Oxidative stress in plants

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During the past few years, considerable progress has been made in understanding how plants protect themselves against oxidative stress. Genes encoding plant antioxidant enzymes have been cloned and characterized. Overproduction of superoxide dismutases and glutathione reductase has been shown to confer increased tolerance to oxidative stress. Several lines of evidence have revealed an important role for hydrogen peroxide in stress-induced signalling.

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Introduction

Every year, environmental stress causes considerable losses in crop quality and productivity. One of the important mechanisms by which plants are damaged during adverse environmental conditions is the excess production of active oxygen species (AOS), such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\bullet}$). Such oxidative stress has been shown to occur in plants exposed to high and low temperatures, particularly in combination with high light intensities, drought, exposure to air pollutants (e.g. ozone or sulphur dioxide), ultraviolet light, and herbicides such as paraquat [1•].

Superoxide and H_2O_2 can inactivate various macromolecules directly, but it is their conversion to $OH^{\bullet}$, catalyzed by transition metals (i.e. the Haber–Weiss reaction), that accounts for their main toxicity. Hydroxyl radicals react instantaneously with proteins, lipids, and DNA, causing rapid cell damage.

Several metabolic processes make use of AOS in a beneficial way. For example, H_2O_2 and $O_2^{\bullet-}$ are involved in lignin formation in cell walls. A large increase in AOS is observed rapidly upon infection of plants with pathogens or upon elicitor treatment. This oxidative burst is thought to be responsible for the hypersensitive cell death and the rapid cross-linking of hydroxyproline-rich glycoproteins in the cell wall associated with active pathogen defense. AOS might also serve as secondary messengers responsible for activating pathogenesis-related genes and genes involved in phytoalexin biosynthesis (see below).

Plants have evolved non-enzymic and enzymic protection mechanisms that efficiently scavenge AOS. Antioxidants, such as ascorbic acid (vitamin C), glutathione, α-tocopherols, and carotenoids, occur in high concentrations in plants. Hydroxyl radicals are too reactive to be eliminated enzymically, but formation is limited by scavenging of $O_2^{\bullet-}$ and H_2O_2. Superoxide dismutases (SODs) are metal-containing enzymes that eliminate $O_2^{\bullet-}$ radicals according to the following reaction:

$$2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$$

Different isoforms can be distinguished on the basis of their metal co-factor. In general, plants contain a mitochondrial MnSOD, as well as a cytosolic and a chloroplastic Cu/ZnSOD [2]. Recently, it has become clear that many plants contain chloroplastic FeSOD [2,3], besides the ubiquitous chloroplastic Cu/ZnSOD.

Hydrogen peroxide is eliminated by catalases and peroxidases. Catalases are peroxisomal enzymes that, in contrast to peroxidases, do not require a reducing substrate for their activity. Ascorbate peroxidases (APXs) are thought to be the most important H_2O_2 scavengers operating both in the cytosol and chloroplasts. They use ascorbic acid as a reducing substrate and form part of a cycle, known as the ascorbate–glutathione or Halliwell–Asada [1•] cycle (see Fig. 1). Other enzymes involved in this oxidation–reduction cycle are monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR). Recently, several cDNA clones encoding glutathione peroxidases (GPXs) have been isolated, suggesting that in plants, as in animals, these proteins might play an important role in scavenging H_2O_2, or the products of lipid peroxidation.

In this review, we summarize current knowledge on the regulation of antioxidant genes and the potential commercial application for engineering stress-tolerant plants.

Abbreviations

AOS—active oxygen species; **APX**—ascorbate peroxidase; **DHAR**—dehydroascorbate reductase; **GPX**—glutathione peroxidase; **GR**—glutathione reductase; **MDAR**—monodehydroascorbate reductase; **sAPX**—stromal APX; **SAR**—systemic acquired resistance; **SOD**—superoxide dismutase; **tAPX**—thylakoid membrane-bound APX.

