Review

The reactive oxygen species network pathways: an essential prerequisite for perception of pathogen attack and the acquired disease resistance in plants

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Availability of complete Arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativa*) genome sequences, together with molecular recourses of functional genomics and proteomics have revolutionized our understanding of reactive oxygen species (ROS) signalling network mediating disease resistance in plants. So far, ROS have been associated with aging, cellular and molecular alteration in animal and plant cells. Recently, concluding evidences suggest that ROS network is essential to induce disease resistance and even to mediate resistance to multiple stresses in plants. ROS are obligatory by-products emerging as a result of normal metabolic reactions. They have the potential to be both beneficial and harmful to cellular metabolism. Their dual effects on metabolic reactions are dosage specific. In this review we focus our attention on cellular ROS level to trigger beneficial effects on plant cells responding to pathogen attack. By exploring the research related contributions coupled with data of targeted gene disruption, and RNA interference approaches, we show here that ROS are ubiquitous molecules of redox-pathways that play a crucial role in plant defence mechanism. The molecular prerequisites of ROS network to activate plant defence system in response to pathogen infections are here underlined. Bioinformatic tools are now available to scientists for high throughput analysis of cellular metabolisms. These tools are used to illustrate crucial ROS-related genes that are involved in the defence mechanism of plants. The review describes also the emerging findings of ROS network pathways to modulate multiple stress resistance in plants.

1. Introduction

The plant pathogens have developed various independent and well-elaborated mechanisms of penetrating and accessing plant cell contents. Stopping the penetration of pathogens during plant infection is generally dependent on the accurate time-course of the pathogen perception by the plant host cells and the activation of networking systems resulting in induction of secondary metabolites, reactive oxygen species (ROS) and pathogenesis related proteins, working often in combination to mount an adequate defence mechanism against the pathogen infection (Bolwell *et al* 2001). This
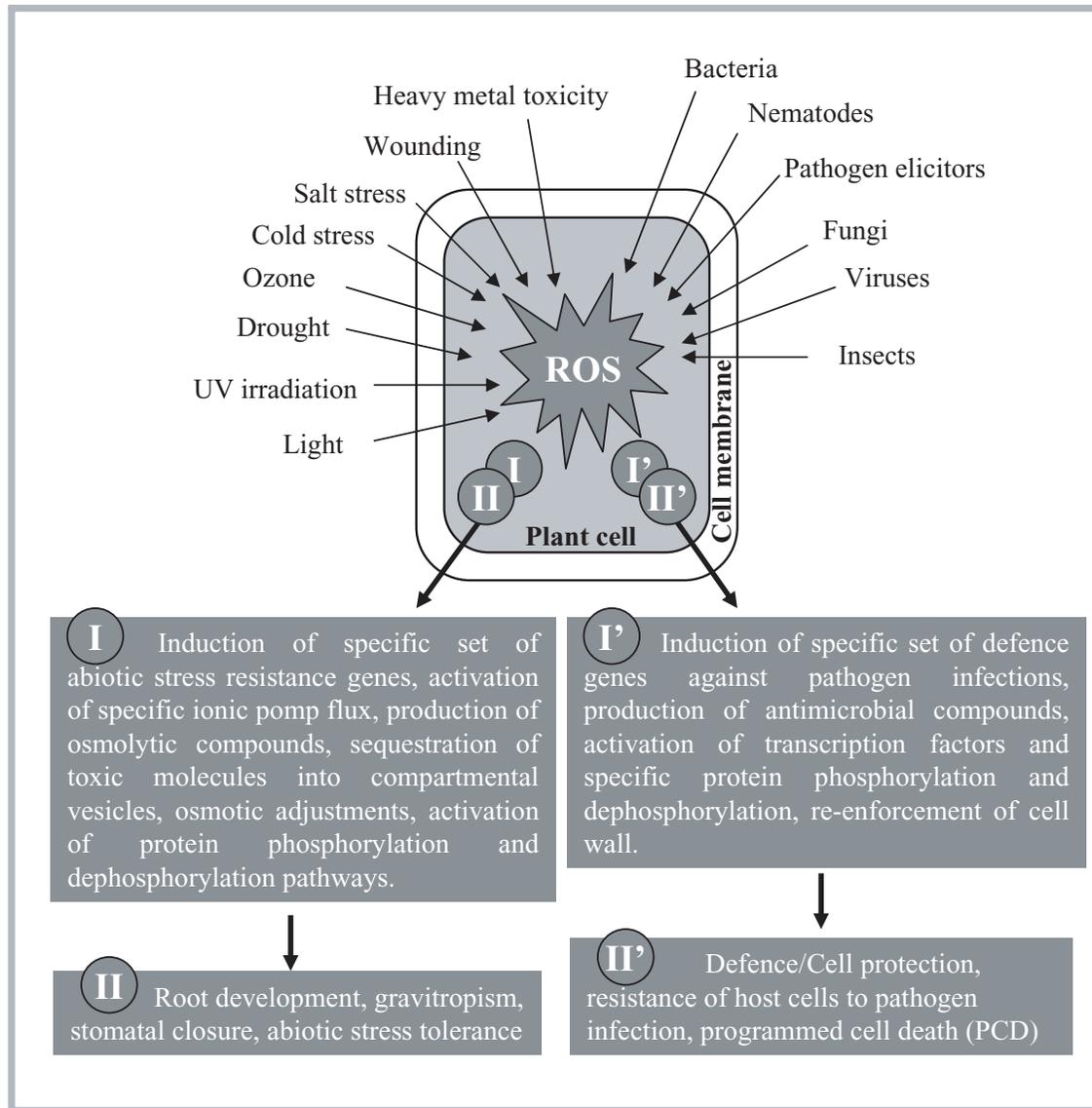
Figure 1.   Involvement of ROS in cellular metabolic processes of plants response to both various environmental stresses. I and II indicate the subsequent downstream events mediating by ROS in plant cells exposed to abiotic stresses, while I' and II' indicate the subsequent downstream events mediating by ROS in plants cells exposed to pathogens and pathogen-elicitors. The resistance of the plant cells to the stress condition is dependent of the intensity and the speed of these downstream events.

mechanism includes modifications of the plant cell wall, deposition of callose containing papillae and production of hydroxyproline rich glycoproteins and phenolic compounds (Mazau and Esquerré-Tugayé 1986; Aist and Bushnell 1991). Being sessile organisms, the plants have evolved various other ways to hinder the pathogens attempting to enter the cells. Here we selectively review the molecular implication of ROS network mediating the perception and induction of defence mechanisms to hinder pathogen infection in plants.

ROS, which include superoxide radical, hydrogen peroxide and singlet oxygen are ubiquitous molecules

produced as a consequence of normal cellular metabolism (Kotchoni 2004). Under normal conditions, ROS are rapidly metabolised with help of constitutive antioxidant enzymes and other metabolites via non-enzymatic pathways such as antioxidant vitamins, proteins and non-protein thiols (Kovtun *et al* 2000; Lim *et al* 1993; Scandalios *et al* 1997). However, when subjected to environmental stresses such as cold, high light, ozone (O_3), drought, salt stress, pathogens, and UV irradiation, excessive ROS is generated (figure 1). This excessive accumulation of ROS necessitates the activation of additional defences (Doke 1997; Scandalios

et al 1997). Unless these ROS are efficiently metabolized, they rapidly oxidize and damage lipids in cellular membranes, proteins, and other cellular components leading consequently to cellular dysfunction and ultimately to cell death, or appearance of necrotic lesions (Doke 1997; Foyer and Noctor 2005).

