Phytochemistry 112 (2015) 33–53

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Review

The metabolomics of oxidative stress

Graham Noctor *, Caroline Lelarge-Trouverie, Amna Mhamdi

Institut de Biologie des Plantes, UMR8618 CNRS, Université de Paris sud, 91405 Orsay Cedex, France

This paper forms part of a special issue of *Phytochemistry* dedicated to the memory and legacy of Professor (Godfrey) Paul Bolwell, MA DSc (Oxon). (1946–2012), internationally-recognised plant biochemist and Regional Editor of *Phytochemistry* (2004–2012). He is much missed by his friends.

ARTICLE INFO

Article history:
Available online 8 October 2014

Keywords:
Antioxidants
Reactive oxygen species
H_2O_2
Pathogenesis
Redox signalling
Redox reactions
Catalase
Peroxidase

ABSTRACT

Oxidative stress resulting from increased availability of reactive oxygen species (ROS) is a key component of many responses of plants to challenging environmental conditions. The consequences for plant metabolism are complex and manifold. We review data on small compounds involved in oxidative stress, including ROS themselves and antioxidants and redox buffers in the membrane and soluble phases, and we discuss the wider consequences for plant primary and secondary metabolism. While metabolomics has been exploited in many studies on stress, there have been relatively few non-targeted studies focused on how metabolite signatures respond specifically to oxidative stress. As part of the discussion, we present results and reanalyze published datasets on metabolite profiles in catalase-deficient plants, which can be considered to be model oxidative stress systems. We emphasize the roles of ROS-triggered changes in metabolites as potential oxidative signals, and discuss responses that might be useful as markers for oxidative stress. Particular attention is paid to lipid-derived compounds, the status of antioxidants and antioxidant breakdown products, altered metabolism of amino acids, and the roles of phytohormone pathways.

Contents

* Corresponding author. Tel.: +33 (0)169153301; fax: +33 (0)169153424.
E-mail address: graham.noctor@u-psud.fr (G. Noctor).

http://dx.doi.org/10.1016/j.phytochem.2014.09.002
0031-9422/

1. Introduction

Most environmental challenges involve redox adjustments linked to enhanced accumulation of reactive oxygen species (ROS), a condition commonly known as oxidative stress. Many developmental processes also involve signalling through ROS that are probably generated at lower, less stressful levels. While still ill-defined, the state of oxidative stress can perhaps be considered as one in which ROS impinge on and produce significant changes in overall cellular redox state, with various, potentially wide-ranging consequences for the metabolic processes that power plant growth. Such a condition can be produced, to varying degrees, by conditions such as excess light, cold or heat, drought, invasion by pathogenic microorganisms, and oxidizing atmospheric pollutants.

In its very broadest sense, metabolomics could describe measurements of any component of the metabolome (ie, metabolite). However, it is often used to refer to large-scale analyses able to measure numerous metabolites at once. In this sense, metabolomics often implies an approach using techniques such as mass spectrometry, which is able to measure several classes of chemical compounds in a relatively non-targeted fashion (Fernie et al., 2004). Because of the chemical diversity of metabolites, which include both hydrophilic and hydrophobic compounds, and the incomplete nature of reference databases, no technique yet exists that is able to measure the majority of them in a single analysis, and comprehensive profiling of specific chemical classes can also fall under the definition of metabolomics.

The lack of a single analytical protocol to measure the majority of metabolites present in an extract of plant tissue contrasts with commonly used methods in transcriptomics, which provide more comprehensive information. Despite this drawback, changes in metabolites could be considered to be potentially more informative on plant function than changes in transcripts because metabolites can contribute directly to plant phenotypes. Thus, non-targeted metabolite profiling is clearly an interesting approach in the analysis of plant stress responses (Shulaev et al., 2008). However, relatively few studies have reported such analyses on plants in which the stress unambiguously originates from increased ROS availability. Examples of such studies include several that have used pharmacologically induced ROS production (Baxter et al., 2007; Obata et al., 2011) and others that have exploited a genetic approach based on mutants deficient in catalase (Chaouch et al., 2010, 2012; Han et al., 2013a).

This review aims to discuss some aspects of how metabolites are involved in oxidative stress and how this stress impacts on their status. Our ever-increasing knowledge of plant metabolic pathways potentially allows changes in individual metabolites or metabolite patterns to be defined as markers that are likely to be useful in assessing the degree or chemical and compartmental specificity of oxidative stress. While certain secondary metabolites or lipid derivatives have long been a focus of studies in this area, growing evidence points to equally important interactions with primary metabolism, leading to a more integrated view of the response of metabolism to increased cellular oxidation. In this review, we pay particular attention to unresolved issues surrounding measurements of ROS themselves, the roles of the key metabolites with which they interact directly, and some of the primary and secondary metabolic pathways that are responsive to oxidative stress. To highlight aspects of the discussion, we present new or reanalyzed data on metabolites measured in the catalase-deficient Arabidopsis *cat2* mutant. Loss of *CATALASE2* function in this line induces changes in cell redox state and metabolites through an unambiguously oxidative trigger (Mhamdi et al., 2010a). Advantages of using such systems are that (1) H_2O_2 is produced inside the cell by physiologically relevant pathways and that (2) the production can be both more readily controlled and more sustainably applied than when oxidative stress is induced pharmacologically.

2. Physiological manifestation and impact of oxidative stress

Numerous reviews have covered ROS and their metabolism in plants (eg, Møller et al., 2007; Van Breusegem et al., 2008; Foyer and Noctor, 2009; Fischer et al., 2013) and so the sources of these molecules will only be briefly introduced here. Primary ROS (ie, direct inorganic derivatives of molecular oxygen) include singlet oxygen, superoxide, hydrogen peroxide (H_2O_2), and the hydroxyl radical. All of these molecules are significantly more reactive than ground state triplet O_2, can modify numerous cellular components (Møller et al., 2007), and can be produced by various cellular sources (Foyer and Noctor, 2009; Fischer et al., 2013). The best known source of singlet oxygen is photodynamic excitation of triplet O_2 in photosystem II during photosynthesis (Fischer et al., 2013). In contrast, production of superoxide or H_2O_2 involves reduction of oxygen, for example by chloroplast and mitochondrial electron transport chains or by enzymes located in compartments such as the peroxisomes (eg, glycolate oxidase) or at the cell surface/apoplast (NADPH oxidases, peroxidases; Torres et al., 2002; Foyer and Noctor, 2003; del Río et al., 2006; Bindschedler et al., 2006; O'Brien et al., 2012a,b). The hydroxyl radical can be produced by reductive cleavage of H_2O_2, notably catalyzed by Fenton reagents such as iron and other transition metals, although it can also be produced from H_2O_2 by radiation, eg, with ultra-violet light (Halliwell, 1996). Among the frequently used pharmacological agents in oxidative stress research are redox cycling quinones like menadione, and paraquat, the latter mainly acting to transfer electrons from the photosynthetic electron transport chain to oxygen. Both of these agents produce superoxide from which other ROS can subsequently be produced, but paraquat is often used as a fairly specific stimulus of ROS production in the chloroplast.

It is now clear that at least some of the effects of ROS are mediated by signalling pathways (Wagner et al., 2004; Achard et al., 2008), and that both the chemical nature and subcellular location of ROS production may be important in determining cellular responses (Gadjev et al., 2006). Thus, it is possible that superoxide/H_2O_2 produced in the mitochondria trigger different signalling pathways from the same molecules produced in the chloroplast. A key distinction may well be the roles of ROS produced inside the cell, where antioxidative capacity and redox buffering are high, and those generated at the cell surface (Foyer and Noctor, 2009; Miller et al., 2009). The apoplast is a significantly more oxidized compartment than much of the cell interior (Pignocchi and Foyer, 2003) and the hydroxyl radical, which is considered to be the most short-lived ROS, is an essential chemical in cell wall

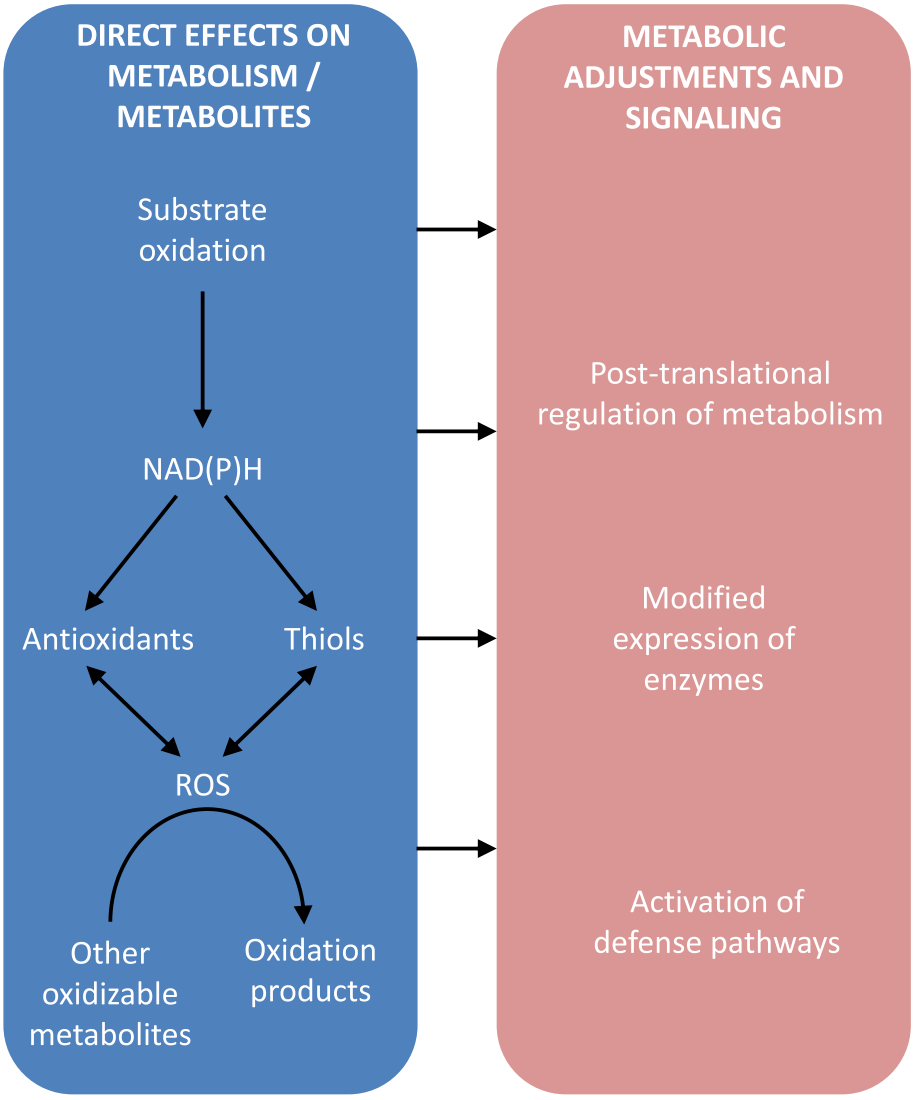

Fig. 1. Some of the multiple ways through which ROS can impact metabolism.

metabolism (Müller et al., 2009). Intracellular and extracellular ROS may be involved in an intricate crosstalk that determines the metabolic and signalling outcome of oxidative stress (Chaouch et al., 2012).

At the phenotypic level, common outcomes of oxidative stress are restricted growth and, in leaves, bleaching. While bleaching may result from a generalized loss of chlorophyll, oxidative stress can also trigger more localized lesions that resemble the hypersensitive response (HR) produced in certain plant–pathogen interactions. For instance, in the *cat2* mutant, HR-like responses can be induced in some conditions whereas, in other conditions, a reversible bleaching is triggered (Mhamdi et al., 2010a; Han et al., 2013a). These observations underscore the complexity of responses to oxidative stress. As well as triggering HR-like cell death, oxidative processes are likely to be involved in autophagy (Hackenberg et al., 2013). However, the relationships between these different ROS-associated cell programmes are still unclear, and metabolite profiling is among the approaches that may help to elucidate them.

Even when driven by a single type of ROS produced in a single compartment, the outcome of oxidative stress may be environmentally conditioned (Queval et al., 2007; Li et al., 2014). For instance, HR-like lesions induced by intracellular redox perturbation in the *cat2* mutant can be prevented by blocking salicylic acid (SA) synthesis, an effect that seems to be independent of the severity of intracellular redox perturbation (Chaouch et al., 2010). Another example is the alleviation of the *cat2* cell death phenotype by auxin-like compounds (Kerchev et al., 2014).

Metabolite profiles can be affected by oxidative stress at multiple levels through diverse mechanisms (Fig. 1). Even in cases where the enzymes or metabolites involved in a given pathway do not themselves react at appreciable rates with ROS, the activity of the pathway could be modified through effects mediated by ROS-responsive regulators such as redox-dependent transcription factors. One direct effect on metabolite profiles is *via* the production of oxidized compounds by chemical reactions of metabolites with ROS (Møller et al., 2007; Farmer and Mueller, 2013). A second effect is through reactions of ROS with enzymes in ways that either inhibit catalysis (eg, the respiratory enzyme, aconitase) or that post-translationally modify activities or protein stability through processes such as carbonylation or thiol oxidation (Fridovich, 1997; Taylor et al., 2002; Davletova et al., 2005; Møller et al., 2007; Oracz et al., 2007; Holtgrefe et al., 2008; Zaffagnini et al., 2012). Another route is *via* metabolic adjustments that do not necessarily involve direct oxidation of enzymes, but that result from increased ROS-dependent engagement of reductant-generating pathways to support increased ROS metabolism (Noctor, 2006; Valderrama et al., 2006;

Dizengremel et al., 2009). All of these types of mechanisms, acting alone or in interaction, may lead to secondary effects as altered metabolite levels modify enzyme activities through transcriptional or post-transcriptional mechanisms that activate signalling, defence, or acclimatory pathways (Fig. 1).

3. Quantifying reactive oxygen species *in vivo* or in extracts: a tricky business

The lack of adequate methods for measuring different ROS with specificity and precision is still a major stumbling-block in oxidative stress research (for critical discussions of this question, see Wardman, 2007; Winterbourn, 2014). On the basis of its relative chemical stability, H_2O_2 can be distinguished from the other primary ROS, whose lesser stabilities make them comparatively short-lived. Theoretically, therefore, H_2O_2 might be best suited as a mobile signal molecule *in vivo* and it should be the easiest of these species to quantify, especially if it accumulates to higher concentrations than other ROS.

3.1. Singlet oxygen and free radicals

Methods for measuring singlet oxygen have recently been reviewed in detail (Fischer et al., 2013). They include the use of "spin-traps" that react with singlet oxygen to produce radicals that can be measured by electron paramagnetic resonance (EPR), as well as probes whose absorbance or fluorescence properties are characteristically altered by reaction with singlet oxygen. As measures of singlet oxygen *in vivo*, many of these techniques have to be used with caution because of limitations with stability, sensitivity, or specificity, or because of possible artefacts linked to distribution of the probe itself within the tissue (Fischer et al., 2013).

While superoxide is less reactive than singlet oxygen, and can be measured in isolated systems like chloroplasts and mitochondria, its quantification in whole tissues or extracts thereof can be problematic (Winterbourn, 2014). Common methods used to detect superoxide in plants rely on formazan dyes like nitroblue tetrazolium (NBT), which is often employed as an *in situ* stain. In the *in vivo* environment, however, this method suffers from a lack of specificity and also, potentially, from artefacts (Fridovich, 1997). Monitoring the hydroxyl radical requires specialized techniques such as EPR. Relative quantification of EPR signals has been used to assess formation of both hydroxyl and superoxide radicals in the cell walls of germinating seeds (Müller et al., 2009).

3.2. Hydrogen peroxide

H_2O_2 is probably the most assayed primary ROS. Methods include purely chemical assays as well as enzyme-based measurements. Staining *in situ* frequently uses diaminobenzidine (DAB), which depends on the activity of peroxidases (Thordal-Christensen et al., 1997). Because peroxidase expression can be altered by oxidative stress, it is not always clear whether changes in staining reflect changes in H_2O_2 concentration, alterations in peroxidase capacity, or both. This limitation also applies to other probes such as Amplex Red (Esposito-Rodriguez et al., 2013). Over recent years, dihydrochlorofluorescin ($DCFH_2$) has become quite widely used as an *in vivo* probe, although it is not specific to H_2O_2 (in fact, it does not react with H_2O_2 directly) and it potentially suffers from several artefacts (Wardman, 2007; Winterbourn, 2014).

While various methods are available to assay H_2O_2 in extracts of plant tissues, several observations suggest that caution is recommended in using the data as a measure of H_2O_2 contents in those compartments that are most active in ROS production. The first is that literature data are extremely variable for what is presumably expected to be a tightly controlled molecule with important signalling functions (Queval et al., 2008). The second is that values in unstressed plants are in excess of what might be predicted from likely concentrations in H_2O_2-producing compartments such as the peroxisomes, chloroplasts, and mitochondria. Reported affinities of intracellular ascorbate- and thiol-dependent peroxidases for H_2O_2 are generally within the range 5–50 μM (Mittler and Zilinskas, 1991; König et al., 2002; Barranco-Medina et al., 2007), making it unlikely that concentrations are much higher in unstressed plants. The volume of expanded leaf mesophyll cells, outside the vacuole and apoplast, probably does not greatly exceed 100 $\mu l\ g^{-1}$ FW (Winter et al., 1994). Mean global concentrations of around 20 μM in this volume would therefore contribute about 2 nmoles $H_2O_2\ g^{-1}$ FW to leaf extracts. This value is far below most reported contents determined in leaf extracts, which are often 100 times higher (Queval et al., 2008).

A third point is that while numerous studies have reported increases in H_2O_2 in stress conditions, these increases can be less apparent in systems that might be considered to be a positive control for excess H_2O_2. One example is deficiency in endogenous catalase. The *cat2* mutant is a conditional mutant that undergoes oxidative stress in air in moderate to high light, when photorespiration is active, but not at low light or at high CO_2, when photorespiration is slow or negligible. In *cat2* leaf extracts, a twofold increase in H_2O_2 was reported by Gao et al. (2014), two days after transferring plants from low to higher light. In our own hands, increases under steady-state conditions are even smaller. For instance, no increased signal was detected in *cat2* using either luminol to measure extracted peroxides or $DCFH_2$ to detect ROS *in situ* (Chaouch et al., 2010, 2012). These analyses were performed on plants exposed to photorespiratory conditions for at least several days: when plants were first grown at high CO_2 to prevent oxidative stress in *cat2*, a slight but significant increase in the luminescence of luminol relative to Col-0 was observed 4 h after transfer to oxidative stress conditions (Han et al., 2013a).

