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Review

The antioxidant systems vis-à-vis reactive oxygen species during plant–pathogen interaction

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Abstract

Plant resistance to pathogens requires the activation of complex metabolic pathways in the infected cells, aimed at recognizing pathogen presence and hindering its propagation within plant tissues. In spite of this both compatible and incompatible responses induce alterations in plant metabolism, only in the latter the plant is able to efficiently block pathogen penetration without suffering excessive damage. One of the most studied incompatible responses is based on the hypersensitive response (HR), in which cells surrounding the site of pathogen penetration switch on genes encoding for phytoalexin synthesis and other pathogenesis related proteins before activating programmed cell death (PCD). The production of reactive oxygen species (ROS) is a key event in HR. Several enzymatic systems have been proposed to be responsible for the oxidative burst characterizing HR. In this review, the involvement of antioxidant redox systems, in particular those related to ascorbate (ASC) and glutathione (GSH), in activating both compatible and incompatible plant responses is analysed. Increasing lines of evidence indicate that alterations in the levels and/or redox state of ASC and/or GSH, as well as in the activity of their redox enzymes, occur during the HR programme. These alterations do not seem to be a mere consequence of the oxidative stress induced by the massive ROS production, but they are induced as part of the transduction pathways triggering defence responses and PCD. The possibility that ASC and GSH systems are links in a redox signalling chain activating defence strategies is also discussed.

Keywords: Antioxidants; Ascorbate; Glutathione; Hypersensitive response; Plant–pathogen interaction; Reactive oxygen species; Redox regulation

1. Introduction

Plants have developed elaborate mechanisms to defend themselves against attack by pathogens such as bacteria, viruses, invertebrates and even other plants. Only a small number of pathogens are able to provoke disease in a certain species or cultivar (compatible response), whereas most potential aggressors are recognised and blocked in their penetration by plant defences (incompatible response). Plants possess physical barriers, such as the cuticle and cell wall and a number of biological and molecular mechanisms to counteract pathogen attacks. Some defences, such as secondary metabolites, are constitutive and located in specific cell compartments ready to be utilised against attack. A sophisticated sensory system enables plants to perceive chemical signals from potential pathogens and to translate them into appropriate biochemical responses. In most incompatible responses the rapid induction of highly localised events determines unfavourable conditions for pathogen growth. This defence response culminates in a localised cell death, called hypersensitive response (HR), designed to impair pathogen spread. In addition, systemic acquired resistance to subsequent attack by normally virulent pathogens develops throughout the rest of the plant. In the last decades the interest for improving the resistance of agronomic plants to phytopathogens has greatly stimulated research aimed at the identification of molecular signals produced in plant–pathogen interaction as well as the steps required for the activation of defence mechanisms. It has been reported that biotic and abiotic stresses often affect the same metabolic pathways. Ozone induces the formation of necrotic spots in the fumigated leaves which have the same characteristics of HR [80]. More-

Abbreviations: APX, ascorbate peroxidase; ASC, ascorbate; CAT, catalase; DHA, dehydroascorbate; GSH, glutathione; GSSG, glutathione disulfide; HR, hypersensitive response; NO, nitric oxide; PCD, programmed cell death; ROS, reactive oxygen species; SA, salicylic acid; SOD, superoxide dismutase; TMV, tobacco mosaic virus.

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over, an alteration in reactive oxygen species (ROS) scavenger enzymes occurs both under abiotic and biotic stress conditions [58].

2. ROS scavenging machinery in plant cells

In plant cells, enzymes and redox metabolites act in synergy to carry out ROS detoxification. SOD catalyses the dismutation of O_2^- to H_2O_2, catalase (CAT) dismutates H_2O_2 to oxygen and water, and ascorbate peroxidase (APX) reduces H_2O_2 to water by utilising ascorbate (ASC) as specific electron donor. These are considered the main enzymatic systems for protecting cells against oxidative damage. These enzymes are present with different isoenzymatic forms in several cell compartments and their expression is genetically controlled and regulated both by developmental and environmental stimuli, according to the necessity to remove ROS produced in cells [26,63,73]. The balance between SOD and APX or CAT activities in cells is crucial for determining the steady-state level of O_2^- and H_2O_2.

Redox metabolites, such as ASC and the tripeptide glutathione (GSH), also protect plant cells against ROS-induced damage, either by directly removing reactive chemical species or blocking the oxidative chain reactions triggered by ROS. Both ASC and GSH are present in all cell compartments that have been analysed.

Several studies on plant responses to abiotic stress conditions (ozone, heavy metals, light, UV radiation) suggest that the activity of ROS-detoxifying enzymes and the levels of their related molecules are tightly coordinated in order to maintain ROS under threshold values that are compatible with the metabolism of each cellular compartment [26,54,62,65]. A coordinated regulation of different antioxidant systems is further confirmed by genetically transformed plants in which the expression of ROS-scavenging enzymes has been altered or by cellular lines identified for their improved capability to synthesise a particular antioxidant. The higher resistance to oxidative stress exhibited by tobacco plants over-expressing Cu/Zn SOD requires a proportional rise in APX. The latter probably occurs as a homeostatic consequence of the increased H_2O_2 production in the transgenic plants [69]. The over-expression of GSH reductase in poplar plants induces a twofold increase in the foliar ASC availability, as a consequence of a higher efficiency in recycling dehydroascorbate (DHA) by means of GSH-dependent DHA reductase [37]. A sunflower cellular line selected for its capability to synthesise high levels of tocopherol is also characterised by increased contents of ASC and GSH [12].

A plethora of data indicate that under abiotic stress conditions, which induce an over-production of ROS into cells, plant resistance is due to the capability to increase the activity of ROS-detoxifying enzymes or the biosynthesis or regeneration of antioxidant metabolites [4,58,63,70]. Results reported in the literature indicate that alteration in the expression/activity of ROS-scavenging enzymes could also be a key step in the activation of phytopathogen defence.

