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植物细胞活性氧种类、代谢及其信号转导*

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摘要：越来越明显的证据表明，植物体十分活跃的产生着活性氧并将之作为信号分子、进而控制着诸如细胞程序性死亡、非生物胁迫响应、病原体防御和系统信号等生命过程，而不仅是传统意义上的活性氧是有氧代谢的附产物。日益增多的证据显示，由脱落酸、水杨酸、茉莉酸与乙烯以及活性氧所调节的激素信号途径，在生物和非生物胁迫信号的“交谈”中起重要作用。活性氧最初被认为是动物吞噬细胞在宿主防御反应时所释放的附产物，现在的研究清楚的表明，活性氧在动物和植物细胞信号途径中均起作用。活性氧可以诱导细胞程序性死亡或坏死、可以诱导或抑制许多基因的表达，也可以激活上述级联信号。近来生物化学与遗传学研究证实过氧化氢是介导植物生物胁迫与非生物胁迫的信号分子，过氧化氢的合成与作用似乎与一氧化氮有关系。过氧化氢所调节的下游信号包括钙“动员”、蛋白磷酸化和基因表达等。

关键词：活性氧；MAPK；H_2O_2；信号转导；胁迫
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Reactive Oxygen Species, Metabolism, and Signal Transduction in Plant Cells

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Abstract: Traditionally, reactive oxygen species (ROS) were considered to be toxic by-products of aerobic metabolism. However, in recent years, it has become apparent that plants actively produce ROS as signaling molecules to control processes such as programmed cell death, abiotic stress responses, pathogen defense and systemic signaling. Emerging evidence suggests that hormone signaling pathways regulated by abscisic acid, salicylic acid, jasmonic acid and ethylene, as well as ROS signaling pathways, play key roles in the crosstalk between biotic and abiotic stress signaling. Reactive oxygen species (ROS) were originally thought to only be released by phagocytic cells during their role in host defence. It is now clear that ROS have a cell signalling role in many biological systems, both in animals and in plants. ROS induce programmed cell death or necrosis, induce or suppress the expression of many genes, and activate cell signalling cascades, such as those involving. Recent biochemical and genetic studies confirm that hydrogen peroxide is a signalling molecule in plants that mediates responses to abiotic and biotic stresses. The synthesis and action of hydrogen peroxide appear to be linked to those of nitric oxide. Downstream signalling events that are modulated by hydrogen peroxide include calcium mobilization, protein phosphorylation and gene expression.

Key words: ROS; MAPK; H_2O_2; Signal transduction; Stress

Ever since the introduction of molecular oxygen (O_2) into our atmosphere by O_2-evolving photosynthetic organisms ～2.7 billion years ago, reactive oxygen species (ROS) have been the unwelcome companions of aerobic life (Halliwell and Gutteridge, 1989). In contrast to O_2, these partially reduced or activated de-

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rivatives of oxygen such as singlet oxygen ($^1O_2$), superoxide radical anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^·$) are highly reactive and toxic, and can lead to the oxidative destruction of cells (Asada and Takahashi, 1987). (Fig. 1)

There are many potential sources of ROS in plants (Table 1). Some are reactions involved in normal metabolism, such as photosynthesis and respiration. These are in line with the traditional concept, considering ROS as unavoidable byproducts of aerobic metabolism (Asada and Takahashi, 1987). Other sources of ROS belong to pathways enhanced during abiotic stresses, such as glycolate oxidase in peroxisomes during photorespiration. However, in recent years, new sources of ROS have been identified in plants, including NADPH oxidases, amine oxidases and cell-wall-bound peroxidases. These are tightly regulated and participate in the production of ROS during processes such as programmed cell death (PCD) and pathogen defense (Dat *et al*. 2000; Grant and Loake, 2000; Hammond-Kosack and Jones, 1996).

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 (Costa and Moradas-Ferreira, 2001; Foreman *et al*. 2003; Jiang *et al*. 2003; Kovtun *et al*. 2000; Kwak *et al*. 2003; Pei *et al*. 2000; Mullineaux and Karpinski, 2002; Mittler, 2004; Neill *et al*. 2002; Overmyer *et al*. 2003; Torres *et al*. 2002). 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 H_2O_2 or O_2^- to act as signaling molecules appears to require a large gene network composed of at least 152 genes in Arabidopsis (Mittler, 2004).

Biotic Strategies to Generate ROS

One of the most rapid defense reactions to pathogens attack is the so-called oxidative burst, which constitutes the production of ROS, primarily superoxide

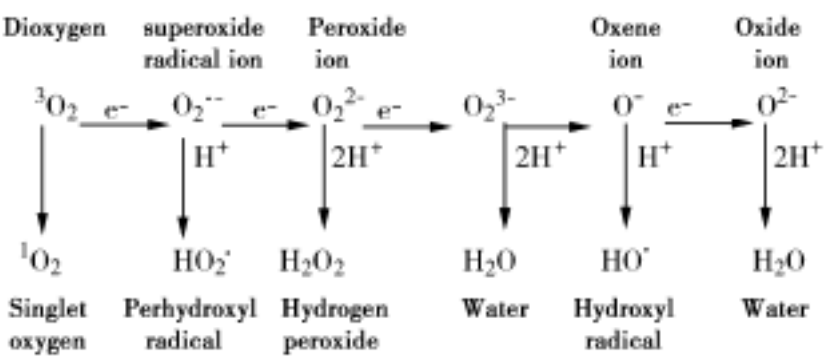

Fig. 1 Generation of different ROS by energy transfer or sequential univalent reduction of ground state triplet oxygen.

Table 1 Producing, scavenging and avoiding reactive oxygen species in plants

Mechanism	Localization	Primary ROS	Mechanism	Localization	Primary ROS
ROS Production			Glutathione peroxidase	Cyt	H_2O_2, ROOH
Photosynthesis ET and PSI or II	Chl	O_2^-	Peroxidases	CW, Cyt, Vac	H_2O_2
Respiration ET	Mit	O_2^-	Thioredoxin peroxidase	Chl, Cyt, Mit	H_2O_2
Glycolate oxidase	Per	H_2O_2	Ascorbic acid	Chl, Cyt, Mit, Per, Apo	H_2O_2, O_2^-
Excited chlorophyll	Chl	O_2^1	Glutathione	Chl, Cyt, Mit, Per, Apo	H_2O_2
NADPH oxidase	PM	O_2^-	α-Tocopherol	Membranes	ROOH, O_2^1
Fatty acid β-oxidation	Per	H_2O_2	Caretenoids	Chl	O_2^1
Oxalate oxidase	Apo	H_2O_2	**Avoidance**		
Xanthine oxidase	Per	O_2^-	Anatomical adaptations	Leaf structure, epidermis	O_2^-, H_2O_2, O_2^1
Peroxidases, Mn^{2+} and NADH	CW	H_2O_2, O_2^-	C_4 or CAM metabolism	Chl, Cyt, Vac	O_2^-, H_2O_2
Amine oxidase	Apo	H_2O_2	Chl movement	Cyt	O_2^-, H_2O_2, O_2^1
ROS Scavenging			Suppression of photosynthesis	Chl	O_2^-, H_2O_2
Superoxide dismutase	Chl, Cyt, Mit, Per, Apo	O_2^-	PS and antenna modulations	Chl	O_2^-, O_2^1
Ascorbate peroxidase	Chl, Cyt, Mit, Per, Apo	H_2O_2	Alternative oxidases	Chl, Mit	O_2^-
Catalase	Per	H_2O_2			

Abbreviations: Apo, apoplast; Chl, chloroplast; CW, cell wall; Cyt, cytosol; ET, electron transport; Mit, mitochondria; O_2^1, singlet oxygen; Per, peroxisome; PM, plasma membrane; PS, photosystem; ROI, reactive oxygen intermediate; Vac, vacuole.

and H_2O_2, at the site of attempted invasion (Apostol *et al* . 1989) . Several enzymes are now recognized as being potentially able to produce ROS, perhaps the most important of these is NADPH oxidase . The NADPH-dependent oxidase system, similar to that present in mammalian neutrophils, has received the most attention . In animals the NADPH oxidase is found in phagocyte and B lymphocytes . It catalyzes the production of superoxide by the one-election reduction of oxygen using NADPH as the election donor .