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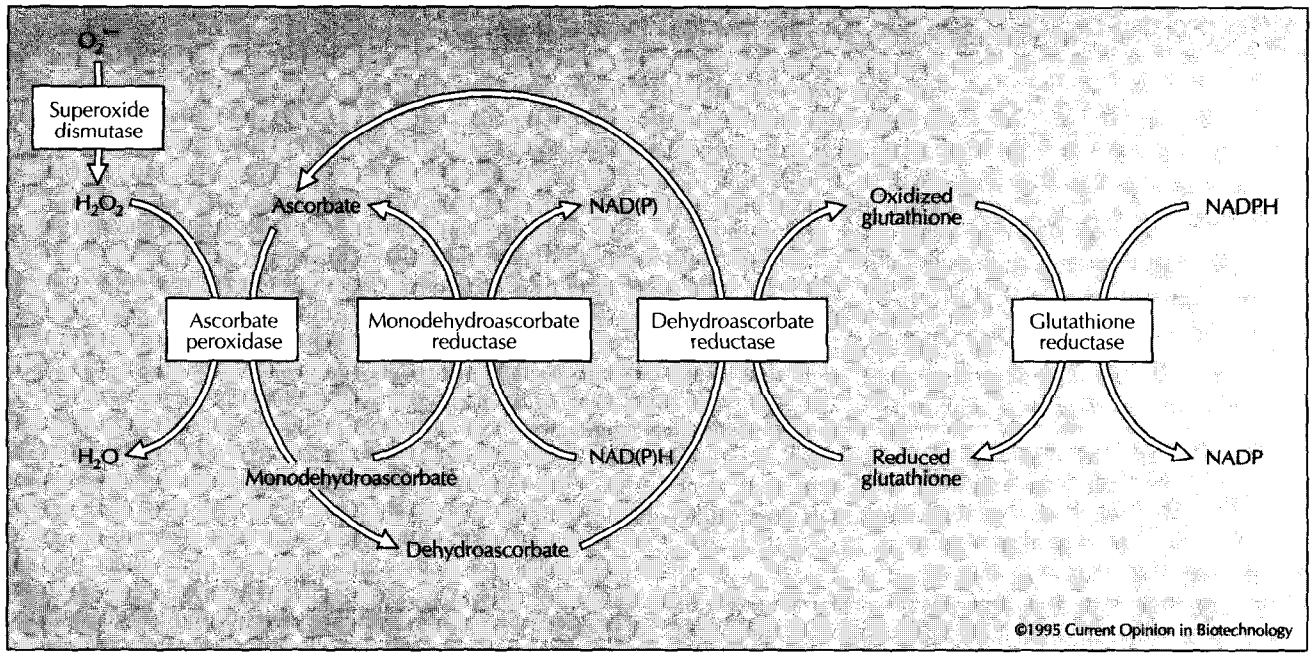

Fig. 1. The ascorbate–glutathione cycle. Hydrogen peroxide is removed by ascorbate peroxidase and ascorbate is regenerated by the ascorbate–glutathione cycle, involving monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase. Ascorbate is first oxidized to monodehydroascorbate. If monodehydroascorbate is not rapidly reduced again to ascorbate by monodehydroascorbate reductase, it will spontaneously disproportionate into ascorbate and dehydroascorbate. Dehydroascorbate recycles ascorbate using reduced glutathione that is regenerated through the action of glutathione reductase in a NADPH-dependent reaction.

Superoxide dismutases

Detailed expression analysis in *Nicotiana plumbaginifolia* [4], tomato [5], and maize ([6] and references therein) show that the *Sod* genes are differentially regulated throughout development and in response to various stress conditions. For example, in *N. plumbaginifolia*, expression of cytosolic Cu/ZnSOD (*SodCc*) mRNA is enhanced most by heat shock, chilling, or paraquat treatment in the dark, whereas expression of FeSOD (*SodB*) mRNA is induced most by chilling and paraquat treatment in the light [4]. In tomato, expression of both the chloroplastic Cu/ZnSOD (*SodCp*) and cytosolic Cu/ZnSOD (*SodCc*) genes is elicited by paraquat in the light, but expression of only the cytosolic isoform is induced following exposure to drought [5]. Using gene-specific probes, it has been shown that the individual members of the maize MnSOD (*SodA*) gene family are differentially expressed during maize development. In general, the pattern of MnSOD mRNA accumulation appears to reflect the mitochondrial activity during plant growth [7]. The large increases in SOD transcript levels upon stress treatment, in general, correlate with much more moderate increases in SOD activity. Possibly, stress causes a more rapid turnover of SOD proteins, thereby necessitating activation of gene expression to maintain SOD levels.