3. Reactive oxygen species and hypersensitive response

The production of ROS is the first response detected within minutes of an attack by virulent or avirulent pathogen [1]. This weak and transient ROS generation is due to a biologically non-specific reaction. After some hours, a second, massive and prolonged ROS production, called oxidative burst, occurs in cells attacked by avirulent pathogens. This two-phase kinetics of ROS production is typical of incompatible plant–pathogen interactions that are characterized by HR [52]. The massive production of ROS occurring during HR is reminiscent of that following neutrophil activation in the mammalian immune system [3].

Although a number of ways to generate intracellular or extra-cellular ROS have been proposed [9,61], many pharmacological, immunological and molecular studies strongly support the idea that the primary ROS generating system in plant cells is a membrane-bound NAD(P)H oxidase analogous, but not identical, to that found in the mammalian phagocytes [10,25,47]. This is also supported by the recent identification of genes in *Arabidopsis* that are homologous to the large subunit of the human NADPH oxidase [10,49,74]. Moreover, Kawasaky et al. [48] report that, in rice, the small GTP-binding protein Rac, known to regulate mammalian NADPH oxidase, may mediate HR-dependent responses in a ROS-dependent manner, thus supporting the involvement of NAD(P)H oxidase as an activator of ROS generation in the oxidative burst preceding HR in plants. Analogously to the situations in mammalian phagocytes, plant NAD(P)H oxidase transfers reducing equivalents from cytosolic NAD(P)H to extra-cellular oxygen, generating superoxide. Apoplastic superoxide dismutase (SOD) isoenzymes are then responsible for H_2O_2 production by means of superoxide (O_2^-) dismutation. Evidence for different sources of ROS has also been provided, as a lipoxygenase acting on polyunsaturated fatty acids derived from membrane lipids [19]. Extra-cellular H_2O_2 could be directly produced by means of apoplastic enzymes such as copper amine oxidase, flavin polyamine oxidases and oxalate oxidase [9,66,72]. Some data suggest that secretory peroxidase is able to produce an oxidative burst under conditions generated by pathogen attack [7,10,39,42].

ROS can act directly against phytopathogen attack by killing the micro-organism. The co-presence of H_2O_2 and O_2^-, or the reaction of H_2O_2 with transition metals, induces the generation of the extremely reactive hydroxyl radical ($OH^\bullet$), the devastating effect on bio-molecules of which is well known. Moreover, H_2O_2 also hinders micro-organism penetration in plant tissues because it contributes to wall stiffening by facilitating peroxidase reactions catalysing intra– and inter-molecular cross-links between structural components of cell walls and lignin polymerisation [67]. The consequent increase in mechanical barriers slows down pathogen penetration allowing plant cells to arrange defences that require more time to be activated. As H_2O_2 is a diffusible

molecule in biological membranes, it also acts as intracellular signal, which is able to activate defence responses [31].

Apart from the oxidative burst, HR is characterised by other metabolic disturbances, such as ion flux across the plasma membrane (Ca^{2+} influx and K^+, Cl^- efflux) as well as changes in pH and plasma membrane depolarisation [45]. Moreover, it has been demonstrated that the cell death occurring during HR is a programmed cell death (PCD) and not a necrotic event. It requires ATP generation and consumption as well as ex novo gene expression and protein synthesis.

4. ROS-scavenging and plant defence against pathogens

4.1. Plant–pathogen interplay

It has been suggested that the oxidative burst occurring during HR produces such large amount of ROS that the cellular antioxidative defences are overwhelmed [52]. On the other hand, an increasing body of data supports the hypothesis that a fine regulation of antioxidant systems is part of the signalling pathways activating defence responses. However, the diversity in the systems used for studying plant–pathogen interplay makes it difficult to formulate a clear picture of whether, and to what extent, changes in antioxidant systems are directly involved in the activation of plant defence responses or are a mere consequence of the oxidative stress occurring in the attacked cells. The involvement of antioxidant systems in plant–pathogen interaction has been studied in plants infected by pathogens which are different (a) from a systematic point of view, such as fungi, bacteria or viruses; (b) in their attack strategies, whether biotrophic or necrotrophic, extra-cellular or intra-cellular micro-organisms; (c) in the virulence of their interaction with plants (Table 1).

The identification of common strategies involving antioxidant systems is further hindered by the fact that only some of the parameters among those working in the ROS-scavenging machinery are analysed in each study. Moreover, the possibility of species-specific responses further complicates the emerging picture.

Among the ubiquitous antioxidants present in plant cells, considerable attention has been given to GSH. An increasing amount of evidence suggests a central role for GSH in plant defence activation. Depletion of GSH or increase in its oxidised form, glutathione disulfide (GSSG), induces accumulation of phytoalexins [40,41,71]. On the other hand, an increase in GSH content has been reported in leaves attacked by avirulent biotrophic pathogens [33,35,75,76]. Since the whole infected leaf was used in the latter experiments, and not only the cells in which the HR was activated, it is difficult to establish whether the increase in GSH is part of the transduction pathway activating HR or the defence responses occurring in the neighbouring cells and aimed at limiting oxidative damage in restrained zones. It is worth noting that an increase in the expression of glutathione *S*–transferase and glutathione peroxidase has been identified in the soybean cells adjacent to those undergoing the hypersensitive cell death induced by an avirulent phytopathogen [53]. Hydrogen peroxide accumulation and GSH oxidation occur in different sites and with different timing in leaves of a resistant barley line attacked by powdery mildew [77]. A decrease in GSH content was observed in tomato leaves infected with the necrotrophic *Botrytis cinerea* [51] as well as in *Avena sativa* leaves inoculated with another virulent necrotrophic fungus [38]. In these cases, the decrease in antioxidant defences could promote the spread of necrotic areas that facilitate the penetration of necrotrophic phytopathogens. In some cases, the GSH increase is followed by its oxidation. In cotyledons of tomato carrying Avr-genes and injected with race-specific elicitors of *Cladosporium fulvum*, almost 90% of GSH pool are present in the oxidised form [55]. GSH seems to act at the transcription level [79]. GSH-responsive elements has been identified on promoters of phenylalanine ammonia-lyase and chalcone synthase [28].