Recently, responses of plants to various environmental stresses have been analysed extensively at biochemical and molecular level (Gachomo *et al* 2003; Rizhsky *et al* 2004, 1996; Kotchoni *et al* 2005). Evidence is increasing that ROS are required for different aspects of the life cycle including adaptation processes to environmental stress conditions (Bartels 2001; Ramanjulu and Bartels 2002). In animals, ROS have been implicated in inflammation, and cancer, but in plant-microbe interactions, ROS have just been recently suggested to participate in plant defence system in a number of ways including acting as signalling agents (Lamb and Dixon 1997; Gachomo and Kotchoni 2006) or causing reinforcement of cell wall through oxidative cross-linking (Brisson *et al* 1994; Brown *et al* 1998; McLusky *et al* 1999; Mellersh *et al* 2002). Various enzymatic mechanisms for generation of ROS, the type and source of ROS have been shown to vary depending on the plant-pathogen combination (Bolwell 1999). ROS have taken a centre stage in signal transduction network of stress-inducible genes in higher plants (Bartels 2001; Mittler *et al* 2004; Davletova *et al* 2005) and it is emerging that a balanced amount of ROS is crucial for many different metabolic processes in plants (Bartels 2001; Kotchoni 2004; Kotchoni *et al* 2006; Davletova *et al* 2005). Manipulating the ROS levels in plants has been proposed as a promising way to generate transgenic plants that can resist multiple environmental stresses (Bartels 2001). We emphasize on the accumulation of ROS in plants response to pathogen infections with the aim of elucidating the mechanisms of disease resistance acquisition in higher plants. This review describes new research trends and highlights several stress-inducible gene families of ROS network pathways that trigger a rapid pathogen attack perception and disease resistance in plants by using bioinformatic tools and software applications (Zimmermann *et al* 2004; Schmid *et al* 2005).

2. Production sites of ROS in plant cells

ROS are generally produced during aerobic phase of photosynthesis and photorespiration (Asada and Takahashi 1987; Mittler 2002; Kotchoni *et al* 2006). Accumulation of these molecules can also be detected in peroxisomes under abiotic stress (Ramanjulu and Bartels 2002; Mittler 2002) and biotic stress (Mittler 2002). During cellular metabolism, oxygen molecules are often converted into several intermediates such as O_2^-, H_2O_2, hydroxyl radical,

which often leak out from electron transport chain (Banerjee *et al* 2003), and can therefore be detected as traces in various cell compartments. New sources of ROS production include cell-wall-bound peroxidase, chloroplasts, and mitochondria (Mittler 2002; Davletova *et al* 2005). The chloroplast is considered to be a focal point of ROS metabolism. It is a major producer of O_2^- and H_2O_2 and contains also a large array of ROS-scavenging mechanisms that have been extensively studied (Davletova *et al* 2005). Most of the oxygen turnover in aerobic organism is utilized in the mitochondria for substrate metabolism and production of ATP. The production of ROS is related to environmental stresses, among which the most predominant are drought/desiccation, salinity, heat shock, heavy metals, UV radiation, ozone, nutrient deprivation, and pathogen attack (Bartels 2001; Davletova *et al* 2005; Foyer and Noctor 2005; Gachomo 2005). Despite being part of a normal process in the life of aerobic organism, accumulation of ROS is the source of oxidative damage and has been considered also as part of a defensive mechanism of cells. The production of ROS is recently shown to be the underlying mechanism of a series of biochemical and physiological changes that occur under environmental stress conditions, which subsequently mediate the disease resistance in plants (Gachomo and Kotchoni 2006).

3. ROS networking beyond the boundary of toxicity

ROS are known to play a dual role depending on their accumulation levels. High intracellular concentration of ROS can cause extensive cell injury or death. The levels of ROS need therefore to be tightly regulated to avoid cell damage (Neill *et al* 2002; Kotchoni 2004; Mittler *et al* 2004). Here we particularly focus on the beneficial steady-state level of ROS production in plants response to pathogen attacks. ROS are partially reduced or excited forms of atmospheric oxygen (O_2) are continuously produced in cells during aerobic metabolism (Halliwell and Gutteridge 1989). A stable and balanced intracellular redox is of vital importance to all organisms. A moderate accumulation of ROS function as key inducers for secondary programmed metabolisms, defence signal, cell wall differentiation and activation of mitogen-activated protein kinases (MAPKs) leading to the environmental stress tolerance (Conrath *et al* 2002; Gachomo *et al* 2003). Different members of gene families involved in the protective mechanism against pathogen attacks were up-regulated in plants under exogenous application of ROS (H_2O_2) (Rizhsky *et al* 2004). Mellersh *et al* (2002) demonstrated that localized generation of H_2O_2 is one of the earliest cytologically detectable defence responses to penetration of plant cell walls by various fungal pathogens. Rapid generation of H_2O_2 in response to cell wall penetration is one of the most

important determinants of pathogen penetration failure in invading epidermal cells (Mellersh *et al* 2002). H_2O_2 generation was the only response detected to account for fungal penetration failure. Enzymatic removal of H_2O_2 resulted in increased penetration success of fungi in the host plant cells (Mellersh *et al* 2002). Although the chemical nature and reactivity of ROS prove them to be potentially harmful to cells, plants use them as secondary messengers in signal transduction cascades regulating diverse processes such as mitosis, tropisms, cell death and defence mechanisms (Pavet *et al* 2005). It is now well accepted that ROS accumulation is crucial to plant development as well as in defence mechanisms (Foyer and Noctor 2005). Exogenous application of H_2O_2 was found to be essential to activate different pathogenesis-related proteins and to provide adequate protection against the pathogenic fungus *Diplocarpon rosae* causing black spot disease of rose leaves (Gachomo and Kotchoni 2006).

Knock-out (KO) plants deficient in ROS-scavenging proteins are of particular interest in elucidating role of ROS in disease defence systems. They have been used to study implication of high ROS content in plants response to pathogens infections. These KO-plants maintain a high steady-state level of H_2O_2 in cells and activate ROS defence mechanisms when grown under control conditions (Pnueli *et al* 2003; Rizhsky *et al* 2004; Davletova *et al* 2005). These null mutant plants (KO) provide an ideal experimental system to study plant responses to ROS accumulation and the effect of ROS mediating the activation of environmental stress-related proteins (Davletova *et al* 2005; Kotchoni *et al* 2006). On the other hand, transgenic plants expressing H_2O_2-generating enzymes have been reported to display increased protection against bacterial and fungal pathogens (Wu *et al* 1995; Schweizer *et al* 1999).