A hypothesis has been formulated that different stress conditions lead to a different extent of oxidative stress in the various subcellular compartments, necessitating the expression of those *Sod* genes encoding the SOD isoform(s) needed to protect a particular compartment [4]. The nature of such signals remains speculative. Organelle-specific lipid peroxidation products are good candidates, although no experimental evidence is currently available in support of this hypothesis. It appears that Ca^{2+} is involved in stress signal transduction. Treatment of tobacco seedlings with H_2O_2 has recently been shown to result in a reduction in SOD activity, coinciding with a transient increase in cytosolic-free Ca^{2+} concentration [8]. Unfortunately, it is unclear which SOD isoforms are under Ca^{2+} control and whether other enzymes involved in AOS-scavenging are affected by these treatments.

Expression of the *N. plumbaginifolia SodCc* expression is under redox control [9,10]. Sulphydryl antioxidants, such as dithiothreitol, cysteine, or reduced glutathione, induce the expression of a promoter *SodCc*–β-glucuronidase gene fusion in protoplasts and in plants, whereas the oxidized forms of glutathione and cysteine have no effects. Levels of reduced glutathione increase under various oxidative stress conditions, such as exposure to ozone, sulphur dioxide, heat shock, or drought [11]. Thus, glutathione can act directly as an antioxidant and simultaneously activate the transcription of a panoply of stress genes, such as Cu/ZnSOD genes [9,10] and genes encoding enzymes of the phenylpropanoid pathway [12].

Catalase and the role of H_2O_2 in signal transduction

Extensive work by Scandalios and co-workers (see review in [1•]) have revealed the presence of three differentially regulated catalases in maize. This situation appears to hold true in dicotyledonous plants. The functional relationship between catalases of monocotyledonous and dicotyledonous plants is currently not clear and, as such, the nomenclature is arbitrary. *N. plumbaginifolia* contains three expressed catalase genes [13•]. The *Cat1* gene product seems to play a role in removing photorespiratory H_2O_2, whereas the expression pattern of *Cat3* suggests the encoded protein has a role in scavenging H_2O_2 generated by the β-oxidation of fatty acids in the glyoxysomes. Interestingly, the *Cat2* gene product most likely has a specific role in protecting the cell from H_2O_2 produced during oxidative stress. Exposure of plants to ambient ozone, sulphur dioxide, and ultraviolet-B radiation, all lead to a rapid decline in *Cat1* steady-state transcript levels and a concomitant rapid increase in *Cat2* transcript levels [14]. GPX transcript levels are also rapidly induced, whereas changes in the *Sod* and APX transcript levels occur in these conditions only with the onset of visible damage [14].

Several other lines of evidence support a central role of catalase in stress tolerance. Maize seedlings exposed to low temperature (4°C) will not survive unless first acclimated at intermediate temperatures (14°C). *Cat3* mRNA levels are induced during acclimation, suggesting that chilling imposes oxidative stress in the seedlings. In accordance, H_2O_2 levels were elevated during chilling of non-acclimated seedlings and during acclimation. It has further been shown that treatment of seedlings with H_2O_2 or menadione, a $O_2^{\bullet-}$-generating compound, at ambient temperature induces chilling tolerance [15••]. These results suggest a dual role for H_2O_2. During acclimation, H_2O_2 levels could be a signal that induces antioxidant enzymes, such as catalase, that subsequently protect the plant from excess H_2O_2 production at chilling temperature.