The fact that GSH acts in synergy with other signals in the activation of defence strategies has been underlined by the investigation on phytopathogen-induced diseases in *Arabidopsis* mutants having GSH levels 70% lower than the wild-type parental ecotype. The infection of the *Arabidopsis* mutant with either virulent or avirulent fungal and bacterial pathogens gives the same responses with wild-type [56]. An explanation of these results could be a GSH compensation with other antioxidant molecules and enzymes. Indeed, the ASC levels are higher in the mutant than in the wild-type [56]. Moreover, in animal systems with depleted GSH, an increase in ASC guarantees resistance against oxidative dis-

Table 1
Plant–pathogen interactions studied for the involvement of antioxidant systems

Host	Plant pathogen	Fungus	Bacterium	Virus	References
Avena sativa	*Blumeria graminis* (biotroph)	+			[76]
A. sativa	*Drechslera* spp (necrotroph)	+			[38]
Arabidopsis thaliana	*Peronospora parasitica* (biotroph)	+			[56]
A. thaliana	*Pseudomonas syringae*		+		[56]
Hordeum vulgare	*Botrytis graminis* (biotroph)	+			[11,33,75,77]
Lactuca sativa	*P. syringae* pv *phaseolicola*		+		[6]
Lycopersicon esculentum	*B. cinerea* (necrotroph)	+			[51]
Nicotiana tabacum	TMV (*tobacco mosaic virus*)			+	[35,59,60,61]
Phaseolus vulgaris	*P. syringae* pv *phaseolicola*		+		[19]
Prunus armeniaca	PPV (*plum pox virus*)			+	[46]

eases. Compensation between GSH and thioredoxin–glutaredoxin systems has also been reported in *Escherichia coli* mutants [57].

The role of the ASC system in regulating ROS level during plant–pathogen interaction has only been sporadically examined. It has recently been suggested that a suppression of APX is required in cells undergoing HR [6,59]. CAT activity has also been reported to decrease in cells undergoing HR. However, the suppression mechanisms of these two H_2O_2–scavenging enzymes are different. CAT is down-regulated at the transcription level [27], whereas, APX regulation in HR involves both transcription and translation (or post-translation) processes. In tobacco leaves, inoculated with tobacco mosaic virus (TMV), a rise in APX mRNA occurs [61], probably as an antioxidant response triggered by the increasing presence of H_2O_2 within cells and similar to that activated under abiotic stress [50]. In spite of the increase in its expression, the activity of the enzyme is strongly suppressed in the TMV-infected cells by a mechanism, still not well characterised, that acts at the transcriptional or post-transcriptional level [59]. The transcriptional/post-transcriptional regulation of APX is unique to HR-related incompatible response and reflects the necessity of accumulating ROS during this defence process. Interestingly, it has been reported that in a compatible response between barley and powdery mildew the cytosolic isoenzyme of APX is up-regulated in both epidermal and mesophyll cells. In these cells, that are not able to trigger a response able to stop pathogens, the APX increase limits the propagation of oxidative processes allowing cells to maintain their viability, a condition required for the penetration of biotrophic powdery mildew in plant tissues [11]. This up-regulation of APX confirms previous results reporting an increase in APX activity during successful infection of barley leaves by biotrophic compatible pathogens [33,51,75] and has also been reported to occur in leaves of susceptible apricot infected by plum pox virus [46].

As far as the alterations during plant–pathogen interaction of the other ASC redox enzymes and ASC/DHA levels are concerned, the available data are still very fragmentary. However, an increase in ASC free radical reductase, the enzyme responsible for the reduction of the first product of ASC oxidation, seems to occur in the compatible responses, thus mimicking the behaviour of APX [11,46]. On the other hand, DHA reductase, the enzyme that reduces DHA to ASC using GSH as reductant, and GSSG reductase, the enzyme responsible for GSH recycling, seem not to show behaviour clearly indicative of resistance or susceptibility [11,46, 51,75]. A decrease in ASC content has been reported to occur in leaves of barley cultivars inoculated with powdery mildew [33]. The decrease in ASC is only transient in the resistant cultivar, since it returns to the values of non-infected plants after HR induction, whereas it is much more evident and irreversible in the susceptible cultivars [33]. However, other results do not agree with the pattern proposed above [35,75]. This is probably due to the fact that the various factors that regulate ASC level and redox state (biosynthesis, oxidation pathways and recycling enzymes) can be differently affected in plant–pathogen interactions, both due to different plant species-specific sensitivity and because, in different plant–pathogen interactions, the suppression or strengthening of ROS detoxification could be obtained in different ways.

4.2. Use of elicitors in studies on the activation of plant defence responses

The complexity of HR and related responses as well as the short time with which they are deployed hinder the identification of the individual pathways, the activation of which is required for successful plant defence. An alternative approach to the analysis of the alterations induced by pathogens is the treatment of plant tissues with molecules that are generated in the interplay between plant and phytopathogens and act as signal molecules. The use of cell cultures has also been of great help for the identification of the signalling pathways and timing of the events activated during plant defence responses. This is because they are uniform systems in which identical cells are equally exposed to a certain stimulus and, thus, simultaneously activate their response. A pioneering study demonstrated that application of exogenous salicylic acid (SA) or its derivatives induces synthesis of pathogenesis related proteins and partial resistance to pathogens [78]. SA is accumulated at high concentrations in the immediate vicinity of incompatible infection sites and is considered a key endogenous regulator of defence responses, being involved both in localised defences and systemically acquired resistance [15]. Despite its mode of action remaining a much debated question, a soluble protein that specifically binds SA has been identified and characterised as a CAT [16,17]. On the basis of these results, it has been proposed that, among other actions, SA elevates the cellular level of H_2O_2 by limiting its CAT-dependent removal. In vitro studies indicate that SA inhibits CAT activity by chelating the heme iron, without specifically binding the enzyme [68]. SA has also been shown to act as a one-electron-donating substrate that shifts the catalytic activity of CAT from dismutation to peroxidation, thereby trapping the enzyme in a less active state and determining a slowing down of H_2O_2 removal [30]. Several proteins containing heme iron in their catalytic site are inhibited in vitro by SA, including APX, another key H_2O_2 scavenger [29,31]. The occurrence of direct in vivo action of SA on these ROS-scavenging enzymes has been questioned by the fact that different CAT isoenzymes have marked differences in SA sensitivity and that in vivo SA has been reported to not significantly affect APX expression at least in some plant systems [14,59]. Moreover, H_2O_2 is produced independently of SA under plant–pathogen interaction and seems to act upstream of SA in the induction of pathogen-related protein expression [8]. Finally, as reported above, down-regulation of CAT expression and post-transcriptional suppression of APX seem to be required events in the pathogen-induced HR. All together, this evidence overshadows the role of SA in increasing ROS

