Journal of Experimental Botany, Vol. 61, No. 15, pp. 4197–4220, 2010
doi:10.1093/jxb/erq282 Advance Access publication 27 September, 2010

Journal of Experimental Botany
www.jxb.oxfordjournals.org

REVIEW PAPER

Catalase function in plants: a focus on *Arabidopsis* mutants as stress-mimic models

Amna Mhamdi[1,2], Guillaume Queval[1], Sejir Chaouch[1], Sandy Vanderauwera[3], Frank Van Breusegem[3] and Graham Noctor[1,*]

[1] Institut de Biologie des Plantes, UMR CNRS 8618, Université de Paris sud, F-91405 Orsay cedex, France
[2] Département de Biologie, Faculté des Sciences de Tunis, Université de Tunis El Manar, Campus Universitaire, 2092 Tunis, Tunisia
[3] Department of Plant Systems Biology, Flanders Institute for Biotechnology, 9052 Gent, Belgium; Department of Plant Biotechnology and Genetics, Gent University, 9052 Gent, Belgium

* To whom correspondence should be addressed: E-mail: graham.noctor@u-psud.fr

Received 21 June 2010; Revised 12 August 2010; Accepted 17 August 2010

Abstract

Hydrogen peroxide (H_2O_2) is an important signal molecule involved in plant development and environmental responses. Changes in H_2O_2 availability can result from increased production or decreased metabolism. While plants contain several types of H_2O_2-metabolizing proteins, catalases are highly active enzymes that do not require cellular reductants as they primarily catalyse a dismutase reaction. This review provides an update on plant catalase genes, function, and subcellular localization, with a focus on recent information generated from studies on *Arabidopsis*. Original data are presented on *Arabidopsis* catalase single and double mutants, and the use of some of these lines as model systems to investigate the outcome of increases in intracellular H_2O_2 are discussed. Particular attention is paid to interactions with cell thiol-disulphide status; the use of catalase-deficient plants to probe the apparent redundancy of reductive H_2O_2-metabolizing pathways; the importance of irradiance and growth daylength in determining the outcomes of catalase deficiency; and the induction of pathogenesis-related responses in catalase-deficient lines. Within the context of strategies aimed at understanding and engineering plant stress responses, the review also considers whether changes in catalase activities in wild-type plants are likely to be a significant part of plant responses to changes in environmental conditions or biotic challenge.

Key words: *Arabidopsis thaliana*, glutathione, H_2O_2, mutants, oxidative stress, pathogens, redox signalling.

Introduction

Increasingly refined and powerful methods of plant breeding and selection produced substantial increases in potential and actual crop yields throughout the last century. Often, however, environmental factors impose constraints on genetic potential so that actual yields are significantly below the theoretically possible yields (Bray *et al.*, 2000). Such limiting factors include the capture of light, water, and nutrients, the driving forces of plant growth (Ahrens *et al.*, 2010). Even when these factors are non-limiting, yield can be compromised by the existence of other factors. While the word 'stress' is widely used in plant biology, interpretations vary as to its exact meaning. Perhaps the simplest definition is a physiological condition caused by any environmental constraint that limits growth, reproductive success, yield, quality, or other traits desirable to humans. In many cases, the effects of stress persist for a certain time even after the removal of the environmental constraint(s). Among the possible causes of such persistent effects are (i) an enduring effect on biomass production following temporary diversion of resources to defence; (ii) deleterious modifications to cell components that take time to repair; and (iii) insufficiently rapid reversibility of developmental programmes (e.g. arrest of cell division, cell death) that are engaged to favour survival over growth and biomass production. All these mechanisms may contribute to what are perceived by humans as the negative effects of stress conditions on plant performance. If insight into the way plants work is to enable crop improvement through the development of

© The Author [2010]. Published by Oxford University Press [on behalf of the Society for Experimental Biology]. All rights reserved.
For Permissions, please e-mail: journals.permissions@oxfordjournals.org

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

rationally designed strategies, it will be necessary to develop accurate concepts of the mechanisms that underlie the effects of stress.

Stress and reactive oxygen

Examples of stress conditions are supra-optimal light, insufficient water supply, atmospheric and soil pollutants, excessive salt, and pathogen attack. In the field, plants often experience more than one of these conditions simultaneously. Depending on the species or variety, plants have the capacity to acquire resistance to stress within the life cycle of the individual (acclimation). Such processes include cold-hardening, adjustment to different growth light intensities and systemic acquired resistance to certain pathogens. Plants exposed to one type of stress condition can also acquire a certain resistance to other stress conditions (cross-tolerance). Inversely stress-specific mechanisms may also compromise responses to other stresses. These observations imply the existence of common and/or antagonistic factors that are involved in responses to different stress conditions. For example, the key stress phytohormone, abscisic acid, is not only important in dormancy and drought stress but also in pathogen responses (Asselbergh *et al.*, 2008), while antagonism has been described between salicylic acid (SA)- and jasmonic acid (JA)-dependent responses to biotic stress (Browse, 2009). A central theme in many stress responses is the accumulation of reactive oxygen species (ROS) and ROS-induced changes in cellular redox state (Foyer and Noctor, 2000; Dat *et al.*, 2001; Pastori and Foyer, 2002).

Changing views of ROS function in plants

Originally considered of little or no importance in biology, then susbequently as rapidly metabolized by-products in reactions such as chloroplast pseudocyclic electron transport (also called the water–water cycle: Asada, 1999), ROS gained importance during the 1980s as key players in both abiotic and biotic stress responses. Until ten years ago, the predominant view on ROS was that they were toxic molecules that cause damage, leading to the notion that (cross-)resistance to stress could be engineered in plants by enhancing antioxidative capacity.

This view of ROS action persists within the literature. Indeed, the reactivity of ROS may inevitably lead to some incidental modifications to cell components (Møller *et al.*, 2007). However, since the beginning of this century, it has been established that the most important physiological effects of ROS are not mediated via indiscriminate damage. Key observations were the ROS-driven activation of gene expression, specific protein kinases and calcium signatures, the 'programmed' production of ROS by NADPH oxidases, the roles of ROS production in sustaining cell growth, the importance of ROS in hormone signal transduction, and the requirement of specific gene modulation for ROS-induced cell death (Kovtun *et al.*, 2000; Desikan *et al.*, 2001; Foreman *et al.*, 2003; Kwak *et al.*, 2003; Vandenabeele *et al.*, 2003; Wagner *et al.*, 2004; Vandenbroucke *et al.*, 2008). Among the many current questions in the field are the nature of the components that perceive ROS, the specificity of the effects of different ROS, the importance of the (sub)cellular location of ROS production, the roles of changes in redox-homeostatic components, such as glutathione, in transmitting or adjusting ROS signals, and the importance of environmental and physiological contexts in determining the outcome of ROS-related redox signalling.

Generation and metabolism of H_2O_2 in plants

The term ROS includes any derivative of molecular oxygen (O_2) that is considered more reactive than O_2 itself. Thus, ROS refers to free radicals such as superoxide (O_2^-) and the hydroxyl radical (OH), but also to non-radicals like singlet oxygen ($^1O_2$) and H_2O_2. Because of its relative stability, H_2O_2 has received particular attention as a signal molecule involved in the regulation of specific biological processes such as plant–pathogen interactions. H_2O_2 is generated by a two-electron reduction of O_2, catalysed by certain oxidases or indirectly via reduction or dismutation of O_2^- that is formed by oxidases, peroxidases, or by photosynthetic and respiratory electron transport chains (Foyer and Noctor, 2000; Mittler *et al.*, 2004; Bindschedler *et al.*, 2006; Sagi and Fluhr, 2006). These reactions generate H_2O_2 at several subcellular compartments of the cell (Fig. 1), and the impact of H_2O_2 will be strongly influenced by the extent to which the potent antioxidative system allows its accumulation. Moreover, oxidative perturbation of components of the antioxidative system is likely to play a part in the initial transmission of H_2O_2 signals. The peroxisomes are important sites of ROS production. In this organelle, H_2O_2 can be formed directly from O_2 by photorespiratory glycolate oxidase (Foyer *et al.*, 2009) or by other enzyme systems such as xanthine oxidase coupled to superoxide dismutase (del Río *et al.*, 2006; Corpas *et al.*, 2008).

Genomic information and approaches have greatly advanced our understanding of plant antioxidant systems (Mittler *et al.*, 2004). Plants contain several types of enzymes that are able to metabolize peroxides such as H_2O_2. These include catalases, ascorbate peroxidases (APX), various types of peroxiredoxins (PRX), glutathione/thioredoxin peroxidases (GPX), and glutathione *S*-transferases (GST) (Willekens *et al.*, 1995; Asada, 1999; Wagner *et al.*, 2002; Dietz, 2003; Mittler *et al.*, 2004; Iqbal *et al.*, 2006). In all cases, these enzymes are encoded by multiple genes. Catalases are most notably distinguished from the other enzymes in not requiring a reductant as they catalyse a dismutation reaction. Together with APX, catalases are also distinguished from many other peroxide-metabolizing enzymes by their high specificity for H_2O_2, but weak activity against organic peroxides. Catalases have a very fast turnover rate, but a much lower affinity for H_2O_2 than APX and PRX, which have K_M values below 100 μM (Mittler and Zilinskas, 1991; König *et al.*, 2002). Although it is difficult to determine the precise kinetic characteristics for catalase, most estimates of the apparent K_M for H_2O_2

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

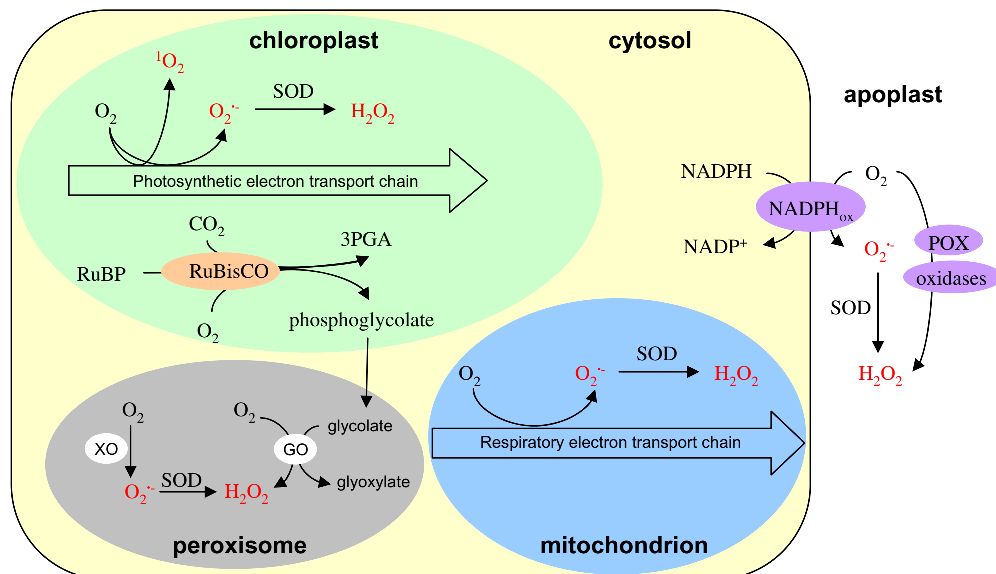

Fig. 1. Major sites of H_2O_2 production in photosynthetic cells. GO, glycolate oxidase. 3PGA, 3-phosphoglycerate. POX, peroxidase. RuBisCO, ribulose 1,5-*bis*phosphate carboxylase/oxygenase. RuBP, ribulose 1,5-*bis*phosphate. SOD, superoxide dismutase. XO, xanthine oxidase.

are in the range 40–600 mM (Chelikani *et al.*, 2004). A value of 190 mM was measured in crude extracts of pea (del Río *et al.*, 1977). This indicates that catalase functions *in vivo* well below its maximal capacity, but that its activity accelerates even if H_2O_2 concentrations increase to relatively high values. Catalases are highly expressed enzymes, particularly in certain plant cell types, and are thus an integral part of the plant antioxidative system. In this review, an overview of catalase function is provided and new results on single and double *Arabidopsis* T-DNA mutants are included as part of a discussion of how studying plants deficient in certain catalases can provide insights into the functional outcome of stress-linked H_2O_2 signalling in plants.

Plant catalases

Biochemistry

Catalase was the first antioxidant enzyme to be discovered and characterized. In giving the enzyme its name, Loew (1900) noted that 'there seems to exist no plant and no animal which is without that particular enzyme' (Kirkman and Gaetani, 2007). Even some anaerobes are known to contain catalase (Zamocky *et al.*, 2008). The typical catalase reaction is the dismutation of two molecules of H_2O_2 to water and O_2. Although some bacterial catalases use manganese as the redox-active co-factor, all known eukaryotic forms are haem-based (Zamocky *et al.*, 2008). The best-characterized type of haem-dependent catalase is found in diverse organisms, including prokaryotes, fungi, animals, and plants, and is composed of 'typical' or 'monofunctional' catalases (Chelikani *et al.*, 2004). These enzymes consist of polypeptides of 50–70 kDa in mass that are organized into tetramers, with each monomer bearing a haem prosthetic group (Regelsberger *et al.*, 2002). A second type of haem-dependent catalase is bifunctional catalase-peroxidases that are structurally distinct proteins found in some fungi and prokaryotes (Mutsada *et al.*, 1996; Regelsberger *et al.*, 2002). The division between monofunctional and bifunctional catalases is not absolute, because the first type can also catalyse some H_2O_2-dependent peroxidation of organic substrates (Zamocky *et al.*, 2008). On the other hand, bifunctional catalase-peroxidases are more similar to the haem-containing peroxidases such as APX and fungal cytochrome *c* peroxidase (Regelsberger *et al.*, 2001). They also have much higher affinities for H_2O_2 than monofunctional catalases and can be distinguished by their relative insensitivity to the inhibitor 3-amino-1,2,4-triazole (3-AT) (Margoliash and Novogrodsky, 1960; Regelsberger *et al.*, 2002). They are the most common type of catalase in cyanobacteria (Zamocky *et al.*, 2008).

Isotope labelling studies have established that O_2 generated in the catalatic (dismutation) reaction comes from a single H_2O_2 molecule (Kato *et al.*, 2004), with the other H_2O_2 being reduced to two water molecules that are formed in distinct steps (Fig. 2). Both haem-dependent catalases and peroxidases reduce H_2O_2 by splitting the O–O bond to generate a first molecule of water with the concomitant production of an oxy-ferryl intermediate (compound I) and a porphyrin cation radical. The specificity of the catalase reaction involves the oxidation of a second H_2O_2 to O_2 as compound I is reduced back to the initial state (Fig. 2B). This entails the release of the bound O, originating from the first H_2O_2, as a second molecule of water (Regelsberger *et al.*, 2001; Alfonso-Prieto *et al.*, 2009). Whereas catalase compound I is very rapidly reduced by two electrons from H_2O_2 (albeit probably in a stepwise manner: Kato *et al.*, 2004; Alfonso-Prieto *et al.*, 2009), reduction of compound I in peroxidases involves successive single-electron oxidations

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

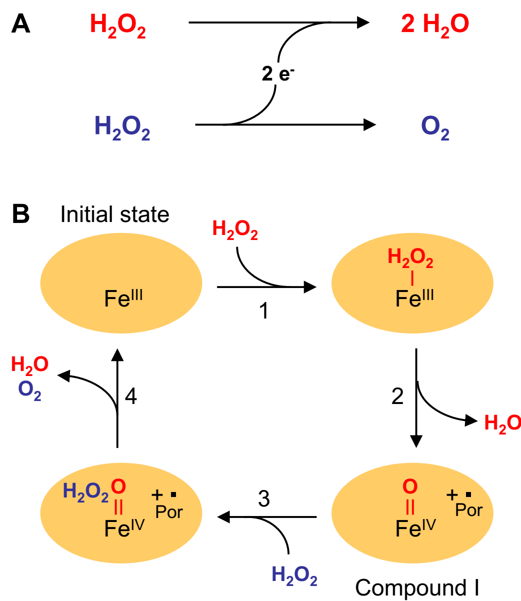

Fig. 2. Simplified scheme of the catalytic mechanism of catalase. (A) Overall substrates and products of the classical dismutation reaction. (B) Scheme of the four-step redox reaction. The roman numerals indicate oxidation state of the haem iron, while $Por^{+\cdot}$ indicates an oxidizing equivalent accumulated as a porphyrin cation radical. The two different H_2O_2 substrates and their respective products are indicated in blue and red. Although not shown, some of the reactions are reversible. Based on Regelsberger *et al.* (2001), Kato *et al.* (2004), and Alfonso-Prieto *et al.* (2009).

of organic compounds to generate two free radicals (e.g. monodehydroascorbate radicals in the case of APX).

As noted above, even monofunctional catalases can catalyse some peroxidation of reducing substrates. In this reaction, the second, reducing H_2O_2 is replaced by small compounds such as ethanol that provide the two electrons required for the reduction of compound I (Zamocky *et al.*, 2008). Rates are slow compared with the dismutation reaction, but this reaction has been suggested to be significant or even predominant when H_2O_2 concentrations in mammalian cells are low (Kirkman and Gaetani, 2007). Catalase-associated peroxidation has also been reported in plants. In partially purified protein extracts from maize, barley, and tobacco leaves, ethanol peroxidase activities co-purified with one of the two fractions containing high catalase dismutation activity (Havir and McHale, 1989).

