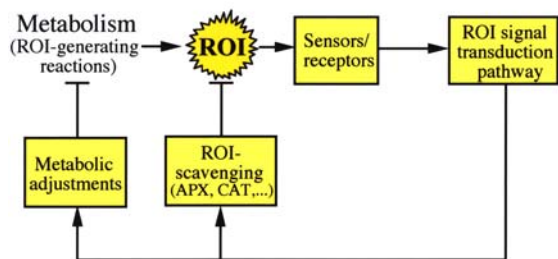


## OXIDATIVE STRESS IN PLANTS

Ever since the introduction of molecular oxygen ( $O_2$ ) into our atmosphere, by  $O_2$ -evolving photosynthetic organisms, about two billion years ago, reactive oxygen intermediates (ROI) have been the unwelcome companions of aerobic metabolism. In contrast to  $O_2$ , these partially reduced or activated derivatives of oxygen ( $O_2^{\cdot-}$ ,  $O_2^{\cdot}$ ,  $H_2O_2$ , and  $HO^{\cdot}$ ) are highly reactive and toxic, and can lead to the oxidative destruction of cells. Consequently, the evolution of all aerobic organisms has been dependent upon the development of efficient ROI-scavenging mechanisms. In recent years a new role for ROI was identified: the control and regulation of biological processes such as programmed cell death, hormonal signaling, stress responses, and development. These studies extend our understanding of ROI and suggest a dual role for ROI in plant biology: **(a)**. Toxic byproducts of aerobic metabolism, and **(b)**. Key regulators of metabolic and defense pathways.

### Production and scavenging of ROI in cells: “the ROI cycle”.

The steady state level of ROI in the different cellular compartments is determined by interplay between multiple ROI-producing pathways, and ROI-scavenging mechanisms. These are controlled by the ROI-signal transduction pathway and constitute the “basic ROI cycle”. During normal growth and development this pathway monitors the level of ROI, produced by aerobic metabolism, and controls the expression and activity of ROI-scavenging pathways (Fig. 1). The basic ROI cycle may also perform fine metabolic tuning, e.g., suppression of photosynthesis, to reduce the production rate of ROI. There are many potential sources of ROI in plants (Table 1). Some are reactions of normal aerobic metabolism, such as photosynthesis and respiration, while others belong to pathways enhanced during abiotic stresses, such as photorespiration. In recent years new sources of ROI were identified in plants, including NADPH oxidases, amine oxidases, and cell wall-bound peroxidases. These are tightly regulated and participate in the control of processes such as programmed cell death, stress response, and pathogen defense.



**Fig. 1.** The basic ROI cycle. This cycle modulates the cellular levels of ROI during normal metabolism. Some of the key ROI scavenging enzymes of plants, ascorbate peroxidase (APX), and catalase (CAT) are indicated.

### Production and scavenging of reactive oxygen intermediates in plants

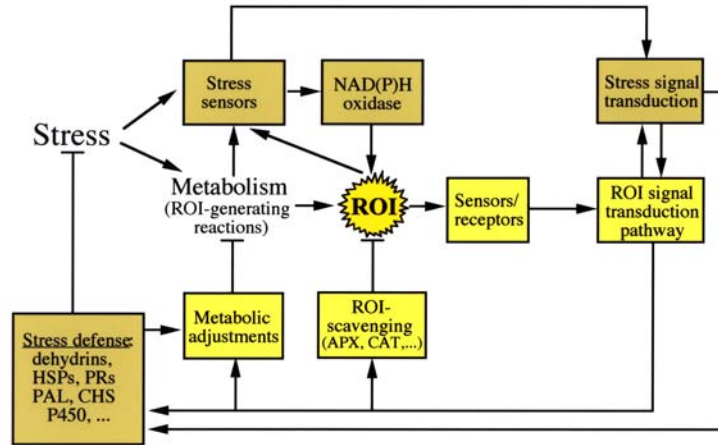
Production of ROI	Localization	Primary ROI
Photosynthesis ET and PSI or II	Chl	$O_2^{\cdot-}$
Respiration ET	Mit	$O_2^{\cdot-}$
Glycolate oxidase	Per	$H_2O_2$
Excited chlorophyll	Chl	$O_2^{\cdot}$
NADPH oxidase	PM	$O_2^{\cdot-}$
Fatty acid $\beta$ -oxidation	Per	$H_2O_2$
Oxalate oxidase	Apo	$H_2O_2$
Xanthine oxidase	Per	$O_2^{\cdot-}$
Peroxidases, $Mn^{2+}$ and NADH	CW	$H_2O_2$ , $O_2^{\cdot-}$
Amine oxidase	Apo	$H_2O_2$
<b>Scavenging of ROI</b>		
SOD	Chl, Cyt, Mit, Per, Apo	$O_2^{\cdot-}$
APX	Chl, Cyt, Mit, Per, Apo	$H_2O_2$
CAT	Per	$H_2O_2$
GPX	Cyt	$H_2O_2$ , ROOH
Peroxidases	CW, Cyt, Vac	$H_2O_2$
Thioredoxin peroxidase	Chl, Cyt, Mit	$H_2O_2$
Ascorbic acid	Chl, Cyt, Mit, Per, Apo	$H_2O_2$ , $O_2^{\cdot-}$
Glutathione	Chl, Cyt, Mit, Per, Apo	$H_2O_2$
$\alpha$ -Tocopherol	Membranes	ROOH, $O_2^{\cdot}$
Carotenoids	Chl	$O_2^{\cdot}$
AOX	Chl, Mit	$O_2^{\cdot-}$