levels through blocking the activity of ROS-scavenging enzymes.

Treatments of leaf tissues with oligogalacturonides, well known elicitors produced during the degradation of plant cell wall pectins by phytopathogen fungi, also induce an increase in H_2O_2 [5]. However, at present it is not known whether these elicitors alter ROS levels by affecting ROS-production or ROS-scavenging machineries or both. Exopolysaccharides purified from phytopathogenic bacteria determined an impairment in the ASC-dependent ROS scavenging system; however, their effects were not strong enough for HR activation [22].

In recent years increasing attention has been paid to nitric oxide (NO) as a signal molecule acting in plant–pathogen interaction [18,23,24,32]. Recently the production of NO in plant cells has been found to occur as a consequence of phytopathogen attack [36]. Results obtained with cultured tobacco cells, in which hypersensitive PCD is induced by simultaneous treatments with NO and H_2O_2 generators, indicate that suppression of APX and decreases in the ascorbate (ASC + DHA) and glutathione (GSH + GSSG) pools are key events in PCD [21]. Moreover, during the HR process, redox balances of ASC and GSH are strongly shifted towards the oxidised forms. These changes in the cellular antioxidant systems are not a mere consequence of the presence of NO and H_2O_2, because neither NO nor H_2O_2 alone induces variations similar to those observed when they are generated together in the cell cultures [21]. Furthermore, these alterations are not a consequence of oxidative processes due to an undefined reactive chemical species generated in the interaction between NO and H_2O_2. Indeed, when PCD is blocked by treatment with protein synthesis inhibitors in the cells in which HR has been triggered by simultaneous generation of NO and H_2O_2, the suppression of APX and the decrease in ASC and GSH pools are also reverted [21]. This supports the hypothesis that changes in the cellular redox balance are not a simple consequence of disease conditions but part of the transduction signalling pathway that triggers defence responses under the opportune stimuli. It has been recently reported that, similar to the GSH/GSSG pair, the level and redox state of ASC play a regulatory role in cell metabolism, both acting at the level of gene expression and altering enzymatic pathways [13,20,34,64]. Even if, at present, no data supporting this hypothesis are yet available, the imbalance in the ASC redox pair produced during HR programme could also supply an additional signal that contributes to triggering the required metabolic alterations.

The involvement of changes in the level or redox balance of specific metabolites in inducing hypersensitive PCD has also been suggested by the release of cytochrome *c* from mitochondria. This event occurs in a very precocious phase of PCD induced by a variety of unrelated stimuli both in animal and plant cells. After its release, cytochrome *c* leads to the activation of caspases, key executioner proteases involved in the onset of PCD [2,54]. It has been reported that, in the cytosol, cytochrome *c* is maintained in the reduced form by GSH or ASC. In conditions of depletion or shift to the oxidised form of these redox molecules, both of which have been reported to occur in cells undergoing HR, the oxidised form of cytochrome *c* increases within cells. Since cytochrome *c* reductase or antioxidant treatments block the cytochrome *c*–dependent PCD processes, it has been hypothesized that the conformational changes, due to cytochrome *c* oxidation, are responsible for caspase activation [44]. Moreover, cytochrome *c* release in the cytoplasm could be an additional cause of the ASC and GSH oxidation during HR.

5. New insights from transgenic plants

Control of plant pathogens by genetic engineering has long been one of the first goals of biotechnology. Several plants, in which resistance to a given pathogen has been induced by genetic transformation, are currently available. Genetic transformation has also been used as a tool for studying steps of the transduction pathway triggering HR, as in the case of *Arabidopsis* or tobacco plants transformed with the bacterial gene encoding for a salicylate hydroxylase [43]. These plants, unable to accumulate SA, are much more sensitive to pathogens. The studies on the effects of pathogens or elicitors in these plants, also supplemented with the use of mutants, has allowed researchers to identify the steps of the HR programme that are SA-dependent [31].

As far as the antioxidant systems is concerned, transgenic antisense tobacco plants with reduced capability to scavenge H_2O_2 have also been obtained. These plants, in which CAT and APX are under-expressed, are hyperresponsive to pathogen attack [60]. This further confirms that the ability of plant cells to regulate the efficiency in their ROS-removal strategies is a key point in their resistance against pathogens.