4.   ROS network mediating gene expression in plants response to pathogen attacks

Response of plants to pathogens depends on the regulatory gene expression at genomic level through complex genetic controls influenced by redox regulation (Pavet *et al* 2005). Control gene expression is one of the key-regulatory mechanisms used by living cells to execute biological functions. Elucidating the gene expression at genomic level in higher plants is still a subject of contemporary research. Microarray technology has provided new insight into the transcriptomes involved in mechanisms of environmental stress tolerance in higher plants. Transcriptomics and proteomics are among the current and most powerful strategies for quantitative, real-time analysis of gene expressions and protein functions in the whole plant. Several robust screening methods such as DNA microarry analysis, mass spectrometry-based proteomics and forward/reverse

genetics (Tyres and Mann 2003; Pavet *et al* 2005) are being now used to identify stress-inducible genes at genomic level. Genomics-based approaches, and bioinformatic tools are now available and being used to identify specific set of genes that are up-regulated by ROS (figure 2a). Bioinformatic tools have facilitated integration of experimental data into a computational framework, thereby allowing structured and systematic processing of information (Zimmermann *et al* 2004). Structured databases and data querying tools provide nowadays the means to assign putative functional information to genes. These tools were used to retrieve ROS-related genes in *Arabidopsis thaliana* which is a model plant with a complete genome sequence (figure 2 a, b), and to view the expression patterns of the selected ROS-related genes in *A. thaliana* exposed to different biotic stresses (table 1). It is now possible to analyse consequences of various stresses by monitoring expression of several genes simultaneously and record subsequent alteration of protein-protein or protein-DNA interactions in the whole plants. As a major tool, the hybridization with cDNA arrays allows for the analysis of thousands of gene expressions, which may be extended to the whole genome of plant. For example, Shimono *et al* (2003) have used the cDNA microarray analysis to elucidate various genes of disease resistance in rice plants. In addition, Scheideler *et al* (2002) have monitored, simultaneously global changes in the *A. thaliana* transcriptome (the whole set of transcripts present in a cell, tissue or organism) after infecting the plant with the incompatible bacteria pathogen *Pseudomonas syringae* pv. tomato by using cDNA arrays comprising 13000 unique expressed tags (ESTs).

Understanding regulatory mechanisms of gene expression under pathogen attack has been the subject of many research efforts. Evidence has shown that pathogen infection leads to oxidative damaging effect in plants (Smirnoff 1998). To prevent oxidative damage, especially in chloroplasts due to excessive ROS accumulation, the chloroplasts containing multiple ROS scavenging systems – such as ascorbate-glutathione cycle (Asada and Takahashi 1987) superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (Asada 1999; Mittler 2002; Apel and Hirt 2004) – must regulate the steady-state level of ROS production in that cell compartment. For example, four different isozymes of SOD – a CuZnSOD (CSD2), three FeSODs, and enzymes of the ascorbate-glutathione cycle capable of reducing oxidized ascorbic acid and glutathione (Asada and Takahashi 1987) – were identified in chloroplasts. However, Davletova *et al* (2005) demonstrated that in the absence of the cytosolic H_2O_2-scavenging enzyme APX1, the entire chloroplastic H_2O_2-scavenging system of *A. thaliana* collapses; H_2O_2 levels increase, and protein oxidation occurs. This suggests that the accumulation of ROS in cytosol and in other cellular compartments must be well regulated to avoid oxidation of macromolecules.
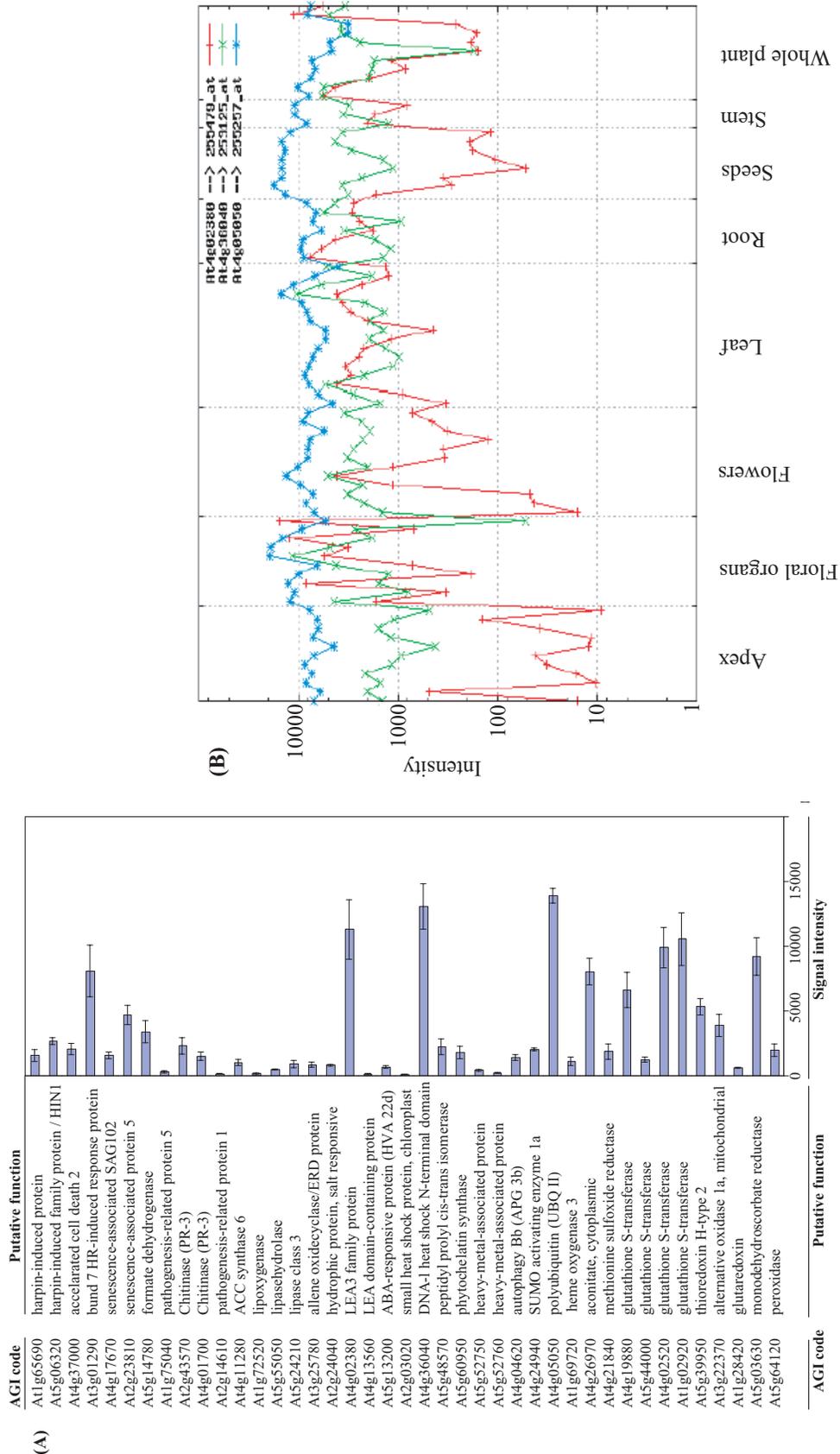
Figure 2. Expression level of ROS inducible genes in *A. thaliana*. Only the ROS-induced genes with an induction rate more than 100 ($\pm$ SE) compared to non-treated plants are shown. (**A**) The Response Viewer of GENEVESTIGATOR database was used to retrieve the expression level of the genes compared to the expression profile (non-induced) in control plants. (**B**) Organ specific expression pattern of selected ROS-induced genes using Weigelworld's database web-browser. The 12 depicted points for each of the three genes in Apex (for example) indicate the time course considered for the probe set used (probe set code) within the life time of this specific organ for the three gene expression profiles addressed in this case.

Table 1. Expression of selected ROS-related genes and their putative functions in plant defence mechanism.

Treatment

For the red-green scheme, red indicates that "the signal intensity of the treatment is higher than signal intensity of the corresponding control", and green means the opposite (see figure below). The ratio is given either in linear or log(2)-scale. The color-coding is fixed for each scale type such as to easily visualize changes in signal intensity levels.