**Abbreviations:** Apo, apoplast; Chl, chloroplast; CW, cell wall; Cyt, cytosol; ET, electron transport; Mit, mitochondria;  $O_2^{\cdot}$ , singlet oxygen; Per, peroxisome; PM, plasma membrane; PS, photosystem; Vac, vacuole.

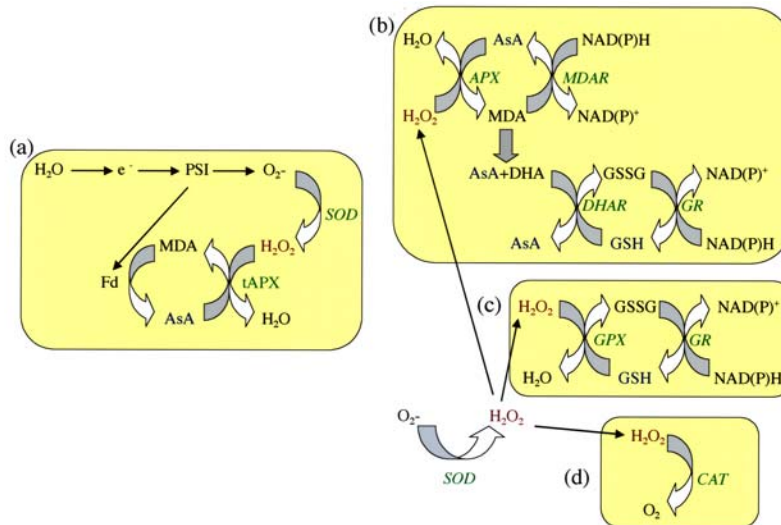
**Table 1.** Mechanisms for production and scavenging of ROI in cells. AOX, alternative oxidase; APX, ascorbate peroxidase; CAT, catalase; GPX, glutathione peroxidase; SOD, superoxide dismutase.

Under optimal growth conditions the production of ROI in cells is estimated at a constant rate of  $240 \mu M s^{-1} O_2^{\cdot-}$ , and a steady state level of  $0.5 \mu M H_2O_2$ . However, stresses that disrupt the cellular homeostasis of cells result in the enhanced production of ROI (up to  $720 \mu M s^{-1} O_2^{\cdot-}$ , and a steady state level of  $5-15 \mu M H_2O_2$ ). These include drought and desiccation, salt, chilling, heat shock, heavy

metals, UV radiation, air pollutants such as ozone and SO<sub>2</sub>, mechanical stress, nutrient deprivation, pathogen attack, and high light. The enhanced production of ROI during stress can pose a threat to cells, and many stress conditions enhance the expression of ROI-scavenging enzymes. However, it is also thought that during stress ROI are actively produced by cells (e.g., by NADPH oxidase), and act as signals for the induction of defense pathways. Thus, ROI may be viewed as cellular byproducts of stress metabolism, as well as secondary messengers involved in the stress-response signal transduction pathway. This view, of the “extended ROI cycle”, is presented in Fig. 2.



**Fig. 2.** The extended ROI cycle. This cycle operates in plants during biotic or abiotic stresses. HSPs, heat shock proteins; PR, pathogenesis related proteins; PAL, phenylalanine ammonia-lyase; CHS, chalcone synthase; P450, cytochrome P450.



**Fig. 3.** The main cellular pathways for ROI removal in plants. (a). SOD and thylakoid-bound APX (tyl-APX) in the chloroplast as part of the water-water cycle. (b). SOD and APX in the stroma, cytosol, mitochondria, and apoplast of plants, as part of the ascorbate-glutathione pathway. (c). Glutathione peroxidase (GPX) and its glutathione regenerating cycle. (d). Catalase in peroxisomes. AsA, ascorbate; GSH and GSSG, reduced and oxidized glutathione; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; MDA, monodehydroascorbate; MDAR, monodehydroascorbate reductase; GR, glutathione reductase; PSI, photosystem I; Fd, ferredoxin; e<sup>-</sup>, electron.