Reduction of compound I by haem-dependent peroxidase involves the formation of compound II, in which the prosthetic group is in an oxidation state intermediate between that of compound I and the initial state. In monofunctional catalases, formation of compound II is associated with enzyme inactivation, which might be mediated by compounds such as ascorbate when H_2O_2 concentrations are low (Davison *et al.*, 1986). Prevention of compound II formation and/or reduction to the initial state involves one molecule of NADPH, which is bound, more or less tightly depending on the species, to each of the four catalase subunit polypeptides (Kirkman *et al.*, 1999). This requirement for NADPH may be the main cause of erythrocyte malfunction in the genetic disease caused by loss of glucose-6-phosphate dehydrogenase activity in humans (Kirkman and Gaetani, 2007). However, comparative structural analyses suggest that the NADPH-binding site is present only in some clade III catalases, such as the human enzyme, but is not found in clade I catalases, the evolutionary group to which most plant catalases belong (Chelikani *et al.*, 2004; Zamocky *et al.*, 2008).

Catalase genes and isoforms

Phylogenetic classification of the numerous available catalase sequences has recently been performed by Zamocky *et al.* (2008). Two catalase genes are found in *Escherichia coli*: *KatE* encodes a monofunctional catalase while *KatG* encodes a catalase-peroxidase. Available genomic information suggests that most animals, including mammals, contain a single catalase gene. Angiosperm species studied to date all contain three catalase genes. This includes monocots and dicots such as tobacco, *Arabidopsis*, maize, pumpkin, and rice (Willekens *et al.*, 1995; Frugoli *et al.*, 1996; Guan and Scandalios, 1996; Esaka *et al.*, 1997; Iwamoto *et al.*, 2000). Information from genome sequencing has confirmed the presence of three catalase genes in *Arabidopsis*, two located on chromosome 1 (*CAT1*, *CAT3*) and one located on chromosome 4 (*CAT2*) (Frugoli *et al.*, 1996). All three translation products consist of 492 amino acids, with high similarity between the sequences (Fig. 3). While nucleotide sequences are sufficiently different to allow construction of gene-specific primers, the similarity of the three polypeptides makes it difficult to produce isoform-specific antibodies, though CAT1-specific antibodies have recently been reported in *Arabidopsis* (Hu *et al.*, 2010). In-gel analysis with antibodies can be performed after electrophoretic separation of CAT2 and CAT3 isoforms (Zimmermann *et al.*, 2006; Smykowski *et al.*, 2010).

Catalase nomenclature is a potential source of confusion when comparing studies on different plant species. Willekens *et al.* (1995) proposed a classification based on the naming of the tobacco genes. According to this grouping, Class I catalases are strongly expressed in photosynthetic tissues, while Class II catalases are associated with vascular tissues. Class III catalases are notably expressed in seeds and reproductive tissues. Although the three catalases may not exactly correspond between species, there is considerable evidence that functional specialization is quite well conserved, and that this classification is useful. Conserved features include tissue and cellular expression, and day–night rhythms in transcript abundance. Comparisons of gene structure (e.g. between maize and rice catalase genes; Iwamoto *et al.*, 2000) also support the division of catalases into three classes (Table 1).

Available evidence from expression patterns and functional analysis suggests that *Arabidopsis CAT1*, *CAT2*, and *CAT3* correspond to Class III, Class I, and Class II catalases, respectively (Table 1). The *CAT1* gene is mainly expressed in pollen and seeds, *CAT2* in photosynthetic

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

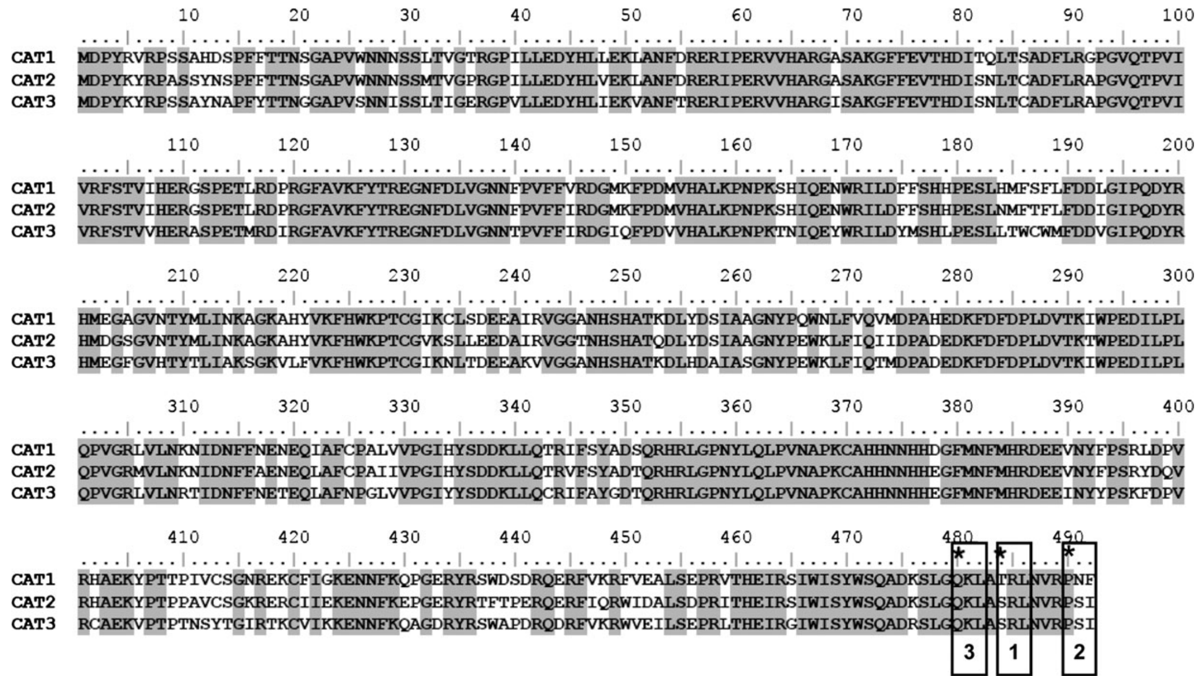

Fig. 3. Alignment of *Arabidopsis* catalase protein sequences. The amino acid sequences of CAT1, CAT2, and CAT3 were aligned using BioEdit software (Hall, 1999). Regions of identical amino acids are shown in grey. Carboxy terminal regions that have been implicated in determining import into peroxisomes are boxed.

Table 1. Probable classification of the three catalases found in different plant species

The division into three classes is based on the classification introduced by Willekens *et al.* (1995) and is shown for five species in which a complete trio of catalase genes have been identified and studied.

	Class I	Class II	Class III
Tobacco	Cat1	Cat2	Cat3
Arabidopsis	CAT2	CAT3	CAT1
Maize	Cat2	Cat3	Cat1
Pumpkin	cat2	cat3	cat1
Rice	CatC	CatA	CatB

tissues but also in roots and seeds, while *CAT3* is associated with vascular tissues but also leaves (Fig. 4A). All three transcripts can be detected in mature *Arabidopsis* rosettes, though *CAT3* and *CAT2* transcripts are much more abundant than those of *CAT1* (Frugoli *et al.*, 1996; McClung, 1997; Fig. 4A). Class I and Class II catalases show a contrasting day–night rhythm in transcript abundance. Thus, in maize, *CAT3* transcripts (encoding Class II catalase) show a circadian rhythm that is opposite to many genes involved in photosynthesis-related metabolism, which have peak expression at the night/day transition (Redinbaugh *et al.*, 1990). Subsequent work in *Arabidopsis* revealed a marked photosynthetic-type rhythm for *CAT2* transcripts (Zhong *et al.*, 1994) and an opposite rhythm for *CAT3* (Fig. 4B). Class III catalases like *Arabidopsis CAT1* show less significant day–night variation in transcript abundance (McClung, 1997). Although some evidence of a day–night rhythm in *CAT3* transcripts was found in tobacco leaves, variations were less marked than for the Class I and II catalases (Dutilleul *et al.*, 2003).

Day–night variations in catalase activity and protein are generally much less evident then changes in transcripts (Dutilleul *et al.*, 2003). This could partly be because Class I and Class II catalases contribute to total foliar activity in a complementary manner throughout the day. However, this view is not supported by analysis of tobacco and *Arabidopsis* knockdown and knockout lines, which suggest that Class I enzymes account for most of the leaf activity (discussed further below). Activity staining on native gels often reveals more than three bands (Scandalios *et al.*, 1980; McClung, 1997; Zimmermann *et al.*, 2006). A recent study in *Arabidopsis* reported seven catalase activity bands (Hu *et al.*, 2010). Based on a comparison between *cat1*, *cat2*, and *cat3* knockouts, four of these were concluded to be heterotetramers (Hu *et al.*, 2010). The *in vivo* significance of these observations remains to be established, given that the three genes are probably mainly expressed in different cells (Zimmermann *et al.*, 2006).

Subcellular localization

Questions remain concerning the subcellular localization of catalases in plants. Of the two genes found in yeast, one encodes a cytosolic isoform, the other an enzyme found mainly in peroxisomes (Petrova *et al.*, 2004). The single gene found in mammals is considered to encode a solely or predominantly peroxisomal enzyme, but some activity may be localized in mitochondria (Zamocky *et al.*, 2008). Unlike most animals studied so far, the worm *Caenorhabditis*

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

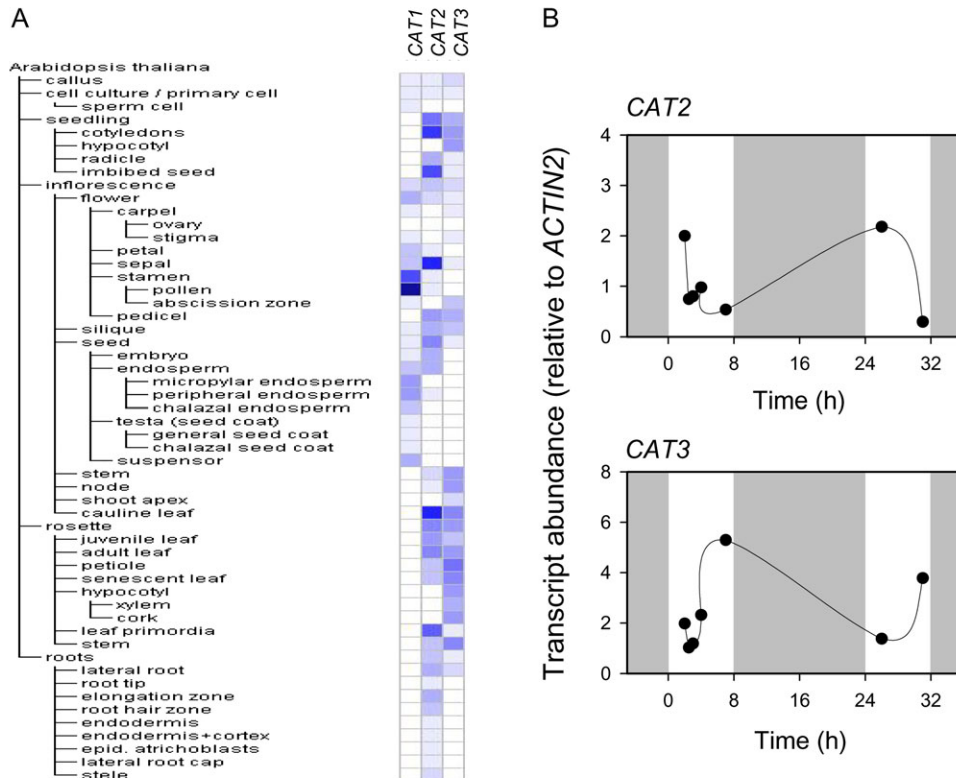

Fig. 4. Expression patterns of the three catalase genes in *Arabidopsis*. (A) Transcript abundance in different tissues and organs. Data were obtained using Genevestigator V3 (https://www.genevestigator.ethz.ch; Hruz *et al.*, 2008) and transcript abundance is represented on a scale from white (low) to deep blue (high). (B) Relative transcript levels of *CAT2* and *CAT3* during day–night cycles in a 16/8 h light/dark photoperiod regime. Transcript abundance was quantified by qRT-PCR and are relative to *ACTIN2*.

elegans was reported to contain three genes, one of which encodes a major peroxisomal form while a second encodes a minor cytosolic enzyme (Petriv and Rachubinski, 2004).

In plants, classical subcellular fractionation studies as well as *in situ* activity staining have established that peroxisomes contain high catalase activity and import of catalase into these organelles has been experimentally demonstrated (Mullen *et al.*, 1997). However, questions concerning the nature of the import mechanism remain. Two principal pathways have been identified for the entry of proteins into peroxisomes. The most common is the Peroxisomal Targeting Sequence 1 (PTS1) pathway (Brown and Baker, 2008; Kaur *et al.*, 2009). Depending on the protein, the PTS1 pathway is completely or partly governed by non-cleaved tripeptide sequences at the extreme C-terminus of the polypeptide (Reumann *et al.*, 2007; Brown and Baker, 2008; Kaur *et al.*, 2009). While one classical PTS1 motif (S-R-L) is found in both *Arabidopsis* CAT2 and CAT3, and CAT1 contains a similar T-R-L sequence at the same position (Fig. 3, box 1), these tripeptides are not located at the extreme C-terminus but at –7 to –9 upstream. Such internally located motifs may enhance import into peroxisomes but are not in themselves considered sufficient (Kaur *et al.*, 2009). Although known C-terminus PTS1 tripeptides show considerable variability between different peroxisomal proteins (Reumann *et al.*, 2007), the corresponding sequences of *Arabidopsis* catalases (Fig. 3, box 2) are not considered to be among them (Kaur *et al.*, 2009). Despite this, the C-terminal tripeptide sequence (Fig. 3, box 2) was necessary for efficient import of cottonseed catalase into tobacco BY-2 cell peroxisomes (Mullen *et al.*, 1997).

As well as the internally located S/T-R-L, a third motif has been implicated in catalase import into peroxisomes (Kamigaki *et al.*, 2003). This is a Q-K-L sequence located further upstream of the S/T-R-L motif (Fig. 3, box 3). This sequence was reported to influence the interaction between CAT1 and the PTS1 receptor protein, Pex5p (Kamigaki *et al.*, 2003). Based on current understanding of PTS1 pathway mechanisms (Lanyon-Hogg *et al.*, 2010), it seems unlikely that the internal Q-K-L sequence interacts directly with Pex5p. Another study concluded that accumulation of pumpkin CAT1 in peroxisomes requires several components of the PTS1 pathway machinery (Oshima *et al.*, 2008). Together, these observations suggest that several motifs within the 13 C-terminal amino acids may act to allow and/or enhance import of catalases into peroxisomes via the PTS1 pathway, although further work is required to resolve this question.

The presence of significant catalase activity in other subcellular compartments is less well established. In the literature, catalase activity in the cytosol and mitochondria has been frequently described. Indeed, catalase activity can be detected in chloroplasts isolated by classical fractionation techniques. It has been accepted for many years,

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

however, that this activity can be attributed to the presence of peroxisomes in the chloroplast preparation or adhesion of contaminating enzyme to the exterior of the chloroplasts (Allen and Whatley, 1977, and references therein for a discussion of concepts prior to the discovery of chloroplast APX in 1979). In cyanobacteria, which are considered to be the closest extant relatives of the earliest oxygen-evolving organisms, both monofunctional catalases and catalase-peroxidases exist, although this varies between cyanobacterial species (Mutsada *et al.*, 1996; Regelsberger *et al.*, 2002). The model unicellular eukaryotic green alga (*Chlamydomonas reinhardtii*), contains several isoforms of catalase. These differ from higher plant catalases because they are dimeric (rather than tetrameric) and localized in the mitochondria (Kato *et al.*, 1997) rather than in peroxisomes, which are generally negligeable or absent in this organism. Significant catalase activity has been detected in washed mitochondria from maize primary leaves (in which Cat3 is the major isoform), and was partly retained in subsequently prepared submitochondrial particles (Scandalios *et al.*, 1980). Interestingly, the putative mitochondrial maize catalase isoform was reported to have higher peroxidase activity than the other maize isoforms and to be relatively sensitive to 3-AT (Havir and McHale, 1989). Proteomic analysis of highly purified mitochondria from *Arabidopsis* cells identified CAT2 and CAT3 peptide sequences (Heazlewood *et al.*, 2004). This finding was interpreted with some caution since catalase activity, used as a marker for peroxisomal contamination, showed a progressive decline throughout the mitochondrial purification procedure, along with the plastid marker enzyme, alkaline pyrophosphatase (Heazlewood *et al.*, 2004). In yeast, peroxisomal catalase was co-localized to mitochondria in a manner that depended on nutritional conditions (Petrova *et al.*, 2004). Dual peroxisomal/mitochondrial targeting of catalases cannot yet be ruled out. However, to date there have been no demonstrations of catalase import into angiosperm mitochondria using either *in vitro* or *in vivo* approaches, and it is possible that contamination may account for reports of catalase activity in this organelle.

Whether some cytosolic catalase activity originates from incomplete import of catalases into the peroxisomes also remains unclear. At least for some peroxisomal proteins, it is known that they can enter peroxisomes as preformed oligomers or as monomers following oligomer disassembly (Kamigaki *et al.*, 2003; Brown and Baker, 2008). Thus, cytosolic catalase activity could possibly arise from assembled tetramers awaiting import. Studies in human cell lines suggest that catalase may be imported less efficiently than typical PTS1-dependent peroxisomal proteins (Koepke *et al.*, 2007). While the issue of cytosolic catalase in plants remains to be resolved, no substantive conclusion can be drawn from the detection of the enzyme in the soluble fraction produced following removal of intact organelles by high-speed centrifugation. Even if such soluble fractions were organelle-free, the activity detected could reflect the presence of enzymes released from organelles during disruption of the original tissue or cells.