Red:
∞ > ratio (linear) > 1
∞ > ratio (log) > 0
("upregulated")

Green:
0 < ratio (linear) < 1
– ∞ < ratio (log) < 0
("downregulated")

257311_at

0.81
3.28
0.8
0.49
0.86

Probeset	Treatment (Biotic +): A. brassiciola, A. tunefaciens, B. cinerea, E. cichoracearum, E. orontii, F. occidentalis, M. persicae, M. persicae, mycorrhiza, nematode, P. infestans, P. rapae, P. syringae, P. syringae	AGI/Links/Annotation
! 266772_s_at		AT2G03020 [MIPS, Tair, Tigr]heat shock protein-related Prosite PS00430: TonB-dependent receptor proteins sig...
248322_at		AT5G52760 [MIPS, Tair, Tigr]heavy-metal-associated domain-containing protein contains Pfam profile PF00403: ...
!! 266746_s_at		AT2G02930 [MIPS, Tair, Tigr][[AT2G02930, glutathione S-transferase, putative]] / AT4G02520 [MIPS, Tair, Tigr][AT4G02520, glutathione S-transferase, putative]
255479_at		AT4G02380 [MIPS, Tair, Tigr]late embryogenesis abundant 3 family protein / LEA3 family protein similar to se...
!! 262119_s_at		AT1G02920 [MIPS, Tair, Tigr][[AT1G02920, glutathione S-transferase, putative similar to glutathione S-transf... / AT1G02930 [MIPS, Tair, Tigr][supported by cDNA GI:443697.]
247327_at		AT5G64120 [MIPS, Tair, Tigr]peroxidase, putative identical to peroxidase [Arabidopsis thaliana] gi|1483222|e...
255595_at		AT4G01700 [MIPS, Tair, Tigr]chitinase, putative similar to peanut type II chitinase GI:1237025 from [Arachis...
249777_at		AT5G24210 [MIPS, Tair, Tigr]lipase class 3 family protein contains Pfam profile PF01764: Lipase]
258452_at		AT3G22370 [MIPS, Tair, Tigr]alternative oxidase 1a, mitochondrial (AOX1A) identical to GB:Q39219 [SP|Q39219]...
250279_at		AT5G13200 [MIPS, Tair, Tigr]GRAM domain-containing protein / ABA-responsive protein-related similar to ABA-r...
255283_at		AT4G04620 [MIPS, Tair, Tigr]autophagy 8b (APG8b) identical to autophagy 8b [Arabidopsis thaliana] GI:1991215...
249385_at		AT5G39950 [MIPS, Tair, Tigr]thioredoxin H-type 2 (TRX-H-2) (Gif2) identical to SP|Q38879 Thioredoxin H-type ...
246595_at		AT5G14780 [MIPS, Tair, Tigr]formate dehydrogenase (FDH) identical to GI:7677266]
253954_at		AT4G26970 [MIPS, Tair, Tigr]aconitate hydratase, cytoplasmic, putative / citrate hydro-lyase/aconitase, puta...
267293_at		AT2G23810 [MIPS, Tair, Tigr]senescence-associated family protein similar to senescence-associated protein 5 ...
250676_at		AT5G06320 [MIPS, Tair, Tigr]harpin-induced family protein / HIN1 family protein / harpin-responsive family p...
254084_at		AT4G24940 [MIPS, Tair, Tigr]SUMO activating enzyme 1a (SAE1a) identical to SUMO activating enzyme 1a [Arabid...
249075_at		AT5G44000 [MIPS, Tair, Tigr]glutathione S-transferase C-terminal domain-containing protein contains Pfam dom...
261494_at		AT1G28420 [MIPS, Tair, Tigr]homeobox transcription factor, putative similar to homeobox transcription factor...
246194_at		AT4G37000 [MIPS, Tair, Tigr]accelerated cell death 2 (ACD2) identical to accelerated cell death 2 (ACD2) GI:...
254716_at		AT4G13560 [MIPS, Tair, Tigr]late embryogenesis abundant domain-containing protein / LEA domain-containing pr...
248118_at		AT5G55050 [MIPS, Tair, Tigr]GDSL-motif lipase/hydrolase family protein similar to family II lipases EXL3 GI:...
266566_at		AT2G24040 [MIPS, Tair, Tigr]hydrophobic protein, putative / low temperature and salt responsive protein, put...
255257_at		AT4G05050 [MIPS, Tair, Tigr]polyubiquitin (UBQ11) identical to GI:304117]
245401_at		AT4G17670 [MIPS, Tair, Tigr]senescence-associated protein-related similar to senescence-associated protein S...
254549_at		AT4G19880 [MIPS, Tair, Tigr]glutathione S-transferase-related contains weak hit to Pfam profile PF00043: Glu...
253125_at		AT4G36040 [MIPS, Tair, Tigr]DNAJ heat shock N-terminal domain-containing protein (J11) identical to dnaJ hea...
254926_at		AT4G11280 [MIPS, Tair, Tigr]1-aminocyclopropane-1-carboxylate synthase 6 / ACC synthase 6 (ACS6) identical...
250916_at		AT5G03630 [MIPS, Tair, Tigr]monodehydroascorbate reductase, putative monodehydroascorbate reductase (NADH), ...
248327_at		AT5G52750 [MIPS, Tair, Tigr]heavy-metal-associated domain-containing protein Pfam profile PF00403: Heavy-me...
! 260419_at		AT1G69720 [MIPS, Tair, Tigr][[AT1G69720, heme oxygenase 3 (HO3) similar to heme oxygenase 3 [Arabidopsis th... / AT1G69730 [MIPS, Tair, Tigr][AT1G69730, protein kinase family protein contains Pfam profile: PF00069 Eukary...
248657_at		AT5G48570 [MIPS, Tair, Tigr]peptidyl-prolyl cis-trans isomerase, putative / FK506-binding protein, putative ...
260399_at		AT1G72520 [MIPS, Tair, Tigr]lipoxygenase, putative similar to lipoxygenase gi:1495804 [Solanum tuberosum], g...
257644_at		AT3G25780 [MIPS, Tair, Tigr]allene oxide cyclase, putative / early-responsive to dehydration protein, putati...
259925_at		AT1G75040 [MIPS, Tair, Tigr]pathogenesis-related protein 5 (PR-5) identical to SP|P28493 Pathogenesis-relate...
247604_at		AT5G60950 [MIPS, Tair, Tigr]phytochelatin synthetase-related contains Pfam profile PF04833: Phytochelatin sy...
262930_at		AT1G65690 [MIPS, Tair, Tigr]harpin-induced protein-related / HIN1-related / harpin-responsive protein-relate...
259272_at		AT3G01290 [MIPS, Tair, Tigr]band 7 family protein similar to hypersensitive-induced response protein [Zea ma...
266385_at		AT2G14610 [MIPS, Tair, Tigr]pathogenesis-related protein 1 (PR-1) identical to GB:M90508 SP|P33154]
260568_at		AT2G43570 [MIPS, Tair, Tigr]chitinase, putative similar to chitinase class IV GI:722272 from [Brassica napus...
!! 254385_s_at		AT4G21830 [MIPS, Tair, Tigr][[AT4G21830, methionine sulfoxide reductase domain-containing protein / SelR do... / AT4G21840 [MIPS, Tair, Tigr][[AT4G21840, methionine sulfoxide reductase domain-containing protein / SelR dom...

Meta analyser (with respect to environmental factors) of GENEVESTIGATOR database was used to retrieve the expression of ROS inducible genes in *A. thaliana* under different pathogen infections. The red colour indicates up-regulation of the genes; the green colour indicates down-regulation of genes, while black colour indicates non-induction.