Hydrogen peroxide appears also to play an important role in signal transduction during plant pathogen interactions. Hypersensitive cell death associated with pathogen–plant interactions is characterized by a rapid oxidative burst. The H_2O_2 produced from the oxidative burst functions as a local trigger of programmed cell death of challenged cells and causes a rapid cross-linking of cell wall proteins [16,17••]. Furthermore, H_2O_2 appears to act as a signal molecule, inducing transcription of defense genes, such as glutathione *S*-transferase and GPX in surrounding cells [17••].

Recent evidence suggests that H_2O_2 may also have a function in the induction of systemically acquired resistance (SAR). For a long time, it has been known that salicylic acid has an important role in the local establishment of SAR. Interestingly, the salicylic acid-binding protein (57 kDa), present in tobacco leaves, has turned out to be a catalase. Salicylic acid inhibits *in vitro* catalase activity and it was hypothesized that, *in vivo*, this property would lead to an increase in H_2O_2 levels which, in turn, would act as a secondary messenger to induce the defense response. Accordingly, it has been shown that H_2O_2 and the catalase inhibitor, 3-amino-1,2,4-triazole induces the accumulation of the pathogenesis-related (PR1) protein [18••].

The mechanism of H_2O_2-controlled gene expression is currently unknown. Hydrogen peroxide is a well known secondary messenger in mammalian cells, functioning through the activation of transcription factors, such as NFκB and AP-1. Possibly, H_2O_2 acts by modulating a redox-sensitive signal transducer. AP-1 only binds to its *cis* DNA element when cysteine residues in the DNA-binding domain are reduced. This reduction is mediated by the nuclear redox factor Ref-1, and a plant homologue of this factor has recently been described [19].

The role of catalases in stress-induced signalling by modulating levels of H_2O_2 is somewhat unexpected in view of the presumed peroxisomal location. Although all three *N. plumbaginifolia* catalases contain a carboxy-terminal peroxisomal targeting sequence [20], their actual location awaits confirmation. The differential pattern of catalase gene expression suggests specific functions, different subcellular locations, and, possibly, different substrate specificities. Biochemical characterization and the analysis of transgenic plants expressing antisense or overexpression constructs will provide further insights into the role of specific catalase isoforms.

Ascorbate–glutathione cycle

Extensive progress has also been made on the characterization and cloning of enzymes involved in the ascorbate–glutathione cycle (see Fig. 1). Complementary DNAs encoding cytosolic APX have been cloned from, amongst other species, *Arabidopsis thaliana* [21] and pea [22]. *Apx* gene expression is rapidly induced by various stress conditions, such as paraquat, abscisic acid, ethylene, drought, and heat shock, suggesting an important role in stress tolerance [23]. In general, a marked discrepancy has been found between the increase in *Apx* steady-state transcript abundance, which is relatively large, and the increase in APX protein and activity, which is relatively small. It appears that the high levels of *Apx* transcript observed in response to drought are restricted from interaction with polysomes [24]. Besides the cytosolic APX, plants also contain a stromal APX (sAPX) and a thylakoid membrane-bound APX (tAPX) [25], yet to be cloned. The spinach tAPX is a 40 kDa monomeric protein, whereas the stromal isoform is approximately 30 kDa in size. The amino-terminal sequence of tAPX has a low degree of similarity with the cytosolic APX [26].

Recently, a full-length cDNA clone encoding MDAR has been isolated from cucumber [27] and pea [28].

DHAR has been purified from spinach chloroplast, the amino-terminal sequence showing surprising similarity with Kunitz-type trypsin inhibitors [29]. The spinach DHAR and soybean trypsin inhibitor are both capable of reducing dehydroascorbate when in the reduced form, but acquire trypsin-inhibiting activity in the oxidized state.

The last enzyme in the ascorbate–glutathione cycle, glutathione reductase (GR), has been extensively studied. Glutathione plays an important role in numerous cellular processes, often resulting in its oxidation to glutathione disulphate and each subcellular compartment recycles the glutathione by GR activities. GR has been cloned from pea and tobacco (see Creissen *et al.* in [1•]). Furthermore, a cDNA encoding the first committed step in glutathione biosynthesis has recently been described [30].