To attempt to resolve this point, we have re-investigated H_2O_2 contents in *cat2* leaf extracts using two different commonly used methods (Fig. 2). In the first, the luminol method was used to measure H_2O_2 in acid extracts from samples taken after transferring plants from high CO_2 to air. In the second, H_2O_2 was measured in plants growing in air from seed using an enzyme-dependent spectrophotometric assay (Veljovic-Jovanovic et al., 2002). In both cases, H_2O_2 contents in *cat2* and Col-0 in the steady-state (24 h after onset of the stress or later) were not significantly different (Fig. 2). Although increased signal was detected with the luminol assay at some early points after the transfer to air, biological variability between *cat2* extracts meant that only the first point (1 h after transfer) was statistically significant at $P < 0.05$ (Fig. 2). In any case, the increases are relatively small, with the additional H_2O_2 in *cat2* not exceeding 200 nmol g^{-1}FW. This compares with an estimated rate of photorespiratory H_2O_2 production of about 15 μmol g $FW^{-1}h^{-1}$. This value is estimated based on a measured leaf mass/area of 260 g m^{-2} and the assumptions that the photorespiratory production of glycolate in our growth conditions is around 1 μmol $m^{-2}s^{-1}$ (derived from typical rates of photosynthesis in Arabidopsis at 22 °C, 200 μmol $m^{-2}\ s^{-1}$ irradiance; Veljovic-Jovanovic et al., 2001) and that its metabolism occurs predominantly through glycolate oxidase. Thus, the maximum measured difference between *cat2* and Col-0 leaf contents could be produced in less than a minute if no other H_2O_2 removal pathways were operating. Even this difference disappears within a few hours, so it seems that the vast majority of H_2O_2 must be metabolized. Although we cannot exclude that some form of down-regulation of H_2O_2 production through the photorespiratory pathways occurs (Schäfer and Feierabend, 2000), it seems clear that catalase deficiency forces this oxidant through alternative

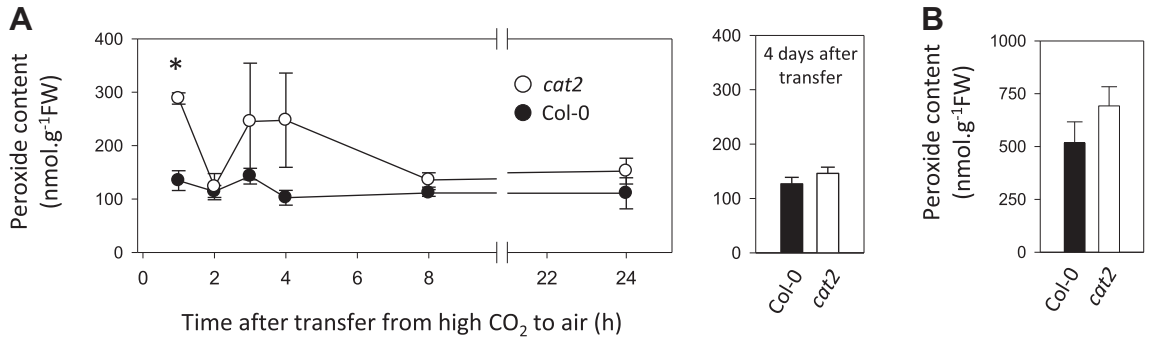

Fig. 2. Peroxide contents in Col-0 (black symbols) and *cat2* mutants (white symbols) using two different analytical methods. (A) Peroxides assayed as luminol chemiluminescence in leaf extracts from plants grown for three weeks in high CO_2 then transferred to air to induce oxidative stress in *cat2*. Left, time course over the first 24 h after transfer. Right, 4 d after transfer. (B) Peroxide assayed enzymatically in leaf extracts from plants grown in air from seed for three weeks. Assay procedures as in Veljovic-Jovanovic et al. (2002). Data are means ± SE of three to six independent biological repeats. *Significant difference from Col-0 at $P < 0.05$.

H_2O_2-reducing pathways that are less active when catalase capacity is high. As we discuss further below, this notably drives significant changes in cell thiol-disulfide status, as well as adjustments in other areas of metabolism.

Observations on the relative stability of H_2O_2 signals in oxidative stress are neither limited to *cat2* mutants nor to H_2O_2 measured in extracts. Exposure of lettuce cells to 3-aminotriazole, a very effective and rapid inhibitor of catalase, did not result in an increase in H_2O_2 staining measured *in situ* using cerium chloride (Bestwick et al., 1997). It is therefore pertinent to ask: what is the signal measured in H_2O_2 assays performed on tissue extracts? In control experiments in which extract aliquots were treated just before the assay with commercially obtained catalase, more than 98% of the absorbance change shown in Fig. 2B was abolished, indicating that the signal is indeed H_2O_2-dependent. Assuming that H_2O_2 is not generated artefactually during sample preparation, one possibility is that H_2O_2 measured in extracts predominantly originates from the vacuole, apoplast, or other compartments with comparatively low antioxidant capacity. Inter-compartmental movement of H_2O_2 could be facilitated by some aquaporins (Henzler and Steudle, 2000; Ye and Steudle, 2005; Bienert et al., 2007; Borisova et al., 2012), although current knowledge suggests that this would tend to attenuate any concentration differences rather than promote them. As well as subcellular heterogeneity, intercellular differences in H_2O_2 signals also exist in wild-type plants exposed to high light (Mullineaux et al., 2006; Galvez-Valdivieso et al., 2009). Assuming they are sufficiently specific and can be calibrated accurately, genetically encoded probes offer a promising approach to provide a more informative picture of localized concentrations of H_2O_2 in a compartment-specific manner (Costa et al., 2010; Esposito-Rodriguez et al., 2013).

4. Lipid peroxides and related molecules

Peroxidation of fatty acid chains is the basic cause of rancidity in fats and oils, and can occur in biological cells during oxidative stress. Indeed, breakdown products of fatty acid peroxides, such as malondialdehyde (MDA), are often used as oxidative stress markers. Non-enzymatic peroxidation of fatty acids occurs though either addition of singlet oxygen or H abstraction (by the hydroxyl radical, by lipid peroxyl radicals) followed by O_2 and H addition. Enzymatic generation can also occur, notably as an early step in the synthesis of jasmonates, catalyzed by chloroplastic lipoxygenases, for instance during wounding stress (Browse, 2009). Lipid peroxides also accumulate during other stresses such as cryptogein-elicited HR-like processes in tobacco (Davoine et al., 2006).

Because of its relatively high capacity for singlet oxygen generation and the presence of lipoxygenases, most attention has focused on the chloroplast as a site of lipid peroxidation. The operation of different pathways can be distinguished based on metabolite signatures measured, for example, by LC–MS/MS or HPLC followed by GC–MS analysis of collected fractions. Such studies have shown that although singlet oxygen can non-enzymatically produce lipid peroxides, it can also induce enzymatic pathways. Excess singlet oxygen in the Arabidopsis *flu* (*fluorescence*) mutant triggered accumulation of lipid peroxide signatures in a conditional manner (Przybyla et al., 2008). Lesions dependent on *EXECUTOR1* (an active cell death pathway) were associated with enzymatic pathways, whereas profiles consistent with non-enzymatic peroxidation were observed in other conditions (Przybyla et al., 2008).

Peroxide profiling has also been used to distinguish between hydroxyl radical- and singlet oxygen-mediated reactions (Triantaphylidès et al., 2008). Based on the predominance of singlet oxygen-related signatures, it was concluded that this molecule was the major ROS causing lipid peroxidation in Arabidopsis subjected to excess light (Triantaphylidès et al., 2008). Comparison of transcriptomic data supports an important role for singlet oxygen during other stresses like drought (Noctor et al., 2014). There is growing interest in the roles of the complex array of compounds that can be produced from lipid peroxidation, many of which are termed "reactive electrophile species" (RES; Farmer and Mueller, 2013).

The biological activity of electrophiles can be regulated by conjugation to glutathione. Conjugation can occur spontaneously but is accelerated by glutathione *S*-transferase (GST) activity. In at least some cases triggering strong accumulation of RES, conjugation can markedly deplete the glutathione pool (Davoine et al., 2006). Among well-studied lipid peroxidation products are 4-hydroxy-2-nonenal (HNE) which, like MDA, is a reactive aldehyde (Taylor et al., 2004). Oxidative stress treatments promoted HNE accumulation in Arabidopsis cells, accompanied by detection of HNE adducts on mitochondrial proteins (Winger et al., 2005). Other reactive aldehydes that have been studied in plastids include acrolein (2-propenal). In isolated chloroplasts, this compound strongly inactivated photosynthesis, accompanied by inactivation of photosynthetic enzymes and the depletion of the glutathione pool, while weaker effects were produced by HNE and MDA (Mano et al., 2009). As well as, or by way of, these inactivating effects, some RES may play important roles in signalling (Farmer and Mueller, 2013). Metabolite profiling will continue to be a key approach in the elucidation of their physiological importance.

5. Chlorophyll catabolism

Chlorophyll breakdown pathways have notably been studied during leaf senescence, but also during dehydration and rehydration of resurrection plants (Christ et al., 2014). Chlorophyll degra-

dation involves loss of magnesium and the phytol chain within the chloroplast, export to the cytosol for further modification by enzymes such as cytochromes P450 (CYP; Christ et al., 2013), and removal to the vacuole for breakdown. During senescence, chlorophyll degradation may be important to avoid excessive ROS production by the electron transport chain under conditions where metabolic capacity is declining or restricted. While non-enzymatic pathways are possible, available evidence suggests that enzyme-dependent degradation is predominant (Hörtensteiner and Kräutler, 2011; Christ et al., 2014).

Free chlorophyll catabolites may sensitize ROS production or otherwise act as stress-related signals to the nucleus, and so their accumulation must be controlled during chlorophyll breakdown. A key feature is metabolite channeling in enzyme complexes. Deregulation of the pathway in mutants for the *Accelerated Cell Death1* (*ACD1*) gene, encoding pheophorbide *a* oxygenase, which catalyzes the opening of the tetrapyrrole macrocycle, causes accelerated cell death (Pružinska et al., 2003; Tanaka et al., 2003). Similar effects have been observed in *acd2* mutants lacking the function of red chlorophyll catabolite reductase, a subsequent enzyme in the pathway, which is induced during pathogenesis responses (Yao and Greenberg, 2006). The ACD2 protein is predominantly chloroplastic but is also found in the mitochondria. Together with evidence that suggests an important role for mitochondria in ROS-dependent cell death, this dual localization led to the hypothesis that "death signals" may involve movement of a sensitizing metabolite out of these energy-generating organelles (Pattanayak et al., 2012).

6. Antioxidants and redox buffers

Many compounds that occur in cells can react with ROS and potentially decrease the levels of these compounds. For a molecule to be considered an effective antioxidant, the oxidized product should be stable and, preferentially, recyclable to the active reduced form, so that the sequence of potentially indiscriminate oxidizing reactions can be effectively interrupted or at least controlled. There has been interest in plant compounds as antioxidants that can improve human health for many years (Halliwell, 1996). Both in the human and plant context, many such compounds may function as "sacrificial scavengers", meaning that their reaction with ROS leads to their irreversible modification and their continued action requires resynthesis. Because of this, it remains unclear whether such compounds are major players in ROS homeostasis in plants. In some cases, however, they could play an important role. One example could be during tomato fruit ripening, which can be delayed by reinforcing anthocyanin contents (Zhang et al., 2013). Similarly, compatible solutes may act to scavenge the hydroxyl radical (Smirnoff and Cumbes, 1989), although evidence is growing that the roles of compounds such as proline may also be important in controlling ROS accumulation more indirectly, for example, through homeostasis of pyridine nucleotide pools (Szabados and Savouré, 2010; Sharma et al., 2011).

6.1. Carotenoids and tocopherols

Within the membrane phase, carotenoids and tocopherols are considered to be the most important compounds that control the accumulation of ROS and derived molecules. In this context, carotenoids can both act to avoid ROS production and as antioxidants by reacting with any ROS that are produced. In addition to their accessory light-harvesting roles, these pigments act in a non-redox role to de-excite triplet chlorophyll or singlet oxygen (Fischer et al., 2013). Moreover, the xanthophyll cycle carotenoids are associated with a specific function in quenching singlet excited chlorophyll, thereby decreasing excitation density that otherwise promotes triplet chlorophyll formation and ROS formation (Ruban et al., 2012). This involves two-step de-epoxidation of violaxanthin to zeaxanthin *via* antheraxanthin in an ascorbate-dependent manner (Demmig-Adams, 1990; Müller-Moulé et al., 2002). Stress conditions such as high light can increase both total xanthophyll cycle pigments and, particularly, the proportion represented by zeaxanthin and antheraxanthin (Grace and Logan, 1996; Verhoeven et al., 1997).

In addition to their biophysical interactions, carotenoids can undergo oxidation: GC–MS analysis of volatile products identified several RES that accumulated after high light treatment of Arabidopsis (Ramel et al., 2012). Treatment of otherwise unstressed Arabidopsis with one of these, β-cyclocitral, induced transcriptomic signatures reminiscent of those previously described in the Arabidopsis singlet oxygen-accumulating *flu* mutant (Ramel et al., 2012). This important study both defines metabolite markers for increased singlet oxygen and provides mechanistic insight into the early steps involved in linking accumulation of this unstable and short-lived ROS to the induction of signalling pathways.

Tocopherols and tocotrienols differ in the presence or absence of double bonds in their side-chains which, like carotenoids, are derived from geranylgeranyl pyrophosphate in the terpenoid pathway. Both tocopherols and tocotrienols contribute to vitamin E activity, acting to directly quench singlet oxygen or to interrupt peroxidation cascades by hydrogen donation to peroxyl radicals, with the main contribution in photosynthetic tissue coming from α-tocopherol (Dellapenna and Mène-Saffrané, 2011). Hydrogen donation leads to the tocopherol radical, which can be re-reduced by compounds such as ascorbate (Halliwell, 1996). Singlet oxygen addition to the chromanol ring generates a relatively stable tocopherolquinone that may be converted back to tocopherols (Dellapenna and Mène-Saffrané, 2011).

Like carotenoids, tocopherols are strongly associated with plastids, but they are found in higher levels in seeds than in leaves, especially in oil-storing seeds. Arabidopsis *vte* mutants deficient in tocopherol show clear signs of oxidative stress associated with enhanced lipid peroxidation and the activation of defense pathways, particularly those associated with pathogenesis, during germination and early seedling establishment (Sattler et al., 2006). In established plants, tocopherols may be less essential, because of the presence of other antioxidative systems (Dellapenna and Mène-Saffrané, 2011), although they may become important in certain stress conditions (Havaux et al., 2005). The results of a study using genetic enrichment of tocopherols in carotenoid-deficient *Chlamydomonas* strains point to overlapping roles of carotenoids and tocopherols in coping with high light stress (Li et al., 2012).

6.2. Ascorbate and glutathione

Important features of core antioxidative systems in the soluble phase of cells are that they either (1) accelerate reactions between primary ROS to produce more stable species (superoxide dismutase, catalase) or (2) involve the oxidation of reducing metabolites to relatively stable forms that do not propagate the stress. An important point is that the first type of antioxidant function does not require accessory reductants. The best studied of the second type is the ascorbate–glutathione pathway, in which regeneration of these antioxidants ultimately requires NAD(P)H in an enzyme-dependent manner. Although these metabolites are involved in multiple cellular reactions (eg, glutathione is required in conjugation reactions), current concepts considers both of them to be key players in controlling concentrations of ROS, notably H_2O_2.

Despite the general acceptance of the ascorbate–glutathione pathway, and direct evidence that enzymes dependent on both reductants become engaged when intracellular H_2O_2 availability

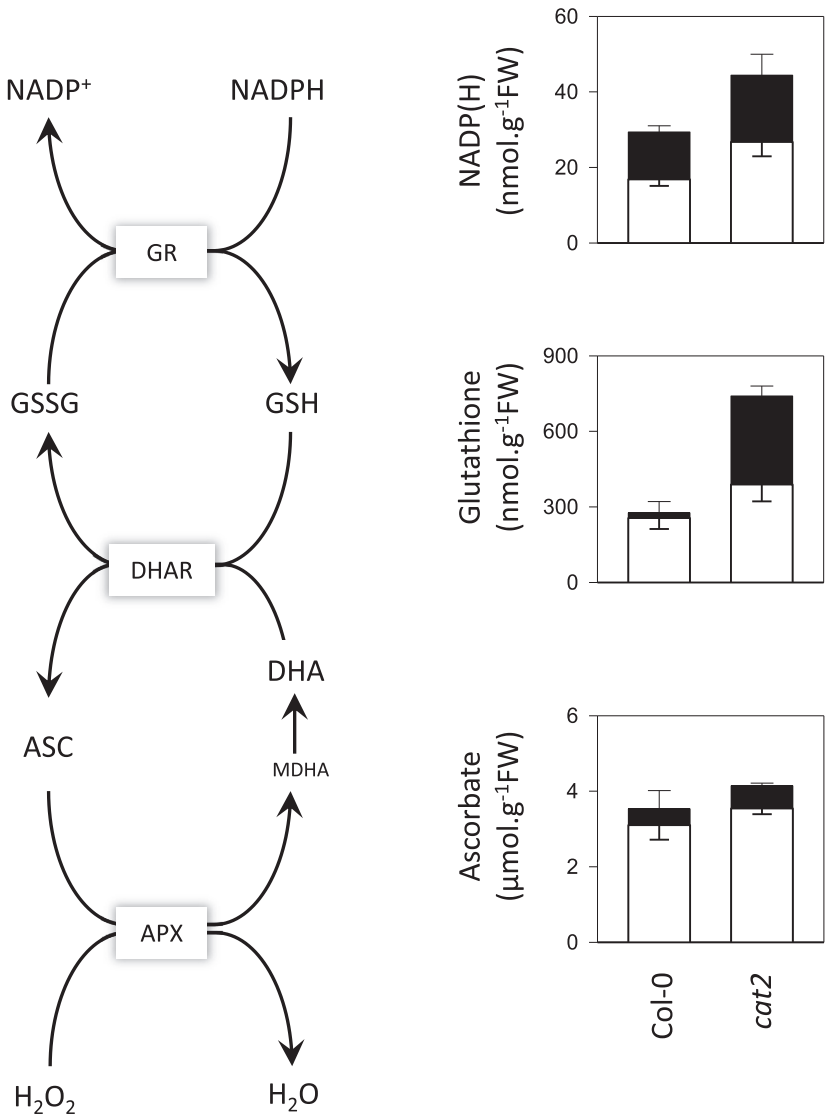

Fig. 3. Ascorbate, glutathione, and NADP(H) contents in Col-0 and *cat2* mutants. Plants were grown for three weeks in longs days, and reduced (white) and oxidized (black) forms of each compound were measured as in Queval and Noctor (2007). Data are means ± SE of three independent biological repeats.

is enhanced (Mhamdi et al., 2010b; Vanderauwera et al., 2011), the exact extent of coupling between the ascorbate and glutathione pools remains to be established (Foyer and Noctor, 2011; Rahantaniaina et al., 2013). Studies in catalase-deficient tobacco and Arabidopsis backgrounds show that cytosolic ascorbate peroxidase (APX) is an important player in H_2O_2 metabolism (Rizhsky et al., 2002; Vanderauwera et al., 2011), but ascorbate pools seem to be much less affected than glutathione in such plants (Fig. 3). Glutathione status therefore seems to be a better marker for changes in cellular redox state, at least those driven by increased H_2O_2 inside the cell. Ascorbate contents are more responsive to factors such as irradiance (Grace and Logan, 1996; Gatzek et al., 2002; Smirnoff, 2011), which affects glutathione somewhat less. Irradiance-dependent changes in ascorbate may be partly related to functions in tocopherol regeneration and the xanthophyll cycle, discussed above.

While depletion of the total glutathione pool can be triggered by stresses such as challenge with cryptogein (Davoine et al., 2006; Hoeberichts et al., 2013), accumulation of glutathione similar to that shown in Fig. 3 can occur, at least transiently, in other conditions that involve oxidative stress (Vanacker et al., 2000; Bick et al., 2001; Gomez et al., 2004a). Such changes in glutathione seem to be of functional importance in linking intracellular H_2O_2 to pathogenesis-related (PR) responses (Han et al., 2013a,b), and several other studies point to an important role of glutathione in determining plant responses to biotic stress (Ball et al., 2004; Parisy et al., 2007; Schlaeppi et al., 2008; Baldacci-Cresp et al., 2012). The mechanisms by which glutathione is involved in oxidative stress signalling remain to be elucidated but may involve processes such as formation of protein disulfides, *S*-glutathionylation, or *S*-nitrosylation (Dixon et al., 2005; Feechan et al., 2005; Tada et al., 2008; Palmieri et al., 2010; Zaffagnini et al., 2012). Calcium signalling may be involved (Gomez et al., 2004b), possibly driven by the sensing of increased glutathione concentrations in the apoplast (Li et al., 2013a).

6.3. Pyridine nucleotides

Maintenance of the reduced forms of ascorbate and glutathione depends on NAD(P)H. Infiltration of these nucleotides into Arabidopsis leaves can induce pathogenesis responses (Zhang and Mou, 2009). This might reflect apoplastic sensing of key compounds that in the absence of stress are mainly confined to the cell interior. Pathogenesis responses can also be induced by enhancing NAD(H) contents through manipulation of a chloroplastic step of the synthesis pathway (Noctor et al., 2011; Pétriacq et al., 2012). Unlike

glutathione, neither NAD(H) nor NADP(H) pools change dramatically in response to intracellular H_2O_2 in *cat2*. Some increase in total NAD(H) and total NADP(H) pools can be observed but changes in redox state are minor (Fig. 3). Thus, while these molecules must be required for oxidative stress responses, assays of their status may not offer a sensitive marker for altered redox states. Given the fast turnover of at least some NAD(P) pools, it is likely that any changes in redox states are transient, similar to changes in H_2O_2 (Fig. 2). Decreases in cellular NADPH:$NADP^+$ ratios were observed when oxidative stress was induced by application of paraquat or menadione (Obata et al., 2011; Benina et al., 2014).

