS and processes such as growth, development, stomatal responses and biotic and abiotic stress responses [7,8,50,52,57,59–62]. These findings demonstrate the complex nature of the ROS gene network in plants and its modulation of key biological processes. Although mutants for all the proteins listed above are viable, demonstrating the redundancy of the ROS network, a clear phenotype is associated with each of the different genes, suggesting that they play a key role in the ROS signaling network of plants.

Based on the analysis of the different mutants, Cat2, Apx1, ChlAOX, CSD2 and 2-cysteine PrxR are essential for the protection of chloroplasts against oxidative damage. Suppression of CSD2, for example, results in the induction of a high-light stress response in Arabidopsis plants grown under a low light intensity [50]. The absence of Apx1 results in reduced photosynthetic activity, augmented induction of heat shock proteins during light stress and altered stomatal responses [52].

Catalase deficiency triggers growth retardation and high sensitivity to ozone and high light stress [57]. By contrast, the absence of the NADPH oxidase genes AtrbohD and AtrbohF suppresses ROS production and the defense response of Arabidopsis against pathogen attack [7], and knockout of AtrbohC has an altered root phenotype [8]. AtrbohD and AtrbohF are also essential for abscisic acid signaling in guard cells [10]. In tomato, suppression of NADPH oxidase results in a highly branched phenotype and fasciated reproductive organs [62]. Characterization of additional mutants and double or triple knockouts for different genes in the network should reveal additional roles for the ROS signaling network of plants and unravel more links between ROS and different signaling pathways. These studies might also identify alternative or redundant components of the network that are unknown at present [63].

**ROS signal transduction pathway of plants**

Recent studies in Arabidopsis have uncovered some of the key components involved in the ROS signal transduction pathway of plants. Although the receptors for ROS are unknown at present, it has been suggested that plant cells sense ROS via at least three different mechanisms (Figure 3): (i) unidentified receptor proteins; (ii) redox-sensitive transcription factors, such as NPR1 or HSFs; and (iii) direct inhibition of phosphatases by ROS [6].

Downstream signaling events associated with ROS sensing involve Ca^{2+} and Ca^{2+}-binding proteins, such as...
calmodulin [65–67], the activation of G-proteins [68], and the activation of phospholipid signaling, which results in the accumulation of phosphatidic acid [69,70]. It is possible that the localization of ROS signals in specific cellular sites is similar to that of Ca$_{2+}$ signals in response to stimuli [67]. The development of intercellular ROS sensors analogous to the fluorescence-based protein sensors for Ca$_{2+}$ would help considerably in studying the spatial and temporal nature of ROS signaling in plants.

A recently identified serine/threonine protein kinase (OXI1) has been shown to play a central role in ROS sensing and the activation of mitogen-activated-protein kinases (MAPKs) 3 and 6 by Ca$_{2+}$ [70]. This kinase is also activated by PDK1 through the phospholipase-C/D–phosphatidic-acid pathway [69]. A MAPK cascade involving MAPK5/6 acts downstream of OXI1 and controls the activation of different defense mechanisms in response to ROS stress [3,20]. The expression of different transcription factors is enhanced by ROS and includes members of the WRKY, Zat, RAV, GRAS and Myb families [50,52,56,57,59,64,71–73]. Recent studies using knockout plants have revealed that the zinc-finger protein Zat12 is required for Apx1 expression and plant protection during oxidative stress [56], and that the highly conserved zinc-finger paralogs LOL1 and LSD1 have antagonistic effects on SOD and O$_2^-$ accumulation [59].

The possible existence of positive amplification loops involving NADPH oxidases in ROS signaling has recently been suggested by pharmacological and genetic studies [27,56]. These loops might be activated by low levels of ROS and result in enhanced production and amplification of the ROS signals in specific cellular locations (Figure 3, localized amplification loop, red broken lines). The accumulation of ROS in cells might activate the ROS-scavenging pathways and result in the suppression of ROS in specific cellular locations or the entire cell (Figure 3, localized or general response, solid green lines). As indicated above, the interplay between the ROS-producing and ROS-scavenging pathways will determine the intensity, duration and localization of the ROS signals (Figures 1, 3). Although O$_2^-$ and H$_2$O$_2$ have been considered to play key roles as ROS signal transduction molecules, recent studies have pointed to the existence of a $^{3}$O$_2$-specific signaling pathway [20,74].

Taking into account the complex nature of the ROS gene network (Figure 2, Table 1) and its integration into the web of plant signaling networks (Figure 1), we face a major challenge in dissecting the genetic network that controls ROS signaling in plants (Box 1). Large-scale transcriptome analyses coupled with proteomic and metabolomic analysis of plants perturbed at the levels of individual or multiple components of the ROS network will be essential for future studies. Integration of results obtained from these studies with the development of computer models [75] and bioinformatics tools [76,77] should enable us to gain a system-level understanding of ROS metabolism in plants.

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**Supplementary material**

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tplants.2004.08.009

**References**

of the coupling of two distantly related Arabidopsis thaliana NADPH-cytochrome P450 reductases with P450 CYP73A5. J. Biol. Chem. 272, 19176–19186


Bowler, C. et al. (1991) Manganese superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants. EMBO J. 10, 1723–1732


Obara, K. et al. (2002) The use of multiple transcription starts causes the dual targeting of Arabidopsis putative monodehydroascorbate reductase to both mitochondria and chloroplasts. Plant Cell Physiol. 43, 697–705


Růžičky, L. et al. (2002) Double antisense plants lacking ascorbate peroxidase and catalase are less sensitive to oxidative stress than single antisense plants lacking ascorbate peroxidase or catalase. Plant J. 32, 329–342


77 Thimm, O. et al. (2004) MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. Plant J. 37, 914–939