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RESEARCH REVIEW

Active oxygen species and antioxidants in seed biology

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Abstract

Active oxygen species (AOS) are involved in various aspects of seed physiology. Their generation, which occurs during seed desiccation, germination and ageing, may lead to oxidative stress and cellular damage, resulting in seed deterioration. However, cells are endowed with detoxifying enzymes and antioxidant compounds that scavenge AOS and participate in seed survival. The detoxifying mechanisms play a key role in acquisition of desiccation tolerance of developing seeds, completion of seed germination and seed storability. However, AOS must also be regarded as molecules intervening in cellular signalling. They are involved in growth processes occurring at early embryogenesis during seed development, and participate in the mechanisms underlying radicle protrusion during seed germination. AOS might also have a regulatory function in the changes in gene expression during seed development, dormancy and germination. Their interplay with other molecules, particularly with hormones such as abscisic acid, suggests that they should be considered as key components of an integrated signalling network involved in many aspects of seed physiology.

Keywords: active oxygen species, antioxidants, cell signalling, detoxifying enzymes, germination, oxidative stress

Introduction

In seed physiology active oxygen species (AOS) are usually considered as toxic molecules, the accumulation of which leads to cell injury and disturbances in seed development or germination processes. The deleterious role of these compounds in seed ageing is now quite well established and documented (Priestley, 1986; Hendry, 1993; McDonald, 1999). AOS may also play a role in desiccation-related damage, particularly in dehydration-intolerant recalcitrant seeds (Pammenter and Berjak, 1999). However, this view is only partially valid. There is now increasing evidence that AOS also function as signalling molecules and are involved in a wide range of responses to various stimuli. The dual function of AOS in plants relies on the cellular antioxidant machinery, which involves detoxifying enzymes and antioxidant compounds. Such mechanisms can eliminate potentially toxic AOS, generally produced under stressful conditions, or rather tightly control AOS concentrations in order to regulate various signalling pathways. The aim of this paper is to review the possible roles of AOS and antioxidant systems in orthodox seeds, focusing attention on the dual function of these compounds.

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AOS: nature and origin

Oxygen: a potentially toxic molecule

Oxygen is a slightly reactive molecule that may give rise, during electron transport processes, to the strongly reactive and potentially harmful active oxygen species (AOS). Reduction of oxygen leads to the formation of the superoxide radical ($O_2^{-\cdot}$), which is a molecule with an uncoupled electron and can react with other molecules to stabilize its energy. However, superoxide itself is not highly reactive and is a short-lived AOS, but it can further form hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\cdot}$). Hydrogen peroxide may result from the non-enzymatic reduction of $O_2^{-\cdot}$ in the presence of H^+ ions, or from the action of catalase on $O_2^{-\cdot}$. H_2O_2 has a strong oxidizing capacity, and its life span is longer than that of superoxide (half-life of 1 ms versus 2 µs). H_2O_2 can also diffuse through membranes and therefore reach target molecules at some distance

from its production site. The Haber–Weiss and Fenton reactions involve superoxide radicals and H_2O_2 and lead, in the presence of iron or other transition metals, to the formation of the hydroxyl radical, $OH^{\cdot}$, which is the most aggressive form of the oxygenated derivatives. Therefore, AOS include the radical derivatives of oxygen ($O_2^{\cdot -}$, $OH^{\cdot}$, but also the peroxyl, alkoxyl or hydroperoxyl radicals), which are termed as free radicals, i.e. molecular species containing one or more unpaired electrons, but they also include non-radical derivatives of oxygen such as H_2O_2, ozone and singlet oxygen (Halliwell and Gutteridge, 1999).

AOS production: the case of orthodox seeds

Many AOS sources have been identified in plants, and any transfer or transport chain of electrons towards oxygen can potentially generate AOS. In this regard, orthodox seeds represent a particular case. Except in the early developmental phase, seeds are devoid of an important source of AOS through photosynthetic electron transport, which leads to the production of superoxide and singlet oxygen. Moreover, from the beginning of development to the end of germination, the seed moisture content and metabolic activity vary dramatically. Therefore, the sources of AOS in seeds also probably vary considerably.