A large proportion of extracted pyridine nucleotides can be bound to proteins *in vivo*. At any one time, therefore, only a fraction of molecules may be contributing to the free pool (Hagedorn et al., 2007). Hence, *in vitro* assays may not necessarily reflect biochemically important changes. Reduced forms of NAD(P) can be measured directly by their intrinsic fluorescence, although this method does not distinguish between NADH and NADPH, which represents a limitation if both forms are present (Kasimova et al., 2006). The relative stability of pyridine nucleotide redox states during sustained oxidative stress may also be due to the plethora of highly active enzymes that can interconvert oxidized and reduced forms. Such enzymes are located notably in chloroplasts but also in the mitochondria, cytosol, and peroxisomes (Møller and Rasmusson, 1998; Valderrama et al., 2006; Mhamdi et al., 2012). For instance, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a central player in photosynthetic and respiratory metabolism. Several isoforms of GAPDH can undergo specific redox modifications that may affect flux through these major pathways during oxidative stress (Hancock et al., 2005; Sparla et al., 2005; Holtgrefe et al., 2008). NADPH-generating dehydrogenases are also predicted to play roles in the response to increased ROS availability, and analysis of mutants is beginning to throw light on their importance in such conditions (Rius et al., 2006; Mhamdi et al., 2010c; Voll et al., 2012; Li et al., 2013b; Dghim et al., 2013; Mhamdi and Noctor, 2014).

Reduced forms of pyridine nucleotides can undergo hydration, rendering them inactive or even inhibitory to essential metabolic reactions. Enzymes that repair the hydrates are known and have recently been described in plants. Loss-of-function Arabidopsis mutants for an epimerase and hydratase, both of which appear to be present as single gene copies in Arabidopsis, showed increases in hydrated forms of NADH and NADPH (Niehaus et al., 2014). However, both mutants showed a wild-type phenotype when grown under standard conditions (Niehaus et al., 2014). It is not yet clear whether these reactions are an important part of oxidative stress responses.

6.4. Compartmentation, synthesis and turnover of NAD(P), glutathione, and ascorbate

Although NAD(P)H, glutathione and ascorbate all have other roles in cell metabolism, they are key to controlling ROS accumulation. The wealth of information that has been generated on synthesis, degradation, and transport pathways in Arabidopsis is crucial to understanding how the status of these metabolites responds to oxidative stress. Knowledge of these pathways may also be useful to identify possible stress markers generated during such responses (Fig. 4).

Although most of the ascorbate pool is intracellular, a large part of the dehydroascorbate (DHA) detected in leaf tissue is probably present in the apoplast so that the 90% reduction state typically measured in leaf extracts (Fig. 3) underestimates the very high reduction states of intracellular pools (Foyer and Noctor, 2011). An important role for extracellular ascorbate pools in resistance to oxidizing pollutants is consistent with the identification of ascorbate-deficient mutants in screens for enhanced ozone sensitivity (Conklin et al., 1996). Changes in apoplastic ascorbate in these mutants may at least partly underlie the activation of PR responses and abscisic acid (ABA) signalling in ascorbate-deficient mutants (Pastori et al., 2003; Pavet et al., 2005; Colville and Smirnoff, 2008). Despite the well-described links between ABA and ROS (Kwak et al., 2003; Hu et al., 2006), it is not clear whether the effect of ascorbate deficiency involves changes in ROS, since ascorbate is also required to maintain optimal activities of various iron-dependent enzymes, including dioxygenases involved in hormone synthesis (including ABA) and histone demethylases (Tsukada et al., 2006; Smirnoff, 2011). In the context of ROS metabolism, it is interesting that the final step of ascorbate synthesis involves an oxidative step that feeds electrons directly into the mitochondrial electron transport chain (Fig. 4). Accordingly, ascorbate synthesis and tissue contents can be influenced by the status of the chain and of the tricarboxylic acid (TCA) cycle (Millar et al., 2003; Nunes-Nesi et al., 2005), both of which may be modified under oxidative stress conditions.

In the apoplast, ascorbate is a substrate for ascorbate oxidase and may also influence H_2O_2 concentrations through various antioxidant and pro-oxidant mechanisms, including acting as a Fenton reagent (Kärkönen and Fry, 2006). In order to be converted to ascorbate by compounds like GSH, at least some apoplastic DHA probably has to be transferred to the more reducing cell interior (Pignocchi and Foyer, 2003). Alternatively, apoplastic DHA can be degraded (Green and Fry, 2005). Current knowledge points to two routes of degradation, one that involves direct oxidation of DHA and one in which oxidative steps are preceded by hydrolysis to 2,3-diketogulonic acid (Parsons et al., 2011). These pathways involve several intermediates, although their unstable nature means that specialized techniques are required to quantify them (Parsons and Fry, 2012). Ultimately, threonate and oxalate are produced (Fig. 4). Accumulation of these compounds may be a marker for oxidative stress because ROS can accelerate not only the formation of DHA from ascorbate but also the oxidation steps in the ensuing degradation pathways (Parsons and Fry, 2012).

One reason why GSSG accumulation seems to be quite a good marker for increased H_2O_2 metabolism through reductive pathways is that a substantial proportion is sequestered in the vacuole, where it escapes the action of glutathione reductase (Queval et al., 2011). This effect may occur through transporters that are able to transfer glutathione *S*-conjugates across the tonoplast (Noctor et al., 2013). Clearance of GSSG from the cytosol is not restricted to plants, but also occurs in some yeasts and in animal cells during stress (Zechmann et al., 2011; Morgan et al., 2012). As well as the vacuole, a significant part of the accumulated GSSG in catalase-deficient Arabidopsis is found in the chloroplast (Queval et al., 2011), a finding that is consistent with an earlier study in barley (Smith et al., 1985). Oxidative stress can therefore drive significant changes in thiol redox states in compartments in which sensitive enzymes are present, perhaps leading to changes in metabolism through post-translational modifications. Pathways that are modulated by the GSH:GSSG ratio may include synthesis of glutathione itself, as well its key precursor, cysteine (Bick et al., 2001; Queval et al., 2009). Degradation of GSSG and GS-conjugates is notably initiated in the vacuole (Grzam et al., 2007; Martin et al., 2007). The pathway involves 5-oxoproline production from the glutathione γ-glutamyl residue (Ohkamu-Ohtsu et al., 2008), and is likely to be increasingly engaged under oxidative stress conditions that promote vacuolar accumulation of GSSG or GS-conjugates (Fig. 4).

Intercompartmental redox transfer through high capacity exchangers and associated dehydrogenases is another important mechanism that may operate to enable ROS homeostasis or support increased ROS metabolism. For instance, the "malate valve" could act to alleviate ROS production in the chloroplast by increas-

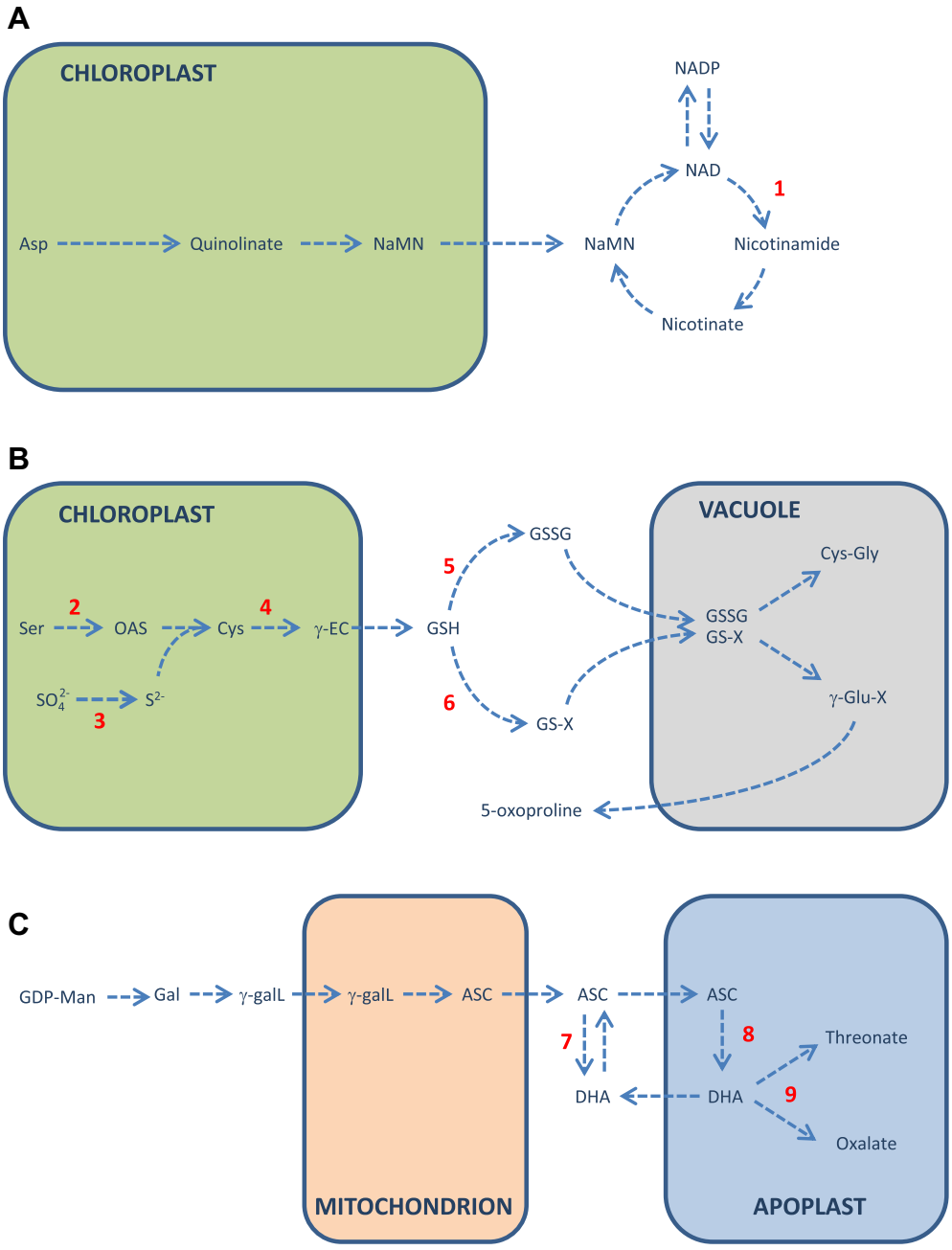

Fig. 4. Simplified schemes of synthesis and degradation pathways of (A) NAD, (B) glutathione, and (C) ascorbate showing some of the steps that may be stimulated by oxidative stress (red numbers). Arrows may represent more than one enzymatic/transport reaction. Glutathione and ascorbate oxidation can occur in many compartments but for simplicity, this is not shown. While other steps are also likely to be sensitive to oxidative stress, the mechanisms depicted are (1) enhanced turnover of NAD by cellular repair enzymes such as poly(ADP-ribose) polymerase; (2) induction of serine acetyltransferase; (3) induction and post-translational activation of some enzymes of sulfate reduction; (4) post-translational activation of γ-glutamylcysteine synthetase; (5) oxidation of GSH by dehydroascorbate reductase or enzymes with glutathione peroxidase activity; (6) induction of glutathione *S*-transferases; (7) oxidation of ascorbate by ascorbate peroxidase and dismutation of monodehydroascorbate; (8) enhanced oxidation of ascorbate by apoplastic ROS; (9) ROS-stimulated degradation of dehydroascorbate. ASC, reduced ascorbate. DHA, dehydroascorbate. γ-EC, γ-glutamylcysteine. Gal, galactose. γ-GalL, galactono-γ-lactone. GDP-Man, GDP-mannose. GSH, glutathione (thiol form). GSSG, glutathione disulphide. GS-X, glutathione *S*-conjugate. NaMN, nicotinic acid mononucleotide. OAS, *O*-acetylserine.

ing $NADP^+$ availability while simultaneously exporting reducing equivalents for ROS metabolism in other compartments (Scheibe et al., 2005). Analysis of knockout mutants for the stromal NADP-malate dehydrogenase did not reveal an obvious phenotype, even under high light, which may be due to a number of metabolic adjustments that compensate for the absence of this enzyme (Hebbelmann et al., 2012).

As well as redox cycling between NAD(P)H and $NAD(P)^+$, pyridine nucleotides can be partly degraded and resynthesized through "salvage" pathways. This type of "non-redox" turnover may be stimulated by increased activities of several types of enzyme that may play roles in oxidative stress, necessitating enhanced rates of synthesis to maintain pool sizes. One notable example is poly(-ADP-ribose)polymerase (De Block et al., 2005; Vanderauwera et al., 2007; Pellny et al., 2009). As well as GSSG, several of the metabolites involved in synthesis, recycling, or degradation pathways of glutathione, ascorbate, and pyridine nucleotides accumulate in response to oxidative stress in catalase-deficient

Arabidopsis. These include nicotinic acid, *O*-acetylserine, cysteine, γ-glutamylcysteine, cysteinylglycine, 5-oxoproline, threonate, and oxalate (Queval et al., 2009; Chaouch et al., 2012; Han et al., 2013a). While the metabolic provenance of some of these compounds could be multiple, their accumulation may reflect stimulation of the mechanisms shown in Fig. 4.

7. Non-targeted profiling of the response to oxidative stress

In the context of studies on oxidative stress, we have published several non-specific metabolite profiling analyses of derivatized polar compounds in *cat2* using GC–MS (Chaouch et al., 2010, 2012; Han et al., 2013a; Li et al., 2014). Within the framework of the present metabolomics-focused paper, we have reanalyzed some of these datasets with the aim of producing a robust description of detected metabolites that respond to oxidative stress in this system. Our analysis is limited to the three studies performed in comparable growth conditions (moderate light, air, long days). This is a condition in which, despite the apparently rather modest and transient increase in H_2O_2 contents (Section 3.2), oxidative stress in *cat2* is sufficient to activate pathogenesis responses in the absence of any external biotic stressor. Up to 117 derivatives were detected and quantified in either Col-0, *cat2*, or both. These are listed in Supplementary Table S1, while Fig. 5 summarizes compounds that were found to be significantly different in *cat2* in all three studies. In total, 29 compounds met this criterion, 28 of which were increased in *cat2* while one was found to be decreased (Fig. 5). The predominance of increased signals probably partly reflects growth restrictions, leading to a relative slowdown in synthesis and/or accelerated degradation of macromolecules and structural compounds, as discussed in the following sections.

7.1. Respiration

The key role of NAD(P)H in cell redox homeostasis and increased energy use in repair processes predict enhanced respiratory flux during oxidative stress. The extractable activities of several enzymes associated with the oxidative pentose phosphate (OPP) pathway and glycolysis are enhanced in response to ozone exposure (Dizengremel et al., 2009). In studies of Arabidopsis cell using pharmacologically induced ROS, a number of glycolytic intermediates accumulated, as well as gluconate (Baxter et al., 2007). In *cat2*, we observed increases in glucose 6-phosphate and fructose 6-phosphate in one study, but these compounds are not routinely detected by our GC–MS procedures (Supplementary Table S1). In agreement with the study of Baxter et al. (2007), gluconate is clearly increased (Fig. 5). In fact, this compound can increase more than 100-fold in *cat2*, suggesting it could be a useful oxidative stress marker (Chaouch et al., 2012). The detected increases in gluconate may either reflect oxidation of glucose or enhanced production of 6-phosphogluconate through the OPP pathway. The latter effect may also possibly contribute to routinely detected increases in ribose (Fig. 5).

Mitochondria play roles in the outcome of oxidative stress, even when the stress does not primarily result from mitochondrial ROS, as in catalase-deficient plants (Dat et al., 2003). Observations in heterotrophic cells exposed to menadione suggest that the TCA cycle is inhibited when ROS are increased (Baxter et al., 2007). Several TCA cycle metabolites were increased in *cat2*. These included 2-oxoglutarate (2-OG), malate and succinate (Fig. 5). These data are consistent with the view that the TCA cycle is impacted significantly by oxidative stress. However, the details of the underlying mechanisms remain to be fully clarified (Obata et al., 2011).

7.2. Amino acids and related molecules

Of the 28 compounds found to be significantly more abundant in *cat2* than in Col-0, 13 were amino acids (Fig. 5). Accumulation of aromatic amino acids may reflect an activation of the shikimate and related pathways to support synthesis of phenolic and indolic compounds, pathways that are known to be important in defence in Arabidopsis (see Section 8). However, there is growing evidence that other areas of amino acid-related metabolism play roles in pathogenesis, for example, to generate respiratory fuel and/or nutritional signals between the host plant and the pathogen (Bolton, 2009; Liu et al., 2010; Stuttmann et al., 2011).

Eleven of the 13 amino acids that increased in *cat2* are among those found in protein. Enhanced protein degradation is probably important to supply respiratory substrates during stress (Araújo et al., 2011). For instance, isovaleryl-CoA dehydrogenase and 2-hydroxyglutarate dehydrogenase are involved in the catabolism of lysine and branched-chain amino acids. They feed electrons into the mitochondrial electron transport chain and play roles in conditions of low carbon availability or senescence (Däschner et al., 2001; Araújo et al., 2010; Engqvist et al., 2011). In oxidative stress conditions, where the TCA cycle may be partly inhibited, these pathways can therefore promote ongoing activity of the mitochondrial electron transport chain and associated ATP generation (Baxter et al., 2007; Obata et al., 2011). Lysine, isoleucine, leucine and valine are all among the metabolites that most reproducibly accumulate in *cat2*, alongside pipecolic acid, a product of lysine breakdown (Fig. 5; Chaouch et al., 2012). Although 2-hydroxyglutarate was not detected in one of the studies, this metabolite accumulated in *cat2* in the other two (Supplementary Table S1). Increases in all these compounds are consistent with enhanced production of lysine and branched-chain amino acids during oxidative stress, possibly following their release from proteins. Pipecolic acid can be produced during lysine degradation (Galili, 1995) and metabolized by a peroxisomal oxidase (Goyer et al., 2004). This metabolite has been implicated in pathogenesis responses (Návarová et al., 2012). As well as responding to peroxisomal-derived H_2O_2 in *cat2*, pipecolic acid accumulates alongside lysine when peroxisomal isocitrate dehydrogenase function is lost (Mhamdi and Noctor, 2014).

Given that glutamate contents are relatively stable under conditions of different nutritional status (Stitt et al., 2002), it is interesting that this amino acid accumulates in *cat2* alongside its carbon skeleton precursor, 2-OG. Proteomic analyses suggest that this may be related to down-regulation of primary ammonia metabolism and recycling through chloroplast glutamine synthetase/glutamate synthase (GS/GOGAT), accompanied by an up-regulation of amino acid remobilization involving the cytosolic GS (Li et al., 2014). Similar effects have been described during pathogenesis responses (Pérez-Garcia et al., 1995). Interestingly, although knocking out the cytosolic isocitrate dehydrogenase, one of the contributors to 2-oxoglutarate production for the GS/GOGAT pathway, produced only a minor growth phenotype, it triggered enhanced resistance to pathogens (Mhamdi et al., 2010c). Given the central role of glutamate in carbon/nitrogen metabolism, this amino acid could play several roles in pathogenesis and other conditions involving oxidative stress (for a recent review, see Seifi et al., 2013).

Although proline has long been implicated in stress responses (Szabados and Savouré, 2010), contents of this amino acid do not generally show strong changes during oxidative stress in *cat2*. Proline can accumulate during drought and related stresses as a compatible solute. However, enhanced sensitivity to drought was observed in mutants for both proline synthesis and degradation (Sharma et al., 2011). An additional possibility is that proline-glutamate cycling plays a role in NADP(H) homeostasis (Sharma et al., 2011 and references cited therein).