6. Concluding remarks

The increasing knowledge of plant defence mechanisms against pathogens is casting light on a process that appears to be more and more complex. In this scenario the antioxidant redox systems seem to have an increasing importance. Differently from what happens during abiotic stress in which ROS-scavenging systems are enhanced to restore ROS at cellular steady-state levels, the success of the HR incompatible response seems to be dependent on the suppression of ROS-scavenging systems and, probably, on the unbalance in the ASC–GSH redox state (Fig. 1). Indeed, these events seem to be an integral part of the incompatible response against biotrophic phytopathogens that allow plant cells to counteract their penetration by surrounding them with an extremely oxidative environment. Moreover, ASC and GSH could per se act as redox sensors able to activate genetic defence responses. However, considering the many interactions between the signal molecules produced in the plant–pathogen interplay, and the possibility that differences in the metabolic alterations could be a consequence of species-specific behaviour rather than the activation of a common defence response,

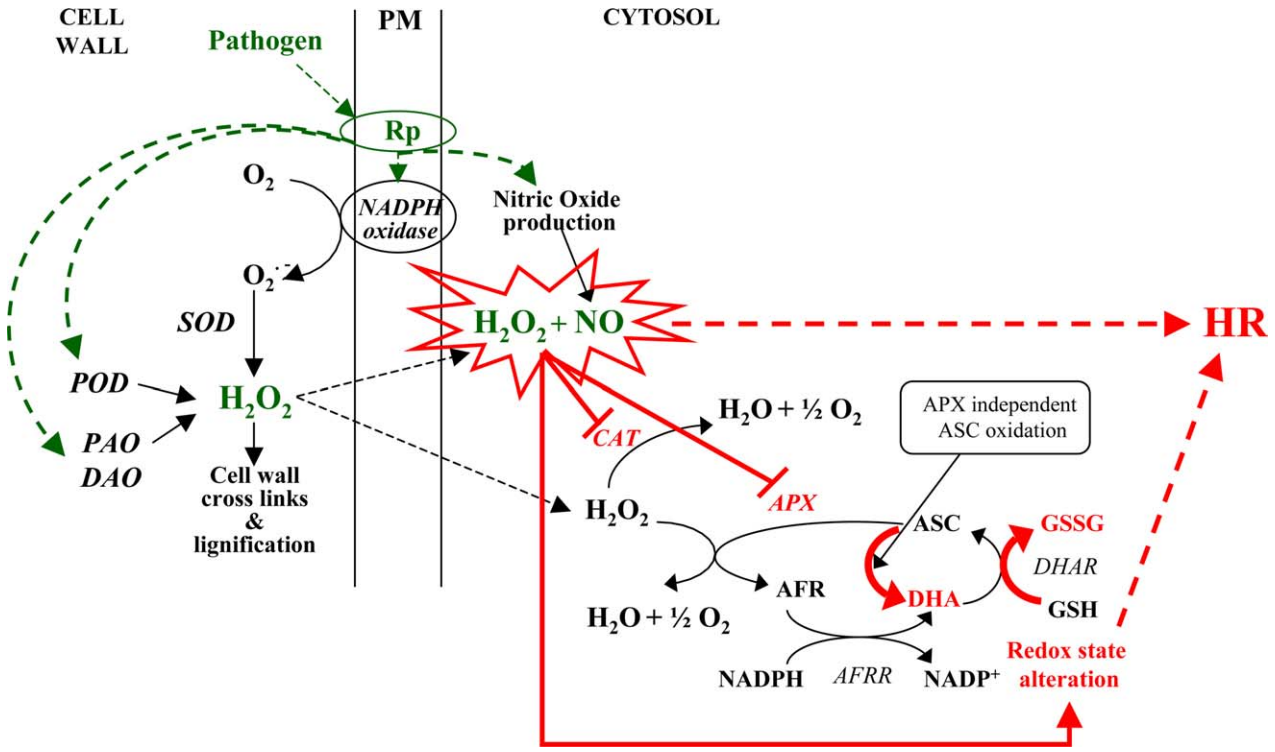

Fig. 1. Changes in the antioxidant system induced during HR. An oxidative burst producing reactive oxygen and/or nitrogen species is a precocious response activated by plant cells against pathogens. These reactive species, in spite of hindering pathogen penetration, also act as signal molecules for the activation of the defence responses leading to HR. The suppression of ROS scavenging enzymes (APX and CAT) contributes to the oxidative burst with ROS producing enzymes activated by pathogen attack. The imbalance of ASC/DHA, GSH/GSSG redox pairs is also part of the transduction pathway triggering HR. APX, ascorbate peroxidase; ASC, ascorbate; AFR, ascorbate free radical; AFRR, ascorbate free radical reductase; CAT, catalase; DAO, diamine oxidase; DHA, dehydroascorbic acid; DHAR, dehydroascorbic acid reductase; GSH glutathione; GSSG, glutathione disulfide; HR, hypersensitive response; NO, nitric oxide; PAO, polyamine oxidase; PM, plasma membrane; POD, secretory peroxidase; Rp, receptor proteins; SOD, superoxide dismutase.

more studies are required in order to clarify how and to what extent the alterations of the antioxidant redox systems represent a common strategy of plant defence against pathogens. The identification of the signals and the transduction pathways that enable plant to increase or decrease antioxidant systems, according to the presence of a virulent or avirulent phytopathogens, is also of great relevance for the possible applications in plant engineering programmes that are focused to increase phytopathogen resistance.

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References

[1] A. Adam, T. Farkas, G. Somlayai, M. Hevesi, Z. Kiraly, Consequence of O_2–generation during bacterially induced hypersensitive reaction in tobacco: deterioration of membrane lipids, Physiol. Mol. Plant Pathol. 34 (1989) 13–26.

[2] E.S. Alnemri, Hidden powers of mitochondria: a primary site for BCL-2 regulation of apoptosis, Nat. Cell Biol. 1 (1999) 40–42.

[3] I. Apostol, P.F. Heinstein, P.S. Low, Rapid stimulation of an oxidative burst during elicitation of cultured plant cells. Role in defence and signal transduction, Plant Physiol 90 (1989) 109–116.

[4] K. Asada, The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons, Annu. Rev. Plant Physiol. Plant Mol. Biol. 50 (1999) 601–639.

[5] D. Bellincampi, N. Dipierro, G. Salvi, F. Cervone, G. De Lorenzo, Extracellular H_2O_2 induced by oligalacturonides is not involved in the inhibition of the auxin-regulated rolB gene expression in tobacco leaf explants, Plant Physiol 122 (2000) 1–7.