In conclusion, genes for all enzymes of the ascorbate–glutathione cycle have now been identified. This will considerably accelerate the study of the occurrence and function of this H_2O_2-detoxification pathway in plants.

Transgenic plants with improved antioxidant systems

Tolerance to a wide variety of environmental stress conditions have been correlated with increased activity of antioxidant enzymes [1•] and levels of antioxidant metabolites [11]. Plants with such enhanced antioxidative properties often show cross-tolerance to several different stresses. The ability to engineer plants that express introduced genes to high levels provides an opportunity to test the role of antioxidant enzymes in stress tolerance.

Tepperman and Dunsmuir [31] obtained transgenic tobacco plants with a 30–50-fold enhancement in chloroplastic Cu/ZnSOD activity. This high increment in SOD activity did not, however, result in any improved resistance against paraquat [31] or ozone [32]. In contrast, overproduction of MnSOD in tobacco chloroplasts has been found to result in a significant, albeit not very pronounced, protection against paraquat [33]. The same transgenic lines display about fourfold less ozone injury than control plants when exposed to ambient levels of ozone (0 ppb at night to 120 ppb during the day) [34••]. Transgenic alfalfa lines with enhanced MnSOD activity in chloroplasts have more rapid regrowth after freezing stress than control plants [35].

Overproduction of either chloroplastic or cytosolic forms of Cu/ZnSOD in potato results in an increased tolerance to paraquat [36]. In addition, transgenic tobacco plants that overproduce chloroplast-located pea Cu/ZnSOD have greater resistance to photooxidative damage and to paraquat-mediated oxidative stress than control plants [37]. Interestingly, these plants exhibit a three to fourfold increase in APX activity and have a corresponding increase in levels of *Apx* mRNA. DHAR or GR activities are not affected in these plants [38]. Similar studies in other transgenic plants have not been reported.

Not only native plant genes, but also genes of bacterial origin have been used to engineer plants for oxidative stress tolerance. Overexpression of the *Escherichia coli* MnSOD gene in chloroplasts of tobacco transgenic plants leads to an increase in tolerance against oxidative stress produced by exposure to low concentrations of methyl viologen or low-temperature stress [39]. The *E. coli gor* gene, encoding GR, has also been expressed in the cytosol of tobacco [40,41]. Although the transgenic plants show a reduction in visible injury caused by paraquat [40], no effects on the protection of the photosynthetic apparatus are observed [40,41]. More recently, in a separate study, the *gor* gene product has been targeted to the chloroplast of transgenic tobacco [42]. These transgenic plants have a GR activity in leaves threefold higher than that in controls. These leaves also have increased tolerance to paraquat, sulphur dioxide, but not ozone.

Perspectives

Research on oxidative stress has progressed rapidly in recent years. Genes encoding enzymes of known antioxidant pathways have been cloned and characterized in some detail. The dual role of H_2O_2 in signal transduction and cell death has become clear and may provide further clues on how plants sense environmentally adverse conditions. Furthermore, convincing evidence now suggests that the manipulation of antioxidant capacities is a valuable route to obtain stress-tolerant plants. Because SOD dismutates $O_2^{\bullet-}$ to H_2O_2, little doubt exists that the overproduction of a H_2O_2-scavenging enzyme alongside SOD will have a cooperatively protective effect on oxidative stress-induced damage. Simultaneous overproduction of a Cu/ZnSOD and a catalase in *Drosophila* extends the life-span by one-third and lowers the amount of oxidative damage to proteins [43••]. It is currently unclear which of the H_2O_2-scavenging enzymes will be most effective for use alongside SOD in plants. Depending on the subcellular compartment, APX or GPX would be ideal because they cooperate with SOD. Their dependence on reduced substrates for activity would, however, demand highly efficient mechanisms for the regeneration of these substrates. Catalases are an alternative, but a possible disadvantage is their light sensitivity and the fact that catalase activity generates oxygen, thus creating more favourable conditions for superoxide formation. Further work is required to test which combination of SOD and H_2O_2-scavenging enzymes provides the best protection.