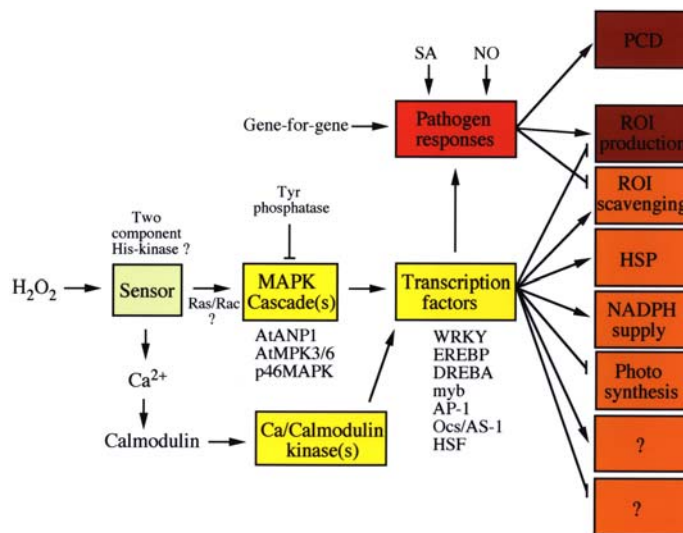
Because ROI are toxic but also participate in key signaling events, plant cells require different mechanisms to regulate their intracellular ROI concentrations by scavenging of ROI. Major ROI scavenging mechanisms of plants are summarized in Table 1. These include superoxide dismutase (SOD; Fig. 3), ascorbate peroxidase (APX; Fig. 3), and catalase (CAT; Fig. 3). The balance between SOD, and APX (and/or CAT) activity in cells is considered to be crucial for determining the steady-

state level of  $O_2^-$  and  $H_2O_2$ . This balance, together with sequestering of metal ions such as Fe and Cu by ferritin and copper-binding proteins, is thought to be important to prevent the formation of the highly toxic  $HO\cdot$  via the metal-dependent Haber–Weiss or the Fenton reactions. Antioxidants such as ascorbic acid and glutathione, found at very high concentrations in chloroplasts and other cellular compartments (5–20 mM ascorbic acid and 1–5 mM glutathione), are also important for the defense of plants against oxidative stress. Consequently, mutants with suppressed ascorbic acid levels, and transgenic plants with suppressed ROI-scavenging enzymes, are hypersensitive to pathogen attack and abiotic stress conditions. In addition, over-expression of ROI-scavenging enzymes increases the tolerance of plants to abiotic stresses. ROI production can also be decreased in cells by the alternative channeling of electrons in the electron transport chains of the chloroplasts and mitochondria by a group of enzymes called alternative oxidases.

### ROI at the interface between biotic and abiotic stresses

ROI play a central role in the defense of plants against pathogens. During this response, ROI are produced by plant cells via the enhanced enzymatic activity of plasma membrane-bound NADPH oxidases, cell wall-bound peroxidases and amine oxidases in the apoplast.  $H_2O_2$  produced during this response is thought to diffuse into cells through aquaporins and together with salicylic acid (SA) and nitric oxide (NO) activate many of the plant defenses, including the induction of programmed cell death. The activity of APX and CAT is suppressed during this response by salicylic acid and nitric oxide, the expression of APX is post-transcriptionally suppressed, and the expression of CAT is down regulated at the steady-state mRNA level. Thus, the plant simultaneously produces more  $H_2O_2$  and diminishes its own capability to scavenge  $H_2O_2$ , resulting in the over-accumulation of  $H_2O_2$  and the activation of PCD. This response serves as an excellent example to how the steady state level of ROI can dramatically increase in cells when the basic ROI cycle is severed.

### The ROI signal transduction pathway



**Fig. 4.** A hypothetical model of the signaling pathway activated in plants in response to external application of oxidants. SA, salicylic acid; NO, nitric oxide; PCD, programmed cell death; HSP, heat shock protein; MAPK, mitogen-activated protein kinase; Tyr, tyrosine; HSF, heat shock transcription factor.

Recent studies identified a number of components that may be involved in the ROI signal transduction of plants. These include the MAPKKK, AtANP1 (also NPK1), the MAPKs, AtMPK3/6, and Ntp46MAPK, and calmodulin. A hypothetical model depicting some of the players involved in this pathway is shown in Fig. 4. A sensor that might be a two component Histidine-kinase, or a receptor-like protein kinase, is thought to sense  $H_2O_2$ . Calmodulin and a MAPK cascade are then

activated resulting in the induction/activation/suppression of a number of transcription factors. These regulate the response of plants to oxidative stress. Cross-talk with the pathogen-response signal transduction pathway (gene-for-gene) also occurs and may involve interactions between different MAPK pathways, feedback loops, and the action of salicylic acid and nitric oxide.