[6] C. Bestwick, A.L. Adam, N. Puri, J.W. Mansfield, Characterization and changes to pro– and anti-oxidant enzyme activities during the hypersensitive reaction in lettuce (*Lactuca sativa* L.), Plant Sci. 161 (2001) 497–506.

[7] C.S. Bestwick, I.R. Brown, J.W. Mansfield, Localized changes in peroxidase activity accompany hydrogen peroxide generation during the development of a non-host hypersensitive reaction in lettuce, Plant Physiol 118 (1998) 1067–1078.

[8] Y.M. Bi, P. Kenton, L. Mur, R. Darby, J. Draper, Hydrogen peroxide does not function downstream of salicylic acid in the induction of PR protein expression, Plant J 8 (1995) 235–245.

[9] G.P. Bolwell, P. Wojtaszek, Mechanisms for the generation of reactive oxygen species in plant defence: a broad perspective, Physiol. Mol. Plant Pathol. 51 (1997) 347–366.

[10] G.P. Bollwell, L.V. Bindschedler, K.A. Blee, V.S. Butt, D.R. Davies, S.L. Gardner, C. Gerrish, F. Minibayeva, The apoplastic oxidative burst in response to biotic stress in plants: a three-component system, J. Exp. Bot. 53 (2002) 1367–1376.

[11] K. Burhenne, L. Gregersen, Up-regulation of the ascorbate-dependent antioxidative system in barley leaves during powdery mildew infection, Mol. Plant Pathol. 1 (2001) 303–314.

[12] S. Caretto, A. Paradiso, L. D'Amico, L. De Gara, Ascorbate and glutathione metabolism in two sunflower cell lines of differing α-tocopherol biosynthetic capability, Plant Physiol. Biochem. 40 (2002) 509–513.

[13] M.V. Catani, A. Rossi, S. Sabatini, M. Levriero, G. Melino, L. Avigliano, Induction of gene expression via activator protein-1 in the ascorbate protection against UV-induced damage, Biochem. J. 356 (2001) 77–85.

[14] Z. Chen, S. Iyer, A. Caplan, D.F. Klessig, B. Fan, Differential accumulation of salicylic acid and salicylic acid-sensitive catalase in different rice tissues, Plant Physiol 114 (1997) 193–201.

[15] Z. Chen, J. Malamy, J. Henning, U. Conrath, P. Sanchez-Casas, H. Silva, J. Ricigliano, D.F. Klessig, Induction, modification and transduction of the salicylic acid signal in plant defence responses, Proc. Natl. Acad. Sci. USA 92 (1995) 4134–4137.

[16] Z. Chen, J. Ricigliano, D.F. Klessig, Purification and characterization of a soluble salicylic acid-binding protein from tobacco, Proc. Natl. Acad. Sci. USA 90 (1993) 9533–9537.

[17] Z. Chen, H. Silva, D.F. Klessig, Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid, Science 262 (1993) 1883–1886.

[18] D. Clark, J. Durner, D.A. Navarre, D.F. Klessig, Nitric oxide inhibition of tobacco catalase and ascorbate peroxidase, Mol. Plant Microbe Interact 13 (2000) 1380–1384.

[19] K.P.C. Croft, C.R. Voisey, A.J. Slusarenko, Mechanisms of hypersensitive cell collapse: correlation of increased lipoxygenase activity with membrane damage in leaves of *Phaseolus vulgaris* L. inoculated with an avirulent race of *Pseudomonas syringae* pv *phaseolicola*, Physiol. Mol. Plant Pathol. 36 (1990) 49–62.

[20] M.C. de Pinto, D. Francis, L. De Gara, The redox state of the ascorbate–dehydroascorbate pair as a specific sensor of cell division in tobacco BY-2 cells, Protoplasma 209 (1999) 90–97.

[21] M.C. de Pinto, F. Tommasi, L. De Gara, Changes in the antioxidant systems as part of the signalling pathway responsible for the programmed cell death activated by nitric oxide and reactive oxygen species in tobacco bright yellow 2 cells, Plant Physiol 130 (2002) 698–708.

[22] M.C. de Pinto, P. Lavermicocca, A. Evidente, M.M. Corsaro, S. Lazzaroni, L. De Gara, Exopolysaccharides produced by plant pathogenic bacteria affect ascorbate metabolism in *Nicotiana tabacum*, Plant Cell Physiol (2003) (in press).

[23] M. Delledonne, Y. Xia, R. Dixon, C. Lamb, Nitric oxide functions as a signal in plant disease resistance, Nature 394 (1998) 585–588.

[24] M. Delledonne, J. Zeier, A. Marocco, C. Lamb, Signal interaction between nitric oxide and reactive oxygen intermediates in plant hypersensitive disease resistance response, Proc. Natl. Acad. Sci. USA 98 (2001) 13454–13459.

[25] R. Desikan, A. Reynolds, J. Hancock, S. Neill, Harpin and hydrogen peroxide both initiate cell death but have differential effects on defence gene expression in *Arabidopsis* suspension cultures, Biochem. J. 330 (1998) 115–120.

[26] R. Di Cagno, L. Guidi, L. De Gara, G.F. Soldatini, Combined cadmium and ozone treatments affects photosynthesis and ascorbate-dependent defences in sunflower, New Phytol 151 (2001) 627–636.

[27] S. Dorey, F. Bailleul, P. Saindrenan, B. Fritig, S. Kauffmann, Tobacco class I and II catalases are differentially expressed during elicitor-induced hypersensitive cell death and localized acquired resistance, Mol. Plant Microb. Int. 11 (1998) 1102–1109.

[28] M. Dron, S.D. Clouse, R.A. Dixon, M.A. Lawton, C.J. Lamb, Glutathione and fungal elicitor regulation of a plant defence promoter in electroporated protoplasts, Proc. Natl. Acad. Sci. USA 85 (1988) 6738–6742.