Genetic manipulation of catalase

Overexpression and ectopic expression of catalase

Catalase capacities in leaf peroxisomes are already very high in many plants, particularly C_3 species. Introduction of the maize *CAT2* gene into tobacco did cause some increase in leaf activity and the lines were used to analyse responses to bacterial infection (Polidoros *et al.*, 2001). An O_2-resistant tobacco mutant line was identified that showed a significant increase in leaf catalase activity (Zelitch, 1992). Using both this mutant line and catalase underexpressors, an inverse correlation was reported between catalase capacities and the leaf compensation point, the ambient CO_2 concentration at which photosynthetic CO_2 uptake equals (photo)respiratory CO_2 release (Brisson *et al.*, 1998). This inverse correlation was explained by catalase-dependent control of H_2O_2-dependent decarboxylation of glyoxylate. Most glyoxylate formed in the peroxisomes during photorespiration is transaminated to glycine, but oxidative decarboxylation triggered by H_2O_2 could also occur. Any such effect would increase the CO_2 yield per glycolate carbon above the accepted value of 0.25. Less direct effects on photosynthesis are also conceivable through inhibition of the Calvin cycle caused by less efficient glycolate recycling. Little evidence of increased glyoxylate decarboxylation was reported in the barley mutant with less than 10% leaf catalase capacity, even though increased decarboxylation was measurable in barley serine:glyoxylate aminotransferase (SGAT) mutants (Leegood *et al.*, 1995, and references therein). In view of the mutant lines and other tools available, this question might be worth revisiting in *Arabidopsis*.

Expression of *E. coli* KatE in the chloroplast improved paraquat and drought tolerance in tobacco and protected thiol-regulated chloroplast enzymes from oxidative inactivation (Shikanai *et al.*, 1998). Introduction of the same catalase gene into rice was reported to increase resistance to salt (Moriwaki *et al.*, 2007). Thus, even though APX and thioredoxin peroxidases are considered to be the main enzymes involved in H_2O_2 metabolism in the chloroplast, ectopically introduced catalase appears be able to make a significant contribution. Characteristics such as efficacy at low H_2O_2 concentrations might explain why peroxidases have replaced catalases during chloroplast evolution.

Catalase mutants and knockdowns

In humans, the autosomal recessive peroxisomal disorder acatalasemia (also known as Takahara's disease) is caused by a complete deficiency in catalase. While this can have several adverse consequences on health, symptoms are confined to specific tissues or physiological conditions (Zamocky *et al.*, 2008). Knockout mice with negligible catalase activity only show differences from wild-type mice under conditions of oxidative challenge (Ho *et al.*, 2004). Likewise, the first plant catalase mutants, isolated in the C_4 plant maize, did not show obvious phenotypes (reviewed in Scandalios, 1994). Subsequently, however, a photorespiratory screen of a mutant collection in the C_3 plant barley

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

identified a stable line with only about 10% wild-type leaf catalase activity (Kendall *et al.*, 1983). When grown under standard conditions (moderate irradiance in air), this mutant displayed leaf bleaching and marked perturbation of glutathione pools, both of which could be prevented by growth at 0.2% CO_2 (Kendall *et al.*, 1983; Smith *et al.*, 1984). Studies using antisense technology confirmed the importance of a specific catalase isoform in C_3 photosynthetic metabolism: tobacco *CAT1* but not *CAT2* knockdowns show spontaneous lesion formation and oxidative perturbation when grown above a threshold irradiance (Chamnongpol *et al.*, 1996, 1998; Willekens *et al.*, 1997).

In contrast to the observations in barley, no photorespiratory catalase mutants were identified using a forward genetics approach in *Arabidopsis* (Somerville, 1986). However, analysis of RNAi lines using a *CAT2*-based sequence revealed phenotypes and changes in gene expression that were dependent on irradiance and that could be opposed by high CO_2 (Vandenabeele *et al.*, 2004). A study of gene-specific T-DNA knockouts confirmed that *CAT2* encodes the major leaf catalase isoform and that the function of this enzyme is closely linked to photorespiration (Queval *et al.*, 2007).

In *cat2* knockouts, leaf catalase activity is only about 10% of Col-0 wild-type plants (Fig. 5) and root catalase activity is also decreased (Bueso *et al.*, 2007). Knockout lines for *cat1* and *cat3* (Fig. 5A) show much less decrease in leaf activity than *cat2* (Fig. 5B). In leaves from 6-week-old plants, catalase activity in *cat3* is reduced about 20%, while in *cat1* it is similar to Col-0. This is consistent with the very low abundance of *CAT1* transcripts in leaves (Fig. 4A). Double *cat2 cat1* and *cat2 cat3* mutants have similar decreases in leaf catalase activity to those in *cat2*.

Figure 5 provides further evidence that CAT2 and CAT3 are the major isoforms in *Arabidopsis* rosette tissue. While the relative contribution of the different genes to overall leaf catalase activity changes with the developmental stage of the plant (Zimmermann *et al.*, 2006), the approximately additive nature of *cat2* (90% decrease in leaf activity) and *cat3* (20% decrease in leaf activity) mutations suggests that the formation of hetero-oligomeric proteins from more than one catalase gene product is a minor phenomenon *in vivo.*

An irradiance of 200 $\mu mol\ m^{-2}\ s^{-1}$ at the *Arabidopsis* leaf surface can be considered moderate since it drives photosynthesis at about 50% of the maximal rate (Veljovic-Jovanovic *et al.*, 2001). When grown in these conditions, *cat2* shows a dwarf phenotype linked to redox perturbation. Phenotypes of *cat2* are apparent at growth irradiances of 50–100 $\mu mol\ m^{-2}\ s^{-1}$ and become more evident with increasing irradiance, demonstrating a close link to the rate of photorespiratory H_2O_2 production (Queval *et al.*, 2007). By contrast, *cat2* is aphenotypic when grown at high CO_2 or at low light (below 50 $\mu mol\ m^{-2}\ s^{-1}$), consistent with studies of tobacco *CAT1* antisense lines (Willekens *et al.*, 1997).

Catalase-deficient barley show leaf bleaching (Kendall *et al.*, 1983) while tobacco CAT1 antisense lines showed necrotic lesions linked to the activation of certain pathogen responses (Chamnongpol *et al.*, 1996; Takahashi *et al.*, 1997). In *Arabidopsis cat2* grown in short days, a dwarf phenotype is observed but no lesions (Fig. 6A). However, when grown in a 16 h photoperiod (long days), *Arabidopsis cat2* shows spreading necrotic lesions (Fig. 6B). The *cat2* phenotypes are accompanied by intracellular redox perturbation as evidenced by the accumulation of oxidized glutathione (GSSG; Fig. 7). However, this perturbation is at least as marked in short-day as in long-day growth conditions (Queval *et al.*, 2007, 2009), indicating that lesion formation in *cat2* in long days is not simply caused by greater oxidative stress than in short days (Chaouch *et al.*, 2010). Thus, increased intracellular oxidation caused by H_2O_2 availability is not sufficient to trigger cell death. Additional signals are required (discussed further below).

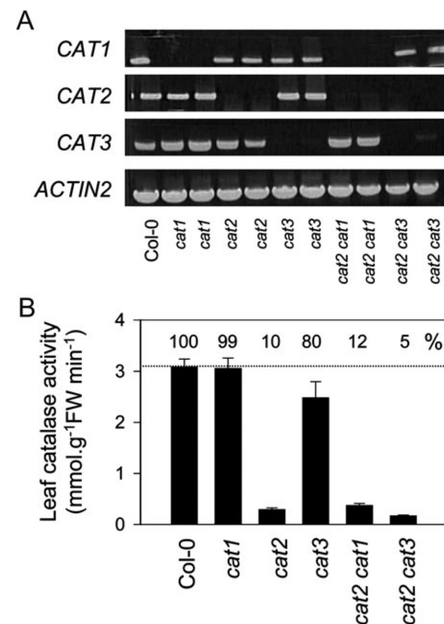

Fig. 5. Catalase transcript abundance and extractable enzyme activity in *Arabidopsis* catalase single and double T-DNA mutants. (A) RT-PCR was performed using gene-specific primers (see Supplementary Table 1 at *JXB* online) and cDNA obtained from duplicate RNA extracts of 6-week-old plants grown in a 8/16 h light/dark regime. (B) Catalase activity was measured according to Queval *et al.* (2007) in leaf extracts from plants sampled in the same conditions as (A). Data are means ±SE of three different extracts. Percentage catalase activity relative to Col-0 (dotted line) is indicated at the top of the frame. T-DNA mutants from the SALK collections were obtained from Nottingham Arabidopsis Stock Centre (NASC), Nottingham University, UK. The polymorphisms were SAIL_760_D05 for *cat1*, SALK_076998 for *cat2* (*cat2-1*; Bueso *et al.*, 2007; Queval *et al.*, 2009, previously named *cat2-2* (Queval *et al.*, 2007)) and SALK_092911 for *cat3*.

In contrast to *cat2*, neither *cat1* nor *cat3* show any obvious rosette phenotype (Fig. 6) or perturbation of glutathione status (Fig. 7) during the vegetative growth stage. Both *cat2 cat3* and *cat2 cat1* double mutants have a slightly exacerbated lesion phenotype in long days (Fig. 6B) but quite similar glutathione status compared to *cat2* (Fig. 7). None of the lines show lethality, and all can survive and set seed, at least when grown under moderate irradiance. Most of these observations on catalase activity

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

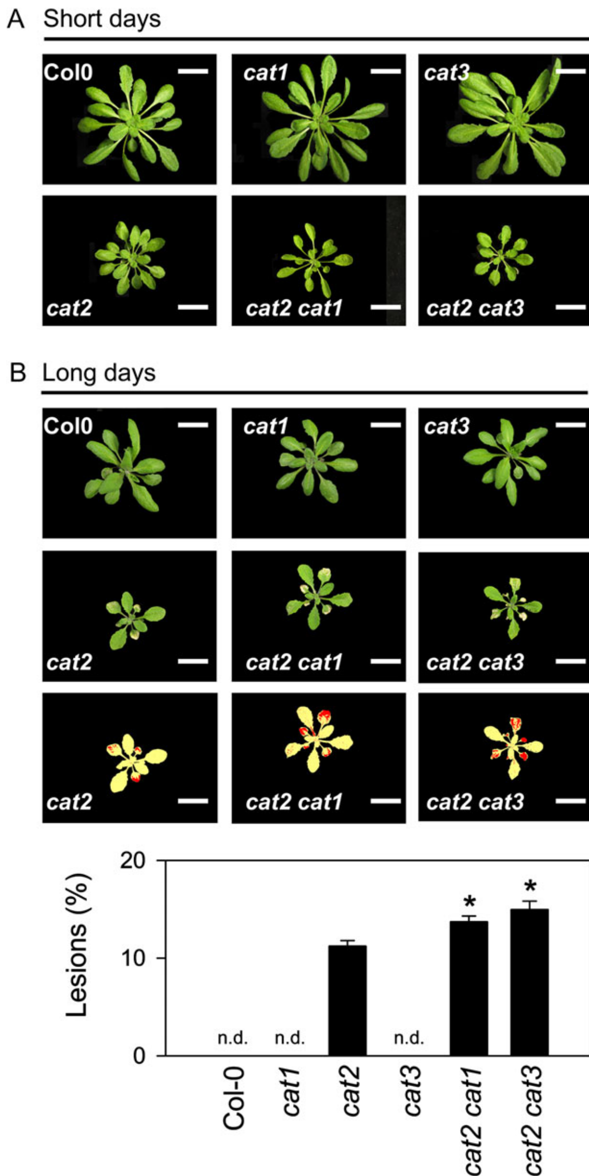

Fig. 6. Daylength-dependent phenotypes of single and double *Arabidopsis* catalase mutants. (A) Plants grown for 6 weeks in an 8/16 h light/dark regime (short days). (B) Plants grown for 3 weeks in a 16/8 h light/dark regime (long days). In both conditions, temperature and irradiance were 20/18 °C (day/night) and 200 μmol m^{-2} s^{-1} at the leaf surface, respectively. In (B) false-colour images of lesion-forming *cat2* genotypes are shown beneath the photographs while the histogram shows the percentage of rosette area with lesions. No lesions were observed in any of the genotypes grown in short days; n.d., not detected. Significant differences between *cat2* and the double mutants *cat2 cat1* and *cat2 cat3* are indicated by *P <0.05 (Student's t test, n=6–8).

and phenotype in the knockouts are in agreement with those of the study of Hu *et al.* (2010). These authors reported that *cat1* and *cat3* knockouts show no phenotype whereas *cat2* rosette growth was decreased. They also nicely showed that CAT2 function is determined by regulation of expression rather than any biochemical specificity, as *cat2*

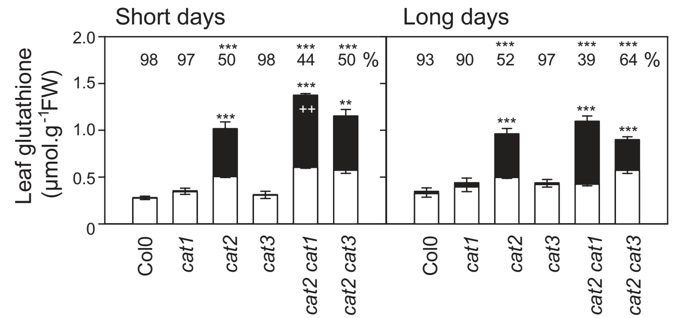

Fig. 7. Leaf glutathione status in Col-0 and catalase mutants. Glutathione was measured in leaf extracts according to Queval and Noctor (2007). Black bars show GSSG (in GSH equivalents) and white bars show GSH. Plants were grown for 6 weeks in short days (8/16 h light/dark) or three weeks in long days (16/8h light/dark). Data are means ±SE of three biological replicates. Percentage reduced glutathione (100 GSH/(GSH+2 GSSG)) is shown at the top of the frame and significant differences from Col-0 are indicated by *P <0.05, **P <0.01, ***P <0.001. No significant differences were observed between *cat2* and *cat2 cat1* or *cat2 cat3*. Asterisks immediately above each column indicate significant difference in total glutathione from Col-0 at *P <0.05, **P <0.01, ***P <0.001. Significant differences in total glutathione between *cat2* and *cat2 cat1* and *cat2 cat3* are indicated by ^{+}P <0.05, ^{++}P <0.01, ^{+++}P <0.001.

could be complemented by either CAT2 or CAT3 expressed under the control of the *cat2* promoter (Hu *et al.*, 2010). Production of the *cat1 cat3* T-DNA combination is not feasible by crossing because of the contiguous position of the two genes. Therefore, it remains to be seen whether plants can survive without any catalase activity at all.

Recently, novel *CAT2* mutant alleles have been identified in a screen of a mutagenized *Arabidopsis* collection for resistance to hydroxyurea, an inhibitor of ribonucleotide reductase (Juul *et al.*, 2010). Seventeen allelic lines were identified with between 15% and 59% wild-type activity, and it was concluded that resistance was probably linked to loss of catalase-dependent activation of hydroxyurea (Juul *et al.*, 2010). Catalase-dependent pre-drug activation is well known in the pathogenic bacterium *Mycobacterium tuberculosis*, where the catalase-peroxidase *katG* activates the anti-tubercular compound, isoniazid (Lei *et al.*, 2000).

Exploiting catalase-deficient plants to understand stress responses

Numerous studies over the last two decades have adopted the strategy of overexpressing antioxidative enzymes with the ultimate aim of enhancing stress resistance in plants. These studies have produced variable effects, though there are many reports of enhanced resistance to artificially induced and relatively short-term oxidative stress generated, for example, by the exogenous addition of ROS or ROS-producing agents. It is less clear whether enhanced expression of antioxidative enzymes would produce desirable effects under agronomically relevant conditions. For example, while overexpression of glutathione reductase (GR) was reported

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

to enhance resistance to paraquat, ozone, and photoinhibitory conditions (Aono et al., 1993; Broadbent et al., 1995; Foyer et al., 1995), it did not lead to improved performance in field conditions (Kornyeyev et al., 2005). There is increasing recognition of the complexity and redundancy of the antioxidative system, as well as the importance of ROS as essential signal molecules whose effects, whether perceived as negative or beneficial, are embedded in cellular and intercellular interactions. These notions are possibly important for attempts to engineer responses to ROS in all complex multicellular organisms. For instance, while mouse life span was increased by the expression of catalase in mitochondria (Schriner et al., 2005), several studies on mammalian cells indicate that catalase overexpression can have undesirable consequences, for example, on wound healing (Zamocky et al., 2008).

In plants, the situation is further complicated by the multiplicity of ROS-producing pathways and the constitutively high capacity of the antioxidative system (Foyer and Noctor, 2009). These factors perhaps make it unlikely that attempts to reinforce front-line metabolic 'defences' will markedly enhance broad-spectrum stress resistance in plants. An alternative approach is to search for ROS-dependent control components (e.g. transcription factors) that act as key nodes in stress responses. Within this objective, plant systems that allow inducible, endogenous increases in ROS availability may be useful tools. In addition to catalase-deficient plants (Dat et al., 2001), these systems include the singlet oxygen-generating *flu* mutant (Baruah et al., 2009) and plants ectopically expressing glycolate oxidase in the chloroplast (Fahnenstich et al., 2008).