5. ROS mediating stress-sensing and network transduction

Sensing and stopping pathogen attack/penetration is among the primary detecting mechanisms associated with disease resistance in plants. The detecting machinery of pathogen infection by the host cells include cross-linking of pre-existing or else induced cell-wall proteins and phenolic compounds (Aist and Bushnell 1991; Brisson *et al* 1994; Thordal-Christensen *et al* 1997; McLusky *et al* 1999; Bolwell *et al* 2001; Davletova *et al* 2005); formation of calcium-pectate gels (Kieffer *et al* 2000); accumulation of glycoproteins (Mazau and Esquerré-Tugayé 1986); silica (Aist and Bushnell 1991); deposition of callose-containing pappille (Aist and Bushnell 1991) and the generation of ROS (Thordal-Christensen *et al* 1997; Gachomo and Kotchoni 2006). Generally, production of these compounds occurs simultaneously to mount a programmed and efficient infection arrest in plants (Perumalla and Hearth 1991). It is widely accepted that ROS may play a central role in many signalling pathways during stress perception, regulation of photosynthesis, pathogen response, hormonal action, and plant growth and development (Dat *et al* 2000; Mittler 2002; Mullineaux and Karpinski 2002; Neill *et al* 2002; Torres *et al* 2002; Foreman *et al* 2003; Kwak *et al* 2003; Apel and Hirt 2004). This assertion was supported by the fact that localized extracellular ROS have been detected during plant-pathogen interations (Doke 1983; Thordal-Christensen *et al* 1997), and has been found to have an antimicrobial effect on phytopathogens (Bestwick *et al* 1997; Gachomo and Kotchoni 2006). H_2O_2 is an electron-accepting substrate for a wide range of plant peroxidases, the well characterized ROS-detoxifying enzymes (Yamasaki *et al* 1997). In this close relation of donor and acceptor of electron, various ion channels are activated, among which the Ca^{2+}-channel is the most predominant of all (Kawano *et al* 2000). These ion-signalling pathways lead to intracellular pH changes needed for subsequent activation of plant protective mechanisms (Kawano *et al* 2000). The regulatory function of the cytosolic pool upon ion fluxes has been well demonstrated by Britto and Kronzucker (2001). Their work brought insight in the pivotal role of ROS in modulating the transmenbrane ion flux regulation of plant protection against external challenges.

Under pathogen attack, ROS metabolism is regulated by a network involving at least 152 genes in *A. thaliana* (Mittler *et al* 2004). The regulatory network controls the rates of ROS production and ROS scavenging enzymes in the different cellular compartments and modulates the steady state level of ROS for signalling as well as defence purposes. The generation of H_2O_2 and the stress inducible genes act within the penetration-inhibiting pathway, possibly through the involvement of phenolic materials (Aist and Brushnell 1991). ROS alone and especially H_2O_2 generation is necessary and sufficient to account for fungal penetration failure in plants (Mellersh *et al* 2002). In addition, ROS have the potential to be directly toxic as exogenous H_2O_2 can inhibit pathogen growth *in vitro* (Lu and Higgins 1999) and can prevent fungal infection of detached leaves as well as leaf disk (Peng and Kuc 1992; Gachomo and Kotchoni 2006). H_2O_2 most likely acts within a pathway involving transcription/translation and the expression of wall-associated responses such as the accumulation of fungal inhibiting compounds (Aist and Brushnell 1991). Recent studies suggest that cross-talk between salicylic acid (SA)-, jasmonic acid (JA)-, and ethylene-dependent signalling pathways regulates plant responses to both biotic and abiotic stress factors. Treating cell cultures with H_2O_2 alone induces defence responses and cell death (Levine *et al* 1994; Solomon *et al* 1999). However, although sublethal H_2O_2 concentrations induce expression of defence genes, complete induction of defence genes and cell death requires additional signalling molecules such as SA at the whole-plant level (Chamnongpol *et al* 1998; Rao and Davis 1999). These studies have established that ROS probably require additional downstream components to transduce or amplify the signal (Van Camp *et al* 1998; Bolwell 1999). Among several molecules proposed to act downstream of ROS, SA, JA, and ethylene are considered to be the major regulators of plant defence responses (Penninckx *et al* 1998; Spoel *et al* 2003; Veronese *et al* 2006). SA is one of the most widely studied stress-signalling molecules, and its role in influencing plant resistance to pathogens and other stress factors is well documented (Draper 1997; Shirasu *et al* 1997; Surplus *et al* 1998; Rao and Davis 1999). Similar to SA, JA is also believed to play an important role in influencing plant resistance to pathogens and other stress factors (Penninckx *et al* 1998; Spoel *et al* 2003).

Production of ROS occurs generally by direct transfer of excitation energy from the chlorophyll to produce singlet oxygen, or by univalent oxygen reduction at photosystem I in the Mehler reaction (Foyer *et al* 1994; Allen 1995). They lead to oxidative injuries, which are mainly caused either by an imbalance of the cellular antioxidant capacity or by a deficiency in the antioxidant system controlling ROS levels (Halliwell and Gutteridge 2002). These damaging effects force the plants to develop complex redox homeostatic mechanisms to cope with the accumulation of ROS (Moon *et al* 2003). A slight alteration in the homeostatic level of intracellular ROS triggers therefore the expression of a specific set of genes generally encoding for antioxidant proteins, compatible molecules, oxidative scavengers, and protein phosporylation cascade via activated MAPK proteins (Borsani *et al* 2003).

On the other hand, accumulation of ROS inhibits mitochondrial electron transport leading to ATP depletion

(Jones 2000). Both ROS accumulation and ATP depletion lead rapidly to Ca^{2+} influx, which triggers programmed cell death (PCD) in plants (Jones 2000). A moderate concentration of ROS activates the cellular defence response (Levine *et al* 1994). Tobacco plants inoculated with the tobacco mosaic virus (TMV) developed systemic acquired resistance (SAR) that was mediated by a burst of ROS (Lamb and Dixon 1997). Rapid production of ROS could also inhibit pathogen growth by restricting pathogen penetration via cross-linking of cell wall glycoproteins (Bradley *et al* 1992), by induction of phytoalexin accumulation (Gachomo *et al* 2003), or by induction of defence-related genes (Desikan *et al* 2001; Gachomo *et al* 2003; Gachomo and Kotchoni 2006). For example, incubation of potato tuber slices with an incompatible race of *Phytophthora infestans* stimulates the NADPH oxidase, which is strictly under the control of O$_2^-$ accumulation (Doke and Miura 1995). This is evidence of potential role of ROS to mediate expression of pathogen defence mechanism in plants.

6. Role of ROS in mitogen-activated protein kinase (MAPK) cascade

The resistance of plants to pathogen infections involves an elaborate system of signal perception and transduction to activate cellular defence. After perception of pathogen attack, the cell host synthesizes various molecules including ROS, protein-kinases and transcription factors, relaying the pathogen-perception signal to activate downstream defence responses (Gachomo and Kotchoni 2006; Veronese *et al* 2006). The speed and intensity of activation of these downstream cascade reactions are crucial for plant resistance to pathogen infection. ROS are one of the most important components of the signalling pathways, which influence plant defence responses, including cell death (Jabs *et al* 1996; Alvarez *et al* 1998; Karpinski *et al* 1999; Solomon *et al* 1999). In plants, the signal transduction is controlled by protein phosphorylation and dephosphorylation involving MAPKs (Khokhlatchev *et al* 1998; Tena *et al* 2001). Induction of MAPK activity has been detected after exposure of plants to various stresses (Kovtun *et al* 2000; Xiong and Yang 2003). It was recently discovered that ROS mediate the activation of MAPK cascade and subsequent responses of plants to external stimuli (Moon *et al* 2003). The MAPK-signalling pathways involve three distinct components of the protein kinase family including (i) the MAPK, (ii) the MAPK kinase (MAPKK) and (iii) the MAPKK kinase (MAPKKK). During signal transduction, the MAPKKKs are phosphorylated and could then be able to activate particular MAPKKs, which become phosphorylated and then activate thereafter specific MAPKs. Activated MAPKs (phosphorylated MAPKs) are often imported into the nucleus, where they phosphorylate and activate specific