In addition to the NADPH oxidase of phagocytes, other NADPH oxidases also associated with plasma membranes are found in a variety of cells (Babior, 1999) . More studies have provided several lines of evidence strongly suggesting a common origin for both mammalian NADPH oxidases and plant NADPH oxidases . Antibodies raised against human NADPH oxidases subunits $p22^{PHOX}$, $p47^{PHOX}$, and $p67^{PHOX}$ cross-reacted with plant proteins of similar size (Desikan *et al* . 1996; Tenhaken *et al* . 1995), and in several plant species *rboh* genes (respiratory burst oxidase homologues) of $p91^{PHOX}$, the catalytic subunit of the NADPH oxidases of phagocytes, have been found (Keller *et al* . 1998; Torres *et al* . 1998) . In addition to a plant specific NADPH oxidase, alternative mechanisms of ROS production have been proposed . For example, many peroxidases are localized in the apoplastic space and are ionically or covalently bound to cell wall polymers . Peroxidases can act in two different catalytic modes . In the presence of H_2O_2 and phenolic substrates they operate in the peroxidatic cycle and are engaged in the synthesis of lignin and other phenolic polymers . Compared with the plant, NADPH-oxidase activity that gives rise to superoxide and hydrogen peroxide, in vitro studies of horseradish peroxidase suggest another activity of this enzyme: generating hydroxyl radicals (Chen and Schopfer, 1999) . Similar to the $Fe^{2+/3+}$ catalyzed Haber-Weiss reaction, horseradish peroxidase can reduce hydrogen peroxide to hydroxyl radicals (Chen and Schopfer, 1999) .

Whereas, under normal growth conditions, the production of ROS in cells is low (240 μM s^{-1} O_2^- and a steady-state level of 0.5 μM H_2O_2 in chloroplasts (Polle, 2001), many stresses that disrupt the cellular homeostasis of cells enhance the production of ROS (240 - 720 μM s^{-1} O_2^- and a steady-state level of 5 - 15 μM H_2O_2 in chloroplasts) (Polle, 2001) . These include drought stress and desiccation, salt stress, chilling, heat shock, heavy metals, ultravioletradiation, air pollutants such as ozone and SO_2, mechanical stress, nutrient deprivation, pathogen attack and high light stress (Allen, 1995; Bowler *et al* . 1992; Dat *et al* . 2000; Desikin *et al* . 2001; Pei *et al* . 2000; Noctor and Foyer, 1998; Orozco-Cardenas and Ryan, 1999) . The production of ROS during these stresses results from pathways such as photorespiration, from the photosynthetic apparatus and from mitochondrial respiration . In addition, pathogens and wounding or environmental stresses (e.g . drought or osmotic stress) have been shown to trigger the active production of ROS by NADPH oxidases (Hammond-Kosack and Jones, 1996; Pei *et al* . 2000; Orozco-Cardenas and Ryan, 1999; Cazale *et al* . 1999) .

Abiotic Strategies to Generate ROS

In plants, ROS are continuously produced predominantly in chloroplasts, mitochondria, and peroxisomes . Production and removal of ROS must be strictly controlled . However, the equilibrium between production and scavenging of ROS may be perturbed by a number of adverse abiotic stress factors such as high light, drought, low temperature, and mechanical stress (Field *et al* . 1998; Malan *et al* . 1990; Prasad *et al* . 1994; Tsugane *et al* . 1999) .

Chloroplasts hydrogen peroxide/superoxide

Oxygen is continuously produced during light-driven photosynthetic electron transport and simultaneously removed from chloroplasts by reduction and assimilation . There are three types of oxygen-consuming processes closely associated with photosynthesis: (a) the oxygenase reaction of ribulose-1, 5-bisphosphate carboxylase (Rubisco), (b) direct reaction of molecular oxygen by photosystem I (PS I) electron transport, and (c) chlororespiration (Keller *et al* . 1998) . (Fig . 2)

Mitochondria as ROS sources (Kirk, 2003)

For years, the chloroplast was considered to be the main source of ROS production in plant cells and consequently one of the main targets for ROS damage during stress . However, it has recently been suggested that the chloroplast is not as sensitive to ROS damage as previously thought (Torres *et al* . 1998) . The mitochondrion is another cellular site of ROS production . However, recent studies suggest that the mitochondrion is also a key regulator of PCD in plants and that enhanced ROS levels

at the mitochondria can trigger PCD (Torres *et al*. 2002). The mitochondrial electron transport chain can produce significant quantities of ROS, primarily because of the presence of the ubisemiquinone radical, which can transfer a single electron to oxygen and produce O_2^-. The mitochondrial alternative oxidase (AOX) catalyses the O_2 dependent oxidation of ubiquinol, limiting the mitochondrial generation of ROS. (Fig. 2)

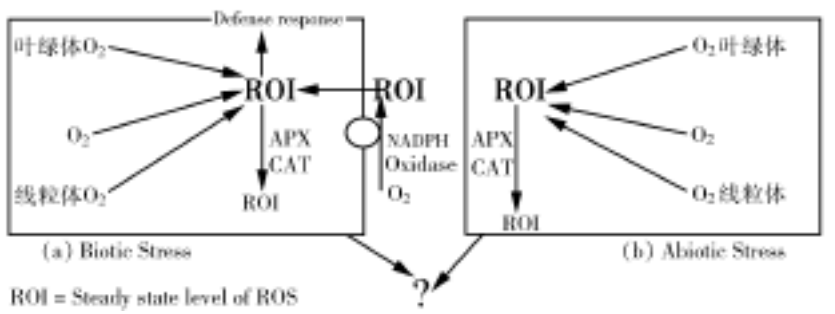

Fig. 2 Differences in the steady-state levels of reactive oxygen intermediates (ROI) during biotic stress and abiotic stress. Biotic stress (a) results in the activation of NADPH oxidase and the suppression of ascorbate peroxidase (APX) and catalase (CAT). This leads to the over-accumulation of ROI and the activation of defense mechanisms. Abiotic stress (b) enhances ROI production by chloroplasts and mitochondria. However, by inducing ROI-scavenging enzymes such as APX and CAT, it reduces ROI levels. The question mark indicates that little is known about the regulation of ROI metabolism during a combination of biotic and abiotic stresses. Chloroplasts are indicated in upper, and mitochondria are in down.

ROS detoxification

Because ROS are toxic but also participate in signaling events, plant cells require at least two different mechanisms to regulate their intracellular ROS concentrations by scavenging of ROS: one that will enable the fine modulation of low levels of ROS for signaling purposes, and one that will enable the detoxification of excess, especially during stress. In addition, the types of ROS produced and the balance between the steady-state levels of different ROS can also be important. These are determined by the interplay between different ROS-producing and ROS scavenging mechanisms, and can change drastically depending upon the physiological condition of the plant and the integration of different environmental, developmental and biochemical stimuli.