Tobacco *CAT1* antisense lines and *Arabidopsis cat2* RNAi lines have been exploited as tools to analyse the effects of H_2O_2 on cell death, pathogen responses, and transcript profiles (Chamnongpol et al., 1996, 1998; Rizshky et al., 2002; Dat et al., 2003; Vandenabeele et al., 2003, 2004; Vanderauwera et al., 2005; Gadjev et al., 2006; Van Breusegem and Dat, 2006). The main advantages of catalase-deficient plants in such studies are the specific intracellular location of H_2O_2 production, its easy manipulation by external conditions, and the predictable effect it produces on cell redox state, which can be assessed using quantitative techniques such as the glutathione assay. As noted previously, catalase-deficient plants are a useful tool because 'perturbation of H_2O_2 homeostasis can be sustained over time, no invasive techniques are needed to modulate H_2O_2 levels, and, more importantly, a potential debate about the physiological relevance of H_2O_2 levels can be avoided' (Dat et al., 2001).

Modification of cell redox state in catalase-deficient plants

H_2O_2 contents in catalase-deficient plants

Given the high flux through the photorespiratory glycolate oxidase reaction, plants deficient in Class I catalase might be expected to accumulate high levels of H_2O_2. Estimations of photorespiratory rates under typical controlled environment growth conditions point to a H_2O_2 generation rate of around 30 μmol g^{-1} FW h^{-1} through this pathway alone (Table 2). Assuming no compensatory metabolism of H_2O_2 by components other than Class I catalase, this would represent a theoretical increase in mean tissue H_2O_2 concentration of about 30 mM h^{-1}. Despite this, available data suggest that H_2O_2 accumulation is relatively minor or undetectable in CAT1-deficient tobacco or plants treated with 3-AT (Ferguson and Dunning, 1986; Willekens et al., 1997; Rizhsky et al., 2002). In *Arabidopsis*, increased diaminobenzidine (DAB) staining was reported in a *cat2* mutant grown at relatively low light (Bueso et al., 2007) while a recent study reported a 2-fold increase in extractable H_2O_2 in *cat2* and *cat2 cat3* knockouts (Hu et al., 2010). Increased DAB staining was also observed in *cat2* RNAi lines exposed to short-term high light stress or ozone (Vandenabeele et al., 2004). While no difference was found in the absence of infection, DAB staining of leaves 24 h after infiltration with avirulent bacteria was about 40% more intense in *cat2* compared with the wild type (Simon et al., 2010). In *Arabidopsis* expressing glycolate oxidase in the chloroplast (in which H_2O_2 production should also be influenced by RuBP oxygenation rates), increased DAB staining was detected above a threshold irradiance (Fahnenstich et al., 2008). However, using DAB staining or assays of extractable H_2O_2 with luminol, no increased signal was observed in *cat2* exposed to moderate irradiances, despite the clearly perturbed cell redox state under these conditions (Chaouch et al., 2010; Mhamdi et al., 2010*a*). Visualization of ROS in the mesophyll cells showed only a slight increase in dichlorofluorescein (DCF) fluorescence in *cat2* compared with Col-0 (Mhamdi et al., 2010*a*). Secondary signals produced by high light, ozone or pathogens may be required to trigger appreciable increases in ROS contents in catalase-deficient plants. Consistent with the notions that secondary production of ROS is important

Table 2. Calculation of approximate typical rates of photorespiratory H_2O_2 production

Net CO_2 uptake rate	7 μmol m^{-2} s^{-1} [a]
RuBP oxygenation rate	2 μmol m^{-2} s^{-1} [b]
Glycolate production	2 μmol.m^{-2} s^{-1}
H_2O_2 production	2 μmol $m^{-2}s^{-1}$ [c]
Fresh weight/leaf area	235 g m^{-2} [d]
H_2O_2 production per second	8.5 nmol g^{-1} FW
per minute	511 nmol g^{-1} FW
per hour	30.6 μmol g^{-1} FW
per 8 h day	245 μmol g^{-1} FW
per 16 h day	490 μmol g^{-1} FW

[a] Typical rate for *Arabidopsis* leaves growing in air at moderate irradiance (Veljovic-Jovanovic et al., 2001).
[b] The carboxylation:oxygenation ratio is taken as four, a conservative estimate of oxygenation (Keys, 2000).
[c] Assuming that all glycolate produced is oxidized by glycolate oxidase.
[d] Measured in expanded *Arabidopsis* rosette leaves.

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

and that catalase can act as a sink for H_2O_2 produced outside the peroxisomes (Willekens *et al.*, 1997), more intense DAB staining can be detected in *cat2* compared to Col-0 when exogenous H_2O_2 is supplied to excised leaves (A Mhamdi, unpublished results).

Minor or undetectable increases in H_2O_2 under conditions in which catalase deficiency triggers significant effects on gene expression and cell redox state may appear surprising. A possible explanation is that H_2O_2 increases in *cat2* are either local and/or transient and that H_2O_2 produced in catalase-deficient plants is rapidly scavenged by other pathways. In the barley catalase mutant, slightly increased H_2O_2 was observed within the first few hours after transfer from high CO_2 to air (Noctor *et al.*, 2002). Nevertheless, extractable leaf H_2O_2 contents measured in this mutant did not exceed 200 nmol g^{-1} FW. Measurements in *Arabidopsis* Col-0 and the *cat2* mutant are also within this range (Chaouch *et al.*, 2010; Mhamdi *et al.*, 2010*a*). When comparing these values with the estimated rate of photorespiratory H_2O_2 production (Table 2), it becomes evident that the majority of H_2O_2 must be metabolized, even in the absence of the major leaf catalase isoform. This view is consistent with the obvious effect of catalase deficiency on glutathione status in C_3 plants (see next section).

Measuring H_2O_2 in plants is not trivial and is further complicated by several interfering factors (Veljovic-Jovanovic *et al.*, 2002; Queval *et al.*, 2008). Most of the currently available methods may preferentially measure H_2O_2 that is sequestered within compartments such as the apoplast, vesicles or other compartments where redox buffering could be relatively low (Leshem *et al.*, 2006; Foyer and Noctor, 2009). Furthermore, the accuracy of many of the techniques commonly used to quantify H_2O_2 is uncertain. One issue is chemical specificity, notably due to interference from other reactive oxidants or from reductants (Wardman, 2007). Additional to this uncertainty are difficulties in the extraction of H_2O_2. Some of these issues may contribute to the very high variability in reported H_2O_2 contents, even in the absence of stress and between studies using the same technique (Queval *et al.*, 2008). If sufficiently specific, the use of *in vivo* probes and emerging nanotechnologies could provide more incisive information on compartment-specific H_2O_2 concentrations *in planta*.

The close link between intracellular H_2O_2 and thiol-disulphide status

Tissue glutathione status is markedly and reproducibly perturbed in catalase-deficient plants, as shown for *cat2* in Fig. 7. This effect is presumably a consequence of the enhanced engagement of catalase-independent pathways to metabolize intracellular H_2O_2 and was first reported in barley (Smith *et al.*, 1984). Very similar effects have been observed in catalase-deficient tobacco and *Arabidopsis* (Willekens *et al.*, 1997; Rizhsky *et al.*, 2002; Queval *et al.*, 2007) and are the most dramatic effects of catalase deficiency on cellular redox state described to date. In *cat2*, perturbation of glutathione redox state occurs within hours after the onset of photorespiratory H_2O_2 production and continues over the following days, with little or no change in global leaf redox states of functionally associated redox compounds through which GSH can be oxidized (ascorbate/dehydroascorbate) or GSSG can be reduced (NADPH/$NADP^+$) (Queval *et al.*, 2007; Mhamdi *et al.*, 2010*a*, *b*). This suggests that reductive pathways appear to compensate quite rapidly for catalase deficiency, leading to a new, more oxidized cellular redox state, notably reflected in adjustments of thiol-disulphide status.

An outstanding question is the functional impact of H_2O_2-triggered changes in glutathione. Despite the association of GSSG accumulation with dormancy and cell death (Creissen *et al.*, 1999; Kranner *et al.*, 2006), this phenomenon does not in itself appear to be part of the signal that leads to lesion formation in catalase-deficient plants (Queval *et al.*, 2007). Extremely dramatic accumulation of GSSG can occur in *cat2 gr1* double mutants without necessarily leading to either cell death or bleaching (Mhamdi *et al.*, 2010*a*). Cell death in *cat2* is reverted by extinction of SA synthesis or by exogenously supplying *myo*-inositol, but reversion is associated with an unchanged or more oxidized glutathione pool (Chaouch and Noctor, 2010; Chaouch *et al.*, 2010). Current evidence from work on catalase-deficient plants suggests that glutathione oxidation may be more important in the decreased growth phenotype rather than in processes associated with cell death. This would be consistent with the role of thiol-disulphide systems in cell cycle and meristem function (Reichheld *et al.*, 1999, 2007; Vernoux *et al.*, 2000; Frendo *et al.*, 2005; Bashandy *et al.*, 2010).

One approach to determine whether the changes in glutathione status provoked by catalase deficiency are part of H_2O_2-triggered signal transduction is to compare responses in *cat2* with those observed in lines in which glutathione contents or reduction states are altered independent of changes in H_2O_2 availability. Mutants in which one of the two *Arabidopsis* GR genes is knocked out (*gr1*) are aphenotypic and show no evidence of oxidative stress. However, this mutation triggers changes in gene expression that partly recapitulate those observed in *cat2* (Mhamdi *et al.*, 2010*a*). Moreover, introduction of the *gr1* mutation into the *cat2* background causes marked modulation of H_2O_2-associated transcript profiles (Mhamdi *et al.*, 2010*a*). This observation points to a significant role for glutathione status in transmitting signals derived from intracellular H_2O_2, though further work is required to resolve this issue fully.

The accumulation of glutathione when catalase is deficient very likely involves up-regulation of biosynthetic pathways. The amount of glutathione that is produced represents a significant increase in sulphur sinks (Queval *et al.*, 2009) and work on barley revealed a marked stimulation of sulphate incorporation into organic compounds when catalase was genetically decreased or biochemically inhibited (Smith *et al.*, 1985). H_2O_2-triggered oxidative accumulation of glutathione possibly involves

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

several mechanisms of sulphate reduction and synthesis of cysteine and glutathione, including enhanced gene expression and post-transcriptional activation (Bick *et al.*, 2001; Queval *et al.*, 2009).

Dissecting the interplay between different antioxidative systems

In systems where catalase is no longer sufficient, H_2O_2 or its derivatives such as organic peroxides must primarily be metabolized by peroxidases, which all require reductants. Catalase-deficient plants therefore provide (i) a means of penetrating the apparent redundancy of some of the different reductant-dependent pathways, and (ii) a genetic background in which the impact of perturbing specific components of these pathways can be evaluated. Recent demonstration of the usefulness of catalase-deficient plants has come from the analysis of *gr1* mutants. Growth of *Arabidopsis* seedlings under non-physiological oxidative stress conditions (H_2O_2 dissolved in agar) produced marked effects in wild-type and in *gr1* plants (Marty *et al.*, 2009). As *gr1* mutants are phenotypically indistinguishable from the wild type in optimal growth conditions (Marty *et al.*, 2009; Mhamdi *et al.*, 2010*a*), this observation provides little evidence for an important role for GR1, even under conditions of oxidative stress. However, introduction of the *gr1* mutation into a *cat2* background drastically exacerbates the *cat2* phenotype, modulates the *cat2* transcriptome, and causes a dramatic acceleration of the onset of leaf bleaching (Mhamdi *et al.*, 2010*a*). As the phenotype of the single *cat2* mutant is less severe compared with wild-type plants grown on H_2O_2, the difference in both studies cannot simply be explained by the dose of H_2O_2 or the severity of oxidative stress. It probably reflects the importance of the location of H_2O_2 production and the fact that in *cat2* and other catalase-deficient lines, the oxidative signal is produced through a physiologically relevant intracellular pathway. Interestingly, a peroxisomal GR activity has been characterized in pea (Romero-Puertas *et al.*, 2006), and studies in *Arabidopsis* show that GR1 is addressed to the peroxisomes as well as the cytosol (Kataya and Reumann, 2010). However, the peroxisomes probably contain only a very minor part of the GR1 gene product (Romero-Puertas *et al.*, 2006), which accounts for 30–60% of the overall leaf enzyme activity in *Arabidopsis* (Marty *et al.*, 2009; Mhamdi *et al.*, 2010*a*).

Differences between *cat2* and *cat2 gr1* transcript profiles could be linked to differences in glutathione redox state. Alternatively, they could be caused by more indirect effects of the *gr1* mutation on H_2O_2 metabolism in *cat2*. Analysis of several oxidative stress factors in *cat2* and *cat2 gr1* double mutants suggest a quite specific effect of H_2O_2 on glutathione redox status (Mhamdi *et al.*, 2010*a*).

One outstanding issue concerns the reductive pathways that metabolize H_2O_2 when catalase is down-regulated (Fig. 8). Ascorbate redox state is generally much less perturbed than that of glutathione in catalase-deficient plants. In part, this could be due to the redox potential of the ascorbate/dehydroascorbate (DHA) couple, which is about 200–300 mV more positive (less reducing) than the GSH/GSSG couple (Noctor, 2006). This predicts that intracellular ascorbate pools should remain highly reduced, even at low GSH:GSSG ratios, as long as both couples are close to redox equilibrium. This situation is observed in double *cat2 gr1* mutants, where whole leaf GSH:GSSG ratios (typically 20 or above in unstressed wild-type plants) can fall well below 0.1 while ascorbate:DHA ratios remain close to wild-type values (Mhamdi *et al.*, 2010*a*).

Increases in intracellular H_2O_2 may impact more on total ascorbate pools than on ascorbate redox states. Decreases in total ascorbate are sometimes observed in *cat2*, although these are relatively minor compared with the marked increase in total glutathione (Queval *et al.*, 2007; Mhamdi *et al.*, 2010*a*; Chaouch and Noctor, 2010; Chaouch *et al.*, 2010). Total ascorbate pools in wild-type plants are highly influenced by growth irradiance (Grace and Logan, 1996; Gatzek *et al.*, 2002). In tobacco *Cat1* knockdowns, increased H_2O_2 stress was triggered by transferring plants from low to moderate light (Willekens *et al.*, 1997). While this treatment caused ascorbate contents to increase 4-fold within 2 d in wild-type tobacco plants, no increase was observed in the catalase-deficient lines (Willekens *et al.*, 1997). Evidence that enhanced availability of intracellular H_2O_2 opposes certain other responses to increased irradiance has been presented in studies of *Arabidopsis CAT2* knockdowns (Vandenabeele *et al.*, 2004; Vanderauwera *et al.*, 2005).

Electron flow between glutathione and ascorbate (Fig. 8) can occur chemically but is also catalysed by dehydroascorbate reductases (DHAR) (Foyer and Mullineaux, 1998). Enhanced activity of the ascorbate–glutathione pathway when catalase is deficient was evidenced by induction of APX and DHARs at both the transcript level and by enzyme activity (Willekens *et al.*, 1997; Vanderauwera *et al.*, 2005; Mhamdi *et al.*, 2010*a*). Changes at transcript level were specific to cytosolic forms of these enzymes in *Arabidopsis cat2* and *cat2 gr1* mutants (Mhamdi *et al.*, 2010*a*), despite the presence of at least one and up to three peroxisome-associated APXs and a dual cytosol/peroxisomal location for GR1 (Narendra *et al.*, 2006; Nyathi and Baker, 2006; Kaur *et al.*, 2009). This points to a close coupling of increased peroxisomal H_2O_2 availability and cytosolic antioxidant systems, consistent with the demonstrated importance of cytosolic APX1 in *Arabidopsis* (Davletova *et al.*, 2005*a*).