A

Metabolite	STUDY 1	STUDY 2	STUDY 3	Class	Main pathway/physiological role(s)
α-Ketoglutaric acid	8.9	2.3	3.5	Organic acid	TCA cycle, amino acid metabolism
Arabinose	1.8	1.5	1.7	Sugar	Cell wall component
Arginine	4.4	5.4	2.4	Amino acid	Protein, N storage
β-Alanine	1.7	1.5	1.8	Amino acid	Product of polyamine oxidation
Gluconic acid	7	95.9	159.9	Organic acid	Product of glucose oxidation
Glutamic acid	2.7	4.4	1.9	Amino acid	Ammonia assimilation, photorespiration
Glycolic acid	2.1	1.8	4.7	Organic acid	Photorespiration
Isoleucine	3.1	3.7	2.6	Amino acid	Protein, respiration (branched chain amino acid)
Leucine	3.7	3.4	2.3	Amino acid	Protein, respiration (branched chain amino acid)
Lysine	2.3	2.0	1.8	Amino acid	Protein, stress response
Maleic acid	3.3	7.7	2.7	Organic acid	Product of lipid/fatty acid oxidation
Malic acid	8.3	4.2	3.1	Organic acid	TCA cycle, redox transfer
Methionine	3.5	6.0	3.5	Amino acid	Protein, S assimilation, C1 metabolism
myo-Inositol	0.3	0.3	0.1	Polyol	Precursor to signaling compounds, membrane lipids
Myristic acid	2.4	2.8	2.1	Fatty acid	Protein localization
Nicotinic acid	2.3	3.9	1.9	Organic acid	Pyridine, NAD(P) metabolism
O-Acetyl-L-serine	2.1	2.8	2.9	Amino acid	Protein, S assimilation, cysteine/glutathione synthesis
Phenylalanine	2.6	4.0	2	Amino acid	Protein, phenylpropanoid precursor
p-hydroxybenzoic acid	2.3	4.9	1.5	Aromatic compound	Phenylpropanoid pathway
Pipecolic acid	23.5	10.0	2.1	Organic acid	Lysine catabolism
Putrescine	3.5	4.1	2.8	Amine	Stress response, H2O2 production
Rhamnose	2	1.6	2.3	Sugar	Cell wall component
Ribose	2.7	2.6	3.6	Sugar	Various (nucleic acid component)
Salicylic acid	3.2	7.2	2.7	Aromatic compound	Pathogen response
Serine	2.4	2.9	3.5	Amino acid	Protein, photorespiration, cysteine precursor
Succinic acid	2.9	+	1.8	Organic acid	TCA cycle
Threonine	1.9	2.8	2.3	Amino acid	Protein
Tryptophan	6.3	+	+	Amino acid	Protein, indole metabolism, camalexin synthesis
Valine	2.1	2.2	2.4	Amino acid	Protein, respiration (branched chain amino acid)

B

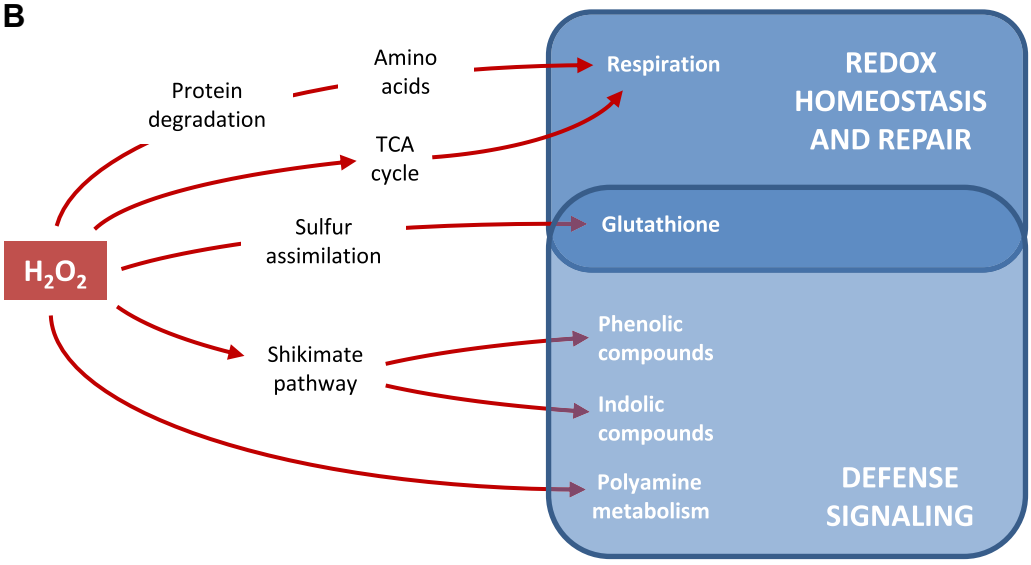

Fig. 5. Summary of metabolite profiling of oxidative stress responses in the *cat2* mutant. Extracts of polar metabolites were analyzed in methanolic extracts by gas chromatography–mass spectrometry (GC–MS). (A) Data show metabolites that were significantly different ($P < 0.05$) from Col-0 in three different studies. Study 1, Chaouch et al. (2010); study 2, Chaouch et al. (2012); study 3, Han et al. (2013a). Plants were sampled during growth in air in long day conditions. Decreases and increases in *cat2* are shown on green and red backgrounds, respectively, and the *cat2*/Col-0-fold-change is indicated. Where no number appears, +indicates detection in *cat2* but not in Col-0. The responses of all metabolites detected in the three studies are listed in Supplementary Table S1a and details of the GC–MS analysis are given in Supplementary Table S1b. (B) Scheme to summarize some of the main pathways impacted by excess intracellular H_2O_2.

Another glutamate-derived compound that has been linked to oxidative stress responses is γ-aminobutyrate (GABA; Bouché et al., 2003). Although GABA accumulated in *cat2* in one study, it remained at wild-type levels in the two others (Table S1). As in the case of proline, it may be that it is not GABA levels *per se*, but rather the metabolic pathway that is important in stress responses (Bao et al., 2014), possibly to provide succinate for the mitochondrial electron transport chain when other steps of the TCA cycle are inactivated (Studart-Guimarães et al., 2007).

Arginine is also derived from the glutamate skeleton, and is reproducibly increased in *cat2* (Fig. 5). Transcripts for enzymes related to arginine metabolism were among those responding to menadione treatment, leading to the proposal that arginine is a "reporter metabolite" for oxidative stress (Baxter et al., 2007). Adjustments in arginine may also be related to synthesis of polyamines, compounds that have long been proposed to play roles in oxidative stress, either as antioxidants or as substrates for ROS-producing oxidases (Bors et al., 1989; Moschou et al., 2008).

Both the simple polyamine, putrescine, and β-alanine, which can be produced from polyamines, were found to be reproducibly increased in *cat2* (Fig. 5).

Cysteine production by serine acetyl transferase activity has been shown to be among the factors limiting glutathione synthesis (Harms et al., 2000). Increases in *O*-acetylserine in *cat2* are very probably related to the marked increase in glutathione production (Figs. 3 and 4). Cysteine detected by GC–MS was increased significantly in *cat2* in two studies but not detected in the other (Supplementary Table S1). However, quantitative HPLC analysis showed that the total cyst(e)ine pool is consistently increased in *cat2*, by about 2-fold (Queval et al., 2009; Han et al., 2013b). Oxidative stress-triggered accumulation of cysteine and glutathione is often accompanied by increased amounts of the glutathione degradation products, Cys-Gly (Han et al., 2013b) and oxoproline (Supplementary Table S1). This probably reflects vacuolar turnover of GSSG and/or GS-conjugates initiated in the vacuole (Fig. 4).

7.3. Sugars and sugar alcohols

Several compounds in these classes are among plant metabolites that have received attention as potential antioxidants, for example, as scavengers of the hydroxyl radical (Smirnoff and Cumbes, 1989; Couée et al., 2006; Nishizawa et al., 2010). Metabolites that directly scavenge ROS might be expected to decrease in oxidative stress, unless their synthesis can be induced to compensate. Intriguingly, the only compound that was reproducibly decreased in *cat2* was *myo*-inositol (Fig. 5). This decrease seems to be necessary for activation of SA-dependent pathogenesis responses in *cat2*, which can be prevented simply by treating plants with *myo*-inositol during the onset of the stress (Chaouch and Noctor, 2010). Other studies provide strong evidence that this metabolite is a potentially important regulator of pathogenesis responses. Mutants for a specific *myo*-inositol phosphate synthase (MIPS1) show a lesion phenotype in long days or at increased growth irradiance (Meng et al., 2009; Donahue et al., 2010) that is similar to a spreading HR previously described in other lesion mimic mutants (Dietrich et al., 1994).

Several other sugar alcohols, including sorbitol, may be oxidized to sugars by NAD(P)-dependent dehydrogenases. Although sorbitol dehydrogenase has been studied more intensively in other species such as pear, an NAD-dependent Arabidopsis enzyme has recently been characterized (Nosarzewski et al., 2012; Aguayo et al., 2013). Interestingly, this enzyme can oxidize other sugar alcohols as well as sorbitol, including ribitol, and the increase in ribose in *cat2* (produced, perhaps, from its isomer, ribulose) could be partly linked to this enhanced activity of this enzyme. Several sugars that accumulate in *cat2* are components in cell wall polymers (arabinose, rhamnose), and this may reflect ROS-driven reorganization of the wall structure as a defense response (Müller et al., 2009).

Redox turnover of the ascorbate pool is likely to be enhanced in conditions of oxidative stress (Davletova et al., 2005). As noted above, changes in ascorbate status in *cat2* are often minor, probably reflecting the presence of more powerful reductants (glutathione, NADPH) that are able to ensure the efficient reduction of DHA inside the cell. However, enhanced ROS availability could promote DHA degradation in the apoplast (Parsons et al., 2011; Fig. 4). Consistent with this notion, threonic acid and oxalate were each significantly increased in at least one study of *cat2* metabolite profiles (Supplementary Table 1). Threonate was also observed to accumulate in menadione-treated Arabidopsis cells (Baxter et al., 2007). Taken together, these changes indicate that intracellular oxidative stress promotes some increase in apoplastic degradation of ascorbate.

8. Phytohormones and secondary metabolites

Increasing evidence suggests that phytohormone pathways are important in mediating oxidative stress signalling. Among the players are the DELLA proteins that regulate gibberellic acid responses (Achard et al., 2008) and abscisic acid (ABA), which is closely entwined with ROS-dependent pathways (Pastori and Foyer, 2002; Hu et al., 2006; Noctor et al., 2014). ABA-induced stomatal closure involves ROS produced by NADPH oxidases (Kwak et al., 2003). ABA is also implicated, together with ROS, in intercellular transmission of high light responses (Galvez-Valdivieso et al., 2009).

8.1. New roles for auxins in oxidative stress

The above findings underscore the tight integration of oxidative stress with signalling involved in growth and development. Recent work by several groups on catalase-deficient plants has uncovered important interactions with auxins. Leaf contents of these key phytohormone compounds are decreased by oxidative stress in *cat2*, an effect that has been nicely shown to be responsible for the hyponastic (upwardly curled) leaf phenotype that can be observed in the mutant in some conditions (Gao et al., 2014). Highly induced genes in catalase-deficient plants include a UDP-glycosyl transferase (UGT) with activity against indolebutyric acid that influences responses to drought (Tognetti et al., 2010). Further, treatment of young *cat2* plants with auxins or auxin-like compounds decreases lesions under conditions promoting high rates of photorespiration (Kerchev et al., 2014).

8.2. Close interactions between H_2O_2 and salicylic acid

Salicylic acid and H_2O_2 are known to interact closely in the response to biotic stress, although most of the research focus in this area has been on H_2O_2 or superoxide produced at the cell surface. While oxidative stress produced in the cell interior in the *cat2* mutant can drive several different phenotypes, the spreading lesion phenotype observed when the mutant is grown at moderate light in long days is dependent on SA. Both free and total (free + glycosylated forms) SA contents are markedly increased, and this is accompanied by strong induction of SA-dependent *PR* genes as well as of the gene encoding Isochorismate Synthase 1 (ICS1; Fig. 6), the key enzyme involved in production of SA during biotic stress (Wildermuth et al., 2001). A very marked increase in camalexin is also observed (Fig. 6), an indole phytoalexin which, like auxins, is produced from tryptophan (Glawischnig, 2007). Together with increases in phenylalanine and tryptophan contents (Fig. 5), this suggests that sustained production of intracellular H_2O_2 in the peroxisomes is sufficient to up-regulate the shikimate and related pathways in the absence of external stress. As well as SA itself, the isochorismate- or phenylalanine-dependent pathways can produce several structurally related metabolites following exposure to pathogens. These include benzoic acid as well as derivatives like *m*-hydroxybenzoic acid. In studies where they were detected, both compounds showed some increase in *cat2* (Supplementary Table S1). A dihydroxybenzoic acid-glycoside produced from isochorismate has been implicated in pathogenesis responses and ageing (Bartsch et al., 2010). This compound could play roles in oxidative stress, possible by controlling the availability of free iron and, therefore, cleavage of H_2O_2 through Fenton reactions (Bartsch et al., 2010).

The close relationship between oxidative stress-triggered induction of these pathways and those observed in response to stressors such as (hemi)biotrophic pathogens is clear. Blocking *ICS1* function using the *sid2* mutation is sufficient to abolish oxida-

tive stress-triggered SA accumulation and to prevent spreading lesions and increases in camalexin (Chaouch et al., 2010). Crucially, introducing the *sid2* mutation into the *cat2* background also prevents much of the GC–MS metabolite signature discussed above, even though other markers suggest that oxidative stress is still occurring. This suggests that a large part of the changes in metabolite profiles triggered by intracellular H_2O_2 is dependent on SA (Chaouch et al., 2010). Taken together, these observations suggest that the oxidative stress triggered by the *cat2* mutation mimics conditions elicited by certain biotic challenges particularly closely (Chaouch et al., 2012). A specific protein phosphatase 2A subunit appears to be a key player in controlling these oxidative stress-triggered metabolite signatures (Trotta et al., 2011; Li et al., 2014).

Perhaps not surprisingly, the activation of SA and related pathways in *cat2* leads to induced resistance to bacteria (Chaouch et al., 2010, 2012). Pathways that are involved in systemic acquired resistance (SAR) also seem to be involved. Azelaic acid is a nine-carbon dicarboxylic acid detected in the leaf exudates of infected plants (Jung et al., 2009). Together with the lipid transfer protein, AZI1, azelaic acid is involved in the activation of SAR (Jung et al., 2009). In one of our studies, azelaic acid was found to be increased (Chaouch et al., 2010; Supplementary Table S1), but the compound is not routinely detectable by GC–MS analysis of whole leaf tissue. Nevertheless, its role in signalling driven by intracellular H_2O_2 receives support from the induction of *AZI1* in *cat2* (Chaouch et al., 2010; Queval et al., 2012).

8.3. Jasmonic acid and oxylipins

As discussed earlier, intracellular oxidative stress can also activate pathways related to jasmonic acid (JA). In *cat2*, however, such effects are less clear than the activation of SA signalling, and are quite dependent on growth conditions. A study using a catalase inhibitor and catalase mutants reported that H_2O_2 produced inside the cell plays a role in potentiating methyl jasmonate-induced stomatal closure (Jannat et al., 2012). Leaf contents of the JA precursor, linolenic acid, showed a tendency to increase in *cat2*, although this effect was only statistically significant in one experiment (Supplementary Table S1). Are contents of JA itself induced by intracellular H_2O_2? To answer this question, an LC–MS protocol was developed that can measure SA at the same time (see Supplementary File S1 for details). Using this technique, an increase in JA of 5.5-fold was observed in wounded Col-0 leaves, a condition that we used as a positive control. In *cat2*, only a more modest increase was apparent, although it was statistically significant (Fig. 7). The trends for SA detected by LC-MS are consistent with those obtained by HPLC-fluorescence (Fig. 6). The *cat2* mutation was sufficient to produce increases in both free SA and SA-glucoside (SAG) to several-fold Col-0 values, in contrast to wounding, which did not affect these compounds (Fig. 7).

Together, the data of Figs. 6 and 7 are broadly consistent with an especially tight link between SA and H_2O_2 generated in the peroxisome, although they also suggest that the ensuing intracellular oxidative stress can simultaneously induce accumulation of JA. This suggests that at least part of the well-known antagonism between the two hormones operates subsequently to the oxidative trigger(s) that drive their accumulation. However, interactions between SA and JA may be complex. For example, synergistic effects can be observed during induction of pathogenesis responses and cell death by relatively low concentrations of the two compounds (Mur et al., 2006). There may also be substantial interplay or overlap between different oxidizing species or different intracellular compartments involved in these responses. For instance, high levels of lipid peroxides in a vitamin E-deficient mutant, whose primary effect is expected to be chloroplastic, can also cause marked accumulation of camalexin (Sattler et al., 2006). Studies on *cat2* carrying secondary mutations that target glutathione status implicate thiol-disulfide signalling (Mhamdi et al., 2010b; Han et al.,

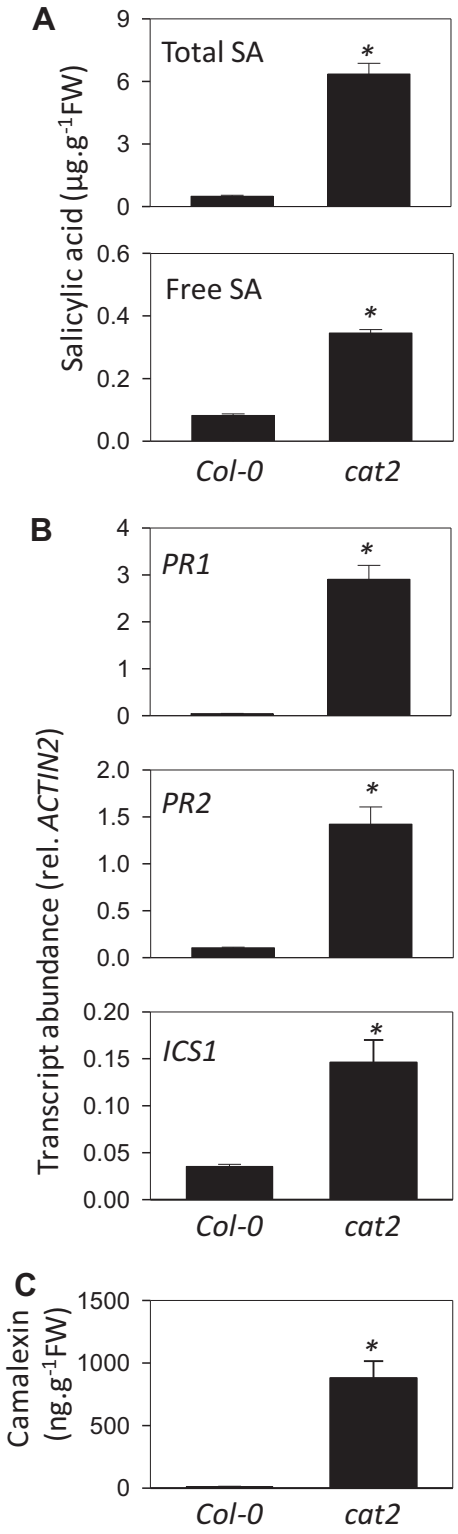

Fig. 6. Activation of salicylic acid (SA) and phytoalexin accumulation and related signaling by intracellular oxidative stress. Col-0 and *cat2* were grown as described in Fig. 3. (A) Total and free SA contents. (B) SA-related gene expression. (C) Camalexin contents. Salicylic acid and camalexin were measured by quantitative HPLC-fluorescence in leaf extracts as in Chaouch et al. (2010). *PR* and *ICS1* transcripts were measured by qPCR using primers described in Chaouch et al. (2010). Data are means ± SE of three to four independent biological repeats. *Significant difference from Col-0 at $P < 0.05$. ICS1, isochorismate synthase. PR, pathogenesis-related gene.

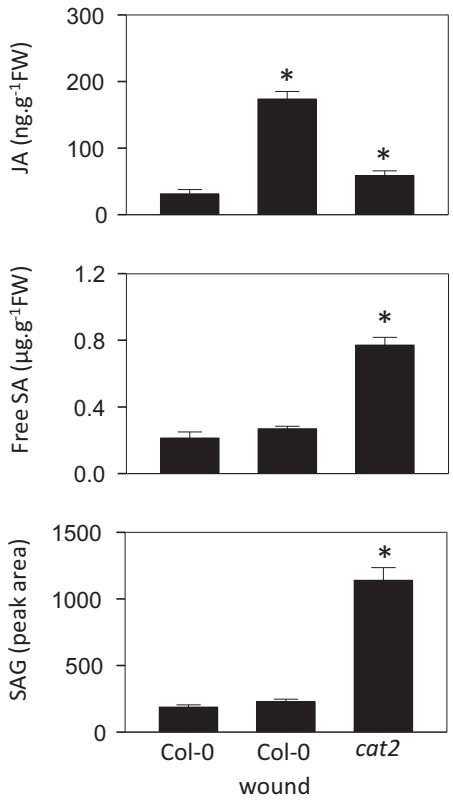

Fig. 7. Simultaneous analysis of jasmonic acid (JA) and salicylic acid (SA) by liquid chromatography–mass spectrometry. SAG, salicylic acid-glucoside. Samples were extracted and analyzed as described in Supplementary File S1. Wounded Col-0 leaves were analyzed as a positive control for JA accumulation, according to the protocol described in Supplementary File S1. Data are means ± SE of four independent biological repeats. *Significant difference from unwounded Col-0 at $P < 0.05$.