In the long term, we need to understand how plants sense oxidative stress in the various compartments and how this stress signal is transduced to turn on the appropriate defense systems. A promising route is the

characterization of *Arabidopsis* mutants that exhibit enhanced sensitivity or tolerance to stress conditions and the molecular analysis of the respective mutated genes. This approach is particularly attractive because it may lead to the characterization of key branch points in stress signalling that orchestrate global regulation of antioxidant defense.

Because oxidative stress occurs in all aerobic organisms, one might expect that the basic defense systems are conserved in evolution. As such, 'shotgun' cloning of plant cDNAs that would render wild-type or mutant yeast strains more resistant to oxidative stress may yield novel defense genes of use in the engineering of stress-tolerant crops.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. • Foyer CH, Mullineaux PM (Eds): *Causes of photooxidative stress and amelioration of defense systems in plants.* Boca Raton: CRC Press; 1994.

An excellent book with comprehensive reviews on biochemical, physiological, and molecular aspects of oxidative stress.

2. Bowler C, Van Camp W, Van Montagu M, Inzé D: **Superoxide dismutase in plants.** *CRC Crit Rev Plant Sci* 1994, **13**:199–218.

3. Van Camp W, Bowler C, Villarroel R, Tsang EWT, Van Montagu M, Inzé D: **Characterization of iron superoxide dismutase cDNAs from plants obtained by genetic complementation in *Escherichia coli*.** *Proc Natl Acad Sci USA* 1990, **87**:9903–9907.

4. Tsang EWT, Bowler C, Hérouart D, Van Camp W, Villarroel R, Genetello C, Van Montagu M, Inzé D: **Differential regulation of superoxide dismutases in plants exposed to environmental stress.** *Plant Cell* 1991, **3**:783–792.

5. Perl-Treves R, Galun E: **The tomato Cu,Zn superoxide dismutase genes are developmentally regulated and respond to light and stress.** *Plant Mol Biol* 1991, **17**:745–760.

6. Scandialos JG: **Oxygen stress and superoxide dismutases.** *Plant Physiol* 1993, **101**:7–12.

7. Zhu D, Scandalios JG: **Maize mitochondrial manganese superoxide dismutases are encoded by a differentially expressed multigene family.** *Proc Natl Acad Sci USA* 1993, **90**:9310–9314.

8. Price AH, Taylor A, Ripley SJ, Griffiths A, Trewavas AJ, Knight MR: **Oxidative signals in tobacco increase cytosolic calcium.** *Plant Cell* 1994, **6**:1301–1310.

9. Hérouart D, Van Montagu M, Inzé D: **Redox-activated expression of the cytosolic copper/zinc superoxide dismutase gene in *Nicotiana*.** *Proc Natl Acad Sci USA* 1993, **90**:3108–3112.

10. Hérouart D, Van Montagu M, Inzé D: **Developmental and environmental regulation of the *Nicotiana plumbaginifolia* cytosolic Cu/Zn-superoxide dismutase promoter in transgenic tobacco.** *Plant Physiol* 1994, **104**:873–880.

11. Alscher RG: **Biosynthesis and antioxidant function of glutathione in plants.** *Physiol Plant* 1989, **77**:457–464.

12. Wingate VPM, Lawton MA, Lamb CJ: **Glutathione causes a massive and selective induction of plant defense genes.** *Plant Physiol* 1988, **87**:206–210.

13. • Willekens H, Langebartels C, Tiré C, Van Montagu M, Inzé D, Van Camp W: **Differential expression of catalase genes in *Nicotiana plumbaginifolia*.** *Proc Natl Acad Sci USA* 1994, **91**:10450—10454.

This paper demonstrates that the three *N. plumbaginifolia* catalase genes are differentially expressed and that the expression of each catalase gene is associated with a specific H_2O_2-producing process.