[29] J. Durner, D.F. Klessig, Inhibition of ascorbate peroxidase by salicylic acid and 2,6–dichloroisonicotinic acid, two inducers of plant defense responses, Proc. Natl. Acad. Sci. USA 92 (1995) 11312–11316.

[30] J. Durner, D.F. Klessig, Salicylic acid is a modulator of tobacco and mammalian catalases, J. Biol. Chem. 271 (1996) 28492–28501.

[31] J. Durner, J. Shah, D.F. Klessig, Salicylic acid and disease in plants, Trends Plant Sci. 2 (1997) 266–274.

[32] J. Durner, D. Wendehenne, D.F. Klessig, Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose, Proc. Natl. Acad. Sci. 95 (1998) 10328–10333.

[33] H.M. El-Zahaby, G. Gullner, Z. Kiraly, Effects of powdery mildew infection of barley on the ascorbate–glutathione cycle and other antioxidant in different host–pathogen interactions, Phytopathology 85 (1995) 1225–1230.

[34] M. Fiorani, R. De Santis, F. Scarlatti, L. Vallorani, R. De Bellis, G. Serafini, M. Bianchi, V. Stocchi, Dehydroascorbic acid irreversibly inhibits hexokinase activity, Mol. Cell Biochem 209 (2000) 145–153.

[35] J. Fodor, G. Gullner, A.L. Adam, B. Barna, T. Komives, Z. Kiraly, Local and systemic responses of antioxidant to tobacco mosaic virus infection and to salicylic acid in tobacco, Plant Physiol 114 (1997) 1443–1451.

[36] I. Foissner, D. Wendehenne, C. Langerbartels, J. Durner, In vivo imaging of an elicitor-induced nitric oxide burst in tobacco, Plant J 23 (2001) 817–824.

[37] C.H. Foyer, N. Souriau, S. Perret, M. Lelandais, K.J. Kunert, C. Pruvost, L. Jouanin, Overexpression of glutathione reductase but not glutathione synthetase leads to increases in antioxidant capacity and resistance to photoinhibition in poplar trees, Plant Physiol 109 (1995) 1047–1057.

[38] M.V. Gonnen, E. Schlösser, Oxidative stress in interaction between *Avena sativa* L. and *Drechlera* spp, Physiol. Mol. Plant Pathol 42 (1993) 221–234.

[39] G.C. Gross, C. Janse, E.F. Elstner, Involvement of malate, monophenols and the superoxide radical in hydrogen peroxide formation by isolated cell wall from horseradish (*Armoraria lapathipholia* Gilib.), Planta 136 (1977) 271–276.

[40] Z.J. Guo, S. Nakagavara, K. Sumitani, Y. Ohta, Effect of intracellular glutathione level on the production of 6-methoxy-mellein in cultured carrot (*Daucus carota*) cells, Plant Physiol 102 (1993) 45–51.

[41] D.L. Gustine, Induction of medicarpin biosynthesis in landino clover by *p*–chloromercuribenzoic acid is reverted by dithiotreitol, Plant Physiol 84 (1987) 3–6.

[42] B. Halliwell, Lignin synthesis: the generation of hydrogen peroxide and superoxide by horseradish peroxidase and its stimulation by manganese (II) and phenols, Planta 140 (1978) 81–88.

[43] K.E. Hammond-Kosack, J.D.G. Jones, Resistance gene-dependent plant defense responses, Plant Cell 8 (1996) 1773–1791.

[44] J.T. Hancock, R. Desikan, S.J. Neil, Does the redox status of cytochrome *c* act as a fail-safe mechanism in the regulation of programmed cell death?, Free Radic. Biol. Med. 31 (2001) 697–703.

[45] M. Heath, Hypersensitive response-related death, Plant Mol. Biol. 44 (2000) 321–334.

[46] J.A. Hernández, J.M. Talavera, P. Martinez-Gomez, F. Dicenta, F. Sevilla, Response of antioxidative enzymes to plum pox virus in two apricot cultivars, Physiol. Plant 111 (2001) 313–321.

[47] V.J. Higgins, H. Lu, T. Xing, A. Gellie, E. Blumwald, The gene-for-gene concept and beyond: interactions and signals, Can. J. Plant Pathol 20 (1998) 150–157.

[48] T. Kawasaky, K. Henmi, E. Ono, S. Hatakeyama, M. Iwano, H. Satoh, K. Shoimamoto, The small GTP-binding protein Rac is a regulator of cell death in plants, Proc. Natl. Acad. Sci. USA 96 (1999) 10922–10926.

[49] T. Keller, H.G. Damude, D. Werner, P. Doerner, R.A. Dixon, C. Lamb, A plant homology of the neutrophil NADPH oxidase gp91^{phox} subunit gene encodes a plasma membrane protein with Ca^{2+} binding motifs, Plant Cell 10 (1998) 255–266.

[50] A. Kubo, H. Saji, K. Tanaka, N. Kondo, Expression of arabidopsis cytosolic ascorbate peroxidase gene in response to ozone of sulfur dioxide, Plant Mol. Biol. 29 (1995) 479–489.

[51] E. Kuzniak, M. Sklodowska, The effect of *Botrytis cinerea* infection on ascorbate glutathione cycle in tomato leaves, Plant Science 148 (1999) 69–76.

[52] C. Lamb, R.A. Dixon, The oxidative burst in plant disease resistance, Annu. Rev. Plant Physiol. Mol. Biol. 48 (1997) 251–275.

[53] A. Levine, R. Tenhaken, R. Dixon, C. Lamb, H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response, Cell 79 (1994) 583–593.

[54] P. Marchetti, M. Castedo, S.A. Susin, N. Zampami, T. Hirsch, A. Macho, A. Haeffner, F. Hirsch, M. Geusken, G. Kroemer, Mitochondrial permeability transition is a central coordinating event in apoptosis, J. Exp. Med. 184 (1996) 1155–1160.