2013a,b). Oxylipin signalling involves a cyclophilin that, on binding 12-oxo-phytodienoic acid (OPDA), activates cysteine synthesis to modulate cell thiol status (Park et al., 2013). Another interaction with glutathione involves possible regulation of OPDA signalling intensity by formation of *S*-conjugates (Ohkamu-Ohtsu et al., 2011). Camalexin accumulation also draws on the glutathione pool, as its sulfur atom is derived from this key intracellular thiol (Su et al., 2011).

8.4. Other pathways

Early steps of the terpenoid synthesis pathway are gaining attention as generators of stress signals. Synthesis of isopentenyl diphosphate in the plastid occurs through the methylerythritol phosphate (MEP) pathway. Starting from a screen for mutants with altered expression of *Hydroperoxide Lyase*, it was shown that accumulation of methylerythritol cyclodiphosphate (MEcPP) triggers SA accumulation and bacterial resistance (Xiao et al., 2012). Levels of MEcPP were also found to be increased by high light and wounding, effects that may be at least partly related to oxidative stress (Xiao et al., 2012).

Although more information is available in animal systems, melatonin occurs in plants and has been implicated in oxidative stress regulation (Hardeland, 2014). In Brassica species, glucosinolates are an important class of stress-related compounds. A mutant deficient in Harmless to Ozone Layer1 (HOL1), so called because it has only low emissions of ozone-damaging methyl halides, shows decreased resistance to bacteria, associated with compromised ability to methylate glucosinolate breakdown products (Nagatoshi and Nakamura, 2009). Together with the glucosinolate-cleaving enzyme (myrosinase) and SA-linked PR proteins (PR2, PR5), HOL1 showed increased phosphopeptide signals in *cat2* and other mutants that show constitutive redox perturbation and increased resistance to pathogens (Trotta et al., 2011; Li et al., 2014). Like camalexin, glucosinolates contain sulfur atoms derived from glutathione (Geu-Flores et al., 2009). Among other things, this might favour coordination of glucosinolate and glutathione synthesis during stress. Genetic deficiency of glutathione in Arabidopsis decreases glucosinolate contents and resistance to insects (Schlaeppi et al., 2008).

9. Clues from oxidative stress transcriptomics

As noted at the beginning of this review, metabolite profiling has technical limitations which mean that no single method currently provides as much coverage of different pathways as transcriptomics analysis through microarray or RNA sequencing. In addition to direct measurements of metabolites, there is considerable evidence from transcriptomics studies that specific secondary metabolism pathways are up-regulated by oxidative stress. In catalase-deficient mutants, the most strongly up-regulated genes typically include those encoding specific UGTs, GSTs, or CYPs. These enzymes are involved in detoxifying and/or regulating the biological activity of metabolites of external or endogenous origin, and some are rapidly induced after transferring catalase-deficient plants to oxidative stress conditions (Vandenabeele et al., 2004; Vanderauwera et al., 2005). In a microarray study of *cat2* (Queval et al., 2012), 35 genes were found to be induced more than 10-fold (Fig. 8). Of these, more than half encoded enzymes, but none of these belonged to the core antioxidative or reductant-generating systems (superoxide dismutase, catalases, peroxidases, ascorbate and glutathione-linked reductases, etc). This underscores the relatively constitutive nature of much of the primary ROS-processing system, which is maybe not surprising given that generation of ROS is a constant feature of primary metabolism and that plants in natural environments have to be prepared for rapid fluctuations in rates of generation.

Glutathione *S*-transferases accelerate the attachment of GSH to electrophilic substrates, including xenobiotic pollutants and endogenous metabolites, although many may also may have other functions, notably as peroxidases (Dixon et al., 2009). All three of the strongly induced GSTs (GSTU1, GSTU24, GSTU25) in *cat2* were also induced in Arabidopsis exposed to herbicides and trinitrotoluene (TNT), alongside genes encoding enzymes of ascorbate regeneration (Ekman et al., 2003; Mezzari et al., 2005). Functional studies suggest that GSUTU24 and U25 are important in TNT degradation (Gunning et al., 2014). The three *GSTU* genes can also be strongly induced by SA treatment (Sappl et al., 2009), although our own studies suggest that their marked induction by oxidative stress does not require increases in SA (Chaouch and Noctor, 2010; Queval et al., 2012).

In other detoxification pathways, CYP catalyze hydroxylation/oxidation reactions, often producing a functional group that is then glycosylated by UGTs. Like GSH conjugation *via* GST action, the introduction of a sugar tag enables recognition by transporters, notably located on the tonoplast. Also like GSTs, CYP and UGT functions extend beyond metabolism of xenobiotics, and include synthesis of secondary metabolites and regulation of secondary signalling molecules produced by ROS-dependent reactions, including phytohormone-related molecules like SA and OPDA (Lim et al., 2002; Ohkamu-Ohtsu et al., 2011; Su et al., 2011; Farmer and Mueller, 2013). At least two CYPs are induced >10-fold in *cat2* (Fig. 8). Both CYPs can be induced by phytoprostanes although their substrates have not been identified (Loeffler et al.,

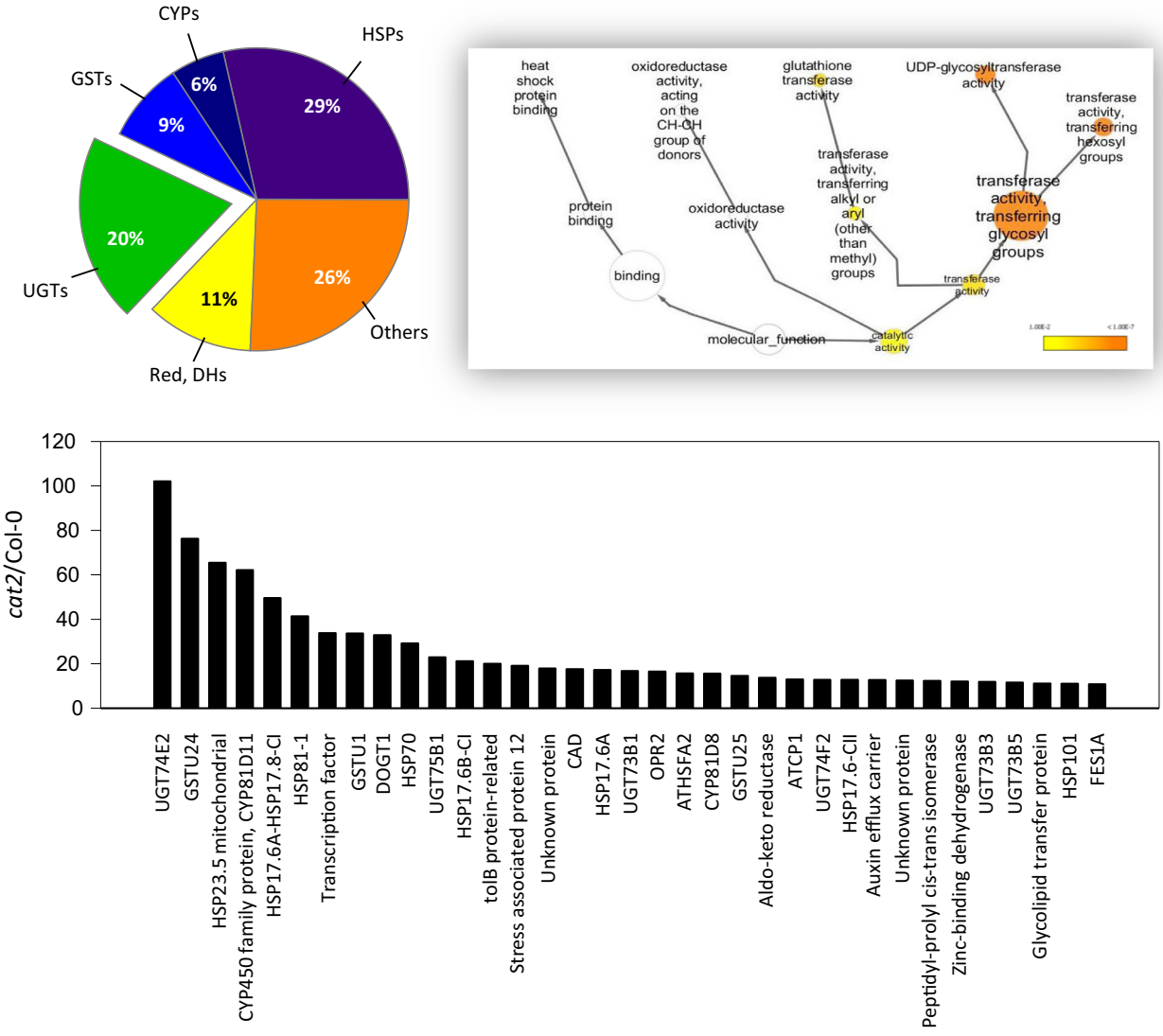

Fig. 8. Genes that are highly induced by oxidative stress. Microarray data published by Queval et al. (2012) were mined for significantly different genes (*cat2*/Col-0, $P < 0.001$, $n = 3$ biological replicates) that were induced more than 10-fold in *cat2*. The histogram shows fold changes for each gene and full descriptions are given in Supplementary Table S2. The pie chart (top left) shows the distribution of the genes between different classes and indicates the marked enrichment in metabolite-conjugating enzymes, also evident from the Biological Networks Gene Ontology tool (BINGO) analysis (top right; Maere et al., 2005). CYP, cytochrome P450. GST, glutathione *S*-transferase. HSP, heat shock protein/factor. Red, DHs, reductase/dehydrogenase. UGT, UDP-glycosyl transferase.

2005; Mueller et al., 2008; Köster et al., 2012). *CYP81D11* is also induced by other compounds such as allelochemicals and herbicides, and by insects as well as biotrophic and necrotrophic pathogens (Matthes et al., 2011; Köster et al., 2012). Induction of *CYP81D11* by both JA and other apparently unrelated compounds required components of the JA signalling pathway to be active (Köster et al., 2012). It is unclear whether H_2O_2 induction of this gene also requires an active JA signalling pathway.

The most highly induced gene in *cat2* was *UGT74E2* (Fig. 8). Functional characterization showed that UGT74E2 is active against indole butyric acid and that the enzyme is implicated in tolerance to drought stress (Tognetti et al., 2010). Attention has been drawn to the role of other H_2O_2-induced UGTs (UGT73b3, UGT73b5) in regulating redox state and metabolism during pathogenesis (Simon et al., 2014), although their substrates remain to be identified. Indeed, four of the seven highly H_2O_2-induced UGTs were among six induced by SA independently of the SA signalling regulator, Nonexpressor Of Pathogenesis-Related Genes1 (NPR1; Blanco et al., 2009), suggesting that they may be part of the early response to this signalling molecule. Of these four, UGT74F2 is one of two able to glycosylate SA (Lim et al., 2002; Dean and Delaney, 2008). Unlike UGT74F1, UGT74F2 is able to glycosylate the SA carboxy hydroxyl group, although it is the less active of the two in glycosylating the 2-hydroxy group (Dean and Delaney, 2008). Interestingly, *UGT74F2* was the only one of these two genes induced in *cat2*, suggesting that the encoded enzyme might be responsible for a significant part of the accumulated SA glycosides driven by intracellular oxidative stress (Figs. 6 and 7).

Another UGT that is also inducible by SA independently of NPR1 is UGT75B1, which has been implicated in callose formation (Hong et al., 2001), an important response against biotic invaders. This UGT was the most effective of three able to glycosylate the folic acid precursor, *p*-aminobenzoic acid (Eudes et al., 2008). The link between folic acid and oxidative stress is not clear. Conceivably, methionine cycle reactions may be important, based on the accumulation of this amino acid in *cat2* (Fig. 5) and the accumulation of the methionine cycle enzyme, *S*-adenosylhomocysteine hydrolase, in mutant lines showing constitutive activation of ROS-associ-

ated pathogenesis-related responses (Trotta et al., 2011; Li et al., 2014). In the *cat2* context, it may also be noteworthy that photorespiratory metabolism relies on folate derivatives for the conversion of glycine to serine in the mitochondria. One of the other H_2O_2-induced UGTs (UGT73C5, or DOGT1) is active against brassinosteroids (Husar et al., 2011). Together with the links to SA, JA, and auxins noted above, this further emphasizes the close relationships between oxidative stress-induced redox signalling and hormone function.

Catalase deficiency and high light act antagonistically in regulating the expression of genes involved in the phenylpropanoid pathway leading to anthocyanin generation (Vanderauwera et al., 2005). This observation underlines the complex interactions involved in oxidative stress responses, since high light is considered to involve increased ROS production, and anthocyanins and flavonoids to have antioxidant activity (Zhang et al., 2013). It may partly reflect antagonism between different ROS, or between ROS produced in different compartments (Gadjev et al., 2006).

While their precise functions remain to be established, the identity of the highly induced reductases and dehydrogenases in *cat2* further emphasizes the links to pathogenesis-induced changes in cell wall metabolism and to regulation of compounds produced secondarily from ROS accumulation. They include a member of the cinnamyl-alcohol dehydrogenase family (At1g09500), which is pathogen-induced, associated with lignin biosynthesis, and negatively controlled by the transcription factor, WRKY7 (Kim et al., 2006). The aldo–keto reductase encoded by At2g37770 (AKR4C9) is active against reactive carbonyls that could be produced from sugar or lipid oxidation (Yamauchi et al., 2011; Saito et al., 2013) while At5g16980 is induced by phytoprostanes (Mueller et al., 2008). Thus, the redox changes triggered by modified H_2O_2 metabolism in *cat2* may induce secondary production of these reactive compounds. If so, this may be important in modulating the phytohormone-related pathways discussed above.

10. Conclusions and perspectives

The impact of oxidative stress on plant metabolism is complex and intricate. Metabolite structure or abundance can be modified through multiple types of mechanism (Fig. 1). Increased ROS may modulate metabolites through both enzymatic and non-enzymatic reactions. The prevailing view is that singlet oxygen drives lipid peroxide signatures mainly through enzymatic pathways under physiological conditions, and that non-enzymatic oxidation of carotenoids is likely to generate important signal metabolites. In the case of H_2O_2, non-enzymatic reactions are unlikely to play an important role, unless the hydroxyl radical is produced. H_2O_2 does not react at significant rates even with highly reducing molecules such as ascorbate and glutathione: catalysts (peroxidases) are required (Rahantaniaina et al., 2013). Inside the cell, peroxidases have a largely antioxidant function that, with catalase, keeps H_2O_2 low under most conditions. The main physiological importance of the many types of metabolite to which direct antioxidant roles have been ascribed is not likely to be in removing H_2O_2, but rather in scavenging the hydroxyl radical that can be generated if H_2O_2 accumulates in the presence of Fenton reagents such as iron. Hence, changes in iron homeostasis may trigger responses that partly recapitulate those elicited by oxidative stress (Sudre et al., 2013). The hydroxyl radical plays an important role in cell well metabolism. Outstanding questions concern the roles of the numerous apoplastic class III peroxidases found in plants, notably which metabolite substrates they oxidize (O'Brien et al., 2012a,b; Daudi et al., 2012).

Whether they be carotenoids, fatty acid chains, or soluble antioxidants, metabolites that interact closely with ROS are likely to be close to the source of signals perceived by the cell. Peptides produced from protein breakdown may also act as indicators of oxidative stress (Møller and Sweetlove, 2010). A potentially important emerging concept is that of metabolite repair (Linster et al., 2013). While such repair reactions may require significant energy expenditure, they could be important in determining cell longevity and survival, particularly in stress conditions. Products of glutathione oxidation may strongly influence H_2O_2-dependent signaling, while peroxide-catalyzed oxidation of sensitive protein thiol groups on regulatory proteins will influence the orchestration of metabolic sequences at transcriptional or post-transcriptional levels. At the level of specific enzymes, post-translational modifications of cysteine and methionine groups could be key in promoting re-direction of metabolism. Significant bottlenecks for progress here are that while many proteomics studies have allowed list of such proteins to be constructed, the extent and impact of the modification *in vivo* are in most cases not yet well characterized. Unresolved questions include effects on protein activity, as well as quantification of what proportion of residues within the population of proteins are affected.

The accumulation of ROS-triggered metabolites may be a truer marker for oxidative stress than ROS signals themselves. One important implication of the close relationship between ROS and phytohormones is that the tightness of the coupling between oxidative stress and downstream responses can be variable. This point is important for research strategies searching to manipulate plant response to oxidative stress because it implies that other avenues may be possible besides one that seeks to enhance stress resistance by reinforcing antioxidant capacity. Rather, it may be possible to search for factors that modulate the outcome of increased ROS, rather than ROS accumulation *per se*. Such factors may include metabolites whose content or nature is influenced by oxidative stress in a way that can be perceived by plants.

Compartmentation remains a key issue, both with respect to metabolomics in general and to redox-dependent changes in particular. Redox gradients between compartments may be important in sensing, possibly driven by membrane-bound proteins with redox-sensitive groups on either side of the membrane. Redox sensors based on green fluorescent proteins that provide information on cell thiol-disulfide status are already available (Jiang et al., 2006; Schwarzländer et al., 2008; Jubany-Mari et al., 2010), and the development of others that are specific for ROS such as H_2O_2 is ongoing (Esposito-Rodriguez et al., 2013). These techniques could help to resolve issues surrounding ROS concentrations in different plant compartments.

Notwithstanding the problems that compartmentation poses for data interpretation, metabolite signatures are interesting in that they may provide an indicator of the extent and influence of oxidative stress in given conditions. One example is the accumulation of branched chain amino acids induced by ABA treatment (Ghassemian et al., 2008), a response that seems to occur reproducibly in oxidative stress (Fig. 5). Nevertheless, just as there is no single oxidative stress transcriptome (Gadjev et al., 2006), it is clear that there cannot be a single "oxidative stress metabolome". Apart from specific effects produced by location and the type of ROS, the metabolic response to increased oxidative load is likely or be dynamic and plastic. Dissecting the regulatory networks that underlie this complexity will require considerable further study.

Acknowledgments

The authors' research in this area is supported by The French Agence Nationale de la Recherche project "Cynthiol", Project No. ANR12–BSV6–0011. We thank Patrick Saindrenan, IBP, Orsay, for fruitful collaborations and HPLC facilities.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2014.09.002.

References

Achard, P., Renou, J.P., Berthomé, R., Harberd, N.P., Genschik, P., 2008. Plant DELLAs restrain growth and promote survival of adversity by reducing the levels of reactive oxygen species. Curr. Biol. 18, 656–660.

Aguayo, M.F., Ampuero, D., Mandujano, P., Parada, R., Munoz, R., Gallart, M., Altabella, T., Cabrera, R., Stange, C., Handford, M., 2013. Sorbitol dehydrogenase is a cytosolic protein required for sorbitol metabolism in *Arabidopsis thaliana*. Plant Sci. 205–206, 63–75.

Araújo, W.L., Ishizaki, K., Nunes-Nesi, A., Larson, T.R., Tohge, T., Krahnert, I., Witt, S., Obata, T., Schauer, N., Graham, I.A., Leaver, C.J., Fernie, A.R., 2010. Identification of the 2-hydroxyglutarate and isovaleryl-CoA dehydrogenases as alternative electron donors linking lysine catabolism to the electron transport chain of Arabidopsis mitochondria. Plant Cell 22, 1549–1563.

Araújo, W.L., Tohge, T., Ishizaki, K., Leaver, C.J., Fernie, A.R., 2011. Protein degradation – an alternative respiratory substrate for stressed plants. Trends Plant Sci. 16, 489–498.

Baldacci-Cresp, F., Chang, C., Maucourt, C., Deborde, C., Hopkins, J., Lecomte, P., Bernillon, S., Brouquisse, R., Moing, A., Abad, P., Hérouart, D., Puppo, A., Favery, B., Frendo, P., 2012. (Homo)glutathione deficiency impairs root-knot nematode development in *Medicago truncatula*. PLoS Pathog. 8, e1002471.

Ball, L., Accotto, G., Bechtold, U., Creissen, G., Funck, D., Jimenez, A., Kular, B., Leyland, N., Mejia-Carranza, J., Reynolds, H., Karpinski, S., Mullineaux, P.M., 2004. Evidence for a direct link between glutathione biosynthesis and stress defense gene expression in Arabidopsis. Plant Cell 16, 2448–2462.