The mitochondrial respiratory chain is one of the major sources of AOS; electron leakage from the transport chain generates superoxide, and subsequently H_2O_2, by dismutation of the former (Moller, 2001). In normoxic conditions, approximately 2–3% of the oxygen used by the mitochondria can be converted into superoxide and H_2O_2 (Chance *et al*., 1973; Puntarulo *et al*., 1988). The amount of H_2O_2 produced is thus directly proportional to respiratory activity (Staniek and Nohl, 2000). In the case of orthodox seeds, respiration is intense during the first stages of embryogenesis, but it strongly decreases during the desiccation phase on the mother plant and becomes nil when seeds are quiescent (Bewley and Black, 1994). It is estimated that mitochondrial respiration ceases at water contents lower than 0.25 g (g DW)$^{-1}$ (Vertucci and Farrant, 1995). Germination, on the other hand, is associated with a strong increase in the respiratory activity and enhanced production of AOS.

Peroxisomes are also a possible source of AOS. Several types of these organelles are frequently distinguished: glyoxysomes (present in oily seeds), leaf-type peroxisomes of photosynthetic tissues, nodule-specific peroxisomes from uninfected cells of *Leguminosae* nodules and gerontosomes found in senescing tissues (Beevers, 1979; Reumann, 2000). Glyoxysomes play a key role in mobilization of lipid reserves of oily seeds because they contain the enzymes of β-oxidation and the glyoxylate cycle, which convert lipid reserves into sugars during the first stages of seedling development (Huang *et al*., 1983). Fatty acid β-oxidation produces H_2O_2, resulting from the activity of enzymes such as glycolate oxidase. In addition, the oxidation of xanthine into uric acid by xanthine oxidase in the peroxisomal matrix is associated with the production of superoxide and seems to be common to all the types of peroxisomes (del Rio *et al*., 1998; Corpas *et al*., 2001). The potential role of these organelles in free-radical biology and oxidative stress is generating increased research interest. Indeed, peroxisomes are also the site of localization of catalase, which eliminates H_2O_2, and of the production of nitric oxide (NO), a compound now considered to play a major role in cellular signalling in plants (Durner and Klessig, 1999; Corpas *et al*., 2001; Neill *et al*., 2003).

Other sources of AOS have been characterized more recently in plants, including NADPH oxidases of the plasma membrane. These enzymes, which transfer electrons from cytoplasmic NADPH to oxygen, give rise to the superoxide radical that subsequently dismutates to H_2O_2. NADPH oxidases are involved in the 'oxidative burst' during plant–pathogen interactions (Lamb and Dixon, 1997; Grant and Loake, 2000), in various plant growth and development processes (Schopfer *et al*., 2001), and plant responses to various forms of abiotic stress (Neill *et al*., 2002). For example, NADPH oxidase is involved in abscisic acid (ABA)-induced generation of AOS during water stress (Jiang and Zhang, 2002a, b). Finally, pH-dependent cell-wall peroxidases and amine oxidases may also lead to the formation of H_2O_2 in the apoplast, particularly during biotic stress (Allan and Fluhr, 1997; Bolwell and Wojtaszek, 1997; Bolwell *et al*., 2002). While it is likely that mitochondria and peroxisomes are the major sources of AOS in non-quiescent orthodox seeds, further work is required to evaluate the contributions of these other sites of AOS production to seed development and germination. It is also necessary to distinguish the production sites from the action sites, because they are often distant from one another. For example, recent work has shown that transmembrane aquaporins and peroxiporins may play a role in the transport of H_2O_2 in vegetative tissue (Henzler and Steudle, 2000; Neill *et al*., 2002), but the mobility of AOS in seeds has not, as yet, been documented.

Finally, non-enzymatic autoxidation of lipids may also represent a potential source of AOS in seeds, particularly during dry storage, when enzymatic activities and metabolism are negligible. Lipid autoxidation would generate free radicals that would be trapped in seed tissues (Priestley, 1986; Wilson and McDonald, 1986; McDonald, 1999).