In the presence of transition metal ions hydrogen peroxide may be reduced to hydroxyl radicals by superoxide. Superoxide and hydrogen peroxide are much less reactive than $OH^{\cdot}$, then the main risk for a cell that produces the two former reactive oxygen intermediates may be posed by the two intermediates interaction, leading to the generaction of highly reactive hydroxyl radicals. Because there are no known scavengers of hydroxyl radicals, the only way to avoid oxidative damage through this radical is to control the reactions that lead to its generation. Thus, cells had to evolve sophisticated strategies to keep the concentrations of superoxide, hydrogen peroxide, and transition metals such as Fe and Cu under tight control.

Nonenzymatic ROS Scavenging Mechanisms

The first pathway to scavenge ROS is nonenzymatic ROS scavenging. Nonenzymatic antioxidants in plant cells include the major cellular redox buffers ascorbate and glutathione (GSH), as well as tocopherol, flavonoids, alkaloids, and carotenoids. Mutants with decreased ascorbic acid levels (Keller *et al*. 1998) or altered GSH content (Kirk, 2003) are hypersensitive to stress. Whereas GSH is oxidized by ROS forming glutathione (GSSG), ascorbate is oxidized to monodehydroascorbate (MDA) and dehydroascorbate (DHA) (Table 1).

Enzymatic ROS Scavenging Mechanisms

The second pathway to scavenge ROS is enzymatic ROS scavenging. Enzymatic ROS scavenging mechanisms in plants include superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), and catalase (CAT) (Table 1).

The role of ROS in signal transduction

Recent studies have identified several components involved in the signal transduction pathway of plants that senses ROS. These include the mitogen-activated protein (MAP) kinase kinase kinases AtANP1 and Nt-NPK1, and the MAP kinases AtMPK3 6 and Ntp46MAPK (Kovtun *et al*. 2000; Samuel *et al*. 2000). In addition, calmodulin has been implicated in ROS signaling (Desikin *et al*. 2001; Harding *et al*. 1997). A hypothetical model depicting some of the players involved in this pathway is shown in Fig. 3. and in Fig. 4. H_2O_2 is sensed by a sensor that might be a two-component histidine kinase, as in yeast (Desikin *et al*. 2001). Calmodulin and a MAP-kinase cascade are then activated, resulting in the activation or suppression of several transcription factors. These regulate the response of plants to oxidative stress (Desikin *et al*. 2001; Maleck *et al*. 2000). Cross-talk with the pathogen-response signal transduction pathway also occurs and might involve interactions between different MAP-kinase pathways, feedback loops and the action of NO and SA as key hormonal regulators. This model (Fig. 3 and Fig. 4) is simplified and is likely to change as research advanc-

es our understanding of this pathway . ROS act as signals that mediate the systemic activation of gene expression in response to pathogen attack (Alvarez *et al* . 1998), wounding (Orozco-Cardenas and Ryan, 1999) and high light (Mullineaux and Karpinski, 2002) . They were suggested to act in conjunction with a compound that travels systemically and activates their production in distal parts of the plant, where they mediate the induction of gene expression (Orozco-Cardenas and Ryan, 1999) . The involvement of ROS in the regulation of stomatal closure (Pei *et al* . 2000) and in other cellular responses involving auxin (Kovtun *et al* . 2000; Klaus and Heribert, 2004) might suggest that more signaling pathways involving ROS as inducers of systemic signals await discovery . It is unlikely that ROS can travel systemically because they are highly reactive and would be scavenged along the way by the many antioxidative mechanisms and antioxidants present in the apoplast . However, it is possible that a wave of activity similar to the ' oxidative burst ' is activated in cells along the systemic path and in distal tissues, resulting in the accumulation of ROS . Future studies using plants with altered levels of ROS-scavenging and or ROS-producing mechanisms might resolve this question .

Reactive oxygen species and hormonal network

Hormone signaling pathways govern biotic and abiotic stress responses through ROS

ABA is a phytohormone that is extensively involved in responses to abiotic stresses such as drought, low temperature, and osmotic stress . ABA also governs a variety of growth and developmental processes, including seed development, dormancy, germination, and stomatal movement . By contrast, the phytohormones SA, JA, and ET play central roles in biotic stress signaling upon pathogen infection . In many cases, ABA