Bao, H., Chen, X., Lv., S., Jiang, P., Feng, J., Fan, P., Nie, L., Li, Y., 2014. Virus-induced gene silencing reveals control of reactive oxygen species accumulation and salt tolerance in tomato by γ-aminobutyric acid metabolic pathway. Plant Cell Environ. http://dx.doi.org/10.1111/pce.12419.

Barranco-Medina, S., Krell, T., Finkemeier, I., Sevilla, F., Lázaro, J.J., Dietz, K.J., 2007. Biochemical and molecular characterization of the mitochondrial peroxiredoxin PsPrxII F from *Pisum sativum*. Plant Physiol. Biochem. 45, 729–739.

Bartsch, M., Bednarek, P., Vivancos, P.D., Schneider, B., von Roepenack-Lahaye, E., Foyer, C.H., Kombrink, E., Scheel, D., Parker, J.E., 2010. Accumulation of isochorismate-derived 2,3-dihydroxybenzoic 3-*O*-β-D-xyloside in *Arabidopsis* resistance to pathogens and ageing of leaves. J. Biol. Chem. 285, 25654–25665.

Baxter, C.J., Redestig, H., Schauer, N., Repsilber, D., Patil, K.R., Nielsen, J., Selbig, J., Liu, J., Fernie, A.R., Sweetlove, L.J., 2007. The metabolic response of heterotrophic Arabidopsis cells to oxidative stress. Plant Physiol. 143, 312–325.

Benina, M., Ribeiro, D.M., Gechev, T.S., Müller-Röber, B., Schippers, J.H.M., 2014. A cell type-specific view on the translation of mRNAs from ROS-responsive genes upon paraquat treatment of *Arabidopsis thaliana* leaves. Plant Cell Environ. http://dx.doi.org/10.1111/pce.12355.

Bestwick, C.S., Brown, I.R., Bennett, M.H.R., Mansfield, J.W., 1997. Localization of hydrogen peroxide accumulation during the hypersensitive reaction of lettuce cells to *Pseudomonas syringae* pv *phaseolicola*. Plant Cell 9, 209–221.

Bick, J.A., Setterdahl, A.T., Knaff, D.B., Chen, Y., Pitcher, L.H., Zilinskas, B.A., Leustek, T., 2001. Regulation of the plant-type 5′-adenylyl sulfate reductase by oxidative stress. Biochemistry 40, 9040–9048.

Bienert, G.P., Moller, A.L., Kristiansen, K.A., Schulz, A., Moller, I.M., Schjoerring, J.K., Jahn, T.P., 2007. Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. J. Biol. Chem. 282, 1183–1192.

Bindschedler, L.V., Dewdney, J., Blee, K.A., Stone, J.M., Asai, T., Plotnikov, J., Denoux, C., Hayes, T., Gerrish, C., Davies, D.R., Ausubel, F.M., Bolwell, G.P., 2006. Peroxidase-dependent apoplastic oxidative burst in Arabidopsis required for pathogen resistance. Plant J. 47, 851–863.

Blanco, F., Salinas, P., Cecchini, N.M., Jordana, X., Van Hummelen, P., Alvarez, M.E., Holuigue, L., 2009. Early genomic responses to salicylic acid in *Arabidopsis*. Plant Mol. Biol. 70, 79–102.

Bolton, M.D., 2009. Primary metabolism and plant defense – fuel for the fire. Mol. Plant Microbe Interact. 22, 487–497.

Borisova, M.M., Kozuleva, M.A., Rudenko, N.N., Naydov, I.A., Klenina, I.B., Ivanov, B.N., 2012. Photosynthetic electron flow to oxygen and diffusion of hydrogen peroxide through the chloroplast envelope via aquaporins. Biochim. Biophys. Acta 1817, 1314–1321.

Bors, W., Langebartels, C., Michel, C., Sandermann, H., 1989. Polyamines as radical scavengers and protectants against ozone damage. Phytochemistry 28, 1589–1595.

Bouché, N., Fait, A., Bouchez, D., Møller, S.G., Fromm, H., 2003. Mitochondrial succinic-semialdehyde dehydrogenase of the γ-aminobutyrate shunt is required to restrict levels of reactive oxygen intermediates in plants. Proc. Natl. Acad. Sci. U.S.A. 100, 6843–6848.

Browse, J., 2009. Jasmonate passes muster: a receptor and targets for the defense hormone. Annu. Rev. Plant Biol. 60, 183–205.

Chaouch, S., Noctor, G., 2010. *Myo*-inositol abolishes salicylic acid-dependent cell death and pathogen defence responses triggered by peroxisomal H_2O_2. New Phytol. 188, 711–718.

Chaouch, S., Queval, G., Vanderauwera, S., Mhamdi, A., Vandorpe, M., Langlois-Meurinne, M., Van Breusegem, F., Saindrenan, P., Noctor, G., 2010. Peroxisomal hydrogen peroxide is coupled to biotic defense responses by ISOCHORISMATE SYNTHASE1 in a daylength-related manner. Plant Physiol. 153, 1692–1705.

Chaouch, S., Queval, G., Noctor, G., 2012. AtRbohF is a crucial modulator of defence-associated metabolism and a key actor in the interplay between intracellular oxidative stress and pathogenesis responses in Arabidopsis. Plant J. 69, 613–627.

Christ, B., Sussenbacher, I., Moser, S., Bichsel, N., Egert, A., Muller, T., Krautler, B., Hortensteiner, S., 2013. Cytochrome P450 CYP89A9 is involved in the formation of major chlorophyll catabolites during leaf senescence in *Arabidopsis*. Plant Cell 25, 1868–1880.

Christ, B., Egert, A., Sussenbacher, I., Krautler, B., Bartels, D., Peters, S., Hortensteiner, S., 2014. Water deficit induces chlorophyll degradation via the "PAO/phyllobilin" pathway in leaves of homoio- (*Craterostigma pumilum*) and poikilochlorophyllous (*Xerophyta viscosa*) resurrection plants. Plant Cell Environ. http://dx.doi.org/10.1111/pce.12308.

Colville, L., Smirnoff, N., 2008. Antioxidant status, peroxidase activity, and PR protein transcript levels in ascorbate-deficient *Arabidopsis thaliana vtc* mutants. J. Exp. Bot. 59, 3857–3868.

Conklin, P.L., Williams, E.H., Last, R.L., 1996. Environmental stress sensitivity of an ascorbic acid-deficient *Arabidopsis* mutant. Proc. Natl. Acad. Sci. U.S.A. 93, 9970–9974.

Costa, A., Drago, A., Behera, S., Zottini, M., Pizzo, P., Schroeder, J.I., Pozzan, T., Lo Schiavo, F., 2010. H_2O_2 in plant peroxisomes: an in vivo analysis uncovers a Ca^{2+}-dependent scavenging system. Plant J. 62, 760–772.

Couée, I., Sulmon, C., Gouesbet, G., El Amrani, A., 2006. Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. J. Exp. Bot. 57, 449–459.

Däschner, K., Couée, I., Binder, S., 2001. The mitochondrial isovaleryl-CoA dehydrogenase of Arabidopsis oxidizes intermediates of leucine and valine catabolism. Plant Physiol. 126, 601–612.

Dat, J.F., Pellinen, R., Beeckman, T., Van De Cotte, B., Langebartels, C., Kangasjärvi, J., Inzé, D., Van Breusegem, F., 2003. Changes in hydrogen peroxide homeostasis trigger an active cell death process in tobacco. Plant J. 33, 1–12.

Daudi, A., Cheng, Z., O'Brien, J.A., Mammarella, N., Khan, S., Ausubel, F.M., Bolwell, G.P., 2012. The apoplastic oxidative burst peroxidase in Arabidopsis is a major component of pattern-triggered immunity. Plant Cell 24, 275–287.

Davletova, S., Rizhsky, L., Liang, H., Shengqiang, Z., Oliver, D.J., Coutu, J., Shulaev, V., Schlauch, K., Mittler, R., 2005. Cytosolic ascorbate peroxidase 1 is a central component of the reactive oxygen gene network of Arabidopsis. Plant Cell 17, 268–281.

Davoine, C., Falletti, O., Douki, T., Iacazio, G., Ennar, N., Montillet, J.L., Triantaphylides, C., 2006. Adducts of oxylipin electrophiles to glutathione reflect a 13 specificity of the downstream lipoxygenase pathway in the tobacco hypersensitive response. Plant Physiol. 140, 1484–1493.

De Block, M., Verduyn, C., De Brouwer, D., Cornelissen, M., 2005. Poly(ADP-ribose) polymerase in plants affects energy homeostasis, cell death and stress tolerance. Plant J. 41, 95–106.

Dean, J.V., Delaney, S.P., 2008. Metabolism of salicylic acid in wild-type, *ugt74f1* and *ugt74f2* glucosyltransferase mutants of *Arabidopsis thaliana*. Physiol. Plant 132, 417–425.

del Río, L.A., Sandalio, L.M., Corpas, F.J., Palma, J.M., Barroso, J.B., 2006. Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging, and role in cell signaling. Plant Physiol. 141, 330–335.

Dellapenna, D., Mène-Saffrané, L., 2011. Vitamin E. Adv. Bot. Res. 59, 179–227.

Demmig-Adams, B., 1990. Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin. Biochim. Biophys. Acta 1020, 1–24.

Dghim, A.A., Mhamdi, A., Vaultier, M.V., Hasenfratz-Sauder, M.P., Le Thiec, D., Dizengremel, P., Noctor, G., Jolivet, E., 2013. Analysis of cytosolic isocitrate dehydrogenase and glutathione reductase 1 in photoperiod-influenced responses to ozone using Arabidopsis knockout mutants. Plant Cell Environ. 36, 1981–1991.

Dietrich, R.A., Delaney, T.P., Uknes, S.J., Ward, E.R., Ryals, J.A., Dangl, J.L., 1994. Arabidopsis mutants simulating disease resistance response. Cell 7, 565–572.

Dixon, D.P., Skipsey, M., Grundy, N.M., Edwards, R., 2005. Stress-induced protein *S*-glutathionylation in Arabidopsis. Plant Physiol. 138, 2233–2244.

Dixon, D.P., Hawkins, T., Hussey, P.J., Edwards, R., 2009. Enzyme activities and subcellular localization of members of the Arabidopsis glutathione transferase superfamily. J. Exp. Bot. 60, 1207–1218.

Dizengremel, P., Le Thiec, D., Hasenfratz-Sauder, M.-P., Vaultier, M.-N., Bagard, M., Jolivet, Y., 2009. Metabolic-dependent changes in plant cell redox power after ozone exposure. Plant Biol. 11, 35–42.

Donahue, J.L., Alford, S.R., Torabinejad, J., Kerwin, R.E., Nourbakhsh, A., Ray, W.K., Hernick, M., Huang, X., Lyons, B.M., Hein, P.P., et al., 2010. The *Arabidopsis thaliana Myo*-inositol 1-phosphate synthase1 gene is required for *Myo*-inositol synthesis and suppression of cell death. Plant Cell 22, 888–903.

Ekman, D.R., Lorenz, W.W., Przybyla, A.E., Wolfe, N.L., Dean, J.F.D., 2003. SAGE analysis of transcriptome responses in Arabidopsis roots exposed to 2,4,6-trinitrotoluene. Plant Physiol. 133, 1397–1406.

Engqvist, M.K., Kuhn, A., Wienstroer, J., Weber, K., Jansen, E.E., Jakobs, C., Weber, A.P., Maurino, V., 2011. Plant D-2-hydroxyglutarate dehydrogenase participates in the catabolism of lysine especially during senescence. J. Biol. Chem. 286, 11382–11390.

Esposito-Rodriguez, M., Laissue, P.P., Littlejohn, G.R., Smirnoff, N., Mullineaux, P.M., 2013. The use of HyPer to examine spatial and temporal changes in H2O2 in high light-exposed plants. Methods Enzymol. 527, 185–201.

Eudes, A., Bozzo, G.G., Waller, J.C., Naponelli, V., Lim, E.L., Bowles, D.J., Gregory, J.F., Hanson, A.D., 2008. Metabolism of the folate precursor *p*-aminobenzoate in plants. Glucose ester formation and vacuolar storage. J. Biol. Chem. 283, 15451–15459.

Farmer, E.E., Mueller, M.J., 2013. ROS-mediated lipid peroxidation and RES-activated signaling. Annu. Rev. Plant Biol. 64, 429–450.

Feechan, A., Kwon, E., Yun, B.W., Wang, Y., Pallas, J.A., Loake, G.J., 2005. A central role for *S*-nitrosothiols in plant disease resistance. Proc. Natl. Acad. Sci. U.S.A. 102, 8054–8059.

Fernie, A.R., Trethewey, R.N., Krotzky, A.J., Willmitzer, L., 2004. Metabolite profiling: from diagnostics to systems biology. Nat. Rev. Mol. Cell Biol. 5, 763–769.

Fischer, B.B., Hideg, E., Krieger-Liszkay, A., 2013. Production, detection, and signaling of singlet oxygen in photosynthetic organisms. Antioxid. Redox Signal. 18, 2145–2162.

Foyer, C.H., Noctor, G., 2003. Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiol. Plant 119, 355–364.

Foyer, C.H., Noctor, G., 2009. Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. Antioxid. Redox Signal. 11, 861–905.

Foyer, C.H., Noctor, G., 2011. Ascorbate and glutathione: the heart of the redox hub. Plant Physiol. 155, 2–18.

Fridovich, I., 1997. Superoxide anion radical, superoxide dismutases, and related matters. J. Biol. Chem. 272, 18515–18517.

Gadjev, I., Vanderauwera, S., Gechev, T.S., Laloi, C., Minkov, I.N., Shulaev, V., Apel, K., Inzé, D., Mittler, R., Van Breusegem, F., 2006. Transcriptomic footprints disclose specificity of reactive oxygen species signaling in Arabidopsis. Plant Physiol. 141, 436–445.

Galili, G., 1995. Regulation of lysine and threonine synthesis. Plant Cell 7, 899–906.

Galvez-Valdivieso, G., Fryer, M.J., Lawson, T., Slattery, K., Truman, W., Smirnoff, N., Asami, T., Davies, W.J., Jones, A.M., Baker, N.R., Mullineaux, P.M., 2009. The high light response in *Arabidopsis* involves ABA signaling between vascular and bundle sheath cells. Plant Cell 21, 2143–2162.

Gao, X., Yuan, H.M., Hu, Y.Q., Li, J., Lu, Y.T., 2014. Mutation of Arabidopsis CATALASE2 results in hyponastic leaves by changes of auxin levels. Plant Cell Environ. 37, 175–188.

Gatzek, S., Wheeler, G.L., Smirnoff, N., 2002. Antisense suppression of L-galactose dehydrogenase in *Arabidopsis thaliana* provides evidence for its role in ascorbate synthesis and reveals light-modulated L-galactose synthesis. Plant J. 30, 541–553.

Geu-Flores, F., Nielsen, M.T., Nafisi, M., Møldrup, M.E., Olsen, C.E., Motawia, M.S., Halkier, B.A., 2009. Glucosinolate engineering identifies a γ-glutamyl peptidase. Nat. Chem. Biol. 5, 575–577.

Ghassemian, M., Lutes, J., Chang, H.S., Lange, I., Chen, W., Zhu, T., Wang, X., Lange, B.M., 2008. Abscisic acid-induced modulation of metabolic and redox control pathways in *Arabidopsis thaliana*. Phytochemistry 69, 2899–2911.

Glawischnig, E., 2007. Camalexin. Phytochemistry 68, 401–406.

Gomez, L., Vanacker, H., Buchner, P., Noctor, G., Foyer, C.H., 2004a. The intercellular distribution of glutathione synthesis and its response to chilling in maize. Plant Physiol. 134, 1662–1671.

Gomez, L.D., Noctor, G., Knight, M., Foyer, C.H., 2004b. Regulation of calcium signaling and gene expression by glutathione. J. Exp. Bot. 55, 1851–1859.

Goyer, A., Johnson, T.L., Olsen, L.J., Collakova, E., Shachar-Hill, Y., Rhodes, D., Hanson, A.D., 2004. Characterization and metabolic function of a peroxisomal sarcosine and pipecolate oxidase from Arabidopsis. J. Biol. Chem. 279, 16947–16953.

Grace, S.C., Logan, B.A., 1996. Acclimation of foliar antioxidant systems to growth irradiance in three broad-leaved evergreen species. Plant Physiol. 112, 1631–1640.

Green, M.A., Fry, S.C., 2005. Vitamin C degradation in plant cells via enzymatic hydrolysis of 4-*O*-oxalyl-L-threonate. Nature 433, 83–87.

Grzam, A., Martin, M.N., Hell, R., Meyer, A.J., 2007. γ-Glutamyl transpeptidase GGT4 initiates vacuolar degradation of glutathione *S*-conjugates in Arabidopsis. FEBS Lett. 581, 3131–3138.

Gunning, V., Tzafestas, K., Sparrow, H., Johnston, E.J., Brentnall, A.S., Potts, J.R., Rylott, E.L., Bruce, N.C., 2014. Arabidopsis glutathione transferases exhibit a range of detoxification activities on the environmental pollutant and explosive 2,4,6-trinitrotoluene. Plant Physiol. 165, 854–865.

Hackenberg, T., Juul, T., Auzina, A., Gwiżdż, S., Małolepszy, A., Lehmann Nielsen, K., Jørgensen, J.E., Hofius, D., Van Breusegem, F., Petersen, M., Andersen, S.U., 2013. Catalase and its regulator NO CATALASE ACTIVITY 1 (NCA1) promote autophagy-dependent cell death in Arabidopsis. Plant Cell 25, 4616–4626.

Hagedorn, P.H., Flyvbjerg, H., Møller, I.M., 2007. Modelling NADH turnover in plant mitochondria. Physiol. Plant 120, 370–385.

Halliwell, B., 1996. Antioxidants in human health and disease. Annu. Rev. Nutr. 16, 33–50.

Han, Y., Chaouch, S., Mhamdi, A., Queval, G., Zechmann, B., Noctor, G., 2013a. Functional analysis of Arabidopsis mutants points to novel roles for glutathione in coupling H_2O_2 to activation of salicylic acid accumulation and signaling. Antioxid. Redox Signal. 18, 2087–2090.

Han, Y., Mhamdi, A., Chaouch, S., Noctor, G., 2013b. Regulation of basal and oxidative stress-triggered jasmonic acid-related gene expression by glutathione. Plant Cell Environ. 36, 1135–1146.

Hancock, J.T., Henson, D., Nyirenda, M., Desikan, R., Harrison, J., Lewis, M., Hughes, J., Neill, S.J., 2005. Proteomic identification of glyceraldehyde 3-phosphate dehydrogenase as an inhibitory target of hydrogen peroxide in Arabidopsis. Plant Physiol. Biochem. 43, 828–835.

Hardeland, R., 2014. Melatonin in plants and other phototrophs – advances and gaps concerning the diversity of functions. J. Exp. Bot. http://dx.doi.org/10.1093/jxb/eru386.

Harms, K., Von Ballmoos, P., Brunold, C., Höfgen, R., Hesse, H., 2000. Expression of a bacterial serine acetyltransferase in transgenic potato plants leads to increased levels of cysteine and glutathione. Plant J. 22, 335–343.

Havaux, M., Eymery, F., Porfirova, S., Rey, P., Dormann, P., 2005. Vitamin E protects against photoinhibition and photooxidative stress in *Arabidopsis thaliana*. Plant Cell 17, 3451–3469.

Hebbelmann, I., Selinski, J., Wehmeyer, C., Goss, T., Voss, I., Mulo, P., Kangasjärvi, S., Aro, E.M., Oelze, M.L., Dietz, K.J., Nunes-Nesi, A., Do, P.T., Fernie, A.R., Talla, S.K., Raghavendra, A.S., Linke, V., Scheibe, R., 2012. Multiple strategies to prevent oxidative stress in Arabidopsis plants lacking the malate valve enzyme NADP-malate dehydrogenase. J. Exp. Bot. 63, 1445–1459.

Henzler, T., Steudle, E., 2000. Transport and metabolic degradation of hydrogen peroxide in *Chara corallina*: model calculations and measurements with the pressure probe suggest transport of H_2O_2 across water channels. J. Exp. Bot. 51, 2053–2066.

Hoeberichts, F.A., Davoine, C., Vandorpe, M., Morsa, S., Ksas, B., Stassen, C., Triantaphylidès, C., Van Breusegem, F., 2013. Cryptogein-induced transcriptional reprogramming in tobacco is light dependent. Plant Physiol. 163, 263–275.