H_2O_2-triggered oxidation of glutathione in *cat2* may also be mediated through ascorbate-independent pathways (Fig. 8). Catalase-deficient tobacco shifted to high light to induce photorespiration showed the accumulation of GPX proteins alongside APX (Willekens *et al.*, 1997) and GPX transcripts are induced in *Arabidopsis cat2* mutants (Queval *et al.*, 2007; Mhamdi *et al.*, 2010*a*). However, thioredoxins are more efficient reductants for annotated GPXs than glutathione (Iqbal *et al.*, 2006). A more significant route for

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

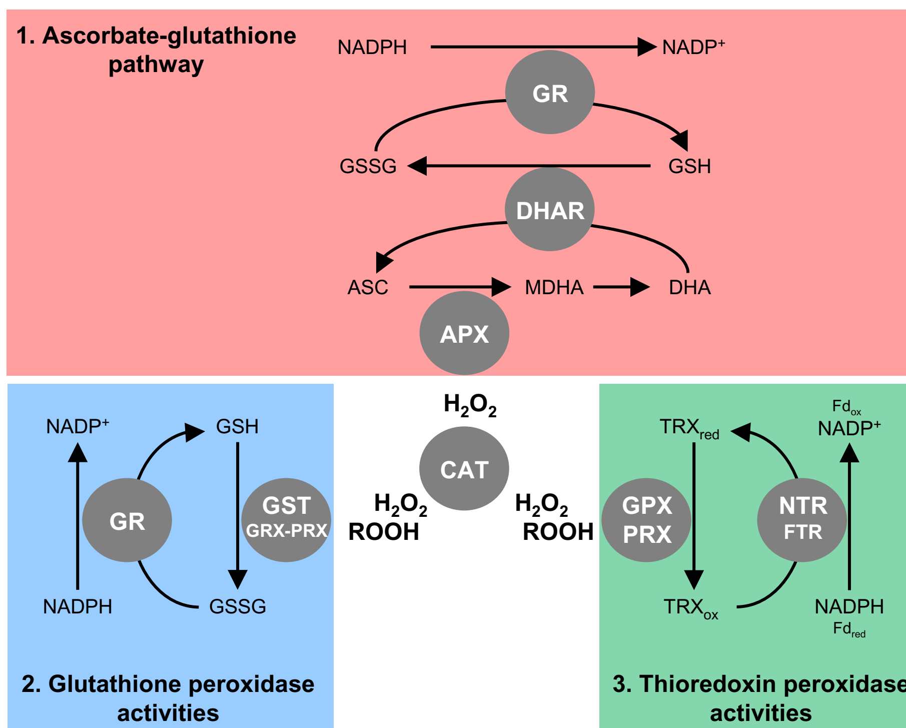

Fig. 8. Interplay between catalase and reductive pathways of peroxide metabolism. The three pathways are represented in a simplified version. Catalase and APX are considered to be H_2O_2-specific, while thiol-dependent peroxidases may metabolize both H_2O_2 and organic peroxides produced secondarily when catalase is deficient. Several interactions are possible between glutathione and thioredoxin peroxidase pathways, and both potentially involve several different types of enzyme. Ascorbate regeneration from MDHA can also occur at the expense of NADPH or other reductants. APX, ascorbate peroxidase. ASC, ascorbate. CAT, catalase. DHAR, dehydroascorbate reductase. Fd_{ox}, oxidized ferredoxin. Fd_{red}, reduced ferredoxin. FTR, ferredoxin-thioredoxin reductase. GPX, glutathione(thioredoxin) peroxidase. GR, glutathione reductase. GRX, glutaredoxin. GSH, glutathione. GSSG, glutathione disulphide. GST, glutathione *S*-transferase. MDHA, monodehydroascorbate reductase. NTR, NADPH-thioredoxin reductase. PRX, peroxiredoxin. ROOH, organic peroxide. TRX_{ox}, oxidized thioredoxin. TRX_{red}, reduced thioredoxin.

direct peroxidation of GSH could be through glutathione *S*-transferases (GSTs), many of which have peroxidase activity (Dixon *et al.*, 2009). Several GSTs are strongly induced in catalase-deficient lines (Vanderauwera *et al.*, 2005; Queval *et al.*, 2007).

The effects of the *gr1* mutation on the *cat2* phenotype contrast intriguingly with the ameliorated phenotype observed when cytosolic APX activity is down-regulated in tobacco *CAT1* knockdowns (Rizhsky *et al.*, 2002). Interestingly, the ameliorated phenotype of *Arabidopsis* mutants that are deficient in both CAT2 and cytosolic APX is correlated with the induction of a network of DNA repair, cell cycle control, and ER cell death pathways (Vanderauwera *et al.*, 2010). Different effects of APX and GR1 deficiency in catalase-deficient backgrounds imply that oxidation of glutathione in catalase-deficient plants occurs only partly through the APX/DHAR route and more direct peroxidation may be catalysed by enzymes such as GSTs that are linked to GR1 but independent of APX1 (Fig. 8). This conclusion is consistent with the observed gene expression patterns. Further work is required to establish which glutathione-associated peroxidases are most important in ascorbate-independent reduction of H_2O_2 or derived peroxides.

Physiological significance of studies of catalase mutants

A major advantage of using C_3 plants deficient in Class I catalases to modify cell redox state is that the rate of intracellular H_2O_2 production can be readily modulated by irradiance and switched off in the light by high CO_2. This feature enables redox state to be conditional and controllably perturbed. Growth of *Arabidopsis cat2* at high CO_2 prevents redox perturbation and hence the marked phenotype observed during growth in air (Fig. 9A). Transferring *cat2* back to air causes the onset of measurable oxidative stress within hours to days, as evidenced by changes in glutathione status (Fig. 9A). However, within a time-scale of several days after transfer, little effect of the oxidative stress on the plant phenotype is observed. Thus, catalase-deficient plants allow early and late events in oxidative signalling pathways to be studied, within the overall aim of distinguishing between H_2O_2-dependent and -independent components involved in stress responses (Fig. 9B). Furthermore, the conditional photorespiratory nature of *cat2* predicts that any effects of secondary mutations should be annulled by growth at high CO_2. For example, *cat2 gr1* double mutants show a phenotype that is much more severe

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

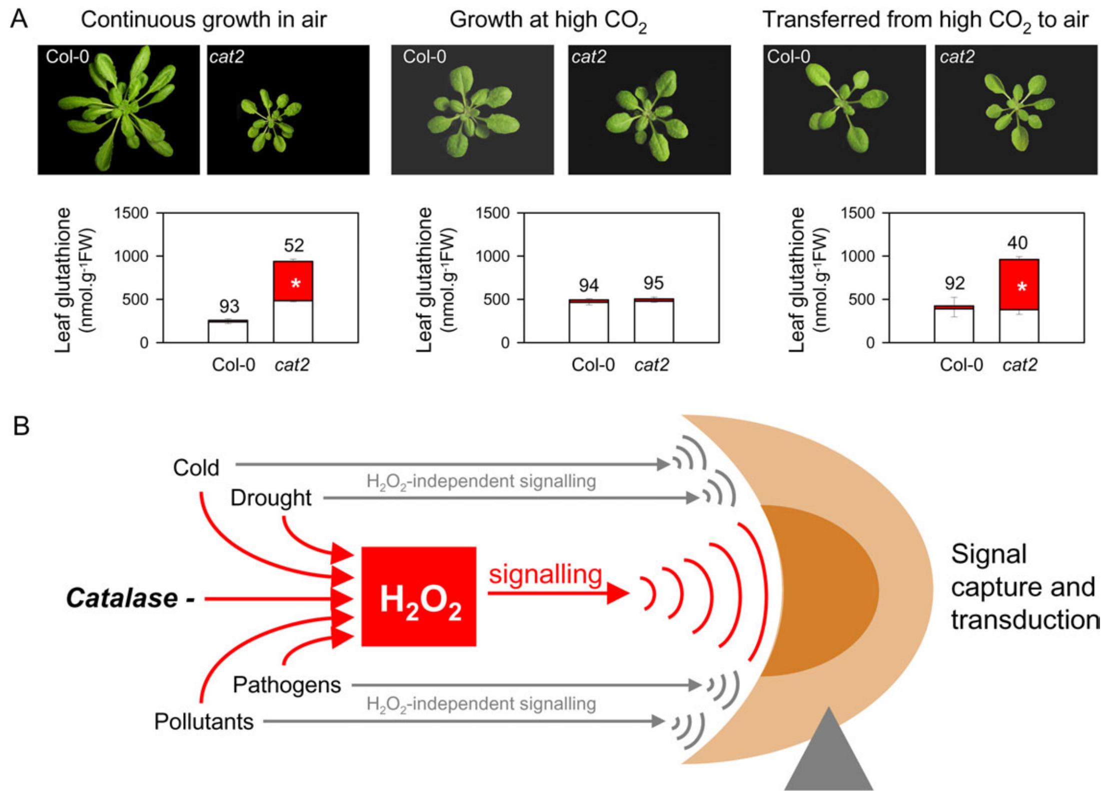

Fig. 9. Conditional photorespiratory nature of *cat2* and its application in the analysis of H_2O_2 signalling processes. (A) Dependence of *cat2* phenotype and leaf redox state on photorespiratory H_2O_2 production. Plants were grown in controlled environments at 8/16h light/dark and irradiance of 200 μmol m^{-2} s^{-1}. Specific conditions were 5 weeks in air (left), 3 weeks at 3000 ppm CO_2 (middle), or 3 weeks at high CO_2 and subsequently transferred for 4 d to air (right). Glutathione was assayed as a marker for leaf redox state in each condition. White and red blocks indicate GSH and GSSG, respectively. Data are means ±SE of three independent leaf extracts. Percentage reduction state (calculated as 100 GSH/(GSH+2GSSG)) is shown above the bars and significant differences between Col-0 and *cat2* are indicated by $^*P$ <0.005. (B) Scheme showing the use of *cat2* and similar plants to identify H_2O_2-specific signalling pathways likely to be important in stress conditions. Although stress conditions are perceived by the plant through H_2O_2-independent pathways (which may be specific to a stress or common between different stresses), most stresses involve redox signalling mediated through components such as H_2O_2. The conditional nature of the catalase-deficient mutants enables H_2O_2-dependent signalling to be studied by varying intensity (irradiance, CO_2 level) and also duration (kinetics studies after transfer from low to high light or from high CO_2 to air).

than *cat2* grown in air. However, when *cat2* and *cat2 gr1* are grown at high CO_2, they display similar phenotypes to wild-type and *gr1* single mutants (Mhamdi *et al.*, 2010*a*).

Two related but distinct questions arise from studies of catalase-deficient plants: (i) How relevant are effects observed in such systems to those occurring during stress? (ii) Does regulation of catalase activity itself play a significant role in H_2O_2-linked signalling in wild-type plants?

How relevant are observations in catalase-deficient models to events occurring during stress conditions?

Considerable information on H_2O_2-induced changes in gene expression has been generated by studies of catalase-deficient tobacco and *Arabidopsis* lines (Vandenabeele *et al.*, 2003, 2004; Vanderauwera *et al.*, 2005; Gadjev *et al.*, 2006; Queval *et al.*, 2007). Analysis of transcription factors that responded rapidly on shifting *Arabidopsis CAT2* knockdowns to high light revealed that several were also affected by abiotic stresses such as cold, heat, and drought (Vanderauwera *et al.*, 2005). Several of these H_2O_2-induced transcription factors are now established regulators of abiotic stress responses (Shinozaki and Yamaguchi-Shinozaki, 2000; Davletova *et al.*, 2005*b*; Vogel *et al.*, 2005; Nishizawa *et al.*, 2006; Ogawa *et al.*, 2007). Furthermore, many of the genes that were strongly induced in *Arabidopsis cat2* knockouts after transfer from high CO_2 to air (Queval *et al.*, 2007) are also induced by abiotic and biotic stresses (Table 3), consolidating the notion that increased availability of H_2O_2 is an important signal in abiotic stress-induced gene expression (Vanderauwera *et al.*, 2005). Furthermore, a complex pattern of different ROS-responsive genes has been identified during various abiotic stresses, suggesting that different abiotic stress conditions provoke the production of different ROS and that transcriptome profiling analyses could predict the degree of involvement of a specific ROS during a specific stress condition (Gadjev *et al.*, 2006).

Phenotypes of tobacco and *Arabidopsis* lines suggest that catalase deficiency mimics biotic stress particularly strongly. Under conditions favouring photorespiration, responses

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

Table 3. Examples of genes that are conditionally induced in *cat2* and a summary of their responses to environmental stresses or hormones

The selected genes represent those most strongly induced in the *cat2* knockout mutant, based on transcriptomic footprinting of about 2000 *Arabidopsis* genes (Queval *et al.*, 2007). Induction is indicated by +. Expression data are taken from https://www.genevestigator.ethz.ch (Hruz *et al.*, 2008). The numbered stress conditions are as follows. 1. H_2O_2. 2. Ozone. 3. Bacteria (*P. syringae*). 4. Fungi (*B. cinerea*). 5. Salicylic acid. 6. Ethylene. 7. Senescence. 8. Drought. 9. Salt. 10. Osmotic. 11. Abscisic acid. 12. Wounding. 13. Methyl jasmonate.

Gene ID	Gene product	Stress condition												
		1	2	3	4	5	6	7	8	9	10	11	12	13
At1g05560	UGT75B1	+	+	+	+	+		+	+	+	+	+	+	+
At1g17170	GSTU24	+	+	+	+	+	+		+	+	+		+	+
At2g15480	UGT73B5	+	+	+	+	+	+	+	+	+	+	+	+	
At2g29500	HSP17.6	+	+	+	+	+	+		+	+	+	+	+	+
At2g30750	Cyt. P450, put.		+	+	+		+	+	+	+		+	+	+
At2g37770	Aldo/keto reductase	+	+	+	+	+		+	+	+	+	+	+	+
At2g43820	UGT74F2	+	+	+	+	+		+		+	+		+	
At2g47730	GSTF8	+	+	+		+				+		+		
At3g25250	OXI1 protein kinase	+	+	+	+	+				+		+	+	+
At3g55430	put. β-1,3-glucanase	+	+	+	+	+		+	+	+	+	+	+	
At4g11600	GPX6		+	+	+	+	+		+		+	+	+	
At4g16690	Esterase/lipase thioest.	+	+	+	+			+		+	+	+		
At4g33540	Lactamase	+	+	+	+	+			+	+	+	+	+	
At5g14730	Unk. Prot.	+	+	+	+	+	+			+			+	+
At5g22300	Nitrilase4	+	+		+		+	+			+		+	+
At5g43450	ACC oxidase, put.	+	+	+	+	+	+	+		+	+	+	+	

include lesion formation, accumulation of SA and induction of *PR* genes (Chamnongpol *et al.*, 1996, 1998; Du and Klessig, 1997; Takahashi *et al.*, 1997; Dat *et al.*, 2003; Chaouch *et al.*, 2010). In *Arabidopsis cat2*, the formation of lesions is daylength-dependent (Fig. 6), as are SA accumulation, *PR* gene induction, activation of camalexin and its synthesis pathway, and enhanced resistance to bacterial challenge (Chaouch *et al.*, 2010). All these responses are absent when oxidative stress occurs under short-day conditions, even though the intracellular thiol-disulphide state is at least as perturbed in short days as in long days. However, responses can be induced in short days by supplementation of exogenous SA and reverted in long days by extinction of SA synthesis through the isochorismate pathway (Chaouch *et al.*, 2010), which is responsible for SA production in response to biotrophic pathogens (Wildermuth *et al.*, 2001). Together, these observations show that *cat2* grown in long-day conditions is a constitutive defence mutant in which peroxisomal H_2O_2 triggers SA-dependent hypersensitive response (HR)-like lesion formation (Chaouch *et al.*, 2010).

While most of the focus on pathogen-associated ROS production has focused on apoplastic events (Bindschedler *et al.*, 2006; Sagi and Fluhr, 2006; Torres *et al.*, 2006), analysis of *cat2* provides further evidence that intracellular ROS play an important role in programmed cell death (Dat *et al.*, 2003; Vlot *et al.*, 2009), particularly the HR, and points to a potentially crucial role for the peroxisomes. These organelles play several potentially important roles in response to stress (Del Río *et al.*, 2006; Nyathi and Baker, 2006; Palma *et al.*, 2009). Besides the production of ROS such as superoxide and H_2O_2, they harbour the terminal steps of JA and SA synthesis. Key peroxisomal biogenesis genes are induced by stresses, including H_2O_2 (Lopez-Huertas *et al.*, 2000) while peroxisome congregation at the invasion site was an early event in cells in response to fungi (Lipka *et al.*, 2005). Indications of a role for photorespiratory H_2O_2 in pathogen responses come from studies of plants with altered serine:glyoxylate aminotransferase activity (Taler *et al.*, 2004) and from work on the *lsd1* lesion-mimic mutant (Mateo *et al.*, 2004).

As discussed above, perturbation of glutathione is one of the clearest responses to increased H_2O_2 availability. Environmental stresses also trigger qualitatively similar changes to those observed in catalase-deficient plants, i.e. decreases in the GSH:GSSG ratio and increases in total glutathione (Vanacker *et al.*, 2000; Bick *et al.*, 2001; Gomez *et al.*, 2004*b*). Redox-sensitive *in vivo* probes revealed perturbation of glutathione redox state in *Arabidopsis* subjected to water stress (Jubany-Mari *et al.*, 2010). Glutathione and related thiols are well known to be involved in pathogen responses, including induction of *PR* genes and phytoalexin synthesis (Edwards *et al.*, 1991; May *et al.*, 1996; Vanacker *et al.*, 2000; Gomez *et al.*, 2004*a*; Parisy *et al.*, 2007). In *Arabidopsis*, the expression of some *PR* genes is under the control of the SA-dependent signalling component, NPR1, which is regulated by cytosolic thiol-disulphide components involving thioredoxins and, potentially, glutathione (Tada *et al.*, 2008). Studies of SA responses in *cat2* backgrounds suggest that catalase deficiency recapitulates at least some of the pathogen-related responses involving cytosolic NADP-dependent thiol-disulphide systems (Chaouch *et al.*, 2010; Mhamdi *et al*, 2010*a*, *b*). Thus, *cat2* and similar systems are likely to be particularly useful in the evaluation of *in vivo* interactions between H_2O_2 and thiol components.

As shown in Fig. 6, growth photoperiod influences the functional outcome of oxidative stress in *cat2*. The absence of an HR-like response in *cat2* in short days is not linked to insufficient oxidative stress. Accumulation of both glutathione (Fig. 7) and H_2O_2-inducible transcripts is at least as pronounced in short days as in long days (Queval *et al.*, 2007; Chaouch *et al.*, 2010). Recovery of lesion formation and a range of other pathogen responses in *cat2* in short days by SA treatment suggests that either daylength or the duration (rather than the intensity) of oxidative stress governs a switch between H_2O_2 responses that do not require SA accumulation in short days and SA-dependent HR-like programmes in long days (Chaouch *et al.*, 2010). Such an interpretation is consistent with observations of other lesion-mimic mutants (Dietrich *et al.*, 1994; Meng *et al.*, 2009), although in many of these mutants the link between oxidative stress and phenotype is less clear than in *cat2*. Links between phytochrome signalling and SA accumulation and between SA, flowering and defence reactions have been reported (Genoud *et al.*, 2002; Martinez *et al.*,

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

2004). Ozone-induced leaf damage was shown to be influenced by the photoperiod context in which plants were subjected to equal-time treatments (Vollsnes *et al.*, 2009).