The dual effect of AOS: from toxicity to signalling

Toxicity of AOS

The uncontrolled accumulation of AOS, particularly of $OH^{\cdot}$, which cannot be eliminated enzymatically, is highly toxic for the cell. AOS can react with the majority of biomolecules, thus resulting in oxidative stress that can become irreversible and cause cellular damage. Many harmful effects of AOS on cellular macromolecules have been identified. Among these, one of the best known is lipid peroxidation, because it has been studied intensively in food science in order to prevent rancidity of fatty products. Lipid peroxidation is a free-radical chain process leading to the deterioration of polyunsaturated fatty acids (PUFAs). It is initiated by free-radical attack upon a lipid, resulting in the removal of a hydrogen atom from a methylene group adjacent to a double bond. In aerobic conditions the carbon radical, originating from the abstraction of hydrogen, is stabilized by oxygen and yields a peroxyl radical ($ROO^{\cdot}$), which is capable of removing a hydrogen atom from another fatty acid chain to form a lipid hydroperoxide (LOOH) in a propagation step (see Halliwell and Gutteridge, 1999, for detailed schemes of lipid peroxidation). Lipid peroxidation is likely to degrade PUFAs present in membranes or in reserve lipids of oily seeds. Nucleic acids and proteins are also potential targets of AOS (Beckman and Ames, 1997). The hydroxyl radical can directly damage both nuclear and organelle DNA because it attacks deoxyribose, purines and pyrimidines (Breen and Murphy, 1995), whereas neither superoxide nor H_2O_2 seem to have such a deleterious effect. Enzymes can be inactivated easily by AOS when amino acids essential for, or close to, the active sites are degraded. Again, the hydroxyl radical seems to be the most reactive species regarding protein sensitivity to oxidative stress, since it can damage a great range of amino acids. Hydrogen peroxide is also known to react with thiol groups, and can lead directly to inactivation of some enzymes, e.g. those of the Calvin cycle (Charles and Halliwell, 1980). Beside these effects on enzymes, AOS can also damage transport proteins, receptors and ion channels and then lead to extensive cellular dysfunction (Halliwell and Gutteridge, 1999).

Revisiting the roles of AOS: cell signalling

Whereas AOS toxicity is well established, cellular antioxidant mechanisms seem to tightly control AOS concentrations, rather than to eliminate them completely, suggesting that some AOS might play normal physiological roles and act as signalling molecules. Following the numerous studies carried out in animal cellular biology, the possible roles of AOS as messengers of various signal transduction pathways are being evaluated in plants. In the early 1970s, it was established that H_2O_2 acts as a second messenger in mammalian cells (reviewed by Wolin and Mohazzab-H., 1997). The role of this molecule was investigated later in plants, and Prasad *et al.* (1994) were among the first to show that H_2O_2 is involved in the tolerance to various abiotic stresses. In 1994, Levine *et al.* also showed that H_2O_2 may elicit cellular defence reactions against pathogens. Since then, many processes involving H_2O_2 have been identified in plants, including programmed cell death (PCD) (Jabs, 1999; Amor *et al.*, 2000, Fath *et al.*, 2001; de Jong *et al.*, 2002, Pellinen *et al.*, 2002), somatic embryogenesis (Cui *et al.*, 1999), response to wounding (Orozco-Cardenas *et al.*, 2001), root gravitropism (Joo *et al.*, 2001) and ABA-mediated stomatal closure (Pei *et al.*, 2000; Zhang *et al.*, 2001). The roles of superoxide and other AOS in signalling pathways are less well described so far; however, $O_2^{-\cdot}$ seems to play a part in cell death and plant defence (Doke *et al.*, 1994; Jabs *et al.*, 1996; Wisniewski *et al.*, 1999). At the cellular level, events regulated by H_2O_2 are beginning to be identified. They include protein phosphorylation through mitogen-activated protein kinase (MAP kinase) cascades (Desikan *et al.*, 1999; Kovtun *et al.*, 2000; Samuel *et al.*, 2000), calcium mobilization (Bowler and Fluhr, 2000; Murata *et al.*, 2001) and regulation of gene expression (Desikan *et al.*, 1998; Desikan *et al.*, 2001).

Control of AOS levels: detoxifying mechanisms

With regard to the possible roles of AOS previously considered, it appears necessary for the cells to be equipped with mechanisms allowing elimination (in the case of oxidative stresses) or homeostasis of AOS (for cellular signalling). Various enzymatic and non-enzymatic mechanisms play these roles in plants. Superoxide dismutase, which can be mitochondrial (MnSOD), cytosolic (Cu/ZnSOD) or chloroplastic (CuZnSOD, FeSOD), dismutates superoxide radicals into H_2O_2 and oxygen (Bowler *et al.*, 1992). Hydrogen peroxide is eliminated by the action of catalase (CAT), which is located in glyoxysomes and peroxisomes (Willekens *et al.*, 1995), except the isoform Cat-3 of maize, which is mitochondrial (Scandalios *et al.*, 1997). The ascorbate–glutathione cycle (also called the Halliwell–Asada cycle) may also take part in H_2O_2 scavenging; it involves ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR). The enzymes of this cycle, which are present in chloroplasts, the cytoplasm, mitochondria, peroxisomes and the apoplast (Mittler, 2002), participate in the regeneration of the powerful

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antioxidants ascorbic acid (vitamin C), reduced glutathione and α-tocopherol (vitamin E). The role of the ascorbate system in seeds has been reviewed recently by De Tullio and Arrigoni (2003). Glutathione peroxidases (GPX) may also catalyse the reduction of H_2O_2 and hydroperoxides (Eshdat *et al.*, 1997). Various compounds, such as polyphenols, flavonoids and peroxiredoxins (Aalen, 1999), also have a strong antioxidant function. The main mechanisms involved in cellular detoxification in plants are shown in Fig. 1.