Holtgrefe, S., Gohlke, J., Starmann, J., Druce, S., Klocke, S., Altmann, B., Wojtera, J., Lindermayr, C., Scheibe, R., 2008. Regulation of plant cytosolic glyceraldehyde 3-phosphate dehydrogenase isoforms by thiol modifications. Physiol. Plant 133, 211–218.

Hong, Z., Zhang, Z., Olson, J.M., Verma, D.P.S., 2001. A novel UDP-glucose transferase is part of the callose synthase complex and interacts with phragmoplastin at the forming cell plate. Plant Cell 13, 769–779.

Hörtensteiner, S., Kräutler, B., 2011. Chlorophyll breakdown in higher plants. Biochim. Biophys. Acta 1807, 977–988.

Hu, X., Zhang, A., Zhang, J., Jiang, M., 2006. Abscisic acid is a key inducer of hydrogen peroxide production in leaves of maize plants exposed to water stress. Plant Cell Physiol. 47, 1484–1495.

Husar, S., Berthiller, F., Fujioka, S., Rozhon, W., Khan, M., Kalaivanan, F., Elias, L., Higgins, G.S., Li, Y., Schuhmacher, R., Krska, R., Seto, H., Vaistij, F.E., Bowles, D., Poppenberger, B., 2011. Overexpression of the UGT73C6 alters brassinosteroid glucoside formation in *Arabidopsis thaliana*. BMC Plant Biol. 11, 51.

Jannat, R., Uraji, M., Hossain, M.A., Islam, M.M., Nakamura, Y., Mori, I.C., Murata, Y., 2012. Catalases negatively regulate methyl jasmonate signaling in guard cells. J. Plant Physiol. 169, 1012–1016.

Jiang, K., Schwarzer, C., Lally, E., Zhang, S.B., Ruzin, S., Machen, T., Remington, S.J., Feldman, L., 2006. Expression and characterization of a redox-sensing green fluorescent protein (reduction–oxidation-sensitive green fluorescent protein) in Arabidopsis. Plant Physiol. 141, 397–403.

Jubany-Mari, T., Alegre-Batlle, L., Jiang, K., Feldman, L.J., 2010. Use of a redox-sensing GFP (c-roGFP1) for real-time monitoring of cytosol redox status in *Arabidopsis thaliana* water-stressed plants. FEBS Lett. 584, 889–897.

Jung, H.W., Tschaplinski, T.J., Wang, L., Glazebrook, J., Greenberg, J.T., 2009. Priming in systemic plant immunity. Science 324, 89–91.

Kärkönen, A., Fry, S.C., 2006. Effect of ascorbate and its oxidation products on H_2O_2 production in cell-suspension cultures of *Picea abies* and in the absence of cells. J. Exp. Bot. 57, 1633–1644.

Kasimova, M.R., Grigiene, J., Krab, K., Hagedorn, P.H., Flyvbjerg, H., Andersen, P.E., Møller, I.M., 2006. The free NADH concentration is kept constant in plant mitochondria under different metabolic conditions. Plant Cell 18, 688–698.

Kerchev, P., Mühlenbock, P., Denecker, J., Morreel, K., Hoeberichts, F., Van Der Kelen, K., Vandorpe, M., Nguyen, L., Audenaert, D., Van Breusegem, F., 2014. Activation of auxin signalling counteracts photorespiratory H_2O_2-dependent cell death. Plant Cell Environ. http://dx.doi.org/10.1111/pce.12250.

Kim, K.C., Fan, B., Chen, Z., 2006. Pathogen-induced Arabidopsis WRKY7 is a transcriptional repressor and enhances plant susceptibility to *Pseudomonas syringae*. Plant Physiol. 142, 1180–1192.

König, J., Baier, M., Horling, F., Kahmann, U., Harris, G., Schürmann, P., Dietz, K.J., 2002. The plant-specific function of 2-Cys peroxiredoxin-mediated detoxification of peroxides in the redox-hierarchy of photosynthetic electron flux. Proc. Natl. Acad. Sci. U.S.A. 99, 5738–5743.

Köster, J., Thurow, C., Kruse, K., Meier, A., Iven, T., Feussner, I., Gatz, C., 2012. Xenobiotic- and jasmonic acid-inducible signal transduction pathways have become interdependent at the Arabidopsis CYP81D11 Promoter1. Plant Physiol. 159, 391–402.

Kwak, J.M., Mori, I.C., Pei, Z.M., Leonhardt, N., Torres, M.A., Dangl, J.L., Bloom, R.E., Bodde, S., Jones, J.D., Schroeder, J.I., 2003. NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in Arabidopsis. EMBO J. 22, 2623–2633.

Li, Z., Keasling, J.D., Niyogi, K.K., 2012. Overlapping photoprotective function of vitamin E and carotenoids in *Chlamydomonas*. Plant Physiol. 158, 313–323.

Li, F., Wang, J., Ma, C., Zhao, Y., Wang, Y., Hasi, A., Qi, Z., 2013a. Glutamate receptor-like channel3.3 is involved in mediating glutathione-triggered cytosolic calcium transients, transcriptional changes, and innate immunity responses in Arabidopsis. Plant Physiol. 162, 1497–1509.

Li, S., Mhamdi, A., Clement, C., Jolivet, Y., Noctor, G., 2013b. Analysis of knockout mutants suggests that Arabidopsis *NADP-MALIC ENZYME2* does not play an essential role in responses to oxidative stress of intracellular or extracellular origin. J. Exp. Bot. 64, 3605–3614.

Li, S., Mhamdi, A., Trotta, A., Kangasjärvi, S., Noctor, G., 2014. The protein phosphatase subunit PP2A-B′ γ is required to suppress daylength-dependent pathogenesis responses triggered by intracellular oxidative stress. New Phytol. 202, 145–160.
Lim, E.L., Doucet, C.J., Li, Y., Elias, L., Worrall, D., Spencer, S.P., Ross, J., Bowles, D.J., 2002. The activity of *Arabidopsis* glycosyltransferases toward salicylic acid, 4-hydroxybenzoic acid, and other benzoates. J. Biol. Chem. 277, 586–592.
Linster, C.L., Van Schaftingen, E., Hanson, A.D., 2013. Metabolite damage and its repair or pre-emption. Nat. Chem. Biol. 9, 72–80.
Liu, G., Ji, Y., Bhuiyan, N.H., Pilot, G., Salvaraj, G., Zou, J., Wei, Y., 2010. Amino acid homeostasis modulates salicylic acid-associated redox status and defense responses in *Arabidopsis*. Plant Cell 22, 3845–3863.
Loeffler, C., Berger, S., Guy, A., Durand, T., Bringmann, G., Dreyer, M., von Rad, U., Durner, J., Mueller, M.J., 2005. B1-phytoprostanes trigger plant defense and detoxification responses. Plant Physiol. 137, 328–340.
Maere, S., Heymans, K., Kuiper, M., 2005. BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. Bioinformatics 21, 3448–3449.
Mano, J., Miyatake, F., Hiraoka, E., Tamoi, M., 2009. Evaluation of the toxicity of stress-related aldehydes to photosynthesis in chloroplasts. Planta 230, 639–648.
Martin, M.N., Saladores, P.H., Lambert, E., Hudson, A.O., Leustek, T., 2007. Localization of members of the γ-glutamyl transpeptidase family identifies sites of glutathione and glutathione *S*-conjugate hydrolysis. Plant Physiol. 144, 1715–1732.
Matthes, M., Bruce, T., Chamberlain, K., Pickett, J., Napier, J., 2011. Emerging roles in plant defense for cis-jasmone-induced cytochrome P450 CYP81D11. Plant Signal. Behav. 6 (4), 563–565.
Meng, P.H., Raynaud, C., Tcherkez, G., Blanchet, S., Massoud, K., Domenichini, S., Henry, Y., Soubigou-Taconnat, L., Lelarge-Trouverie, C., Saindrenan, P., et al., 2009. Crosstalks between *myo*-inositol metabolism, programmed cell death and basal immunity in *Arabidopsis*. PLoS One 4, e7364.
Mezzari, M.P., Walters, K., Jelinkova, M., Shih, M.C., Just, C.L., Schnoor, J.L., 2005. Gene expression and microscopic analysis of Arabidopsis exposed to chloroacetanilide herbicides and explosive compounds. A phytoremediation approach. Plant Physiol. 138, 858–869.
Mhamdi, A., Noctor, G., 2014. Analysis of the roles of the Arabidopsis peroxisomal isocitrate dehydrogenase in leaf metabolism and oxidative stress. Environ. Exp. Bot. http://dx.doi.org/10.1016/j.envexpbot.2014.07.002.
Mhamdi, A., Queval, G., Chaouch, S., Vanderauwera, S., Van Breusegem, F., Noctor, G., 2010a. Catalase in plants: a focus on Arabidopsis mutants as stress-mimic models. J. Exp. Bot. 61, 4197–4220.
Mhamdi, A., Hager, J., Chaouch, S., Queval, G., Han, Y., Taconnat, Y., Saindrenan, P., Issakidis-Bourguet, E., Gouia, H., Renou, J.P., Noctor, G., 2010b. *Arabidopsis* GLUTATHIONE REDUCTASE 1 is essential for the metabolism of intracellular H_2O_2 and to enable appropriate gene expression through both salicylic acid and jasmonic acid signaling pathways. Plant Physiol. 153, 1144–1160.
Mhamdi, A., Mauve, C., Gouia, H., Saindrenan, P., Hodges, M., Noctor, G., 2010c. Cytosolic NADP-dependent isocitrate dehydrogenase contributes to redox homeostasis and the regulation of pathogen responses in *Arabidopsis* leaves. Plant Cell Environ. 33, 1112–1123.
Mhamdi, A., Noctor, G., Baker, A., 2012. Plant catalases: peroxisomal redox guardians. Arch. Biochem. Biophys. 525, 181–194.
Millar, A.H., Mittova, V., Kiddle, G., Heazlewood, J.L., Bartoli, C.G., Theodoulou, F.L., Foyer, C.H., 2003. Control of ascorbate synthesis by respiration and its implications for stress responses. Plant Physiol. 133, 443–447.
Miller, G., Schlauch, K., Tam, R., Cortes, D., Torres, M.A., Shulaev, V., Dangl, J.L., Mittler, R., 2009. The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. Science Signal. 2, ra45.
Mittler, R., Zilinskas, B.A., 1991. Purification and characterization of pea cytosolic ascorbate peroxidase. Plant Physiol. 97, 962–968.
Møller, I.M., Rasmusson, A.G., 1998. The role of NADP in the mitochondrial matrix. Trends Plant Sci. 3, 21–27.
Møller, I.M., Sweetlove, L., 2010. ROS signaling – specificity is required. Trends Plant Sci. 15, 370–374.
Møller, I.M., Jensen, P.E., Hansson, A., 2007. Oxidative modifications to cellular components in plants. Annu. Rev. Plant Biol. 58, 459–481.
Morgan, B., Ezerina, D., Amaoko, T.N.E., Riemer, J., Seedorf, M., Dick, T.P., 2012. Multiple glutathione disulfide removal pathways mediate cytosolic redox homeostasis. Nat. Chem. Biol. 9, 119–125.
Moschou, P.N., Paschalidis, K.A., Delis, I.D., Andriopoulou, A.H., Lagiotis, G.D., Yakoumakis, D.I., Roubelakis-Angelakis, K.I., 2008. Spermidine exodus and oxidation in the apoplast induced by abiotic stress is responsible for H_2O_2 signatures that direct tolerance responses in tobacco. Plant Cell 20, 1708–1724.
Mueller, S., Hilbert, B., Dueckershoff, K., Roitsch, T., Krischke, M., Mueller, M.J., Berger, S., 2008. General detoxification and stress responses are mediated by oxidized lipids through TGA transcription factors in Arabidopsis. Plant Cell 20, 768–785.
Müller, K., Linkies, A., Vreeburg, R.A.M., Fry, S.C., Krieger-Liszkay, A., Leubner-Metzger, G., 2009. In vivo cell wall loosening by hydroxyl radicals during cress seed germination and elongation growth. Plant Physiol. 150, 1855–1865.
Müller-Moulé, P., Conklin, P.L., Niyogi, K.K., 2002. Ascorbate deficiency can limit violaxanthin de-epoxidase activity in vivo. Plant Physiol. 128, 970–977.
Mullineaux, P.M., Karpinski, S., Baker, N.R., 2006. Spatial dependence for hydrogen peroxide-directed signaling in light-stressed plants. Plant Physiol. 141, 346–350.
Mur, L.A.J., Kenton, P., Atzorn, R., Miersch, O., Wasternack, C., 2006. The outcomes of concentration–specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. Plant Physiol. 140, 249–262.
Nagatoshi, Y., Nakamura, T., 2009. *Arabidopsis* HARMLESS TO OZONE LAYER protein methylates a glucosinolate breakdown product and functions in resistance to *Pseudomonas syringae* pv. *maculicola*. J. Biol. Chem. 284, 19301–19309.
Návarová, H., Bernsdorff, F., Döring, A.C., Zeier, J., 2012. Pipecolic acid, an endogenous mediator of defense amplification and priming, is a critical regulator of inducible plant immunity. Plant Cell 24, 5123–5141.
Niehaus, T.D., Richardson, L.G.L., Gidda, S.K., ElBadawi-Sidhu, M., Meissen, J.K., Mullen, R.T., Fiehn, O., Hanson, A.D., 2014. Plants utilize a highly conserved system for repair of NADH and NADPH hydrates. Plant Physiol. 165, 52–61.
Nishizawa, A., Yabuta, Y., Shigeoka, S., 2010. Galactinol and raffinose constitute a novel function to protect plants from oxidative damage. Plant Physiol. 147, 1251–1263.
Noctor, G., 2006. Metabolic signalling in defence and stress: the central roles of soluble redox couples. Plant Cell Environ. 29, 409–425.
Noctor, G., Hager, J., Li, S., 2011. Biosynthesis of NAD and its manipulation in plants. Adv. Bot. Res. 58, 153–201.
Noctor, G., Mhamdi, A., Queval, G., Foyer, C.H., 2013. Regulating the redox gatekeeper: vacuolar sequestration puts glutathione disulfide in its place. Plant Physiol. 163, 665–671.
Noctor, G., Mhamdi, A., Foyer, C.H., 2014. The roles of reactive oxygen metabolism in drought stress: not so cut and dried. Plant Physiol. 164, 1636–1648.
Nosarzewski, M., Downie, A.B., Wu, B., Archbold, D.D., 2012. The role of SORBITOL DEHYDROGENASE in *Arabidopsis thaliana*. Funct. Plant Biol. 39, 462–470.
Nunes-Nesi, A., Carrari, F., Lytovchenko, A., Smith, A.M.O., Loureiro, M.E., Ratcliffe, R.G., Sweetlove, L.J., Fernie, A.R., 2005. Enhanced photosynthetic performance and growth as a consequence of decreasing mitochondrial malate dehydrogenase activity in transgenic tomato plants. Plant Physiol. 137, 611–622.
Obata, T., Matthes, A., Koszior, S., Lehmann, M., Araújo, W.L., Bock, R., Sweetlove, L.J., Fernie, A.R., 2011. Alteration of mitochondrial protein complexes in relation to metabolic regulation under short-term oxidative stress in Arabidopsis seedlings. Phytochemistry 72, 1081–1091.
O'Brien, J.A., Daudi, A., Butt, V.S., Bolwell, G.P., 2012a. Reactive oxygen species and their role in plant defence and cell wall metabolism. Planta 236, 765–779.
O'Brien, J.A., Daudi, A., Finch, P., Butt, V.S., Whitelegge, J.P., Souda, P., Ausubel, F.M., Bolwell, G.P., 2012b. A peroxidase-dependent apoplastic oxidative burst in cultured Arabidopsis cells functions in MAMP-elicited defense. Plant Physiol. 158, 2013–2027.
Ohkamu-Ohtsu, N., Oikawa, A., Zhao, P., Xiang, C., Saito, K., Oliver, D.J., 2008. A γ-glutamyl transpeptidase-independent pathway of glutathione catabolism to glutamate via 5-oxoproline in Arabidopsis. Plant Physiol. 148, 1603–1613.
Ohkamu-Ohtsu, N., Sasaki-Sekimoto, Y., Oikawa, A., et al., 2011. 12-oxo-phytodienoic acid-glutathione conjugate is transported into the vacuole in Arabidopsis. Plant Cell Physiol. 52, 205–209.
Oracz, K., Bouteau, H.E.M., Farrant, J.M., Cooper, K., Belghazi, M., Job, C., Job, D., Corbineau, F., Bailly, C., 2007. ROS production and protein oxidation as a novel mechanism for seed dormancy alleviation. Plant J. 50, 452–465.
Palmieri, M.C., Lindermayr, C., Bauwe, H., Steinhauser, C., Durner, J., 2010. Regulation of plant glycine decarboxylase by *S*-nitrosylation and glutathionylation. Plant Physiol. 152, 1514–1528.
Parisy, V., Poinssot, B., Owsianowski, L., Buchala, A., Glazebrook, J., Mauch, F., 2007. Identification of PAD2 as a γ-glutamylcysteine synthetase highlights the importance of glutathione in disease resistance in Arabidopsis. Plant J. 49, 159–172.
Park, S.W., Li, W., Viehhauser, A., He, B., Kim, S., Nilsson, A.K., Andersson, M.X., Kittle, J.D., Ambavaram, M.M., Luan, S., Esker, A.R., Tholl, D., Cimini, D., Ellerström, M., Coaker, G., Mitchell, T.K., Pereira, A., Dietz, K.J., Lawrence, C.B., 2013. Cyclophilin 20-3 relays a 12-oxo-phytodienoic acid signal during stress responsive regulation of cellular redox homeostasis. Proc. Natl. Acad. Sci. U.S.A. 110, 9559–9564.
Parsons, H.T., Fry, S.C., 2012. Oxidation of dehydroascorbic acid and 2,3-diketogulonate under plant apoplastic conditions. Phytochemistry 75, 41–49.
Parsons, H.T., Yasmin, T., Fry, S.C., 2011. Alternative pathways of dehydroascorbic acid degradation in vitro and in plant cell cultures: novel insights into vitamin C catabolism. Biochem. J. 440, 375–383.
Pastori, G.M., Foyer, C.H., 2002. Common components, networks, and pathways of cross-tolerance to stress. The central role of "redox" and abscisic acid-mediated controls. Plant Physiol. 129, 460–468.
Pastori, G.M., Kiddle, G., Antoniw, J., Bernard, S., Veljovic-Jovanovic, S., Verrier, P.J., Noctor, G., Foyer, C.H., 2003. Leaf vitamin C contents modulate plant defense transcripts and regulate genes that control development through hormone signaling. Plant Cell 15, 939–951.
Pattanayak, G.K., Venkataramani, S., Hortensteiner, S., Kunz, L., Christ, B., Moulin, M., Smith, A.G., Okamoto, Y., Tamiaki, H., Sugishima, M., Greenberg, J.T., 2012. Accelerated cell death 2 suppresses mitochondrial oxidative bursts and modulates cell death in Arabidopsis. Plant J. 69, 589–600.
Pavet, V., Olmos, E., Kiddle, G., Mowla, S., Kumar, S., Antoniw, J., Alvarez, M.E., Foyer, C.H., 2005. Ascorbic acid deficiency activates cell death and disease resistance responses in Arabidopsis. Plant Physiol. 139, 1291–1303.
Pellny, T.K., Locato, V., Vivancos, P.D., Markovic, J., De Gara, L., Pallardo, F.V., Foyer, C.H., 2009. Pyridine nucleotide cycling and control of intracellular redox state in relation to poly (ADP-ribose) polymerase activity and nuclear localization of