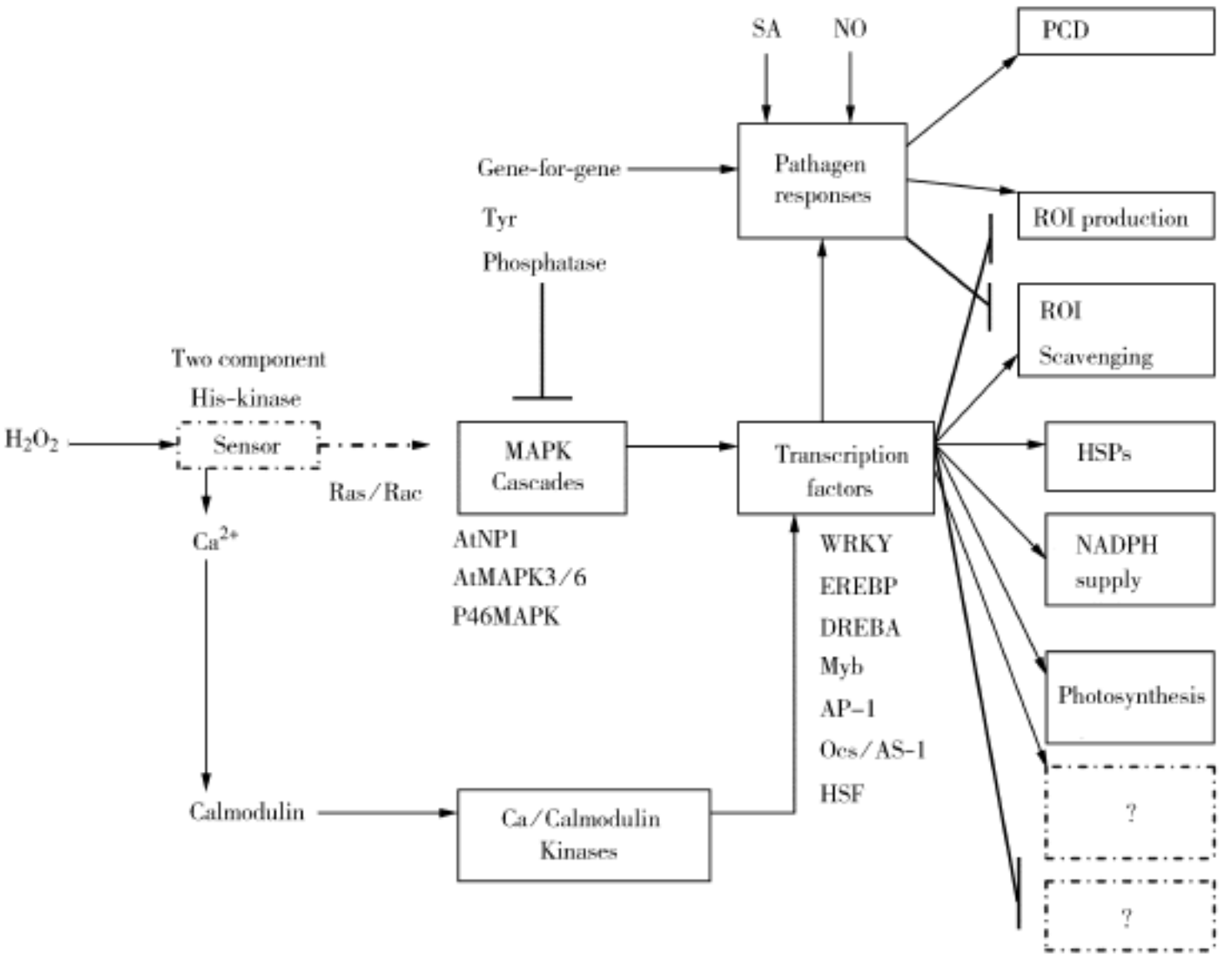

Fig . 3　A suggested model for the activation of signal transduction events during oxidative stress . H_2O_2 is detected by a cellular receptor or sensor . Its detection results in the activation of a Mitogen activated-protein kinase (MAPK) cascade and a group of transcription factors that control different cellular pathways . H_2O_2 sensing is also linked to changes in the levels of Ca^{2+} and calmodulin, and to the activation or induction of a Ca^{2+}-calmodulin kinase that can also activate or suppress the activity of transcription factors . The regulation of gene expression by the different transcription factors results in the induction of various defense pathways, such as reactive oxygen intermediate (ROI) scavenging and heat-shock proteins (HSPs), and in the suppression of some ROI-producing mechanisms and photosynthesis . There is also cross-talk with the plant-pathogen signal transduction pathway, which might depend on pathogen recognition by the gene-for-gene mechanism and can result in an inverse effect on the regulation of ROS-production and ROI-scavenging mechanisms, as well as on the activation of programmed cell death (PCD) . The plant hormones nitric oxide (NO)、abscisic acid (ABA) and salicylic acid (SA) are key regulators of this response .

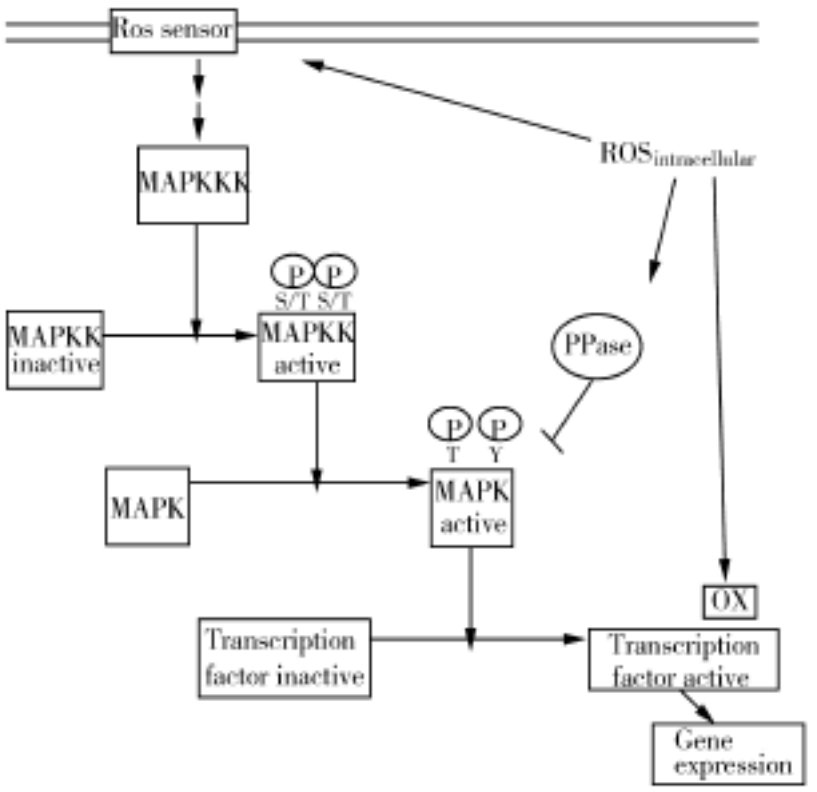

Fig . 4 Schematic depiction of cellular ROS sensing and signalling mechanisms . ROS sensors such as membrane-localized histidine kinases can sense extracellular and intracellular ROS . Intracellular ROS can also influence the ROS-induced mitogen-activated protein kinase (MAPK) signalling pathway through inhibition of MAPK phosphates (PPases) or downstream transcription factors . Whereas MAP kinases regulate gene expression by altering transcription factor activity through phosphorylation of serine and threonine residues, ROS regulation occurs by oxidation of cysteine residues .

acts as a negative regulator of disease resistance (Mauch-Mani and Mauch, 2005) . For example, the ABA-deficient tomato mutant sitiens has increased resistance to pathogens and application of exogenous ABA restored the susceptibility of sitiens (Audenaert *et al* . 2002; Thaler and Bostock, 2004) . The sitiens mutant has greater SA-mediated responses, suggesting that high ABA concentrations inhibit the SA-dependent defense response in tomato . ABA and ET are well known to interact, mostly antagonistically, in a number of developmental processes and in vegetative tissues (Beaudoin *et al* . 2000; Ghassemian *et al* . 2000) . Genetic analysis of enhanced response to ABA3 (*era*3) alleles revealed that ERA3 is allelic to ETHYLENE INSENSITIVE2 (*EIN*2), which encodes a membrane-bound putative divalent cation sensor that may represent a crosstalk point that intersects the ABA and ET signaling pathways (Ghassemian *et al* . 2000) . Furthermore, jasmonic acid resistance1 (*jar*1) and jasmonic acid insensitive4 (*jin*4) mutants, which are hypersensitive to ABA-mediated inhibition of germination, exhibit antagonistic effects of ABA and JA (Lorenzo and Solano, 2005; Anderson *et al* . 2004) . Additionally, exogenous application of ABA resulted in the downregulation of JA- or ET-responsive defense gene expression in wildtype plants, whereas higher expression levels of these defense genes were observed in ABA deficient mutants without any treatments (Anderson *et al* . 2004) . Taken together with the findings that exogenous application of methyl-JA and ET cannot restore the defense gene expression that is suppressed by exogenous ABA application, these data suggest that the ABA-mediated abiotic stress response is a dominant process (Anderson *et al* . 2004) .

Additionally, recent studies identified other molecular entities that significantly impact crosstalk among stress response pathways via hormone signaling . For instance, both nitric oxide (Wendehenne *et al* . 2004) and Ca^{2+} signaling play an important role in plant defense responses, ABA-dependent stomatal movements, and drought stress responses (Ludwig *et al* . 2004) . Calcium dependent protein kinases in tobacco might control biotic and abiotic stress responses via signaling pathways that are mediated by hormones such as SA, ET, JA, and ABA (Ludwig *et al* . 2005; Chung *et al* . 2004; Ludwig *et al* . 2004) . In addition, fungal elicitors can activate a branch of the ABA signaling pathway in guard cells that regulates plasma membrane Ca^{2+} channels (Klusener *et al* . 2002) . Moreover, a battery of studies examining the induction of resistance by the non-protein amino acid *b*-aminobutyric acid revealed that ABA considerably enhances plant resistance to fungal pathogens through its positive effect on callose deposition (Mauch-Mani and Mauch, 2005; Ton *et al* . 2005; Ton and Mauch-Mani, 2004) (Fig . 5) .

Roles of ROS at points of convergence between biotic and abiotic stress response pathways

The tight regulation of the steady-state levels of ROS is involved in multiple cellular processes in plants (Zhang and Klessig, 2001) . Some ROS species are toxic byproducts of aerobic metabolism, whereas ROS also function as signaling molecules (Zhang and Klessig, 2001) . Rapid ROS production plays a pivotal role in both ABA signaling and disease resistance responses (Guan *et al* . 2000; Laloi *et al* . 2004) . Several lines of evidence suggests that the NADPH-dependent respiratory burst oxidase homolog genes (*AtrbohD and AtrbohF*) are required for ROS generation, leading to ABA-induced stomatal closure and to hypersensitive cell death in response to avirulent pathogen attack (Kwak *et al* . 2003; Torres and Dangl, 2005; Torres *et al* .