AOS and seed physiology

The seed developmental pattern is associated with marked changes in AOS content

Embryogenesis, reserve accumulation and maturation/drying are the three typical stages of orthodox seed development on the mother plant, leading from a zygotic embryo to a mature, quiescent seed. Most research contributions dealing with AOS and seed development have, up to now, concerned the final stage of seed desiccation in relation to acquisition of dehydration tolerance, a common feature of all orthodox seeds. However, some studies have also shown that AOS metabolism might also be

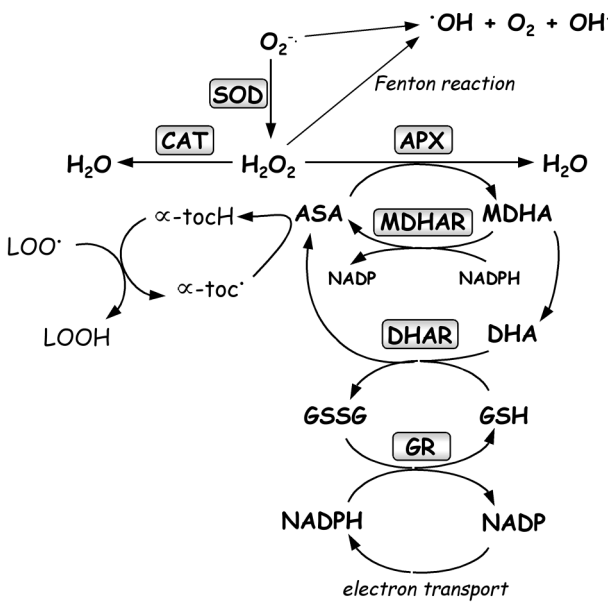

Figure 1. Main detoxifying systems in plants. CAT, catalase; SOD, superoxide dismutase; APX, ascorbate peroxidase; MDHAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase, GR, glutathione reductase. ASA, ascorbate; MDHA, monodehydroascorbate; DHA, dehydroascorbate; GSSG, oxidized glutathione; GSH, reduced glutathione; α-tocH, α-tocopherol; α-toc·, α-tocopheryl; LOOH, lipid peroxide; LOO·, lipid radical.

important during initial embryogenesis and seed filling.

Metabolic activity and mitochondrial respiration are high during embryo development. Moreover, some developing embryos contain functional chloroplasts with photosynthetic activity, but the contribution of the latter to seed filling seems to vary greatly among species (Bewley and Black, 1994). This suggests that developing embryos have the potential to generate significant amounts of AOS, necessitating tight control by antioxidant mechanisms. The ascorbate system seems to play a central role in embryogenesis and cell growth (reviewed by De Tullio and Arrigoni, 2003), mainly because ascorbate may control cell-cycle progression (Kato and Esaka, 1999). Recently, it has also been proposed that ascorbate content could influence cell growth by modulating the expression of genes involved in hormonal signalling pathways (Pastori *et al.*, 2003). Studies carried out in the fields of *in vitro* micropropagation and somatic embryogenesis may yield complementary insights on the roles of AOS in embryo development. During zygotic and somatic embryogenesis, activities and/or expression of the main antioxidant enzymes, i.e. CAT and SOD, vary greatly during cell differentiation. In *Arabidopsis*, MnSOD expression increases during early embryo development (Otegui *et al.*, 2002). SOD and CAT activities also increase during development of horse chestnut somatic embryos (Bagnoli *et al.*, 1998) or during development of oak microcuttings (Racchi *et al.*, 2001). Totipotency of plant protoplasts has also been related to the activity of the cell antioxidant machinery, since a direct correlation exists between high AOS content and repressed expression of totipotency (Papadakis *et al.*, 2001 and references therein). Conversely, AOS may also play a positive role in growth and development. For example, the differentiation of embryogenic cells of *Lycium barbarum* is promoted by a transient decrease in CAT activity, resulting in high cellular H_2O_2 (Cui *et al.*, 1999). It has also been postulated that hydroxyl radicals are involved in cell-wall extension during cell growth, by causing oxidative scission of polysaccharides (Fry, 1998; Schweikert *et al.*, 2000, 2002; Schopfer *et al.*, 2002; Liszkay *et al.*, 2003). This aspect, which has been mainly studied in seedling growth, will be discussed further regarding the roles of AOS in seed germination.