downstream signalling components such as transcription factors (Khokhlatchev *et al* 1998). A connection between the activation of plant MAPK cascades and the mediating action of ROS (H$_2$O$_2$) has been demonstrated using the *Arabidopsis* protoplast transient expression assay (Tena *et al* 2001). This interactive process underlines the pivotal role of ROS as the key activator of the MAPK cascade and the subsequent expression of the targeted genes. Kovtun *et al* (2000) showed that the ANP (*Nicotinama* protein kinase) class of MAPK kinase-kinases (MAPKKKs) from *Arabidopsis* is induced specifically by H$_2$O$_2$, which subsequently induced the activation of other set of specific classes of MAPKs under environmental stresses. Moon *et al* (2003) demonstrated that increased accumulation of ROS in transgenic plants activates several MAPKs, which regulates the state of cellular redox and enhances tolerance to multiple stresses. Their results demonstrated that NDP kinase (NDPK) is associated with hydrogen peroxide-mediated MAPK signalling in plants. In addition, H$_2$O$_2$ induces the expression of the NADPK2 gene in *A. thaliana* (AtNDPK2) and that of AtMAPK3 and AtMAPK6 (Moon *et al* 2003) and proteins from transgenic plants overexpressing AtNDPK2 showed high levels of autophosphorylation and NDPK activity than the-wild type plants (Moon *et al* 2003).

7. Nitric oxide in plants: another fascinating ROS molecule

ROS (H$_2$O$_2$, O$_2^-$ and nitric oxide) have been shown to serve as diffusible intra and intercellular signals for activation of various defence genes in animals, bacteria and plants (Durner *et al* 1998). Nitric oxide (NO) in particular has recently emerged as one of the pivotal ROS mediators of various physiological, biochemical and stress responses such as inflammation, acute phase responses, PCD and disease resistance in plants (Durner *et al* 1998; Zeidler *et al* 2004). The various activities of NO, including redox signalling have been explored principally in animals. Based on its molecular stability and reactivity, NO and its redox-activated derivates are regarded as the ROS that can fulfill the requirements of a true intra- and intercellular signalling molecule (Stamler 1994). A major advance in our understanding of the multiple functions of NO in plants has been the identification of enzymes that catalyze NO synthesis. Nitrate reductase (NR) was the first enzymatic source of NO to be identified in plants (Yamasaki and Sakihama 2000). In addition to its role in nitrate reduction, NR catalyzes the reduction of nitrite to NO using NAD(P)H as co-factor (Meyer *et al* 2004). The observation that NO produced by NR is required for abscisic acid (ABA)-induced stomatal closure in *A. thaliana* guard cells (Desikan *et al* 2002), suggests the involvement of NO in signalling processes leading to abiotic stress tolerance such as drought and osmotic stress through

the ABA-regulatory pathway. A similar result of drought and salt stress tolerance was also observed in *A. thaliana* after H_2O_2 treatment (Kotchoni *et al* 2006). Another enzymatic source of NO in plants, the *Nicotiana*-nitric oxide reductase (Ni-NOR), has been identified (Stöhr *et al* 2001). Ni–NOR, a 310 kDa plasma membrane-bound enzyme from tobacco has been shown to catalyze the reduction of nitrite to NO using cytochrome C as an electron donor (Stöhr *et al* 2001). Recently, a protein displaying NO synthase (NOS) activity has also been identified in *A. thaliana*. This protein, termed as 'AtNOS1' (Guo *et al* 2003), is unrelated to previously described mammalian NOS proteins, and belongs to a novel family of putative NOS which is conserved in eukaryotes and bacteria (Zemojtel *et al* 2004). These proteins contain a GTP-binding domain and possess a GTPase activity which is Ca^{2+}-CaM/NADPH-dependent, but FAD, FMN, BH_4, and heme-independent. The characterization of AtNOS1 and *Atnos*1 mutant mark an important step in elucidating the multiple functions of NO in plants (Guo *et al* 2003). In addition, the identification of *nox1*, an *A. thaliana* mutant overproducing NO (He *et al* 2004), paved a way for the functional analysis of NO. AtNOS1 was therefore confirmed as a source of NO in ABA-induced stomatal closure (Guo *et al* 2003), in the repression of flowering (He *et al* 2004), and in lipopolysaccharides-induced defence responses (Zeidler *et al* 2004). In animals, NO is known to act as a redox transmitter in the regulation of a diverse array of physiological processes, and NOS plays a central role in host responses to pathogen infection. Particularly two key enzymes involved in mammalian macrophage action, NADPH oxidase and NOS, have been found to play a crucial role in plant defence system during plant-microbe interactions (Durner *et al* 1998). The analysis of the phenotypic traits of *Atnos*1 mutants with an impaired NO synthesis indicated that AtNOS1 also plays a central role in plant development as *Atnos*1 plants showed reduced shoot, root, growth, and fertility compared to the wild type plants (Guo *et al* 2003). Several arguments suggest that AtNOS1 could also be involved in NO synthesis in the process of senescence of pea leaves (Lamotte *et al* 2005). In addition to AtNOS1, another NOS-like protein has been reported in plant peroxisomes (Corpas *et al* 2004). It has been observed that NO production occurs within the same time frame with that of H_2O_2, and a critical balance between the two ROS regulates cellular outcomes such as sensitivity or resistance to a given stress condition (Delledonne *et al* 2001), because H_2O_2 and NO have been shown to regulate in tandem the expression of a number of common genes whose products are involved in limiting pathogen growth, in cellular protection or in other signalling responses (Desikan *et al* 2001; Huang *et al* 2002). For example, a correlating accumulation of H_2O_2 or O_2^- and NO is often observed during the oxidative burst preceding the hypersensitive response (HR), cell death and

disease resistance (Grün *et al* 2006). The evidence that NO, in combination with other ROS, is required for cell death and induced disease resistance was given by Delledonne *et al* (1998). NO was found to react with O_2^- and/or H_2O_2 to the non-HR-inducing peroxynitrite ($ONOO^-$). The importance of the fine-tuning of NO/H_2O_2 balance was demonstrated using thylakoidal ascorbate peroxidases (tAPX) mutants with enhanced or reduced expression of tAPX gene (Murgia *et al* 2004; Tarantino *et al* 2005). Antisense reduction of tAPX in *A. thaliana* enhances paraquat-induced H_2O_2 accumulation and NO-induced cell death and disease resistance (Tarantino *et al* 2005), confirming therefore the correlation of H_2O_2 and NO acting in tandem to induce multiple signal transductions. Understanding the mechanisms by which NO-based signals are initiated, processed, and propagated in plant cells will require the elucidation of subcellular location of the enzymes implicated in NO synthesis and the identification of their co-factors. NO modulates their activities by nitrosylation or, in certain cell types including endothelial and neuronal cells, via indirect signalling pathways involving cyclic GMP and/or cyclic ADP ribose (cADPR) (Stamler *et al* 2001; Willmott *et al* 1996). In plants, NO treatment has indeed been shown to increase cGMP levels both in tobacco and *A. thaliana* (Durner *et al* 1998; Clarke *et al* 2000). NO primary targets in plant cells include Ca^{2+} channels and MAPK, because artificially generated NO stimulated MAPK in both tobacco and *A. thaliana* leaves (Clarke *et al* 2000; Kumar *et al* 2000; Capone *et al* 2004). How is NO integrated in these various plant physiological processes? Microarrays analyses of NO-responsive transcripts in *A. thaliana*, show that NO governs the regulation of expression of numerous genes via Ca^{2+} channels, cGMP, cADPR and phytohormones. These genes encode proteins related to defense responses, metabolism, cellular detoxification, transport, iron homeostasis, signalling, flowering, and lignin biosynthesis (Durner *et al* 1998; Polverari *et al* 2003; Parani *et al* 2004). NO also modulates the synthesis of SA, JA, and ethylene involved in wound responses (Huang *et al* 2004; Parani *et al* 2004).