[55] M.J. May, K.E. Hammond-Kosack, J.D.G. Jones, Involvement of reactive oxygen species, glutathione metabolism, and lipid peroxidation in the Cf-gene-dependent defence response of tomato cotyledons induced by race-specific elicitors of *Cladosporium fulvum*, *Plant Physiol* 110 (1996) 1367–1379.

[56] M.J. May, J.E. Parker, M.J. Daniels, C.J. Leaver, S. Christopher, An *Arabidopsis* mutant depleted in glutathione shows unaltered responses to fungal and bacterial pathogens, Mol. Plant Microb. Int. 9 (1996) 349–356.

[57] A. Miranda-Vizute, E. Martinez-Glisteo, F. Aslund, J. Lopez-Barea, C. Pueyo, A. Holmgren, Null thioredoxin and glutaredoxin *Escherichia coli* K–12 mutants have no enhanced sensitivity to mutagens due to a new GSH-dependent hydrogen donor and high increases in ribonucleotide reductase activity, J. Biol. Chem. 269 (1994) 16631–16637.

[58] R. Mittler,, Oxidative stress, antioxidants and stress tolerance, Trends Plant Sci. 7 (2002) 405–410.

[59] R. Mittler, X. Feng, M. Cohen, Post-transcriptional suppression of cytosolic ascorbate peroxidase expression during pathogen-induced programmed cell death in tobacco, Plant Cell 10 (1998) 461–473.

[60] R. Mittler, E.H. Herr, B.L. Orvar, W. Van Camp, H. Willekens, D. Inzé, B.E. Ellis, Transgenic tobacco plants with reduced capability to detoxify reactive oxygen species intermediates are hyperresponsive to pathogen infection, Proc. Natl. Acad. Sci. USA 96 (1999) 14165–14170.

[61] R. Mittler, E. Lam, V. Shulaev, M. Cohen, Signal controlling the expression of cytosolic ascorbate peroxidase during pathogen-induced programmed cell death in tobacco, Plant Mol. Biol. 39 (1999) 1025–1035.

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

[63] G. Noctor, C.H. Foyer, Ascorbate and glutathione: keeping active oxygen under control, Annu. Rev. Plant Physiol. Plant Mol. Biol. 49 (1998) 249–279.

[64] G. Pastori, G. Kiddle, J. Antoniw, S. Bernard, S. Veljovic-Jovanovic, P.J. Terrier, G. Noctor, C.H. Foyer, Leaf vitamin C contents modulate plant defense transcripts and regulate genes controlling development through hormone signaling, Plant Cell 15 (2003) 939–951.

[65] M.V. Rao, C. Paliyath, D.P. Ormrod, Ultraviolet-B– and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*, Plant Physiol 110 (1996) 125–136.

[66] G. Rea, M. Laurenzi, E. Tranquilli, R. D'Ovidio, R. Federico, R. Angelini, Developmentally and wound-regulated expression of the gene encoding a cell wall copper amine oxidase in chickpea seedlings, FEBS Lett 437 (1998) 177–182.

[67] A. Ros Barceló, Lignification in plant cell walls, Int. Rev. Citol. 176 (1997) 87–132.

[68] M. Rüffer, B. Steipe, M.H. Zenk, Evidence against specific binding of salicylic acid to plant catalase, FEBS Lett 377 (1995) 175–180.

[69] A. Sen Gupta, R.B. Webb, A.S. Holaday, R.D. Allen, Overexpression of superoxide-dismutase protects plants from oxidative stress. (Induction of ascorbate peroxidase in superoxide dismutase-overexpressing plants), Plant Physiol 103 (1993) 1067–1073.

[70] N. Smirnoff, Ascorbic acid: metabolism and functions of a multi-facetted molecule, Curr. Opin. Plant Biol. 3 (2000) 229–235.

[71] P. Stossel, Regulation of sulphydryl groups of glyceollin accumulation in soybean hypocotyls, Planta 160 (1984) 314–319.

[72] P. Tavladoraky, M.E. Schininà, F. Cecconi, S. Di Agostino, F. Manera, G. Rea, P. Mariottini, R. Federico, R. Angelini, Maize polyamine oxidase: primary structure from protein and cDNA sequencing, FEBS Lett 426 (1998) 62–66.

[73] F. Tommasi, C. Paciolla, M.C. de Pinto, L. De Gara, A comparative study of glutathione and ascorbate metabolism during germination of *Pinus pinea* L. seeds, J. Exp. Bot. 52 (2001) 1647–1654.

[74] M.A. Torres, H. Onouchi, S. Hamada, C. Machita, K.E. Hammond-Kosack, J.J. Jones, Six *Arabidopsis thaliana* homologues of the human respiratory burst oxidase ($gp91^{phox}$), Plant J 14 (1998) 365–370.

[75] H. Vanacker, T.L.W. Carver, C.H. Foyer, Pathogen-induced changes in the antioxidant status of the apoplast in barley leaves, Plant Physiol 117 (1998) 1103–1114.

[76] H. Vanacker, C.H. Foyer, T.L.W. Carver, Changes in apoplastic antioxidants induced by powdery mildew attack in oat genotypes with race non-specific resistance, Planta 208 (1999) 444–452.

[77] H. Vanacker, T.L.W. Carver, C.H. Foyer, Early H_2O_2 accumulation in mesophyll cells leads to induction of glutathione during hypersensitive response in the barley-powdery mildew interaction, Plant Physiol 123 (2000) 1289–1300.

[78] R.F. White, Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco, Virology 99 (1979) 410–412.

[79] W.P.M. Wingate, M.A. Lawton, C.J. Lamb, Glutathione causes a massive and selective induction of plant defence genes, Plant Physiol 87 (1988) 206–210.

[80] H. Wohlgemuth, K. Mittelstrass, S. Kschieschan, J. Bender, H.J. Weigel, K. Overmyer, J. Kangasjarvi, H. Sandermann, C. Langebartels. Activation of an oxidative burst is a general feature of sensitive plants exposed to the air pollutant ozone, Plant Cell Environ 25 (2002) 717–726.