In both animals and plants, ROS have been implicated in ageing and catalase may play a role in senescence. However, transgenic mice in which the single mammalian catalase gene is knocked out show normal development (Ho *et al.*, 2004). Knockout of peroxisomal catalase in *C. elegans* causes premature ageing (Petriv and Rachubinski, 2004) while overexpression in mice mitochondria or cardiac-specific expression was reported to alleviate symptoms of ageing (Schriner *et al.*, 2005; Wu *et al.*, 2007). In humans, catalase deficiency has recently been implicated in greying of hair (Wood *et al.*, 2009) and another interesting observation is that ageing of human cells can be alleviated by engineering improved peroxisomal import of catalase (Koepke *et al.*, 2007). Several studies implicate peroxisomes and ROS in senescence in plants (for a review, see del Río *et al.*, 1998). In *Arabidopsis*, *CAT2* expression decreases after flowering and this decrease has been proposed to be an integral part of H_2O_2-triggered leaf senescence (Zimmerman *et al.*, 2006; Smykowski *et al.*, 2010). Treatment of non-senescent *Arabidopsis* with 3-AT induced the expression of H_2O_2-responsive genes, including *PR1* and *GPX6*, that were also highly expressed in senescent leaves (Navabpour *et al.*, 2003). However, the senescence marker *SAG12* was not among the genes induced by catalase inhibition (Navabpour *et al.*, 2003).

Genes induced by photorespiratory H_2O_2 in *cat2* include some that are induced during senescence (Table 3). Despite this, the *cat2* phenotypes do not provide strong evidence for a co-ordinated activation of senescence programmes by peroxisomal H_2O_2, and this view is supported by other data from transcriptomic profiling. For instance, WRKY53 is a transcription factor involved in senescence that is induced in leaves within hours after spraying with H_2O_2 and that can interact with the promoters of *CAT1*, *CAT2*, and *CAT3* (Miao *et al.*, 2004, 2007). Analysis of transcription factors rapidly induced (within 8 h) of transferring *cat2* RNAi lines to high light identified several *WRKY* genes, but not *WRKY53* (Vanderauwera *et al.*, 2005). Experiments over a longer time-scale (2–4 d after transfer of *cat2* knockouts from high CO_2 to air) also show that *WRKY53* is not significantly induced in response to increases in peroxisomal H_2O_2 (Mhamdi *et al.*, 2010*a*; authors' unpublished results). On the contrary, *WRKY53* was found to be strongly and commonly up-regulated in KD-SOD, KO-Apx1, and *flu* mutants, and upon treatment with ozone or methyl viologen (Gadjev *et al.*, 2006). The lesion phenotype and other responses of *cat2* in long days are clearly more similar to HR-like processes than to senescence, while in short days *cat2* plants have a decreased growth rather than altered senescence. Furthermore, *CAT3* transcripts are not increased in *cat2* (Queval *et al.*, 2007; Fig. 5), although expression of *CAT3* is enhanced in older *Arabidopsis* leaves (Zimmermann *et al.*, 2006). The growth restraint observed in *cat2* possibly involves mechanisms common to many environmental stresses, reflecting the centrality of redox state in these conditions. The link between H_2O_2 and senescence appears to be complex and future studies will further unravel the roles of catalase regulation in this process.

How important is catalase regulation in response to stress?

Numerous studies of plant responses to various stresses have included data on changes in catalase activity or expression. A detailed analysis of this literature is beyond the scope of the present review. The following discussion focuses on potential mechanisms by which regulation of catalase could contribute to oxidative signalling, with a particular focus on increases in H_2O_2 availability that could result from down-regulation of catalase.

A first mode of possible regulation is through changes in transcript abundance. Potato Class II catalase was induced in roots exposed to nematodes and bacteria and by SA in stem tissue (Niebel *et al.*, 1995). Similar induction of Class II catalase was observed in tobacco leaves treated with tobacco mosaic virus or fungal elicitor (Dorey *et al.*, 1998). Intriguingly, in view of the observations in catalase-deficient plants, Class I catalase transcripts were down-regulated by these treatments (Dorey *et al.*, 1998). *CAT1* expression was strongly induced by treatment of *Arabidopsis* seedlings with H_2O_2 (Xing *et al.*, 2008), though a similar effect on *CAT1* transcripts has not been observed in *cat2* mutant backgrounds, whether measured by qPCR (Queval *et al.*, 2007), semi-quantitative RT-PCR (Fig. 5) or microarray analysis (Mhamdi *et al.*, 2010*a*). This discrepancy may reflect the importance of developmental stage or the (sub)cellular localization of H_2O_2 signals. *CAT1* transcripts were also induced by abscisic acid through a pathway dependent on mitogen-activated protein kinase-dependent signalling (Xing *et al.*, 2008). As well as potential catalase interactions with WRKY53 (Miao *et al.*, 2004), a G-box binding factor (GBF1) was found to interact with the *CAT2* promoter in yeast 1-hybrid experiments and this interaction may play a role in regulating the onset of leaf senescence (Smykowski *et al.*, 2010). However, as discussed above, phenotypic and other effects observed in *cat2* knockouts do not point to a simple relationship between *CAT2* expression and senescence.

A second potential mode of regulation of catalase activity is at the post-transcriptional level. Selective degradation of catalase was reported to be a trigger of autophagic cell death in animal cells (Yu *et al.*, 2006). One well-described mechanism in plants is diminished re-synthesis of catalase in stress conditions. Catalase is a light-sensitive protein that has a high turnover rate, and stresses such as cold, salt, and high light can cause decreases in the total protein through accelerated inactivation or decreased capacity to replace the protein (Volk and Feierabend, 1989; Hertwig *et al.*, 1992; Streb and Feierabend, 1996). Re-synthesis of rye Cat1 is post-transcriptionally controlled by mRNA methylation, which is stimulated by blue light and peroxides (Schmidt *et al.*, 2002, 2006).

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

Other potential mechanisms that could down-regulate catalase activity include SA and nitric oxide (Vlot *et al.*, 2009), as well as an unknown inhibitor that accumulated in the medium of *Arabidopsis* cells treated with fusicoccin (Beffagna and Lutzu, 2007). As noted above, it is also possible that ROS homeostasis is affected by catalase targeting efficiency and potential changes in subcellular distribution. In *Chlamydomonas*, which lack peroxisomes, mitochondrial catalase activity is inhibited by light via a redox-dependent mechanism involving chloroplast thioredoxins (Shao *et al.*, 2008).

Several proteins have been identified that could interact with catalase polypeptides. *Arabidopsis* CAT3 has been reported to bind calmodulin (Yang and Poovaiah, 2002). Calmodulin was shown to be present in peroxisomes and to increase the activity of tobacco catalase but not catalases from a bacterium, a fungus, or humans (Yang and Poovaiah, 2002). Catalases have also been found to interact with nucleoside disphosphate kinase 1 (NDK1), a cytosolic protein (Fukamatsu *et al.*, 2003). This interaction may also increase catalase activity (Fukamatsu *et al.*, 2003). Another cytosolic protein reported to interact with both CAT2 and CAT3 is SOS2, a SNF-related kinase (Verslues *et al.*, 2007). Both associations could modulate the activity of any catalase that may be found in the cytosol. However, it remains unclear whether these interactions reflect *in vivo* phenomena because NDPK2, which was also found to interact with SOS2 (Verslues *et al.*, 2007), was reported to be located in the stroma rather than in the cytosol (Bölter *et al.*, 2007).

It remains unclear whether natural genetic variability in catalase could contribute to differences in stress responses, though there is sufficient allelic variability at the maize Cat3 locus to encode electrophoretically distinguishable proteins (Scandalios *et al.*, 1980). A maize line showing increased resistance to *Aspergillus flavus* infection had a Cat3 allele with a deletion of 20 amino acids compared to the control line (Magbanua *et al.*, 2007).

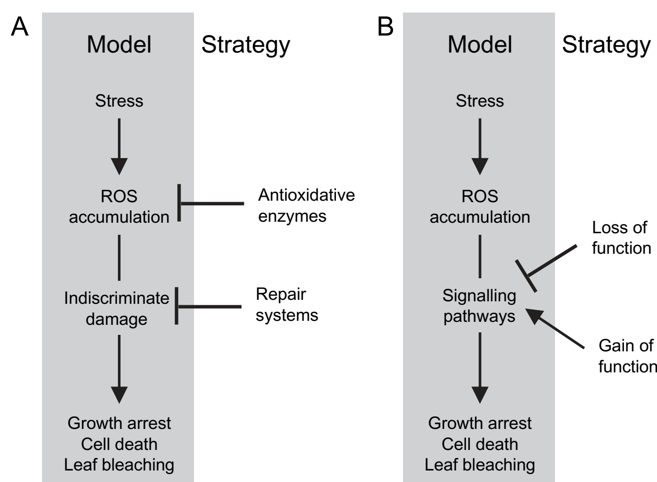

Fig. 10. Alternative models of oxidative stress-mediated effects and potential strategies to modulate them. (A) 'Damage/toxicity' model of ROS action during stress. (B) 'Signalling' model of ROS action.

Conclusions and perspectives

Concepts of ROS as toxic compounds have been qualified over recent years by the realization that they are also important signal molecules. It is often considered that concentration is a key factor in determining which of these modes ROS operate through. However, observations that singlet oxygen-induced bleaching can be genetically reverted (Wagner *et al.*, 2004) have been complemented by the finding that plants deficient in the major catalase isoform not only recapitulate a wide range of pathogen responses, but that these responses, including cell death, can also be reverted (Chaouch *et al.*, 2010). It is therefore likely that ROS signalling is a more useful conceptual paradigm than damage, even when this involves marked intracellular redox perturbation that results from the loss of a highly active H_2O_2-metabolizing enzyme. Further, analysis of biochemical markers of redox state in *cat2* and *cat2 gr1* mutants points to a rather specific perturbation of glutathione, suggesting that altered status of this key redox buffer may act as an important channel for H_2O_2-triggered signal transduction.

To exploit processes in which ROS act through widespread damage, preferred strategies would be to minimize ROS accumulation or the probability of interaction with sensitive components (Fig. 10A), because it would presumably be too energetically costly or too difficult to engineer directly resistance against a plethora of indiscriminate modifications. However, if the physiological effects of ROS work primarily through a more limited number of signalling mechanisms, other approaches could be feasible, such as the selection of gain-of-function or loss-of-function mutants (Fig. 10B). Although there could be overlap and interdependence between the two approaches (enhancing defence or adjustment of signalling), the choice of experimental strategies will inevitably be influenced by which concept is dominant. Hence, whether ROS act primarily through 'damage or signalling' is more than a merely semantic issue. Whichever view is favoured, it is not likely to be simple to achieve the desired modifications in plant performance without less attractive trade-off effects. However, this objective will probably be aided by improved knowledge on how redox signalling interfaces and interacts with the network of signalling pathways through components such as phytohormones. The growing awareness of the crucial role of redox-dependent signalling in plant development and function suggests that conditional catalase-deficient mutants are likely to continue to be useful tools to this end.

Supplementary data

Supplementary data can be found at *JXB* online.

Supplementary Table S1. PCR and RT-PCR primers used to characterize *cat1* and *cat3* T-DNA mutants.

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

Acknowledgements

The Orsay group thanks Emmanuelle Issakidis-Bourguet (IBP, Orsay, France), for expert advice and help during the characterization of the *cat2* mutant, while GN expresses his gratitude to Christine Foyer (University of Leeds, UK), for many stimulating discussions over the last two decades. We thank Alison Baker (University of Leeds, UK) for expert comments on catalase localization and targeting. Collaboration between the Orsay and Gent groups was supported by a Scientific Exchange program Flanders-France (grant Tournesol T.2008.21 to FVB and GN). Work in Orsay was also supported by the French Agence Nationale de la Recherche programmes 'Redoxome' and 'Vulnoz'. SC was supported by a Bourse de Docteur Ingénieur grant from the French Centre National de la Recherche Scientifique. AM was supported in part by PhD grants from the Tunisian Ministry of Higher Education and Scientific Research, Tunisia, and the Université de Paris sud 11, France. SV is a Postdoctoral Fellow of the Research Foundation-Flanders.

References

Ahrens TD, Lobell DB, Ortiz-Monasterio JI, Li Y, Matson PA. 2010. Narrowing the agronomic yield gap with improved nitrogen use efficiency: a modeling approach. *Ecological Applications* **20,** 91–100.

Alfonso-Prieto M, Biarnés X, Vidossich P, Rovira C. 2009. The molecular mechanism of the catalase reaction. *Journal of the American Chemical Society* **131,** 11751–11761.

Allen JF, Whatley FR. 1977. Effects of inhibitors of catalase on photosynthesis and on catalase activity in unwashed preparations of intact chloroplasts. *Plant Physiology* **61,** 957–960.

Aono M, Kubo A, Saji H, Tanaka K, Kondo N. 1993. Enhanced tolerance to photooxidative stress of transgenic *Nicotiana tabacum* with high chloroplastic glutathione reductase activity. *Plant and Cell Physiology* **34,** 129–135.

Asada K. 1999. The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Annual Review of Plant Physiology and Plant Molecular Biology* **50,** 601–639.

Asselbergh D, De Vleesschauwer D, Hofte M. 2008. Global switches and fine-tuning: ABA modulates plant-pathogen defense. *Molecular Plant–Microbe Interactions* **21,** 709–719.

Baruah A, Simková K, Apel K, Laloi C. 2009. Arabidopsis mutants reveal multiple singlet oxygen signaling pathways involved in stress response and development. *Plant Molecular Biology* **70,** 547–563.

Bashandy T, Guilleminot J, Vernoux T, Caparros-Ruiz D, Ljung K, Meyer Y, Reichheld JP. 2010. Interplay between the NADP-linked thioredoxin and glutathione systems in Arabidopsis auxin signaling. *The Plant Cell* **22,** 376–391.

Beffagna N, Lutzu I. 2007. Inhibition of catalase activity is an early response of *Arabidopsis thaliana* cultured cells to the phytotoxin fusicoccin. *Journal of Experimental Botany* **58,** 4183–4194.

Bick JA, Setterdahl AT, Knaff DB, Chen Y, Pitcher LH, Zilinskas BA, Leustek T. 2001. Regulation of the plant-type 5′-adenylyl sulphate reductase by oxidative stress. *Biochemistry* **40,** 9040–9048.

Bindschedler LV, Dewdney J, Blee KA, *et al.* 2006. Peroxidase-dependent apoplastic oxidative burst in Arabidopsis required for pathogen resistance. *The Plant Journal* **47,** 851–863.

Bölter B, Sharma B, Soll J. 2007. Localisation of Arabidopsis NDPK2 – revisited. *Planta* **226,** 1059–1065.

Brisson LF, Zelitch I, Havir EA. 1998. Manipulation of catalase levels produces altered photosynthesis in transgenic tobacco plants. *Plant Physiology* **116,** 259–269.

Bray EA, Bailey-Serres J, Weretilnyk E. 2000. Responses to abiotic stresses. In: Buchanan B, Gruissem W, Jones R, eds. *Biochemistry and molecular biology of plants*. American Society of Plant Physiologists, 1158–1203.

Broadbent P, Creissen GP, Kular B, Wellburn AR, Mullineaux PM. 1995. Oxidative stress responses in transgenic tobacco containing altered levels of glutathione reductase activity. *The Plant Journal* **8,** 247–255.

Brown LA, Baker A. 2008. Shuttles and cycles: transport of proteins into the peroxisome matrix. *Molecular Membrane Biology* **25,** 363–375.

Browse J. 2009. Jasmonate passes muster: a receptor and targets for the defense hormone. *Annual Review of Plant Biology* **60,** 183–205.

Bueso E, Alejandro S, Carbonell P, Perez-Amador MA, Fayos J, Bellés JM, Rodriguez PL, Serrano R. 2007. The lithium tolerance of the Arabidopsis *cat2* mutant reveals a cross-talk between oxidative stress and ethylene. *The Plant Journal* **52,** 1052–1065.

Chamnongpol S, Willekens H, Langebartels C, Van Montagu M, Inzé D, Van Camp W. 1996. Transgenic tobacco with a reduced catalase activity develops necrotic lesions and induces pathogenesis-related expression under high light. *The Plant Journal* **10,** 491–503.

Chamnongpol S, Willekens H, Moeder W, Langebartels C, Sandermann Jr. H, Van Montagu M, Inzé D, Van Camp W. 1998. Defense activation and enhanced pathogen tolerance induced by H_2O_2in transgenic plants. *Proceedings of the National Academy of Sciences, USA* **95,** 5818–5823.

Chaouch S, Noctor G. 2010. *myo*-Inositol abolishes salicylic acid-dependent cell death and pathogen defence responses triggered by peroxisomal H_2O_2. *New Phytologist* doi: 10.1111/j.1469-8137.2010.03435.x.

Chaouch S, Queval G, Vanderauwera S, Mhamdi A, Vandorpe M, Langlois-Meurinne M, Van Breusegem F, Saindrenan P, Noctor G. 2010. Genetic reversion of cell death in the Arabidopsis *cat2* knockout mutant shows that peroxisomal H_2O_2 is coupled to biotic defense responses by isochorismate synthase 1 in a daylength-related manner. *Plant Physiology* **153,** 1692–1705.

Chelikani P, Fita I, Loewen PC. 2004. Diversity of structures and properties among catalases. *Cell and Molecular Life Sciences* **61,** 192–208.