The possible involvement of AOS in seed-filling processes is less well documented. Hydrogen peroxide is suspected to participate in lignin deposition in the cell walls in a peroxidase-catalysed reaction. In developing barley grains, the involvement of a diamine oxidase in H_2O_2 production has been demonstrated along with lignin deposition in the chalazal cells (Asthir *et al.*, 2002). Apoplast

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lignification, and its subsequent separation from the symplast, ensures that assimilates move into the endosperm via the symplast only (Cochrane *et al*., 2000), which suggests that H_2O_2 might play a role in the control of grain filling. The regulation of CAT gene expression has been studied intensively throughout the development of maize kernels by Scandalios and co-workers (reviewed by Scandalios *et al*., 1997). These authors have shown that a temporal and spatial distribution of CAT isoforms occurs. Among the three genes, *Cat1*, *Cat2* and *Cat3*, which code for the three CAT isoforms in maize, *Cat3* is expressed during very early post-pollination kernel development, whereas *Cat1* and *Cat2* are expressed later (Scandalios *et al*., 1997). The same authors have suggested that differential catalase gene expression during embryo development might be regulated by the phytohormones ABA and auxin (Guan and Scandalios, 1998, 2002). De Gara *et al*. (2003) have followed the changes in detoxifying enzyme activities during maturation of *Triticum durum* kernels. Their results show that seed filling is associated with a high potential of the H_2O_2 detoxification machinery, mainly due to CAT and APX activities.

As mentioned previously, studies dealing with the involvement of AOS in seed development have focused mainly on the acquisition of desiccation tolerance. The ability of developing orthodox seeds to withstand severe desiccation generally appears during the phase of reserve accumulation, approximately midway through development, but it depends on the drying rate, which affects seed survival after drying (Kermode, 1995; Pammenter and Berjak, 1999; Kermode and Finch-Savage, 2002). AOS generation is known to occur during dehydration of various plant tissues (Smirnoff, 1993) and in recalcitrant seeds (Hendry *et al*., 1992); it might result from metabolic imbalances leading to leakage of high-energy intermediates from plastids and mitochondria (Puntarulo *et al*., 1988, 1991; Foyer *et al*., 1994, Vertucci and Farrant, 1995). Therefore, desiccation tolerance might be related, at least in part, to the cellular ability to scavenge these compounds to avoid deleterious AOS-related damage (Hendry *et al*., 1992; Leprince *et al*., 1993; Vertucci and Farrant, 1995). Desiccation damage or tolerance of developing orthodox seeds is largely suspected to be related to oxidative processes. But the possible role of antioxidant systems in desiccation tolerance has been studied more during dehydration of recalcitrant seeds (Pammenter and Berjak, 1999) or during drying of germinated seeds (Leprince *et al*., 1990; Farrant *et al*., 2004) than during seed development *in planta*, probably because the latter studies are not easy to implement. Nevertheless, in bean seeds, acquisition of drying tolerance seems clearly to be associated with a reorientation of the enzymatic antioxidant defence systems. Dried, mature, desiccation-tolerant seeds display high CAT and GR activities and low SOD and APX activities, whereas the reverse is the case in immature, desiccation-intolerant seeds (Bailly *et al*., 2001). The decrease in APX activity during seed desiccation seems to be common to seeds of other species, such as *Vicia faba* (Arrigoni *et al*., 1992) and *Triticum durum* (De Gara *et al*., 2003), as already mentioned by De Tullio and Arrigoni (2003), suggesting that the ascorbate system is probably not involved in desiccation tolerance. Interestingly, it has also been demonstrated that desiccation of developing sunflower seeds is associated with an increase in CAT activity, thus leading to decreased H_2O_2 content and lipid peroxidation damage (Bailly *et al*., 2003, 2004). This study has made it possible to identify the catalase gene as being finely regulated at the transcriptional level by the loss of water, which is in accordance with the data obtained with cotton (Kunce and Trelease, 1986) and maize (Scandalios *et al*., 1997) seeds. Although several other ROS-scavenging enzyme genes, such as those coding for SOD and GR, are up-regulated by dehydration in plants (Ingram and Bartels, 1996; Sherwin and Farrant, 1998), data about their regulation during seed desiccation are less clear than for CAT and APX, and do not permit us to construct a clear picture of their possible roles in this process. For example, GR activity increases at the onset of dehydration tolerance in French bean seeds (Bailly *et al*., 2001), whereas it does not change significantly during desiccation of sunflower seeds (Bailly *et al*., 2003), and decreases in the case of wheat seeds (De Gara *et al*., 2003).