A full understanding of NO signalling in plants also requires information about mechanisms that switch off NO signals and/or protect cells from excess of NO. For example, Hausladen *et al* (1998) reported that the bacterial flavohaemoglobin functions as a dioxygenase, metabolizing NO into nitrate, controlling therefore the excessive accumulation of NO. In plants, overexpression of class 1 non-symbiotic haemoglobin was shown to reduce NO levels and consequently protects alfalfa root cultures challenged by hypoxic conditions (Dordas *et al* 2003). Besides the haemoglobin, the involvement of S-nitrosoglutathione (GSNO) reductase (also named glutathione dependent formaldehyde dehydrogenase, or FALDH in plants) has also been reported (Sakamoto *et al* 2002; Diaz *et al* 2004). In humans, GSNO, which is one of the major metabolites of

NO formed primarily by nitrosylation of GSH, serves as a reservoir of NO and is believed to mediate some of its action. By converting GSNO to GSSG and NH_3, GSNO reductase controls intracellular levels of GSNO and limits NO toxicity. Like its mammalian counterpart, FALDH from *A. thaliana* displays GSNO-reductase activity *in vitro* (Sakamoto *et al* 2002), suggesting its involvement in the cellular protection against nitrosative stress. The NO donors GSNO and S-nitroso-N-acetyl-penicillamine (SNAP), as well as the membrane analog of the NO second messenger cGMP were found to induce phenylalanine ammonia lyase (PAL) expression in tobacco suspension cells. PAL is involved in the biosynthesis of SA (Durner *et al* 1998) confirming that NO indirectly mediate the synthesis of SA. Administration of NO donors or recombinant mammalian NOS to tobacco plants triggered expression of the defence-related gene encoding pathogenesis-related 1 (PR-1) protein. PR-1 exhibits antifungal activity and considered as an excellent marker of plant disease resistance against pathogens.

One of the major questions to answer is how the correct specific response is evoked and how ROS particularly NO and H_2O_2, which shared common secondary messengers are coordinated in the plants to induce multiple signal transductions. Based on overwhelming research contributions, NO has been shown to trigger the expression of a wide range of genes and transcription factors through cGMP/cADPR and S-nitosylation via direct regulation of ion channels and phytohormones such as ABA, gibbereline, SA and others (Stamler 1994). Several lines of evidence point to an interrelationship between NO, SA and wounding/JA-signalling pathway, because NO donors are found to affect both wounding-induced H_2O_2 synthesis and wounding- or JA-induced expression of defence genes (Grün *et al* 2006). The beneficial effects of this fascinating molecule, NO, are overwhelming and far from exhausted. Till now, our understanding of how NO participates in signalling function remains rudimentary. NO affects several aspects of the plant development, including seed germination, primary and lateral root growth, pollen tube growth regulation, fertility, circadian clock regulation, flowering, senescence, defence response and abiotic stress. Discussing these in detail will require a book chapter but because of the page limitation, we have chosen to focus on NO-mediating stress responses and signal transduction pathways of physiological importance in plants and selectively discussed here the molecular implication of NO in different metabolic pathways that fits the scope of this topic.

8. Role of peroxisomal ROS in plant signal transduction

Peroxisomes are subcellular organelles like chloroplasts and mitochondria, with a potential of generation and integration of ROS into signal transduction regulation the expression of various genes (del Río *et al* 2002). Plants peroxisomes have been demonstrated as source of superoxide radicals, and H_2O_2 production (Corpas *et al* 2001; del Río *et al* 2002). At least, two sites of superoxide generation have been identified and characterized in peroxisomal organelles: one in the organelle matrix; the generating system being xanthine oxidase, and the other site is located in the peroxisomal membrane and depends on NAD(P)H. In this second site, integrated peroxisomal membrane polypeptides (PMPs) are the generating sources of the ROS especially the superoxide radicals. Three PMPs with 18, 29 and 32 kDa have been characterized in peroxisomal membrane and proved to produced essential superoxide radicals necessary for the reducing power to transform NAD(P)H to $NAD(P)^+$ initiating therefore the peroxisomal metabolism in the matrix of the organelle, which includes various cascade reactions such as ascorbate-glutathione cycle, catalase-peroxidase cycle, generation of GSNO, conversion of xanthine into $ONOO^-$ and production of NO via peroxisomal NOS using L-arginine. Identification of NOS in plant peroxisomes (del Río *et al* 2002) confirms that these organelles, with their ability to produce superoxide radicals and H_2O_2 could function in plant cells as a source of signal molecules like NO and S-nitrisoglutathione (GSNO) to trigger various metabolic reactions including plants' response to external stimuli. Research on NO has gained considerable attention due to the function of NO in plant growth and response to the environment, and as a key-signalling molecule in different intracellular processes. For instance, the physiological function of NO in plants mainly involves the induction of different processes, including the expression of defence-related genes against pathogens and apoptosis/PCD, maturation and senescence, stomatal closure, seed germination, root development and the induction of ethylene emission (Durner *et al* 1998; Guo *et al* 2003, He *et al* 2004). Different experimental evidences suggested that peroxisomes function as subcellular source of ROS production and particularly NO required as signal molecules to mediate plant disease resistance, plant growth, and resistance to multiple environmental stresses (Corpas *et al* 2001; del Río *et al* 2004). In addition to peroxisomal organelles, research contributions indicate that there are other potential enzymatic sources of ROS particularly NO in plants, including cytosolic xanthine oxidoreductase, peroxidase, cytochrome P450, and some hemeproteins, but the important role of plant peroxisomes in a variety of metabolic reactions is here underlined. NO and H_2O_2 can permeate the peroxisomal membrane and superoxide radicals can be produced on the cytosolic side of the membrane. The generating signal molecules (NO, H_2O_2 and superoxide-radicals) of peroxisomes have important implications for cellular metabolism, particularly under abiotic and biotic stress.