Corpas FJ, Palma, Sandalio LM, Valderrama R, Barroso JB, Del Río LA. 2008. Peroxisomal xanthine oxidoreductase:

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

characterization of the enzyme from pea (*Pisum sativum* L.) leaves. *Journal of Plant Physiology* **165,** 1319–1330.

Creissen G, Firmin J, Fryer M, *et al*. 1999. Elevated glutathione biosynthetic capacity in the chloroplasts of transgenic tobacco paradoxically causes increased oxidative stress. *The Plant Cell* **11,** 1277–1291.

Dat JF, Inzé D, Van Breusegem F. 2001. Catalase-deficient tobacco plants: tools for in planta studies on the role of hydrogen peroxide. *Redox Reports* **6,** 37–42.

Dat JF, 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. *The Plant Journal* **33,** 1–12.

Davison AJ, Kettle AJ, Fatur DJ. 1986. Mechanism of the inhibition of catalase by ascorbate. Roles of active oxygen species, copper and semidehydroascorbate. *Journal of Biological Chemistry* **261,** 1193–1200.

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

Davletova S, Schlauch K, Coutu J, Mittler R. 2005 *b*. The zinc-finger protein Zat12 plays a central role in reactive oxygen and abiotic stress signaling in Arabidopsis. *Plant Physiology* **139,** 847–856.

del Río LA, Ortega MG, López AL, Gorgé JL. 1977. A more sensitive modification of the catalase assay with the Clark oxygen electrode: application to the kinetic study of the pea leaf enzyme. *Analytical Biochemistry* **80,** 409–415.

del Río LA, Pastori GM, Palma JM, Sandalio LM, Sevilla F, Corpas FJ, Jiménez A, López-Huertas E, Hernández JA. 1998. The activated oxygen role of peroxisomes in senescence. *Plant Physiology* **116,** 1195–1200.

del Río LA, Sandalio LM, Corpas FJ, Palma JM, Barroso JB. 2006. Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging, and role in cell signaling. *Plant Physiology* **141,** 330–335.

Desikan R, Mackerness SAH, Hancock JT, Neill SJ. 2001. Regulation of the Arabidopsis transcriptome by oxidative stress. *Plant Physiology* **127,** 159–172.

Dietrich RA, Delaney TP, Uknes SJ, Ward ER, Ryals JA, Dangl JL. 1994. Arabidopsis mutants simulating disease resistance response. *Cell* **77,** 565–577.

Dietz KJ. 2003. Plant peroxiredoxins. *Annual Review of Plant Biology* **54,** 93–107.

Dixon DP, Hawkins T, Hussey PJ, Edwards R. 2009. Enzyme activities and subcellular localization of members of the *Arabidopsis* glutathione transferase superfamily. *Journal of Experimental Botany* **60,** 1207–1218.

Dorey S, Baillieul F, Saindrenan P, Fritig B, Kauffmann S. 1998. Tobacco class I and II catalases are differentially expressed during elicitor-induced hypersensitive cell death and localized acquired resistance. *Molecular Plant–Microbe Interactions* **11,** 1102–1109.

Du H, Klessig DF. 1997. Role for salicylic acid in the activation of defense responses in catalase-deficient transgenic tobacco. *Molecular Plant–Microbe Interactions* **10,** 922–925.

Dutilleul C, Garmier M, Noctor G, Mathieu CD, Chétrit P, Foyer CH, De Paepe R. 2003. Leaf mitochondria modulate whole cell redox homeostasis, set antioxidant capacity and determine stress resistance through altered signaling and diurnal regulation. *The Plant Cell* **15,** 1212–1226.

Edwards R, Blount JW, Dixon RA. 1991. Glutathione and elicitation of the phytoalexin response in legume cell cultures. *Planta* **184,** 403–409.

Esaka M, Yamada N, Kitabayashi M, Setoguchi Y, Tsugeki R, Kondo M, Nishimura M. 1997. cDNA cloning and differential gene expression of three catalases in pumpkin. *Plant Molecular Biology* **33,** 141–155.

Fahnenstich H, Scarpeci TE, Valle EM, Flügge UI, Maurino VG. 2008. Generation of hydrogen peroxide in chloroplasts of Arabidopsis overexpressing glcyolate oxidase as an inducible system to study oxidative stress. *Plant Physiology* **148,** 719–729.

Ferguson IB, Dunning SJ. 1986. Effect of 3-amino-1,2,4-triazole, a catalase inhibitor, on peroxide content of suspension-cultured pear fruit cells. *Plant Science* **43,** 7–11.

Foreman J, Demidchik V, Bothwell JH, *et al*. 2003. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature* **27,** 442–446.

Foyer CH, Bloom AJ, Queval G, Noctor G. 2009. Photorespiratory metabolism: genes, mutants, energetics, and redox signaling. *Annual Review of Plant Biology* **60,** 455–484.

Foyer CH, Mullineaux PM. 1998. The presence of dehydroascorbate and dehydroascorbate reductase in plant tissues. *FEBS Letters* **425,** 528–529.

Foyer CH, Noctor G. 2000. Oxygen processing in photosynthesis: regulation and signalling. *New Phytologist* **146,** 359–388.

Foyer CH, Noctor G. 2009. Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. *Antioxidants and Redox Signaling* **11,** 861–905.

Foyer CH, Souriau N, Perret S, Lelandais M, Kunert KJ, Pruvost C, Jouanin L. 1995. Overexpression of glutathione reductase but not glutathione synthetase leads to increases in antioxidant capacity and resistance to photoinhibition in poplar trees. *Plant Physiology* **109,** 1047–1057.

Frendo P, Harrison J, Norman C, Hernandez-Jimenez MJ, Van de Sype G, Gilabert A, Puppo A. 2005. Glutathione and homoglutathione play a critical role in the nodulation process of *Medicago truncatula*. *Molecular Plant–Microbe Interactions* **18,** 254–259.

Frugoli JA, Zhong HH, Nuccio ML, McCourt P, McPeek MA, Thomas TL, McClung CR. 1996. Catalase is encoded by a multigene family in *Arabidopsis thaliana* (L.) Heynh. *Plant Physiology* **112,** 327–336.

Fukamatsu Y, Yabe N, Hasunuma K. 2003. *Arabidopsis* NDK1 is a component of ROS signaling by interacting with three catalases. *Plant and Cell Physiology* **44,** 982–989.

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

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

Gatzek S, Wheeler GL, 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. *The Plant Journal* **30,** 541–553.

Genoud T, Buchala AJ, Chua N-H, Métraux J-P. 2002. Phytochrome signalling modulates the SA–perceptive pathway in *Arabidopsis*. *The Plant Journal* **31,** 87–95.

Gomez L, Noctor G, Knight M, Foyer CH. 2004*a*. Regulation of calcium signalling and gene expression by glutathione. *Journal of Experimental Botany* **55,** 1851–1859.

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

Grace SC, Logan BA. 1996. Acclimation of foliar antioxidant systems to growth irradiance in three broad-leaved evergreen species. *Plant Physiology* **112,** 1631–1640.

Guan L, Scandalios JG. 1996. Molecular evolution of maize catalases and their relationship to other eukaryotic and prokaryotic catalases. *Journal of Molecular Evolution* **42,** 570–579.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41,** 95–98.

Havir EA, McHale NA. 1989. Enhanced peroxidatic activity in specific catalase isozymes of tobacco, barley, and maize. *Plant Physiology* **91,** 812–815.

Heazlewood JL, Tonti-Filipinni JS, Gout AM, Day DA, Whelan J, Millar AH. 2004. Experimental analysis of the Arabidopsis mitochondrial proteome highlights signaling and regulatory components, provides assessment of targeting prediction programs, and indicates plant-specific mitochondrial proteins. *The Plant Cell* **16,** 241–256.

Hertwig B, Streb P, Feierabend J. 1992. Light dependence of catalase synthesis and degradation in leaves and the influence of interfering stress conditions. *Plant Physiology* **100,** 1547–1553.

Ho YS, Xiong Y, Ma W, Spector A, Ho DS. 2004. Mice lacking catalase develop normally but show differential sensitivity to oxidant tissue injury. *Journal of Biological Chemistry* **279,** 32804–32812.

Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, Oertle L, Widmayer P, Gruissem W, Zimmermann P. 2008. Genevestigator V3: a reference expression database for the meta-analysis of transcriptomes. *Advances in Bioinformatics* Vol. 2008. Article ID 420747, 5 pages. doi:10.1155/2008/420747.

Hu YQ, Liu S, Yuan HM, Li J, Yan DW, Zhang JF, Lu YT. 2010. Functional comparison of catalase genes in the elimination of photorespiratory H_2O_2 using promoter- and 3'-untranslated region exchange experiments in the *Arabidopsis cat2* photorespiratory mutant. *Plant, Cell and Environment* **33,** 1656–1670.

Iqbal A, Yabuta Y, Takeda T, Nakano Y, Shigeoka S. 2006. Hydroperoxide reduction by thioredoxin-specific glutathione peroxidase isoenzymes of *Arabidopsis thaliana*. *FEBS Journal* **273,** 5589–5597.

Iwamoto M, Higo H, Higo K. 2000. Differential diurnal expression of rice catalase genes: the 5'-flanking region of *CatA* is not sufficient for circadian control. *Plant Science* **151,** 39–46.

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

Juul T, Malolepszy A, Dybkaer K, Kidmose R, Rasmussen JT, Andersen GR, Johnsen HE, Jørgensen JE, Andersen SU. 2010. The *in vivo* toxicity of hydroxyurea depends on its direct target catalase. *Journal of Biological Chemistry* **285,** 21411–21415.

Kamigaki A, Mano S, Terauchi K, Nishi Y, Tachibe-Kinoshita Y, Nito K, Kondo M, Hayashi M, Nishimura M, Esaka M. 2003. Identification of peroxisomal targeting signal of pumpkin catalase and the binding analysis with PTS1 receptor. *The Plant Journal* **33,** 161–175.

Kataya AMR, Reumann S. 2010. Arabidopsis glutathione reductase 1 is dually targeted to peroxisomes and the cytosol. *Plant Signaling and Behaviour* **5,** 171–175.

Kato J, Yamahara T, Tanaka K, Takio S, Satoh T. 1997. Characterization of catalase from green algae *Chlamydomonas reinhardtii*. *Journal of Plant Physiology* **151,** 262–268.

Kato S, Ueno T, Fukuzumi S, Watanabe Y. 2004. Catalase reaction by myoglobin mutants and native catalase. Mechanistic investigation by kinetic isotope effect. *Journal of Biological Chemistry* **279,** 52376–52381.

Kaur N, Reumann S, Hu J. 2009. Peroxisome biogenesis and function. In: *The Arabidopsis Book*. Rockville, USA: American Society of Plant Biologists.

Kendall AC, Keys AJ, Turner JC, Lea PJ, Miflin BJ. 1983. The isolation and characterisation of a catalase-deficient mutant of barley (*Hordeum vulgare* L.). *Planta* **159,** 505–511.

Keys AJ. 1999. Biochemistry of photorespiration and the consequences for plant performance. In: Bryant JA, Burrell MM, Kruger NJ, eds. *Plant carbohydrate biochemistry*. Oxford, UK: BIOS Scientific,147–161.

Kirkman HN, Gaetani GF. 2007. Mammalian catalase: a venerable enzyme with new mysteries. *Trends in Biochemical Sciences* **32,** 44–50.

Kirkman HN, Rolfo M, Ferraris AN, Gaetani GF. 1999. Mechanisms of protection of catalase by NADPH. Kinetics and stoichiometry. *Journal of Biological Chemistry* **278,** 13908–13914.

Koepke JI, Nakrieko KA, Wood CS, Boucher KK, Terlecky LJ, Walton PA, Terlecky SR. 2007. Restoration of peroxisomal catalase import in a model of human cellular aging. *Traffic* **8,** 1590–1600.

König J, Baier M, Horling F, Kahmann U, Harris G, Schürmann P, Dietz KJ. 2002. The plant-specific function of 2-Cys peroxiredoxin-mediated detoxification of peroxides in the redox-hierarchy of photosynthetic electron flux. *Proceedings of the National Academy of Sciences, USA* **99,** 5738–5743.

Kornyeyev D, Logan BA, Allen RD, Holaday AS. 2005. Field-grown cotton plants with elevated activity of chloroplastic glutathione

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

reductase exhibit no significant alteration of diurnal and seasonal patterns of excitation energy partitioning and CO_2 fixation. *Field Crops Research* **94,** 165–175.

Kovtun Y, Chiu WL, Tena G, Sheen J. 2000. Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. *Proceedings of the National Academy of Sciences, USA* **97,** 2940–2945.

Kranner I, Birtic S, Anderson KM, Pritchard HW. 2006. Glutathione half-cell reduction potential: a universal stress marker and modulator of programmed cell death. *Free Radical Biology and Medicine* **40,** 2155–2165.

Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, Dangl JL, Bloom RE, Bodde S, Jones JD, Schroeder JI. 2003. NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in Arabidopsis. *EMBO Journal* **22,** 2623–2633.

Lanyon-Hogg T, Warriner SL, Baker A. 2010. Getting a camel through the eye of a needle: the import of folded proteins by peroxisomes. *Biology of the Cell* **102,** 245–263.

Leegood RC, Lea PJ, Adcock MD, Häusler RE. 1995. The regulation and control of photorespiration. *Journal of Experimental Botany* **46,** 1397–1414.

Lei B, Wei CJ, Tu SC. 2000. Action mechanism of antitubercular isoniazid. Activation by *Mycobacterium tuberculosis KatG*, isolation, and characterization of inhA inhibitor. *Journal of Biological Chemistry* **275,** 2520–2526.

Leshem Y, Melamed-Book, Cagnac O, Ronen G, Nishri Y, Solomon M, Cohen G, Levine A. 2006. Suppression of *Arabidopsis* vesicle-SNARE expression inhibited fusion of H_2O_2-containing vesicles with tonoplast and increased salt tolerance. *Proceedings of the National Academy of Sciences, USA* **103,** 18008–18013.

Lipka V, Dittgen J, Bednarek P, *et al*. 2005. Pre- and postinvasion defenses both contribute to nonhost resistance in Arabidopsis. *Science* **310,** 1180–1183.

Loew O. 1990. A new enzyme of general occurrence in organisms. *Science* **11,** 701–702.

Lopez-Huertas E, Charlton WL, Johnson B, Graham IA, Baker A. 2000. Stress induces peroxisome biogenesis genes. *EMBO Journal* **19,** 6770–6777.

Magbanua ZV, De Moraes CM, Brooks TD, Williams WP, Luthe DS. 2007. Is catalase activity one of the factors associated with maize resistance to *Aspergillus flavus? Molecular Plant–Microbe Interactions* **20,** 697–706.

Margoliash E, Novogrodsky A. 1960. Irreversible reaction of 3-amino-1,2,4-triazole and related inhibitors with the protein of catalase. *Biochemical Journal* **74,** 339–348.

Martínez C, Pons E, Prats G, León J. 2004. Salicylic acid regulates flowering time and links defence responses and reproductive development. *The Plant Journal* **37,** 209–217.

Marty L, Siala W, Schwarzländer M, Fricker MD, Wirtz M, Sweetlove LJ, Meyer Y, Meyer AJ, Reichheld JP, Hell R. 2009. The NADPH-dependent thioredoxin system constitutes a functional backup for cytosolic glutathione reductase in Arabidopsis. *Proceedings of the National Academy of Sciences, USA* **106,** 9109–9114.

Mateo A, Mühlenbock P, Rustérucci C, Chi–Chen Chang C, Miszalski Z, Karpinska B, Parker JE, Mullineaux PM, Karpinski S. 2004. *LESION SIMULATING DISEASE 1* is required for acclimation to conditions that promote excess excitation energy. *Plant Physiology* **136,** 2818–2830.

May MJ, Hammond-Kosack KE, Jones DG. 1996. Involvement of reactive oxygen species, glutathione metabolism and lipid peroxidation in the *Cf*-gene-dependent defence response of tomato cotyledons induced by race-specific elicitors of *Cladosporium fulvum*. *Plant Physiology* **110,** 1367–1379.

McClung CR. 1997. Regulation of catalases in Arabidopsis. *Free Radical Biology and Medicine* **23,** 489–496.

Meng PH, Raynaud C, Tcherkez G, *et al*. 2009. Crosstalks between myo–inositol metabolism, programmed cell death and basal immunity in *Arabidopsis*. *PLoS One* 4, e7364.

Mhamdi A, Hager J, Chaouch S, *et al*. 2010*a*. *Arabidopsis* GLUTATHIONE REDUCTASE 1 plays a crucial role in leaf responses to intracellular $H_2O_2$and in ensuring appropriate gene expression through both salicylic acid and jasmonic acid signaling pathways. *Plant Physiology* **153,** 1144–1160.

Mhamdi A, Mauve C, Houda G, Saindrenan P, Hodges M, Noctor G. 2010*b*. Cytosolic NADP-dependent isocitrate dehydrogenase contributes to redox homeostasis and the regulation of pathogen responses in *Arabidopsis* leaves. *Plant, Cell and Environment* **33,** 1112–1123.

Miao Y, Laun TM, Smykowski A, Zentgraf U. 2007. Arabidopsis MEKK1 can take a short cut: it can directly interact with senescence-related WRKY53 transcription factor on the protein level and can bind to its promoter. *Plant Molecular Biology* **65,** 63–76.