2002) . ROS scavengers are thought to detoxify the cytotoxic effects of ROS under various stress conditions (Klaus and Heribert, 2004; Mittler, 2004) . Large-scale transcriptome analyses of plants that had been subjected to various abiotic and biotic stress treatments revealed the induction of a large set of genes that encode ROS-scavenging enzymes under these conditions (Seki *et al* . 2002; Schenk *et al* . 2000; Mittler *et al* . 2004) . Moreover, scavenging enzymes (e . g . superoxide dismutase, glutathione peroxidase and ascorbate peroxidase) have been utilized to engineer plants that are tolerant of abiotic stresses (Bartels and Sunkar, 2005; Umezawa *et al* . 2006) . Microarray analysis using Arabidopsis cultured cells reveal that many ABA inducible genes are induced by oxidative stress (Takahashi *et al* . 2004) . Recently, it has been suggested that a C_2H_2-type zincfinger transcription factor, Zat12, might be a regulator in the ROS scavenging mechanism that is involved in biotic and abiotic stress responses . Deficiency in Zat12, which is highly responsive to multiple stresses including wounding, pathogen infection and abiotic stresses (Seki *et al* . 2002; Zimmermann *et al* . 2004; Davletova *et al* . 2005; Vogel *et al* . 2005) , suppresses the expression of the ASCORBATE PEROXIDASE 1 (*APX*1) gene, which is induced by H_2O_2 and increases the level of H_2O_2-induced protein oxidation (Rizhsky *et al* . 2004) . Overexpression of Zat12 results in upregulation of oxidative- and light-stress-responsive genes and in enhanced tolerance of high light, freezing, and oxidative stresses (Davletova *et al* . 2005; Lida *et al* . 2000; Rizhsky *et al* . 2004; Vogel *et al* . 2005; Zimmermann *et al* . 2004) . Interestingly, the expression of Zat12 is regulated by a redox-sensitive transcription factor, HEAT SHOCK FACTOR (HSF) 21, which is likely to be an initial sensor for H_2O_2 that accumulates in response to various stresses (Davletova *et al* . 2005) . These findings suggest that the ROS might mediate crosstalk between biotic and abiotic stress-responsive gene-expression networks (Fig . 5) .

Reactive oxygen species signaling networks of plants

ROS can be detected by at least three mechanisms (ROS receptors, redox sensitive transcription factors and phosphatases) . Detection of ROS by receptors results in the generation of Ca^{2+} signals and the activation of a phospholipase C/D (PLC/PLD) activity that generates phosphatidic acid (PA) . PA and Ca^{2+} are thought to activate the protein kinase OXI1 . Activation of OXI1 results in the activation of a mitogen-activatedprotein kinase (MAPK) cascade (MAPK3/6) and the induction or activation of different transcription factors that regulate the ROS-scavenging and ROS-producing pathways . The activation or inhibition of redox-sensitive transcription factors by ROS might also affect the expression of

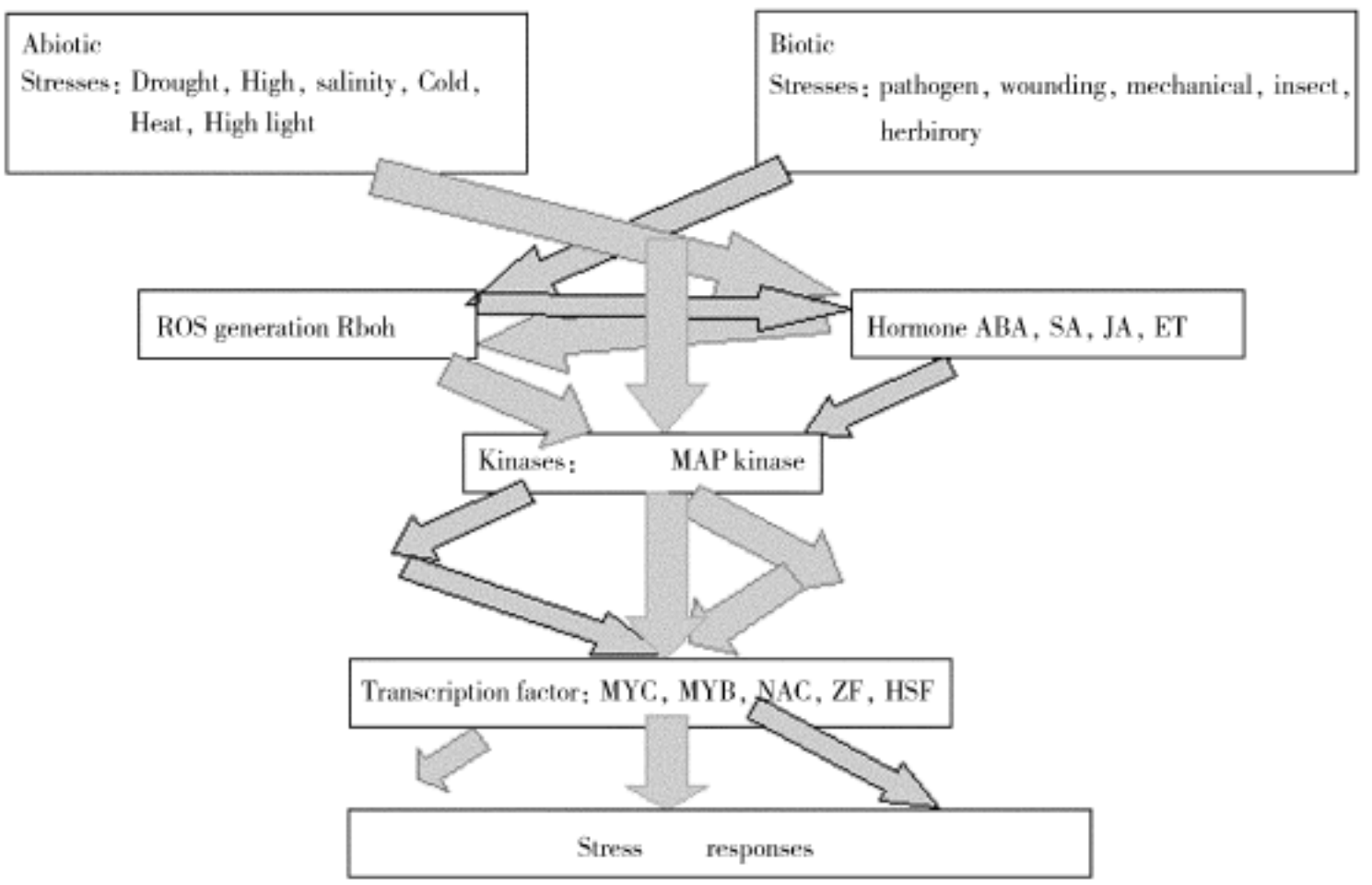

Fig . 5 Convergence points in abiotic and biotic stress signaling networks .

OXI1 or other kinases and/or the induction of ROS-specifictranscription factors . Inhibition of phosphatases by ROS might result in the activation of kinases such as OXI1 or MAPK3/6 . Two different loops are shown to be involved in the ROS signal transduction pathway . A localized or general defense response (a negative feedback loop; solid green line) can be activated to suppress ROS, whereas a localized amplification loop (positive feedback loop; red dashed line) can be activated to enhance ROS signals via the activity of NADPH oxidases . Salicylic acid (SA) and nitric oxide (NO) might be involved in this amplification loop as enhancers . (Fig . 6) .