14. Willekens H, Van Camp W, Van Montagu M, Inzé D, Sandermann H Jr, Langebartels C: **Ozone, sulfur dioxide, and ultraviolet B have similar effects on mRNA accumulation of antioxidant genes in *Nicotiana plumbaginifolia* (L.).** *Plant Physiol* 1994, **106**:1007–1014.

15. •• Prasad TK, Anderson MD, Martin BA, Stewart CR: **Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide.** *Plant Cell* 1994 **6**:65–74.

Strong support is provided for the dual role of H_2O_2 in chilling stress. At acclimation temperatures, H_2O_2 acts as a signal to induce catalase gene expression and possibly also the expression of other antioxidant genes. As a result, the antioxidant capacity at chilling temperatures is enhanced, thereby providing protection against the excess production of H_2O_2.

16. Bradley DJ, Kjellbom P, Lamb CJ: **Elicitor- and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: a novel, rapid defense response.** *Cell* 1992, **70**:21–30.

17. •• Levine A, Tenhaken R, Dixon R, Lamb C: **H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response.** *Cell* 1994, **79**:583–593.

A particularly interesting paper because it provides evidence that H_2O_2 produced during hypersensitive cell death has a twofold function. Hydrogen peroxide not only acts as a local trigger of programmed death in challenged cells, but it is also a diffusible signal inducing defense genes in adjacent cells.

18. •• Chen Z, Silva H, Klessig DF: **Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid.** *Science* 1993, **262**:1883–1886.

Shows that salicylic acid binds and inhibits catalase as well as inducing an increase in H_2O_2 concentration *in vivo*. Hydrogen peroxide in itself, or other inhibitors of catalase, induces expression of pathogen-related genes. Thus, the action of salicylic acid in systemically acquired resistance is probably mediated by elevated amounts of H_2O_2.

19. Babiychuk E, Kushnir S, Van Montagu M, Inzé D: **The *Arabidopsis thaliana* apurinic endonuclease Arp reduces human transcription factors Fos and Jun.** *Proc Natl Acad Sci USA* 1994, **91**:3299–3303.

20. Willekens H, Villarroel R, Inzé D, Van Montagu M, Van Camp W: **Molecular identification of catalases from *Nicotiana plumbaginifolia*.** *FEBS Lett* 1994, **352**:79–83.

21. Kubo A, Saji H, Tanaka K, Tanaka K, Kondo N: **Cloning and sequencing of a cDNA encoding ascorbate peroxidase from *Arabidopsis thaliana*.** *Plant Mol Biol* 1992, **18**:691–701.

22. Mittler R, Zilinskas BA: **Molecular cloning and nucleotide sequence analysis of a cDNA encoding pea cytosolic ascorbate peroxidase.** *FEBS Lett* 1991, **289**:257–259.

23. Mittler R, Zilinskas BA: **Molecular cloning and characterization of a gene encoding pea cytosolic ascorbate peroxidase.** *J Biol Chem* 1992, **267**:21802–21807.

24. Mittler R, Zilinskas BA: **Regulation of pea cytosolic ascorbate peroxidase and other antioxidant enzymes during the progres-**

sion of drought stress and following recovery from drought. *Plant J* 1994, 5:397–405.

25. Miyake C, Asada K: **Thylakoid-bound ascorbate peroxidase in spinach chloroplasts and photoreduction of its primary oxidation product monodehydroascorbate radicals in thylakoids.** *Plant Cell Physiol* 1992, **33**:541–553.

26. Miyake C, Cao W-H, Asada K: **Purification and molecular properties of the thylakoid-bound ascorbate peroxidase in spinach chloroplasts.** *Plant Cell Physiol* 1993, **34**:881–889.

27. Sano S, Asada K: **cDNA cloning of monodehydroascorbate radical reductase from cucumber: a high degree of homology in terms of amino acid sequence between this enzyme and bacterial flavoenzymes.** *Plant Cell Physiol* 1994, **35**:425–437.