9. Role of ROS as potential mediators in apoptosis and acquired disease resistance in plants

Eukaryotes such as plants, animals and yeast have evolved ways of cellular suicide that are known as PCD, a restricted zone of cell death to effectively contain pathogens at their site of entry, with the purpose of removing unwanted, damaged or infected cells. The term 'apoptosis' generally referred to a PCD observed in biological systems during their life cycle, and especially under environmental stress or pathogen attack (Kerr *et al* 1972). In other words, apoptosis/PCD is a general biological phenomenon of targeted self-cell suicide with the aim of overcoming the invading organism/system or maintaining homeostasis of the cellular metabolism at a normal biological status (Beers and McDowell 2001). The well-characterized model system for study of plant-PCD/apoptosis is the HR, which is often observed during plant-microbe interactions (Wyllie *et al* 1980; Beers and McDowell 2001). The cytological network events that mediate cell-death activation in several plant-PCD model systems include various phytohormones such as SA, JA, ABA, ethylene, the ROS (NO, H_2O_2, O_2^-), the lipid related signals (sphyncolipids) and the ion flux signal pathways (Guo *et al* 2003; Khurana *et al* 2005). With respect to the topic addressed in this review, we singularly focused our attention on the potential role of ROS to mediate apoptosis and subsequent disease resistance acquisition in plants. Khurana *et al* (2005) have elegantly discussed the molecular basis of apoptosis induction in various organisms including animals, yeast and plants. The involvement of ROS in the activation of apoptosis/PCD was demonstrated as a consequence of ROS accumulation detected during the early and late phases of plant-pathogen interaction (Durner *et al* 1998; Beers and McDowell 2001). Recent observations also support the role for ROS in cell-death signalling (Kachroo *et al* 2003). Recently, mutations of *A. thaliana* genes, *rbohD* and *rbohF*, that encode orthologues of the mammalian *GP91^phox* NADPH oxidase catalytic subunit, have revealed insights into the role of ROS in PCD formation (Torres *et al* 2002). Loss-of-function of *rbohD* and *rbohF* in *A. thaliana* mutants resulted in decreased ROS production during the HR, with a corresponding decrease in cell death (Torres *et al* 2002). These results provided the first direct evidence of ROS implication in cell-death response via plant NADPH oxidases. The role of ROS as potential mediator of apoptosis and subsequent acquired disease resistance has been well documented (Khurana *et al* 2005). As discussed above, ROS and NO generally interfere in PAL activity and ascorbate, and glutathione (GSH) metabolisms. Based on current research contributions, the simultaneous implication of induced PAL, GSH-metabolisms and APX mediates the induction of PCD (Khurana *et al* 2005). It has been proposed that the balance between intracellular NO and hydrogen peroxide (H_2O_2),

but not superoxide, concentrations is the key determinant for the HR cell-death response (Delledonne *et al* 2001). In addition, NO cooperates with SA to induce HR cell death and activate defence, which is analogous to its role in animal systems (Durner *et al* 1998). Increased NO production is sufficient to induce cell death in an *A. thaliana* cell culture (Clarke *et al* 2000). However, Khurana *et al* (2005) recently reported that O_2^- rather than H_2O_2 was identified as the primary signal molecule for pathogen inducing GST, and the rates of production and dismutation of O_2^- generated during the oxidative burst is crucial for the modulation and integration of NO/H_2O_2 signalling in HR inducing plant programmed, localized cell death at the site of infection limits pathogen spread (Levine *et al* 1994). H_2O_2 is also involved in signal transduction leading to SAR, a phenomena in which localized infection confers systemic resistance to subsequent attacks by the same or unrelated pathogens (Conrath *et al* 2002). The ROS detected in plant pathogen interactions are $\cdot O_2^-$ (O_2H), H_2O_2 and $\cdot OH$ (Scheel 2002). Plants also respond to aggression of other parasites such as insects whose feeding causes wounding by producing ROS in the damaged tissue (Kessler and Baldwin 2002). In addition to direct wounding by herbivory insects, insects' enzymes have also been linked to increased production of ROS in plant tissues (Felton and Eichenseer 1999). These ROS are believed to work together with NO to induce a hypersensitive response cell death as a defence reaction against avirulent pathogens (Delledonne *et al* 1998). Successful pathogens have to overcome or suppress the host defence mechanisms for example, the secretion of superoxide dismutase and catalase converting the ROS into less reactive molecules.

10. Current research trends in plants response to environmental stress

A major challenge in biology is the large-scale determination of gene function (Boyes *et al* 2001). Plants rarely experience stressful conditions caused by a single environmental constraint. Pathogen infection under field conditions is often accompanied by various additional abiotic stresses such as wound, water deficit, mineral stresses, and others (figure 1), causing multistress interactions. Databases and web-browser data mining interface for Affymetrix GeneChips are now available to scientists. As shown in figure 2 users can query the database to retrieve the expression patterns of individual genes throughout chosen environmental conditions, growth stages, or organs (Zimmermann *et al* 2004; Schmid *et al* 2005). Different genes specifically expressed during selected stresses, growth stages, or in particular organs can now be identified at any growing time point of the plant development. Thousands of arrays have since been processed, of which a significant number are publicly available through services and repositories such

as Nottingham Arabidopsis Stock Centre Transcriptomics Service (NASCArrays), ArrayExpress at the European Bioinformatics Institute (EBI), or Gene Expression Omnibus (GEO) at the National Center for Biotechnology Information (NCBI) (Edgar *et al* 2002; Brazma *et al* 2003; Craigon *et al* 2004). Public repositories such as GEO, ArrayExpress and GENEVESTIGATOR provide to scientists some potential storage tools and retrieval of heterogeneous data sets as well as coherent data from a single organism generated on a common hybridisation platform. Advantage of the designed bioinformatic tools is to retrieve biologically meaningful results from the highly diverse experiments represented in the database, which can be easily interpreted to generate new experimental hypotheses or more precise understanding of the behaviour of plants in their environment. Despite these major research contributions toward elucidating the molecular network pathways mediating environmental resistant mechanism in plants, we still have only a fragmented view of the relevant pathways. Some of the remaining questions include the identities of up and downstream receptors, substrates of various kinases, which loci interact directly or indirectly during ROS signal network and the identities of additional signalling elements linking the yet known and characterized elements to complete network pathway to mount the appropriate defence mechanism in plants. The interaction of pathogen infection with other stress factors must be investigated in field trials in order to really understand plant defence mechanism beyond the boundary of laboratory trials.

11. Conclusion

It is now clear that ROS is involved in physiological and biochemical mechanisms of disease resistance in plants. Plant responses to ROS are dose dependent. They interact both positively and negatively during plants responses to external challenges. High dosage of ROS results to hypersensitive reactions and oxidative damage in cells, whereas biologically balanced levels of ROS interact with other major signalling pathways including those regulated by ABA, JA, SA, ethylene, ion channels to trigger a complex metabolism leading to up regulation of stress inducible genes. *Arabidopsis thaliana* adopted as model plant (Meinke *et al* 1998) has broadened our understanding in the pivotal role of ROS to trigger the disease resistance in plants. Our current understanding of ROS signal transduction in plant responses to various pathogen attacks is still limited, but essential to successfully engineer plants with enhanced disease resistance. Complexity of plant responses to multiple stresses has shown a need to develop new research approaches to elucidate the overwhelming benefit of ROS in plant defence mechanisms. With the advance of biotechnology, ribosome-inactivating proteins (RIPs) can now be used as a

potential tool to engineer plants resistant to various stresses. RIPs are a group of RNA N-glycosidases with an extremely high site-specific deadenylation activity towards ribosomal RNA (Nielson and Boston 2001). Due to the removal of a single adenine by RIP, the ribosomes are no longer capable of binding the elongation factors EF-1 and EF-2, and therefore loose their elongation capacity. Based on this property, an overexpression of specific RIP genes can arrest the synthesis of targeted proteins that enhance the excessive accumulation of ROS, and thereby regulate ROS levels in plants during multiple environmental challenges. Desmyter *et al* (2003) have used this approach to demonstrate that the expression of the type-1 ribosome-inactivating protein from iris bulbs (IRIP) in transgenic tobacco increases the resistance of the plants against infection with TMV. Introduction of mechanisms that keep the concentration of ROS at normal redox status in response to pathogen attacks is a promising way to engineer transgenic plants that can resist pathogen infections and even other environmental stresses (Bartels 2001). The notion of ROS accumulation mediating the up-regulation of specific set of stress inducible genes is a positive aspect of plant defence systems, which needs to be evaluated in various agricultural important crops and the ROS network pathways may be adopted as a highly beneficial pre-requisite for disease resistance in plants.

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