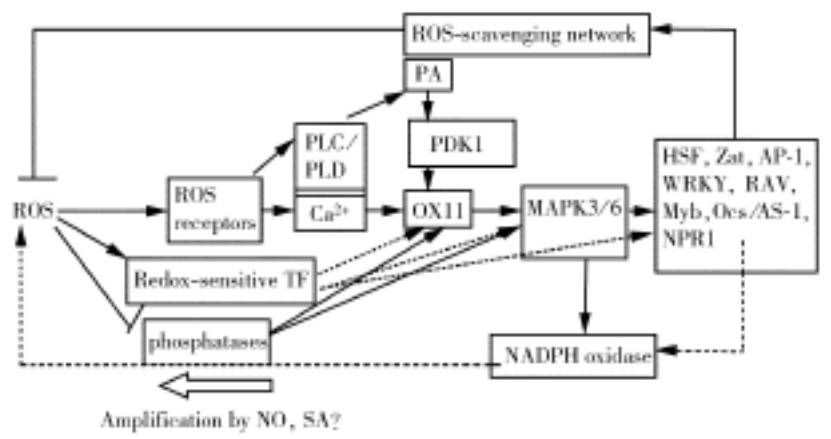

Fig . 6 Generalized model of the reactive oxygen species (ROS) signal transduction pathway .

Abbreviations: HSF, heat shock factor; PDK, phosphoinositide-dependent kinase; TF, transcription factor; SA, Salicylic; NO, nitric oxide .

Conclusions

During the evolution of organisms that are adapted to aerobic life conditions, ROS seem to have undergone several modifications of their biological activities . The continuous production of ROS, an unavoidable consequence of aerobic metabolic processes such as respiration and photosynthesis, has necessitated the evolution of ROS scavengers in order to minimise the cytotoxic effects of ROS within the cell . Disturbances is used by plants to activate stress responses that help the plant to cope with environmental changes . Finally, the genetically controlled production of H_2O_2 (e.g . by NADPH oxidases) is apparently used by plants to release an intracellular signal that, often together with nitric oxide, controls a variety of processes (Guo *et al* . 2003; Neill *et al* . 2002) . Detailed information on how these two signaling molecules interact and how they are sensed are still scarce . Key issues to be addressed in the future concern the questions of how ROS are integrated into the general signaling network of a cell, how the chemical identity of a given ROS and/or its intracellular production sites affect its signalling, and what factors determine the specificity of the biological activities of ROS .

For example, the role of mitogen-activated protein kinase (MAPK) in abscisic acid (ABA) -induced antioxidant defense was investigated extensively in leaves of maize (*Zea mays* L .) plants . Treatments with ABA or H_2O_2 induced the activation of a 46 kDa MAPK and enhanced the expression of the antioxidant genes CAT1, cAPX and GR1 and the total activities of the antioxidant enzymes catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and superoxide dismutase (SOD) (Aying *et al* . 2006) . The histochemical and cytochemical localization of abscisic acid (ABA) -induced H_2O_2 production in leaves of maize (*Zea mays* L .) plants were examined (Xiu *et al* . 2005) .

Current evidence supports the concept that ROS represent a significant point of convergence between pathways that respond to biotic and abiotic stresses . Nevertheless, our current understanding of ROS participation in crosstalk between these pathways is very limited . Thus, dissecting the genetic network that regulates ROS signaling in response to biotic and abiotic stresses merits extensive future study . When combined, the results of large-scale transcriptome, proteome, and metabolome analyses in plants will enable the elucidation of the ROS network components that govern multiple stress signaling pathways . In particular, the Genevestigator software should yield powerful clues, allowing us to connect these key molecular players and to discover novel crosstalk networks (Zimmermann *et al* . 2005) . A significant body of research suggests an antagonistic interaction between ABA-mediated abiotic stress signaling and disease resistance . This relationship may simply suggest that plants have developed strategies to avoid simultaneously producing proteins that are involved in abiotic stress and disease resistance responses (Anderson *et al* . 2004) . In nature, simultaneous exposure of plants to drought and necrotrophic pathogen attack is actually rare, as successful pathogen infection requires relatively humid conditions . The finding that high humidity and high temperature weaken plant resistance to pathogen attack is consistent with this concept . Moreover, the view that the ABA mediated abiotic stress signaling potentially takes precedence over biotic stress signaling (Anderson *et al* . 2004) also supports the notion that water stress

more significantly threatens plant survival than does pathogen infection . To date, the biological significance of crosstalk between signaling pathways that operate under stress conditions and the mechanisms that underlie this crosstalk remain obscure . We are just beginning to dissect key factors governing the crosstalk between these signaling pathways under various stress conditions .

Conclusions and future challenges

Powerful genetic strategies driven by the use of Arabidopsis have resulted in the elucidation of many hormone and other signaling pathways in plants . As illustrated by the studies reviewed here, the application of this knowledge and, in particular, the use of signaling mutants have allowed the delineation of signals involved in cell death regulation . Similarly, genetic approaches involving mutants have been key in identifying novel plant pathways, such as the MAP kinase cascades involved in the regulation of ROS responses and cell death regulation . The picture is also more complicated, considering that even more hormones (e.g . abscisic acid and gibberellic acid) are likely to be involved in cell death regulation (Klusener *et al* . 2002; Bethke *et al* . 1999) . Continued work with these powerful systems should result in the further molecular definition of these pathways and poses the challenge to produce an integrated map that connects these pathways at themolecular level .

Future challenges and questions

The cause of cell death induced in plants by oxidative stress is not well known . Is it simply the toxicity of ROS that damages cells or is it the activation of a PCD pathway by ROS ? It is possible that the level of H_2O_2 that is currently thought to kill cells by direct cellular damage actually induces PCD (Lam *et al* . 2001; Mitsuhara *et al* . 1999), and it might require a higher level of ROS to kill cells by direct oxidation . Perhaps future studies applying oxidative stress to mutants deficient in different PCD pathways will answer this question . Many questions related to ROS metabolism remain unanswered . We are currently at an exciting time, when most of the technologies required to answer these questions are in place . Thus, a comprehensive analysis of gene expression using microarrays and chips, coupled with proteomics and metabolomics to follow different antioxidants and related compounds during oxidative stress, should answer many of these questions . This analysis can be performed on plants responding to abiotic stresses, biotic insults or combinations of both, and can be complemented by using mutants with altered ability to produce or scavenge ROS . In addition, the development of cellular markers that enable the nondestructive quantification of different ROS in the different cellular compartments, like the markers used for Ca^{2+} imaging, will considerably advance our understanding of ROS metabolism .

Abbreviations: ABA: abscisic acid; AOX: alternative oxidase; AtMPK3: Arabidopsis thaliana MAPK3; AtNDPK2: Arabidopsis thaliana NUCLEOTIDE DIPHOSPHATE KINASE2; MAPK: mitogen-activated protein kinase; $^1O_2$: singlet oxygen; ost1: open stomata1; PCD: programmed cell death; PR: pathogenesis-related; PTP: protein tyrosine phosphatases; Rboh: respiratory burst oxidase homologue; ROS: reactive oxygen species; SA: salicylic acid; NO: nitric oxide; JA: jasmonic acid; ET: ethylene; TF: transcription factor; ABI1: ABA-INSENSITIVE1; CaM: calmodulin; GA: gibberellin; H_2O_2: hydrogen peroxide; PP: protein phosphatase

References:

Allen R, 1995 . Dissection of oxidative stress tolerance using transgenic plants [J] . *Plant Physiol*, **107**: 1049—1054

Alvarez ME, Pennell RI, Meiker PJ *et al* . 1998 . Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity [J] . *Cell*, **92**: 773—784

Anderson JP, Badruzsaufari E, Schenk PM *et al* . 2004 . Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in *Arabidopsis* [J] . *Plant Cell*, **16**: 3460—3479