28. Murthy SS, Zilinskas BA: **Molecular cloning and characterization of a cDNA encoding pea monodehydroascorbate reductase.** *J Biol Chem* 1994, **269**:31129–31133.

29. Trümper S, Follmann H, Häberlein I: **A novel dehydroascorbate reductase from spinach chloroplasts homologous to plant trypsin inhibitor.** *FEBS Lett* 1994, **352**:159–162.

30. May MJ, Leaver CJ: ***Arabidopsis thaliana* γ-glutamylcysteine synthetase is structurally unrelated to mammalian, yeast, and *Escherichia coli* homologs.** *Proc Natl Acad Sci USA* 1994, **91**:10059–10063.

31. Tepperman JM, Dunsmuir P: **Transformed plants with elevated levels of chloroplastic SOD are not more resistant to superoxide toxicity.** *Plant Mol Biol* 1990, **14**:501–511.

32. Pitcher LH, Brennan E, Hurley A, Dunsmuir P, Tepperman JM, Zilinskas BA: **Overproduction of petunia chloroplastic copper/zinc superoxide dismutase does not confer ozone tolerance in transgenic tobacco.** *Plant Physiol* 1991, **97**:452–455.

33. Bowler C, Slooten L, Vandenbranden S, De Rycke R, Botterman J, Sybesma C, Van Montagu M, Inzé D: **Manganese superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants.** *EMBO J* 1991, **10**:1723–1732.

34. •• Van Camp W, Willekens H, Bowler C, Van Montagu M, Inzé D, Reupold-Popp P, Sandermann H Jr, Langebartels C: **Elevated levels of superoxide dismutase protect transgenic plants against ozone damage.** *Biotechnology* 1994, **12**:165–168.

The authors demonstrate that SOD overproduction in chloroplasts results in a 3–4-fold reduction of visible ozone injury. This result also indicates that oxidative stress is an important constituent of ozone stress.

35. McKersie BD, Chen Y, De Beus M, Bowley SR, Bowler C, Inzé D, D'Halluin K, Botterman J: **Superoxide dismutase enhances tolerance of freezing stress in transgenic alfalfa (*Medicago sativa* L.).** *Plant Physiol* 1993, **103**:1155–1163.

36. Perl A, Perl-Treves R, Galili S, Aviv D, Shalgi E, Malkin S, Galun E: **Enhanced oxidative-stress defense in transgenic potato expressing tomato Cu,Zn superoxide dismutase.** *Theor Appl Genet* 1993, **85**:568–576.

37. Sen Gupta A, Heinen JL, Holaday AS, Burke JJ, Allen RD: **Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase.** *Proc Natl Acad Sci USA* 1993, **90**:1629–1633.

38. Sen Gupta A, Webb RP, Holaday AS, Allen RD: **Overexpression of superoxide dismutase protects plants from oxidative stress.** *Plant Physiol* 1993, **103**:1067–1073.

39. Foyer CH, Descourvières P, Kunert KJ: **Protection against oxygen radicals: an important defence mechanism studied in transgenic plants.** *Plant Cell Environ* 1994, **17**:507–523.

40. Aono M, Kubo A, Saji H, Natori T, Tanaka K, Kondo N: **Resistance to active oxygen toxicity of transgenic *Nicotiana tabacum* that expresses the gene for glutathione reductase from *Escherichia coli*.** *Plant Cell Physiol* 1991, **32**:691–697.

41. Foyer C, Lelandais M, Galap C, Kunert KJ: **Effects of elevated cytosolic glutathione reductase activity on the cellular glutathione pool and photosynthesis in leaves under normal and stress conditions.** *Plant Physiol* 1991, **97**:863–872.

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

43. •• Orr WC, Sohal RS: **Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*.** *Science* 1994, **263**:1128–1130.

This very interesting article shows that simultaneous overexpression of Cu/ZnSOD and catalases considerably extends the life-span of transgenic *Drosophila*. It clearly underlines the importance of an optimum balance between SOD and a H_2O_2-scavenging enzyme.

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