Miao Y, Laun TM, Zimmermann P, Zentgraf U. 2004. Targets of the WRKY53 transcription factor and its role during leaf senescence in Arabidopsis. *Plant Molecular Biology* **55,** 853–867.

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F. 2004. The reactive oxygen gene network in plants. *Trends in Plant Science* **9,** 490–498.

Mittler R, Zilinskas BA. 1991. Purification and characterization of pea cytosolic ascorbate peroxidase. *Plant Physiology* **97,** 962–968.

Møller IM, Jensen PE, Hansson A. 2007. Oxidative modifications to cellular components in plants. *Annual Review of Plant Biology* **58,** 459–481.

Moriwaki T, Yamamoto Y, Aida T, Funashi T, Shishido T, Asada M, Prodhan SH, Komanine A, Motohashi T. 2007. Overexpression of the *Escherichia coli* catalase *katE*, enhances tolerance to salinity stress in the transgenic indica rice cultivar, BR5. *Plant Biotechnology Reports* **2,** 41–46.

Mullen RT, Lee MS, Trelease RN. 1997. Identification of the peroxisomal targeting signal for cottonseed catalase. *The Plant Journal* **12,** 313–322.

Mutsada M, Ishikawa T, Takeda T, Shigeoka S. 1996. The catalase-peroxidase of Synechococcus PCC 7942: purification, nucleotide sequence analysis and expression in *Escherichia coli*. *Biochemical Journal* **316,** 251–257.

Narendra S, Venkataramani S, Shen G, Wang J, Pasapula V, Lin Y, Korneyev D, Holaday AS, Zhang H. 2006. The *Arabidopsis*

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

ascorbate peroxidase 3 is a peroxisomal membrane-bound antioxidant enzyme and is dispensable for *Arabidopsis* growth and development. *Journal of Experimental Botany* **57,** 3033–3042.

Navabpour S, Morris K, Allen R, Harrison E, Mackerness SAH, Buchanan-Wollaston V. 2003. Expression of senescence-enhanced genes in response to oxidative stress. *Journal of Experimental Botany* **54,** 2285–2292.

Niebel A, Heungens K, Barthels N, Inzé D, Van Montagu M, Gheysen G. 1995. Characterization of a pathogen-induced potato catalase and its systemic expression upon nematode and bacterial infection. *Molecular Plant–Microbe Interactions* **8,** 371–378.

Nishizawa A, Yabuta Y, Yoshida E, Maruta T, Yoshimura K, Shigeoka S. 2006. Arabidopsis heat shock transcription factor A2 as a key regulator in response to several types of environmental stress. *The Plant Journal* **48,** 535–547.

Noctor G. 2006. Metabolic signalling in defence and stress: the central roles of soluble redox couples. *Plant, Cell and Environment* **29,** 409–425.

Noctor G, Veljovic-Jovanovic S, Driscoll S, Novitskaya L, Foyer CH. 2002. Drought and oxidative load in the leaves of C_3 plants: a predominant role for photorespiration? *Annals of Botany* **89,** 841–850.

Nyathi Y, Baker A. 2006. Plant peroxisomes as a source of signalling molecules. *Biochimica et Biophysica Acta* **1763,** 1478–1495.

Ogawa D, Yamaguchi K, Nishiuchi T. 2007. High-level overexpression of the *Arabidopsis HsfA2* gene confers not only increased thermotolerance but also salt/osmotic stress tolerance and enhanced callus growth. *Journal of Experimental Botany* **58,** 3373–3383.

Oshima Y, Kamigaki A, Nakamori C, Mano S, Hayashi M, Nishimura M, Esaka M. 2008. Plant catalase is imported into peroxisomes by Pex5p but is distinct from typical PTS1 import. *Plant and Cell Physiology* **49,** 671–677.

Palma JM, Corpas FJ, del Río LA. 2009. Proteome of plant peroxisomes: new perspectives on the role of these organelles in cell biology. *Proteomics* **9,** 2301–2312.

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. *The Plant Journal* **49,** 159–172.

Pastori GM, Foyer CH. 2002. Common components, networks, and pathways of cross-tolerance to stress. The central role of 'redox' and abscisic acid-mediated controls. *Plant Physiology* **129,** 460–468.

Petriv OI, Rachubinski RA. 2004. Lack of peroxisomal catalase causes a progeric phenotype in *Caenorhabditis elegans*. *Journal of Biological Chemistry* **279,** 32804–32812.

Petrova VY, Drescher D, Kudjumdzieva AV, Schmidt MJ. 2004. Dual targeting of yeast catalase A to peroxisomes and mitochondria. *Biochemical Journal* **380,** 393–400.

Polidoros AN, Mylona PV, Scandalios JG. 2001. Transgenic tobacco plants expressing the maize *cat2* gene have altered catalase levels that affect plant–pathogen interactions and resistance to oxidative stress. *Transgenic Research* **10,** 555–569.

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. *Journal of Experimental Botany* **59,** 135–146.

Queval G, Issakidis-Bourguet E, Hoeberichts FA, 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. *The Plant Journal* **52,** 640–657.

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. *Analytical Biochemistry* **363,** 58–69.

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. *Molecular Plant* **2,** 344–356.

Redinbaugh MG, Sabre M, Scandalios JG. 1990. Expression of the maize *Cat3* catalase gene is under the influence of a circadian rhythm. *Proceedings of the National Academy of Sciences, USA* **87,** 6853–6857.

Regelsberger G, Jakopitsch C, Furtmüller PG, Rueker F, Switala J, Loewen PC, Obinger C. 2001. The role of distal tryptophan in the bifunctional activity of catalase-peroxidases. *Biochemical Society Transactions* **29,** 99–105.

Regelsberger G, Jakopitsch C, Plasser L, Schwaiger H, Furtmüller PG, Peschek GA, Zámocky M, Obinger C. 2002. Occurrence and biochemistry of hydroperoxidase in oxygenic phototrophic prokaryotes (cyanobacteria). *Plant Physiology and Biochemistry* **40,** 479–490.

Reichheld JP, Vernoux T, Lardon F, Van Montagu M, Inzé D. 1999. Specific checkpoints regulate plant cell cycle progression in response to oxidative stress. *The Plant Journal* **17,** 647–656.

Reichheld JP, Khafif M, Riondet C, Droux M, Bonnard G, Meyer Y. 2007. Inactivation of thioredoxin reductases reveals a complex interplay between thioredoxin and glutathione pathways in Arabidopsis development. *The Plant Cell* **19,** 1851–1865.

Reumann S, Babujee L, Ma C, Wienkoop S, Siemson T, Antonicelli GE, Rasche N, Luder F, Weckwerth W, Jahn O. 2007. Proteome analysis of Arabidopsis leaf peroxisomes reveals novel targetting peptides, metabolic pathways, and defense mechanisms. *The Plant Cell* **19,** 3170–3193.

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. *The Plant Journal* **32,** 329–342.

Romero-Puertas MC, Corpas FJ, Sandalio LM, Leterrier M, Rodriguez-Serrano M, Del Rio LA, Palma JM. 2006. Glutathione reductase from pea leaves: response to abiotic stress and characterization of the peroxisomal isozyme. *New Phytologist* **170,** 43–52.

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

Sagi M, Fluhr R. 2006. Production of reactive oxygen species by plant NADPH oxidases. *Plant Physiology* **141,** 336–340.

Scandalios JG. 1994. Regulation and properties of plant catalases. In: Foyer CH, Mullineaux PM, eds. *Causes of photooxidative stress and amelioration of defense systems in plants*. Boca Raton, Florida: CRC Press, 275–315.

Scandalios JG, Tong WF, Roupakias DG. 1980. *Cat3*, a third gene locus coding for a tissue-specific catalase in maize: genetics, intracellular location, and some biochemical properties. *Molecular and General Genetics* **179,** 33–41.

Schmidt M, Dehne S, Feierabend J. 2002. Post-transcriptional mechanisms control catalase synthesis during its light-induced turnover in rye leaves through the availability of the hemin cofactor and reversible changes of the translation efficiency of mRNA. *The Plant Journal* **31,** 601–613.

Schmidt M, Grief J, Feierabend J. 2006. Mode of translational activation of the catalase (*cat1*) mRNA of rye leaves (*Secale cereale* L.) and its control through blue light and reactive oxygen. *Planta* **223,** 835–846.

Schriner SE, Linford NJ, Martin GM, *et al*. 2005. Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* **308,** 1909–1911.

Shao N, Beck CF, Lemaire SD, Krieger-Liszkay A. 2008. Photosynthetic electron flow affects H_2O_2signalling by inactivation of catalase in *Chlamydomonas reinhardtii. Planta* **228,** 1055–1066.

Shikanai T, Takeda T, Yamauchi H, Sano S, Tomizawa KI, Yokota A, Shigeoka S. 1998. Inhibition of ascorbate peroxidase under oxidative stress in tobacco having bacterial catalase in chloroplasts *FEBS Letters* **428,** 47–51.

Shinozaki K, Yamaguchi-Shinozaki K. 2000. Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. *Current Opinion in Plant Biology* **3,** 217–223.

Simon C, Langlois-Meurinne M, Bellvert F, *et al*. 2010. The differential spatial distribution of secondary metabolites in *Arabidopsis* leaves reacting hypersensitively to *Pseudomonas syringae* pv. *tomato* is dependent on the oxidative burst. *Journal of Experimental Botany* **61,** 3355–3370.

Smith IK, Kendall AC, Keys AJ, Turner JC, Lea PJ. 1984. Increased levels of glutathione in a catalase-deficient mutant of barley (*Hordeum vulgare* L.). *Plant Science Letters* **37,** 29–33.

Smith IK, Kendall AC, Keys AJ, Turner JC, Lea PJ. 1985. The regulation of the biosynthesis of glutathione in leaves of barley (*Hordeum vulgare* L.). *Plant Science* **41,** 11–17.

Smykowski A, Zimmermann P, Zentgraf U. 2010. G-box binding factor 1 reduces *CATALASE2* expression and regulates the onset of leaf senescence in *Arabidopsis thaliana*. *Plant Physiology* **153,** 1321–1331.

Somerville CR. 1986. Analysis of photosynthesis with mutants of higher plants and algae. *Annual Review of Plant Physiology* **37,** 467–507.

Streb P, Feierabend J. 1996. Oxidative stress responses accompanying photoinactivation of catalase in NaCl-treated rye leaves. *Botanica Acta* **109,** 125–132.

Tada Y, Spoel SH, Pajerowska-Mukhtar K, Mou Z, Song J, Wang C, Zuo J, Dong X. 2008. Plant immunity requires conformational changes of NPR1 via S-nitrosylation and thioredoxins. *Science* **321,** 952–956.

Takahashi H, Chen Z, Du H, Liu Y, Klessig DF. 1997. Development of necrosis and activation of disease resistance in transgenic tobacco plants with severely reduced catalase levels. *The Plant Journal* **11,** 993–1005.

Taler D, Galperin M, Benjamin I, Cohen Y, Kenigsbuch D. 2004. Plant *eR* genes that encode photorespiratory enzymes confer resistance against disease. *The Plant Cell* **16,** 172–184.

Torres MA, Jones JDG, Dangl JL. 2006. Reactive oxygen species signaling in response to pathogens. *Plant Physiology* **141,** 373–378.

Van Breusegem F, Dat JF. 2006. Reactive oxygen species in plant cell death. *Plant Physiology* **141,** 384–390.

Vanacker H, Carver TLW, Foyer CH. 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 Physiology* **123,** 1289–1300.

Vandenabeele S, Van Der Kelen K, Dat J, *et al*. 2003. A comprehensive analysis of hydrogen peroxide-induced gene expression in tobacco. *Proceedings of the National Academy of Sciences, USA* **100,** 16113–16118.

Vandenabeele S, Vanderauwera S, Vuylstecke M, *et al*. 2004. Catalase deficiency drastically affects gene expression induced by high light in *Arabidopsis thaliana*. *The Plant Journal* **39,** 45–58.

Vandenbroucke K, Robbens S, Vandepoele K, Inzé D, Van de Peer Y, Van Breusegem F. 2008. Hydrogen peroxide-induced gene expression across kingdoms: a comparative analysis. *Molecular Biology and Evolution* **25,** 507–516.

Vanderauwera S, Suzuki N, Miller G, *et al*. 2010. Extra-nuclear protection of chromosomal DNA from oxidative stress. *21st International Conference on Arabidopsis Research* abstract no. 03002.

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 Physiology* **139,** 806–821.

Veljovic-Jovanovic SJ, Pignocci C, Noctor G, Foyer CH. 2001. Low ascorbic acid in the *vtc-1* mutant of Arabidopsis is associated with decreased growth and intracellular redistribution of the antioxidant system. *Plant Physiology* **127,** 426–435.

Veljovic-Jovanovic SJ, Noctor G, Foyer CH. 2002. Are leaf hydrogen peroxide concentrations commonly overestimated? The potential influence of artefactual interference by tissue phenolics and ascorbate. *Plant Physiology and Biochemistry* **40,** 501–507.

Vernoux T, Wilson RC, Seeley KA, *et al*. 2000. The *ROOT MERISTEMLESS1/CADMIUM SENSITIVE2* gene defines a glutathione-dependent pathway involved in initiation and maintenance of cell division during postembryonic root development. *The Plant Cell* **12,** 97–110.

Verslues PE, Batelli G, Grillo S, Agius F, Kim YS, Zhu J, Agarwal M, Katiyar-Agarwal S, Zhu JK. 2007. Interactions of

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022

SOS2 with nucleoside diphosphate kinase 2 and catalases reveals a point of connection between salt stress and H_2O_2 signaling in *Arabidopsis thaliana*. *Molecular and Cellular Biology* **27,** 7771–7780.

Vlot AC, Dempsey DMA, Klessig DF. 2009. Salicylic acid, a multifaceted hormone to combat disease. *Annual Review of Phytopathology* **47,** 177–206.

Vogel JT, Zarka DG, Van Buskirk HA, Fowler SG, Thomashow MF. 2005. Roles of the CBF2 and ZAT12 transcription factors in configuring the low temperature transcriptome of Arabidopsis. *The Plant Journal* **41,** 195–211.

Volk S, Feierabend J. 1989. Photoinactivation of catalase at low temperature and its relevance to photosynthetic and peroxide metabolism in leaves. *Plant, Cell and Environment* **12,** 701–712.

Vollsnes AV, Erikson AB, Otterholt E, Kvaal K, Oxaal U, Futsaether C. 2009. Visible foliar injury and infrared imaging show that daylength affects short-term recovery after ozone stress in *Trifolium subterraneum*. *Journal of Experimental Botany* **60,** 3677–3686.

Wagner U, Edwards R, Dixon DP, Mauch F. 2002. Probing the diversity of the Arabidopsis glutathione *S*-transferase gene family. *Plant Molecular Biology* **49,** 515–532.

Wagner D, Przybyla D, op den Camp R, *et al*. 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 Radical Biology and Medicine* **43,** 995–1022.

Wildermuth MC, Dewdney J, Wu G, Ausubel FM. 2001. Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature* **414,** 562–565.

Willekens H, Inzé D, Van Montagu M, Van Camp W. 1995. Catalases in plants. *Molecular Breeding* **1,** 207–228.

Willekens H, Chamnongpol S, Davey M, Schraudner M, Langebartels C, Van Montagu M, Inzé D, Van Camp W. 1997. Catalase is a sink for H_2O_2 and is indispensable for stress defense in C_3 plants. *EMBO Journal* **16,** 4806–4816.

Wood JM, Decker H, Hartmann H, *et al*. 2009. Senile hair graying: H_2O_2-mediated oxidative stress affects human hair color by blunting methionine sulphoxide repair. *The FASEB Journal* **23,** 2065–2075.

Wu S, Li Q, Du M, Li SY, Ren J. 2007. Cardiac-specific overexpression of catalase prolongs lifespan and attenuates ageing-induced cardiomyocyte contractile dysfunction and protein damage. *Clinical and Experimental Pharmacology and Physiology* **34,** 81–87.

Xing Y, Jia W, Zhang J. 2008. AtMKK1 mediates ABA-induced CAT1 expression and H_2O_2 production via AtMPK6-coupled signaling in Arabidopsis. *The Plant Journal* **54,** 440–451.

Yang T, Poovaiah BW. 2002. Hydrogen peroxide homeostasis: activation of plant catalases by calcium/calmodulin. *Proceedings of the National Academy of Sciences, USA* **99,** 4097–4102.

Yu L, Wan F, Dutta S, Welsh S, Liu Z, Freundt E, Baehrecke EH, Lenardo M. 2006. Autophagic programmed cell death by selective catalase degradation. *Proceedings of the National Academy of Sciences, USA* **103,** 4952–4957.

Zamocky M, Furtmüller PG, Obinger C. 2008. Evolution of catalases from bacteria to humans. *Antioxidants and Redox Signaling* **10,** 1527–1547.

Zelitch I. 1992. Factors affecting expression of enhanced catalase activity in a tobacco mutant with O_2-resistant photosynthesis. *Plant Physiology* **98,** 1330–1335.

Zhong HH, Young JC, Pease EA, Hangarter RP, McClung CR. 1994. Interactions between light and the circadian clock in the regulation of *CAT2* expression in *Arabidopsis*. *Plant Physiology* **104,** 889–898.

Zimmermann P, Heinlein C, Orendi G, Zentgraf U. 2006. Senescence-specific regulation of catalase in *Arabidopsis thaliana* (L.) Heynh. *Plant, Cell and Environment* **29,** 1049–1056.

Downloaded from https://academic.oup.com/jxb/article/61/15/4197/436494 by U.S. Department of Justice user on 16 August 2022