Apostol I, Heinstein PF, Low PS, 1989 . Rapid stimulation of an oxidative burst during elicidation of cultured plant cells . Role in defense and signal transduction [J] . *Plant physiol*, **90**: 106—116

Asada K, Takahashi M, 1987 . Production and scavenging of active oxygen in photosynthesis [A] . In: Kyle DJ *et al* ., eds, Photoinhibition (Topics in Photosynthesis) [M] . Vol. 9, 227—287, Elsevier

Audenaert K, De Meyer GB, Hofte MM, 2002 . Abscisic acid determines basal susceptibility of tomato to *Botrytis cinerea* and suppresses salicylic acid-dependent signaling mechanisms [J] . *Plant Physiol*, **128**: 491—501

Babior BM, 1999 . NADPH oxidase: an update [J] . *Blood*, **93**: 1464—1476

Bartels D, Sunkar R, 2005 . Drought and salt tolerance in plants [J] . *Crit Rev Plant Sci*, **24**: 23—58

Beaudoin N, Serizet C, Gosti F *et al* . 2000 . Interactions between abscisic acid and ethylene signaling cascades [J] . *Plant Cell*, **12**: 1103—1115

Bethke PC, Lonsdale JE, Fach A *et al* .1999 . Hormonally regulated programmed cell death in barley aleurone cells [J] . *Plant Cell*, **11**: 1033—1045

Bowler C, Montagu MV, Inzé D, 1992 . Superoxide dismutase and stress tolerance [J] . *Annu Rev Plant Physiol Plant Mol Biol*, **43**: 83—

116

Cazale AC, Drollard MJ, Wilson C *et al* . 1999 . MAP kinase activation by hypoosmotic stress of tobacco cell suspensions: towards the oxidative burst response ? [J] . *Plant J*, **19**: 297—307

Chen SX, Schopfer P, 1999 . Hydroxyl radical production in physiological reactions . A novel function of peroxidase [J] . *Eur J Biochem*, **260**: 726—735

Chung E, Park JM, Oh SK *et al* . 2004 . Molecular and biochemical characterization of the *Capsicum annuum* calcium-dependent protein kinase3 (CDPK3) gene induced by abiotic and biotic stresses [J] . *Planta*, **220**: 286—295

Costa V, Moradas-Ferreira P, 2001 . Oxidative stress and signal transduction in Saccharomyces cerevisiae: insights into ageing, apoptosis and diseases [J] . *Mol Aspects of Med*, **22** (4-5): 217—246

Dat J, Vandenabeele S, Vranová E *et al* . 2000 . Dual action of the active oxygen species during plant stress responses [J] . *Cell Mol Life Sci*, **57**: 779—795

Davletova S, Schlauch K, Coutu J *et al* . 2005 . The zinc-finger protein Zat12 plays a central role in reactive oxygen and abiotic stress signaling in *Arabidopsis* [J] . *Plant Physiol*, **139**: 847—856

Davletova S, Rizhsky L, Liang H *et al* . 2005 . Cytosolic ascorbate peroxidase 1 is a central component of the reactive oxygen gene network of *Arabidopsis* [J] . *Plant Cell*, **17**: 268—281

Desikan R, Hancock JT, Coffey MJ *et al* . 1996 . Generation of active oxygen in elicited cells of *Arabidopsis thaliana* is mediated by a NADPH oxidase-like enzyme [J] . *FEBS Lett*, **382**: 213—217

Desikin R, A-H-Mackerness S, Hancock JT *et al* . 2001 . Regulation of the *Arabidopsis* transcriptosome by oxidative stress [J] . *Plant Physiol*, **127**: 159—172

Field TS, Nedbal L, Ort DR, 1998 . Nonphotochemical reduction of the plastoquinone pool in sunflower leaves originate from chlororespiration [J] . *Plant Physiol*, **116**: 1209—1218

Foreman J, Demidchik V, Bothwell JHF *et al* . 2003 . Reactive oxygen species produced by NADPH oxidase regulate plant cell growth [J] . *Nature*, **422**: 442—446

Ghassemian M, Nambara E, Cutler S *et al* . 2000 . Regulation of abscisic acid signaling by the ethylene response pathway in *Arabidopsis* [J] . *Plant Cell*, **12**: 1117—1126

Grant JJ, Loake GJ, 2000 . Role of reactive oxygen intermediates and cognate redox signaling in disease resistance [J] . *Plant Physiol*, **124**: 21—29

Guan LM, Zhao J, Scandalios JG, 2000 . Cis-elements and transfactors that regulate expression of the maize Cat1 antioxidant gene in response to ABA and osmotic stress: H_2O_2 is the likely intermediary signaling molecule for the response [J] . *Plant J*, **22**: 87—95

Guo FQ, OkamotoM, Crawford NM, 2003 . Identification of a plant nitric oxide synthase gene involved in hormonal signaling [J] . *Science*, **302**: 100—103

Halliwell B, Gutteridge JMC, 1989 . Free Radicals in Biology and Medicine, Clarendon Press

Hammond-Kosack KE, Jones JDG, 1996 . Resistance gene-dependent plant defense responses [J] . *Plant Cell*, **8**: 1773—1791

Harding SA, Oh Suk-Heung, Roberts DM, 1997 . Transgenic tobacco expressing a foreign calmodulin gene shows an enhanced production of active oxygen species [J] . *EMBO J*, **16**: 1137—1144

Hu XL, Jiang MY, Zhang AY *et al* . 2005 . Abscisic acid-induced apoplastic H_2O_2 accumulation up-regulates the activities of chloroplastic and cytosolic antioxidant enzymes in maize leaves [J] . *Planta*, **223**: 57—68

Iida A, Kazuoka T, Torikai S *et al* . 2000 . A zinc finger protein RHL41 mediates the light acclimatization response in *Arabidopsis* [J] . *Plant J*, **24**: 191—203

Jiang K, Meng YL, Feldman LJ, 2003 . Quiescent center formation in maize roots is associated with an auxin-regulated oxidizing environment [J] . *Development*, **130**: 1429—1438

Keller T, Danude HG, Werner D *et al* . 1998 . A plant homolog of the neutrophil NADPH oxidase gp91phox subunit gene encodes a plasma membrane protein with Ca^{2+} binding motifs [J] . *Plant Cell*, **10**: 255—266

Kirk Overmyer, 2003 . Reactive oxygen species and hormonal control of cell death [J] . *Trends in Plant Science*, **8**: 335—342

Klaus Apel, Heribert Hirt, 2004 . REACTIVE OXYGEN SPECIES: Metabolism, Oxidative Stress, and Signal Transduction [J] . *Annual Review of Plant Biology*, **55**: 373—399

Klusener B, Young JJ, Murata Y *et al* . 2002 . Convergence of calcium signaling pathways of pathogenic elicitors and abscisic acid in *Arabidopsis* guard cells [J] . *Plant Physiol*, **130**: 2152—2163

Kovtun Y, Chiu WL, Tena Guillaume *et al* . 2000 . Functional analysis of oxidative stress activated mitogen-activated protein kinase cascade in plants [J] . *Proc Natl Acad Sci USA*, **97**: 2940—2945

Kwak JM, Mori IC, Pei ZM *et al* . 2003 . NADPH oxidase AtrbohD and AtrbohF genes function in ROS-dependent ABA signaling in *Arabidopsis* [J] . *EMBO J*, **22**: 2623—2633

Laloi C, Apel K, Danon A, 2004 . Reactive oxygen signaling: the latest news [J] . *Curr Opin Plant Biol*, **7**: 323—328

Lam E, Kato N, Lawton M, 2001 . Programmed cell death, mitochondria and the plant hypersensitive response [J] . *Nature*, **411**: 848—853