Accumulation of non-enzymatic antioxidant components might also play a role in protecting cells against AOS during desiccation. Indeed, it must be emphasized that the *in vivo* enzyme activities are closely related to the cell water content. At low moisture contents water is tightly bound on to macromolecular structures, thus decreasing molecular mobility and accessibility of enzymes to their substrates. As enzymatic activities are usually measured *in vitro* in aqueous media, they do not necessarily reflect their behaviour *in situ*. This suggests that prevention of oxidative damage at low moisture contents might be more likely related to AOS scavenging by antioxidant compounds. The reduced glutathione/oxidized glutathione (GSH/GSSG) ratio is suspected to be involved in withstanding dehydration tolerance, but it has been little investigated during acquisition of desiccation tolerance of orthodox seeds. Nevertheless, high GSSG contents have been observed in dry seeds of pea (Kranner and Grill, 1993) and tomato (de Vos *et al*., 1994). Peroxiredoxins (Prxs) are thiol-dependent antioxidants capable of reducing H_2O_2 and $OH^{\cdot}$. They accumulate in seeds during maturation drying (reviewed by Aalen, 1999). 1Cys-peroxiredoxin seems

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to be expressed only in those barley seed tissues that survive during desiccation (Stacy *et al*., 1999). Finnie *et al*. (2002) have also shown that the protein 1Cys-peroxiredoxin occurs at the onset of drying in barley ears. Furthermore, Prxs would play a particular role in protecting nuclear integrity, thus preserving genetic information during desiccation (Stacy *et al*., 1999). LEA (late embryogenesis abundant)-related proteins are the proteins most often cited as accumulating during drying. Their presence generally correlates with desiccation tolerance, but their biological functions remain unclear (Buitink *et al*., 2002). Interestingly, it has been demonstrated recently that dehydrins, a group-2 LEA class of proteins, could act as free-radical scavengers (Hara *et al*., 2003). If this role was ubiquitous, it would, without any doubt, strengthen the importance of AOS scavenging in dehydration tolerance mechanisms.

Complementary data on putative roles of AOS in desiccation damage and tolerance are available from studies on recalcitrant seeds, resurrection plants and germinated seeds. They generally provide information similar to those observed with orthodox seeds, i.e. acquisition and loss of desiccation tolerance are closely related to the capacity of cells to scavenge AOS. Loss of viability during drying of recalcitrant seeds of *Quercus robur* (Finch-Savage *et al*., 1994), *Shorea robusta* (Chaitanya and Naithani, 1994) and *Theobroma cocoa* (Li and Sun, 1999) is accompanied by a loss of the cellular antioxidant potential and an accumulation of free radicals. Desiccation and rehydration of the resurrection plant *Xerophyta viscosa* (Sherwin and Farrant, 1998), and germinated maize (Leprince *et al*., 1990) or wheat seeds (Farrant *et al*., 2004), are also associated with changes in the balance of AOS content and detoxifying enzyme activities.

Taken together, these data suggest a critical role of antioxidants in preventing dehydration-related damage and allowing acquisition of desiccation tolerance. They also imply that the ability of cells to withstand loss of water might be closely related to AOS scavenging. However, other protective mechanisms must not be ruled out, and the cell antioxidant machinery must be considered as a part of a wider arsenal of weapons against desiccation stress.

When dealing with involvement of AOS in seed development, one often considers only the 'dark side' of these compounds, i.e. their potentially toxic effects. Nevertheless, they may have a beneficial role in embryo growth, as mentioned previously, and they are increasingly considered as playing a key part in cell signalling. Regarding the variations in AOS contents observed during seed maturation, these could, therefore, be involved in the shift of gene function from a developmental to a germinative mode, which is supposed to be initiated by seed dehydration (Kermode, 1995). Indeed, AOS are known to regulate the expression of many genes. In *Arabidopsis*, for example, H_2O_2 induces 113 genes and represses 62 others (Desikan *et al*., 2001). However, the mechanisms allowing control of gene expression by AOS in plants are still largely unknown. One of the most cited possibilities concerns the activation of transcription factors by redox status changes (Vranova *et al*., 2002; Foyer and Noctor, 2003). Alternatively, gene promoter regions may possess antioxidant response elements (ARE motifs) suspected to play a role in either H_2O_2 or antioxidant sensing, as is the case for the maize catalase *Cat1* gene (Scandalios *et al*., 1997).