glutathione during exponential growth of Arabidopsis cells in culture. Mol. Plant 2, 442–456.
Pérez-Garcia, A., Canovas, F.M., Gallardo, F., Hirel, B., De Vicente, A., 1995. Differential expression of glutamine synthetase isoforms in tomato detached leaflets infected with *Pseudomonas syringae* pv. *tomato*. Mol. Plant Microbe Interact. 8, 96–103.
Pétriacq, P., de Bont, L., Hager, J., Didierlaurent, L., Mauve, C., Guérard, F., Noctor, G., Pelletier, S., Renou, J.P., Tcherkez, G., Gakière, B., 2012. Inducible NAD overproduction in Arabidopsis alters metabolic pools and gene expression correlated with increased salicylate content and resistance to Pst-AvrRpm1. Plant J. 70, 650–665.
Pignocchi, C., Foyer, C.H., 2003. Apoplastic ascorbate metabolism and its role in the regulation of cell signalling. Curr. Opin. Plant Biol. 6, 379–389.
Pružinska, A., Anders, I., Tanner, G., Roca, M., Hortensteiner, S., 2003. Chlorophyll breakdown: pheophorbide *a* oxygenase is a Rieske-type iron-sulfur protein, encoded by the *accelerated cell death 1* gene. Proc. Natl. Acad. Sci. U.S.A. 100, 15259–15264.
Przybyla, D., Göbel, C., Imboden, A., Hamberg, M., Feussner, I., Apel, K., 2008. Enzymatic, but not non-enzymatic, $^1O_2$-mediated peroxidation of polyunsaturated fatty acids forms part of the EXECUTER1-dependent stress response program in the *flu* mutant of *Arabidopsis thaliana*. Plant J. 54, 236–248.
Queval, G., Noctor, G., 2007. A plate reader method for the measurement of NAD, NADP, glutathione, and ascorbate in tissue extracts: application to redox profiling during Arabidopsis rosette development. Anal. Biochem. 363, 58–69.
Queval, G., Issakidis-Bourguet, E., Hoeberichts, F.A., Vandorpe, M., Gakière, B., Vanacker, H., Miginiac-Maslow, M., Van Breusegem, F., Noctor, G., 2007. Conditional oxidative stress responses in the *Arabidopsis* photorespiratory mutant *cat2* demonstrate that redox state is a key modulator of daylength-dependent gene expression and define photoperiod as a crucial factor in the regulation of H_2O_2-induced cell death. Plant J. 52, 640–657.
Queval, G., Hager, J., Gakière, B., Noctor, G., 2008. Why are literature data for H_2O_2 contents so variable? A discussion of potential difficulties in quantitative assays of leaf extracts. J. Exp. Bot. 59, 135–146.
Queval, G., Thominet, D., Vanacker, H., Miginiac-Maslow, M., Gakière, B., Noctor, G., 2009. H_2O_2-activated up-regulation of glutathione in Arabidopsis involves induction of genes encoding enzymes involved in cysteine synthesis in the chloroplast. Mol. Plant 2, 344–356.
Queval, G., Jaillard, D., Zechmann, B., Noctor, G., 2011. Increased intracellular H_2O_2 availability preferentially drives glutathione accumulation in vacuoles and chloroplasts. Plant Cell Environ. 34, 21–32.
Queval, G., Neukermans, J., Vanderauwera, S., Van Breusegem, F., Noctor, G., 2012. Daylength is a key regulator of transcriptomic responses to both CO_2 and H_2O_2 in *Arabidopsis*. Plant Cell Environ. 35, 374–387.
Rahantaniaina, M.-S., Tuzet, A., Mhamdi, A., Noctor, G., 2013. Missing links in understanding redox signaling via thiol/disulfide modulation: how is glutathione oxidized in plants? Front. Plant Sci. 4, 477.
Ramel, F., Birtic, S., Ginies, C., Soubigou-Taconnat, L., Triantaphylides, C., Havaux, M., 2012. Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. Proc. Natl. Acad. Sci. U.S.A. 109, 5535–5540.
Rius, S.P., Casati, P., Iglesias, A.A., Gomez-Casati, D.F., 2006. Characterization of an *Arabidopsis thaliana* mutant lacking a cytosolic non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase. Plant Mol. Biol. 61, 945–957.
Rizhsky, L., Hallak-Herr, E., Van Breusegem, F., Rachmilevitch, S., Barr, J., Rodermel, S., Inzé, D., Mittler, R., 2002. Double antisense plants lacking ascorbate peroxidase and catalase are less sensitive to oxidative stress than single antisense plants lacking ascorbate peroxidase or catalase. Plant J. 32, 329–342.
Ruban, A.V., Johnson, M.P., Duffy, C.D.P., 2012. The photoprotective molecular switch in the photosystem II antenna. Biochim. Biophys. Acta 1817, 167–181.
Saito, R., Shimakawa, G., Nishi, A., Iwamoto, T., Sakamoto, K., Yamamoto, H., Amako, K., Makino, A., Miyake, C., 2013. Functional analysis of the AKR4C subfamily family of Arabidopis thaliana: model structures, substrate specificity, acrolein toxicity, and responses to light and [CO_2]. Biosci. Biotechnol. Biochem. 77, 2038–2045.
Sappl, P.G., Carroll, A.J., Clifton, R., Lister, R., Whelan, J., Millar, A.H., Singh, K.B., 2009. The *Arabidopsis* glutathione transferase gene family displays complex stress regulation and co-silencing multiple genes results in altered metabolic sensitivity to oxidative stress. Plant J. 58, 53–68.
Sattler, S.E., Mene-Saffrane, L., Farmer, E.E., Krischke, M., Mueller, M.J., DellaPenna, D., 2006. Nonenzymatic lipid peroxidation reprograms gene expression and activates defense markers in Arabidopsis tocopherol-deficient mutants. Plant Cell 18, 3706–3720.
Schäfer, L., Feierabend, J., 2000. Photoinactivation and protection of glycolate oxidase in vitro and in leaves. Z. Naturforsch. C 55, 361–372.
Scheibe, R., Backhausen, J.E., Emmerlich, V., Holtgrefe, S., 2005. Strategies to maintain redox homeostasis during photosynthesis under changing conditions. J. Exp. Bot. 56, 1481–1489.
Schlaeppi, K., Bodenhausen, N., Buchala, A., Mauch, F., Reymond, P., 2008. The glutathione-deficient mutant *pad2-1* accumulates lower amounts of glucosinolates and is more susceptible to the insect herbivore *Spodoptera littoralis*. Plant J. 55, 774–786.
Schwarzländer, M., Fricker, M.D., Müller, C., Marty, L., Brach, T., Novak, J., Sweetlove, L.J., Hell, R., Meyer, A.J., 2008. Confocal imaging of glutathione redox potential in living plant cells. J. Microsc. 231, 299–316.
Seifi, H.S., Van Bockhaven, J., Angenon, G., Höfte, M., 2013. Glutamate metabolism in plant disease and defense: friend or foe. Mol. Plant Microbe Interact. 26, 475–485.
Sharma, S., Villamor, J.G., Verslues, P.E., 2011. Essential role of tissue-specific proline synthesis and catabolism in growth and redox balance at low water potential. Plant Physiol. 157, 292–304.
Shulaev, V., Cortes, D., Miller, G., Mittler, R., 2008. Metabolomics for plant stress response. Physiol. Plant 132, 199–208.
Simon, C., Langlois-Meurinne, M., Didierlaurent, L., Chaouch, S., Bellvert, F., Massoud, K., Garmier, M., Thareau, V., Comte, G., Noctor, G., Saindrenan, P., 2014. The secondary metabolism glycosyltransferases UGT73B3 and UGT73B5 are components of redox status in resistance of Arabidopsis to *Pseudomonas syringae* pv. Tomato. Plant Cell Environ 37, 1114–1129. http://dx.doi.org/10.1111/pce.12221.
Smirnoff, N., 2011. Vitamin C: the metabolism and functions of ascorbic acid in plants. Adv. Bot. Res. 59, 107–177.
Smirnoff, N., Cumbes, Q., 1989. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry 28, 1057–1060.
Smith, I.K., Kendall, A.C., Keys, A.J., Turner, J.C., Lea, P.J., 1985. The regulation of the biosynthesis of glutathione in leaves of barley (*Hordeum vulgare* L.). Plant Sci. 41, 11–17.
Sparla, F., Zaffagnini, M., Wedel, N., Scheibe, R., Pupillo, P., Trost, P., 2005. Regulation of photosynthetic GAPDH dissected by mutants. Plant Physiol. 138, 2210–2219.
Stitt, M., Müller, C., Matt, P., Gibon, Y., Carillo, P., Morcuende, R., Scheible, W.R., Krapp, A., 2002. Steps towards an integrated view of nitrogen metabolism. J. Exp. Bot. 53, 959–970.
Studart-Guimarães, C., Fait, A., Nunes-Nesi, A., Carrari, F., Usadel, B., Fernie, A.R., 2007. Reduced expression of succinyl-coenzyme A ligase can be compensated for by up-regulation of the γ-aminobutyrate shunt in illuminated tomato leaves. Plant Physiol. 145, 626–639.
Stuttmann, J., Hubberten, H.-M., Rietz, S., Kaur, J., Muskett, P., Guerois, R., Bednarek, P., Hoefgen, R., Parker, J.E., 2011. Perturbation of Arabidopsis amino acid metabolism causes incompatibility with the adapted biotrophic pathogen *Hyaloperonospora arabidopsidis*. Plant Cell 23, 2788–2803.
Su, T., Xu, J., Li, Y., Lei, L., Zhao, L., Yang, H., Feng, J., Liu, G., Ren, D., 2011. Glutathione-indole-3-acetonitrile is required for camalexin biosynthesis in *Arabidopsis thaliana*. Plant Cell 23, 364–380.
Sudre, D., Gutierrez-Carbonell, E., Lattanzio, G., Rellán-Alvarez, R., Gaymard, F., Wohlgemuth, G., Fiehn, O., Alvarez-Fernández, A., Zamarreno, A.M., Bacaicoa, E., Duy, D., Garcia-Mina, J.-M., Abadia, J., Philippar, K., Lopez-Millan, A.-F., Briat, J.-F., 2013. Iron-dependent modifications of the flower transcriptome, proteome, metabolome, and hormonal content in an Arabidopsis ferritin mutant. J. Exp. Bot. 64, 2665–2688.
Szabados, L., Savouré, A., 2010. Proline: a multifunctional amino acid. Trends Plant Sci. 15, 89–97.
Tada, Y., Spoel, S.H., Pajerowska-Mukhtar, K., Mou, Z., Song, J., Wang, C., Zuo, J., Dong, X., 2008. Plant immunity requires conformational charges of NPR1 via S-nitrosylation and thioredoxins. Science 321, 952–956.
Tanaka, R., Hirashima, M., Satoh, S., Tanaka, A., 2003. The *Arabidopsis-accelerated cell death* gene *ACD1* is involved in oxygenation of pheophorbide *a*: inhibition of pheophorbide *a* oxygenase activity does not lead to the "stay-green" phenotype in *Arabidopsis*. Plant Cell Physiol. 44, 1266–1274.
Taylor, N.L., Day, D.A., Millar, A.H., 2002. Environmental stress causes oxidative damage to plant mitochondria leading to inhibition of glycine decarboxylase. J. Biol. Chem. 277, 42663–42668.
Taylor, N.L., Day, D.A., Millar, A.H., 2004. Targets of stress-induced oxidative damage in plant mitochondria and their impact on cell carbon/nitrogen metabolism. J. Exp. Bot. 55, 1–10.
Thordal-Christensen, H., Zhang, Z., Wei, Y., Collinge, D.B., 1997. Subcellular localisation of H_2O_2 in plants. H_2O_2 accumulation in papillae and hypersensitive response during the barley–powdery mildew interaction. Plant J. 11, 1187–1194.
Tognetti, V.B., Van Aken, O., Morreel, K., Vandenbroucke, K., van de Cotte, B., De Clercq, I., Chiwocha, S., Fenske, R., Prinsen, E., Boerjan, W., Genty, B., Stubbs, K.A., Inzé, D., Van Breusegem, F., 2010. Perturbation of indole-3-butyric acid homeostasis by the UDP-glucosyltransferase UGT74E2 modulates Arabidopsis architecture and water stress tolerance. Plant Cell 22, 2660–2679.
Torres, M.A., Dangl, J.L., Jones, J.D., 2002. *Arabidopsis* $gp91^{phox}$ homologues *AtrbohD* and *AtrbohF* are required for accumulation of reactive oxygen intermediates in the plant defense response. Proc. Natl. Acad. Sci. U.S.A. 99, 517–522.
Triantaphylidès, C., Krischke, M., Hoeberichts, F.A., Ksas, B., Gresser, G., Havaux, M., Van Breusegem, F., Mueller, M.J., 2008. Singlet oxygen is the major reactive oxygen species involved in photooxidative damage to plants. Plant Physiol. 148, 960–968.
Trotta, A., Wrzaczek, M., Scharte, J., Tikkanen, M., Konert, G., Rahikainen, M., Holmström, M., Hiltunen, H.M., Rips, S., Sipari, N., et al., 2011. Regulatory subunit B'gamma of protein phosphatase 2A prevents unnecessary defense reactions under low light in Arabidopsis. Plant Physiol. 156, 1464–1480.
Tsukada, Y., Fang, J., Erdjument-Bromage, H., Warren, M.E., Borchers, C.H., Tempst, P., Zhang, Y., 2006. Histone demethylation by a family of JmjC domain-containing proteins. Nature 16, 811–821.
Valderrama, R., Corpas, F.J., Carreras, A., Gómez-Rodríguez, M.V., Chaki, M., Pedrajas, J.R., Fernández-Ocaña, A., del Río, L.A., Barroso, J.B., 2006. The dehydrogenase-mediated recycling of NADPH is a key antioxidant system against salt-induced oxidative stress in olive plants. Plant Cell Environ. 29, 1449–1459.

Van Breusegem, F., Bailey-Serres, J., Mittler, R., 2008. Unraveling the tapestry of networks involving reactive oxygen species in plants. Plant Physiol. 147, 978–984.
Vanacker, H., Carver, T.L.W., Foyer, C.H., 2000. Early H_2O_2 accumulation in mesophyll cells leads to induction of glutathione during the hypersensitive response in the barley–powdery mildew interaction. Plant Physiol. 123, 1289–1300.
Vandenabeele, S., Vanderauwera, S., Vuylstecke, M., Rombauts, S., Langebartels, C., Seidlitz, H.K., Zabeau, M., Van Montagu, M., Inzé, D., Van Breusegem, F., 2004. Catalase deficiency drastically affects gene expression induced by high light in *Arabidopsis thaliana*. Plant J. 39, 45–58.
Vanderauwera, S., Zimmermann, P., Rombauts, S., Vandenabeele, S., Langebartels, C., Gruissem, W., Inzé, D., Van Breusegem, F., 2005. Genome-wide analysis of hydrogen peroxide-regulated gene expression in Arabidopsis reveals a high light-induced transcriptional cluster involved in anthocyanin biosynthesis. Plant Physiol. 139, 806–821.
Vanderauwera, S., De Block, M., Van de Steene, N., De Cottet, B.V., Metzlaff, M., Van Breusegem, F., 2007. Silencing of poly(ADP-ribose) polymerase in plants alters abiotic stress signal transduction. Proc. Natl. Acad. Sci. U.S.A. 104, 15150–15155.
Vanderauwera, S., Suzuki, N., Miller, G., van de Cotte, B., Morsa, S., Ravanat, J.L., Hegie, A., Triantaphylidès, C., Shulaev, V., Van Montagu, M.C.E., Van Breusegem, F., Mittler, R., 2011. Extranuclear protection of chromosomal DNA from oxidative stress. Proc. Natl. Acad. Sci. U.S.A. 108, 1711–1716.
Veljovic-Jovanovic, S.D., Pignocchi, C., Noctor, G., Foyer, C.H., 2001. Low ascorbic acid in the *vtc-1* mutant of Arabidopsis is associated with decreased growth and intracellular redistribution of the antioxidant system. Plant Physiol. 127, 426–435.
Veljovic-Jovanovic, S.D., Noctor, G., Foyer, C.H., 2002. Are leaf hydrogen peroxide concentrations commonly overestimated? The potential influence of artefactual interference by tissue phenolics and ascorbate. Plant Physiol. Biochem. 40, 501–507.
Verhoeven, A.S., Demmig-Adams, B., Adams, W.W., 1997. Enhanced employment of the xanthophyll cycle and thermal energy dissipation in spinach exposed to high light and N stress. Plant Physiol. 113, 817–824.
Voll, L.M., Zell, M.B., Engelsdorf, T., Saur, A., Wheeler, M.G., Drincovich, M.F., Weber, A.P.M., Maurino, V.G., 2012. Loss of cytosolic NADP-malic enzyme 2 in *Arabidopsis thaliana* is associated with enhanced susceptibility to *Colletotrichum higginsianum*. New Phytol. 195, 189–202.
Wagner, D., Przybyla, D., Op den Camp, R., Kim, C., Landgraf, F., Lee, K.P., Würsch, M., Laloi, C., Nater, M., Hideg, E., Apel, K., 2004. The genetic basis of singlet oxygen-induced stress responses of *Arabidopsis thaliana*. Science 306, 1183–1185.
Wardman, P., 2007. Fluorescent and luminescent probes for measurement of oxidative and nitrosative species in cells and tissues: progress, pitfalls, and prospects. Free Radic. Biol. Med. 43, 995–1032.
Wildermuth, M.C., Dewdney, J., Wu, G., Ausubel, F.M., 2001. Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature 414, 562–565.
Winger, A.M., Millar, A.H., Day, D.A., 2005. Sensitivity of plant mitochondrial terminal oxidases to the lipid peroxidation product 4-hydroxy-2-nonenal (HNE). Biochem. J. 387, 865–870.
Winter, H., Robinson, D.G., Heldt, H.W., 1994. Subcellular volumes and metabolite concentrations in spinach leaves. Planta 193, 530–535.
Winterbourn, C., 2014. The challenges of using fluorescent probes to detect and quantify specific reactive oxygen species in living cells. Biochim. Biophys. Acta 1840, 730–738.
Xiao, Y., Savchenko, T., Baidoo, E.E.K., Chehab, W.E., Hayden, D.M., Tolstikov, V., Corwin, J.A., Kliebenstein, D.J., Keasling, J.D., Dehesh, K., 2012. Retrograde signaling by the plastidial metabolite MEcPP regulates expression of nuclear stress-response genes. Cell 149, 1525–1535.
Yamauchi, Y., Hasegawa, A., Taninaka, A., Mizutani, M., Sugimoto, Y., 2011. NADPH-dependent reductases involved in the detoxification of reactive carbonyls in plants. J. Biol. Chem. 286, 6999–7009.
Yao, N., Greenberg, J.T., 2006. Arabidopsis ACCELERATED CELL DEATH2 modulates programmed cell death. Plant Cell 18, 397–411.
Ye, Q., Steudle, E., 2005. Oxidative gating of water channels (aquaporins) in corn roots. Plant Cell Environ. 29, 459–470.
Zaffagnini, M., Bedhomme, M., Lemaire, S.D., Trost, P., 2012. The emerging roles of protein glutathionylation in chloroplasts. Plant Sci. 185–186, 86–96.
Zechmann, B., Liou, L.C., Koffler, B.E., Horvat, L., Tomasic, A., Fulgosi, H., Zhang, Z., 2011. Subcellular distribution of glutathione and its dynamic changes under oxidative stress in the yeast *Saccharomyces cerevisiae*. FEMS Yeast Res. 11, 631–642.
Zhang, X., Mou, 2009. Extracellular pyridine nucleotides induce *PR* gene expression and disease resistance in Arabidopsis. Plant J. 57, 302–312.
Zhang, Y., Butelli, E., De Stefano, R., Schoonbeek, H.-J., Magusin, A., Pagliarani, C., Wellner, N., Hill, L., Orzaez, D., Granell, A., Jones, J.D.G., Martin, C., 2013. Anthocyanins double the shelf life of tomatoes by delaying overripening and reducing susceptibility to gray mold. Curr. Biol. 23, 1094–1100.

Graham Noctor has worked on plant physiology and biochemistry in different positions in the UK and France. Since 2003, he has been a Professor at the Université de Paris sud, Orsay, France, where he teaches courses on photosynthesis, primary and secondary plant metabolism, plant stress responses, and metabolite analysis. He has been involved in research in all these areas although a key theme over the last two decades has focused on the roles of antioxidants in oxidative stress. As of 2014, he is a member of the American Society of Plant Biologists and the Society for Experimental Biology, and acts as an editor for Plant Physiology, Plant Biology, and Plant, Cell & Environment.

Caroline Lelarge-Trouverie is a chemistry engineer at the Université de Paris sud, where she has worked since 1999. She is specialized in isotope and mass spectrometry-based analyses, and contributes to the development of methods using both gas and liquid chromatography to study the plant metabolome.

Amna Mhamdi obtained her PhD in biology at the Université de Paris sud in 2010, where she has continued as a post-doctoral researcher. Her work has focused especially on roles of glutathione and NADPH in H_2O_2 signalling in plants, for which she regularly uses transcriptomic and metabolomic profiling. She is a member of the Society for Experimental Biology and the American Society of Plant Biologists, and collaborates with several groups in France, Belgium, the UK, and Finland.