Lorenzo O, Solano R, 2005 . Molecular players regulating the jasmonate signaling network [J] . *Curr Opin Plant Biol*, **8**: 532—540

Ludwig AA, Romeis T, Jones JDG, 2004 . CDPK-mediated signaling pathways: specificity and cross-talk [J] . *J Exp Bot*, **55**: 181—188

Ludwig AA, Saitoh H, Felix G *et al* . 2005 . Ethylene-mediated cross-talk between calcium-dependent protein kinase and MAPK signaling controls stress responses in plants [J] . *Proc Natl Acad Sci USA*, **102**: 10736—10741

Malan C, Gregling MM, Gressel J, 1990 . Correlation between CuZn superoxide dismutase and glutathione reductase and environmental and xenobiotic stress tolerance in maize inbreds [J] . *Plant Sci*, **69**: 157—166

Maleck K, Levine A, Eulgem T *et al* . 2000 . The transcriptome of Arabidopsis thaliana during systemic acquired resistance [J] . *Nat Genet*, **26**: 403—410

Mauch-Mani B, Mauch F, 2005 . The role of abscisic acid in plant-pathogen interactions [J] . *Curr Opin Plant Biol*, **8**: 409—414

Miki Fujita, Yasunari Fujita, 2006 . Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks [J] . *Current Opinion in Plant Biology*, **9**: 436—442

Mitsuhara I, Malik KA, Miura M *et al* . 1999 . Animal cell-death suppressors Bcl-xL and Ced-9 inhibit cell death in tobacco plants [J] . *Curr Biol*, **9**: 775—778

Mittler R, 2002 . Oxidative stress, antioxidants and stress tolerance [J] . *Trends Plant Sci*, **7**: 405—410

Mittler R, Vanderauwera S, Gollery M *et al* . 2004 . Reactive oxygen gene network of plants [J] . *Trends Plant Sci*, **9**: 490—498

Mullineaux P, Karpinski S, 2002 . Signal transduction in response to excess light: getting out of the chloroplast [J] . *Curr Opin Plant Biol*, **5**: 43—48

Neill S, Desikan R, Hancock JT, 2002 . Hydrogen peroxide signalling [J] . *Curr Opin Plant Biol*, **5**: 388—395

Neill SJ, Desikan R, Hancock J *et al* . 2002 . Hydrogen peroxide and nitric oxide as signalling molecules in plants [J] . *J Exp Bot*, **53**: 1237—1247

Noctor G, Foyer C, 1998 . Ascorbate and glutathione: keeping active oxygen under control [J] . *Annu Rev Plant Physiol Plant Mol Biol*, **49**: 249—279

Orozco-Cardenas M, Ryan CA, 1999 . Hydrogen peroxide is generated systemically in plant leaves by wounding and systemin via the octadecanoid pathway [J] . *Proc Natl Acad Sci USA*, **96**: 6553—6557

Overmyer K, Brosché M, Kangasj rvi J, 2003 . Reactive oxygen species and hormonal control of cell death [J] . *Trends Plant Sci*, **8**: 335—342

Pei ZM, Benning G, Thomine S *et al* . 2000 . Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells [J] . *Nature*, **406**: 731—734

Polle A, 2001 . Dissecting the superoxide dismutase-ascorbate peroxidase-glutathione pathway in chloroplasts by metabolic modeling . Computer simulations as a step towards fluxanalysis [J] . *Plant Physiol*, **126**: 445—462

Prasad TK, Anderson MD, Martin BA *et al* . 1994 . Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide [J] . *Plant Cell*, **6**: 65—74

Rizhsky L, Davletova S, Liang H *et al* . 2004 . The zinc finger protein Zat12 is required for cytosolic ASCORBATE PEROXIDASE 1 expression during oxidative stress in *Arabidopsis* [J] . *J Biol Chem*, **279**: 11736—11743

Samuel MA, Miles GP, Ellis BE, 2000 . Ozone treatment rapidly activates MAP kinase signalling in plants [J] . *Plant J*, **22**: 367—376

Schenk PM, Kazan K, Wilson I *et al* . 2000 . Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis [J] . *Proc Natl Acad Sci USA*, **97**: 11655—11660

Seki M, Narusaka M, Ishida J *et al* . 2002 . Monitoring the expression profiles of 7000 *Arabidopsis* genes under drought, cold and high-salinity stresses using a full-length cDNA microarray [J] . *Plant J*, **31**: 279—292

Takahashi S, Seki M, Ishida J *et al* . 2004 . Monitoring the expression profiles of genes induced by hyperosmotic, high salinity, and oxidative stress and abscisic acid treatment in *Arabidopsis* cell culture using a full-length cDNA microarray [J] . *Plant Mol Biol*, **56**: 29—55

Tenhaken R, Levine A, Brisson LF *et al* . 1995 . Function of the oxidative burst in hypersensitive disease resistance [J] . *Proc Natl Acad Sci USA*, **92**: 4158—4163

Thaler JS, Bostock RM, 2004 . Interactions between abscisic-acidmediated responses and plant resistance to pathogens and insects [J] . *Ecology*, **85**: 48—58

Ton J, Mauch-Mani B, 2004 . Beta-amino-butyric acid-induced resistance against necrotrophic pathogens is based on ABA-dependent priming for callose [J] . *Plant J*, **38**: 119—130

Ton J, Jakab G, Toquin V *et al* . 2005 . Dissecting the *b*-aminobutyric acid-induced priming phenomenon in *Arabidopsis* [J] . *Plant Cell*, **17**: 987—999

Torres MA, Onouchi H, Hamada S *et al* . 1998 . Six *Arabidopsis* thaliana homologues of the human respiratory burst oxidase (gp91phox) [J] . *Plant J*, **14**: 365—370

Torres MA, Dangl JL, Jones JDG, 2002 . *Arabidopsis* gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response [J] . *Proc Natl Acad Sci USA*, **99**: 517—522

Torres MA, Dangl JL, 2005 . Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development [J] . *Curr Opin Plant Biol*, **8**: 397—403

Tsugane K, Kobayashi K, Niwa Y *et al* . 1999 . A recessive *Arabidopsis* mutant that grows enhanced active oxygen detoxification [J] . *Plant Cell*, **11**: 1195—1206

Umezawa T, Fujita M, Fujita Y *et al* . 2006 . Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future [J] . *Curr Opin Biotechnol*, **17**: 113—122

Vogel JT, Zarka DG, Van Buskirk HA *et al* . 2005 . Roles of the CBF2 and ZAT12 transcription factors in configuring the low temperature transcriptome of *Arabidopsis* [J] . *Plant J*, **41**: 195—211

Wendehenne D, Durner J, Klessig DF, 2004 . Nitric oxide: a new player in plant signaling and defense responses [J] . *Curr Opin Plant Biol*, **7**: 449—455

Zhang S, Klessig DF, 2001 . MAPK cascades in plant defense signaling [J] . *Trends Plant Sci*, **6**: 520—527

Zhang AY, Jiang MY, Zhang JH *et al* . 2006 . Mitogen-Activated Protein Kinase Is Involved in Abscisic Acid-Induced Antioxidant Defense and Acts downstream of Reactive Oxygen Species Production in Leaves of Maize Plants1 Plant Physiology March 10, 2006, as DOI: 10.1104/pp . 105.075416

Zimmermann P, Hirsch-Hoffmann M, Hennig L *et al* . 2004 . Genevestigator . *Arabidopsis* microarray database and analysis toolbox [J] . *Plant Physiol*, **136**: 2621—2632

Zimmermann P, Hennig L, Gruissem W, 2005 . Gene-expression analysis and network discovery using Genevestigator [J] . *Trends Plant Sci*, **10**: 407—409