Finally, seed development is generally required to allow the embryo to produce a viable and vigorous seed, capable of germinating in a wide range of environmental conditions and permitting species survival. Changes in antioxidant compounds or enzymes during seed development should also be regarded as a prerequisite for obtaining a vigorous seed. We will see below that a vigorous seed has to be endowed with a full antioxidant machinery to avoid oxidative stresses that occur during germination.

Seed germination: a potentially harmful process

Germination *sensu stricto* is associated with many metabolic, cellular and molecular events, rendering the radicle able to emerge from the seed. We shall consider here only this phase of the germination process, which precedes visible signs of radicle extension, keeping in mind that other AOS-generating mechanisms, such as fatty acid oxidation, may occur during early seedling growth. The reactivation of metabolism following seed imbibition may provide an important source of AOS. For example, H_2O_2 is produced at the early imbibition period of soybean (Puntarulo *et al*., 1988, 1991; Gidrol *et al*., 1994), radish (Schopfer *et al*., 2001), maize (Hite *et al*., 1999), sunflower (Bailly *et al*., 2002), wheat (Caliskan and Cumming, 1998) and tomato (Morohashi, 2002) seeds. Accumulation of other AOS, such as NO (Caro and Puntarulo, 1999), hydroxyl radicals (Schopfer *et al*., 2001) and superoxide radicals (Puntarulo *et al*., 1991; Gidrol *et al*., 1994; Schopfer *et al*., 2001) also occurs during germination of seeds of various species. Nevertheless, the exact sites of AOS generation during germination are not known precisely; embryonic axes, seed coats and aleurone layers have been proposed as such putative sites of synthesis.

The production of AOS by germinating seeds has often been regarded as a cause of stress that might affect the success of germination. Therefore, antioxidant compounds and enzymes have been

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widely considered as being of particular importance for the completion of germination. The antioxidant compounds α-tocopherol (Simontacchi *et al.*, 1993, 2003 ; Yang *et al.*, 2001), flavonoids and phenolics (Simontacchi *et al.*, 1993; Andarwulan *et al.*, 1999; Yang *et al.*, 2001) increase during germination. Ascorbate and reduced glutathione, two related antioxidants, also increase during early seed imbibition (Kranner and Grill, 1993; De Gara *et al.*, 1997; Tommasi *et al.*, 2001; Yang *et al.*, 2001, De Tullio and Arrigoni, 2003 and references therein). The two latter compounds might play a wider role than the sole scavenging of AOS, through control of the cellular redox balance (Tommasi *et al.*, 2001) or protein synthesis (Kranner and Grill, 1996). Protection against oxidative stress during imbibition has also been suggested for peroxiredoxins (Aalen, 1999). 1-Cys Prxs are synthesized during rehydration of the desiccation-tolerant moss *Tortula ruralis* (Oliver, 1996) and in germination of buckwheat seeds (Lewis *et al.*, 2000).

The other battery of AOS scavenging mechanisms, i.e. the detoxifying enzymes, also displays important changes during seed imbibition and germination. In germinating sunflower seeds, GR and CAT activities increase prior to radicle protrusion, the latter being concomitant with the elimination of H_2O_2 and the limitation of lipid peroxidation (Bailly *et al.*, 2000, 2002). Similar stimulation of CAT activity and/or expression during germination has also been reported in seeds of maize (Scandalios *et al.*, 1997; Hite *et al.*, 1999, Guan and Scandalios, 2002), soybean (Puntarulo *et al.*, 1991; Gidrol *et al.*, 1994) and *Arabidopsis* (Gallardo *et al.*, 2001). Interestingly, in sunflower seeds, a quite tight relationship between CAT activity and germination rate exists (Bailly *et al.*, 1998). The enhancement of seed germination by priming has also been associated with stimulation of CAT activity in sunflower (Bailly *et al.*, 1998, 2000), soybean (Posmyk *et al.*, 2001) and sweet corn (Chiu *et al.*, 2002), and of CAT expression in *Arabidopsis* (Gallardo *et al.*, 2001). In rice grains, Tanida (1996) has demonstrated that germination rate at a suboptimal temperature is positively correlated with CAT activity. Conversely, slow germination of aged seeds seems to be associated with low CAT activity in sunflower (Bailly *et al.*, 1996, 2002), soybean (Sung, 1996) and maize (Bernal-Lugo *et al.*, 2000). Changes in other detoxifying enzymes during seed imbibition and germination are less well-documented than for CAT, although there is a general trend for stimulation of the activities of these enzymes. This is the case for SOD (Puntarulo *et al.*, 1991; Gidrol *et al.*, 1994), APX (De Tullio and Arrigoni, 2003) and GR (Tommasi *et al.*, 2001).

Comparative analysis of changes in antioxidant enzymes or compounds and in AOS during germination, therefore, brings together several lines of evidence supporting a role for AOS scavenging in seed germination. In many cases radicle protrusion occurs at the time when AOS content reaches a steady-state level, as illustrated in Fig. 2. In this regard, AOS production, resulting from tissue rehydration, appears to be a negative event that has to be counteracted. Even though this aspect is plausible and is quite well documented in the literature, AOS production during germination should also be regarded from a different point of view. In light of the increasing progress made in the understanding of cellular mechanisms driven by AOS, the role of AOS in seed germination perhaps needs to be revisited. To date, at least four putative distinct roles for AOS, apart from their toxic effects, have been identified.

As mentioned previously, AOS, and particularly H_2O_2, may induce expression of many genes, including those coding for defence-related proteins, transcription factors, phosphatases, kinases and enzymes involved in AOS synthesis or degradation (Desikan *et al.*, 2001; Neill *et al.*, 2002). Additionally,

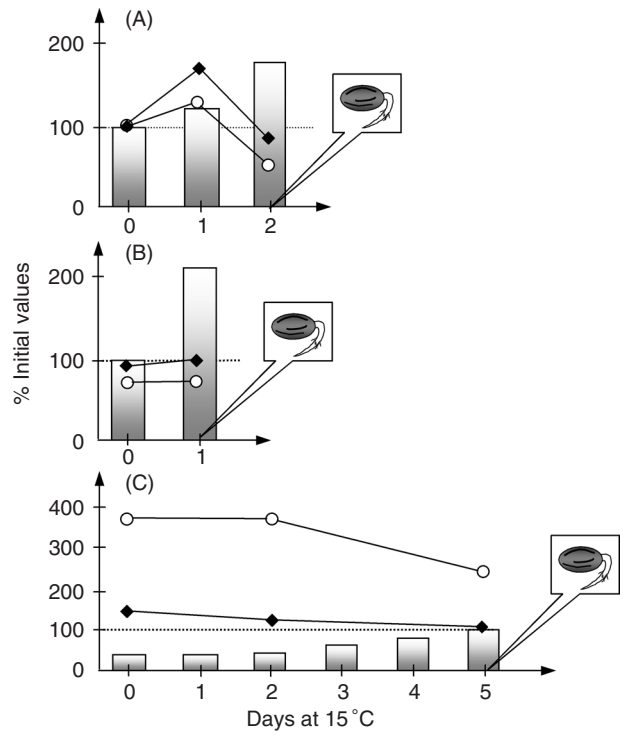

Figure 2. Changes in catalase (CAT) activity (bars) and in hydrogen peroxide (H_2O_2) (◆) and malondialdehyde (○) contents in sunflower seeds incubated at 15°C. (A) Control unprimed and unaged seeds; (B) seeds primed for 7 d; (C) seeds aged (accelerated ageing) for 5 d. Results are expressed as percentage of the values obtained with the dry control seeds. The graphs show that there exists a clear-cut relationship between the time to radicle protrusion (indicated by the schemes on the graphs) and the balance H_2O_2 content/CAT activities. (Data from Bailly *et al.*, 1998, 2001, 2002.)

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AOS may also regulate genes through changes in cellular redox status (Foyer and Noctor, 2003).

AOS might also intervene in the cell-wall modification required for elongation of the radicle, the first sign of the completion of germination. Hydroxyl radicals, produced from $O_2^{-\cdot}$ and H_2O_2 by cell-wall peroxidases *in vivo* (Chen and Schopfer, 1999), can lead to cell-wall loosening processes underlying cell expansion (Schweikert *et al*., 2000, 2002). Hydroxyl radicals may break down polysaccharides by an oxidative scission of backbone bonds (Schweikert *et al*., 2000, 2002), a process that could be involved in radicle protrusion. In tomato seeds, for example, radicle protrusion requires weakening of the micropylar region of the endosperm (Bradford *et al*., 2000). Several hydrolases (mannanase, cellulase, glucanase) have been suspected to contribute to cell-wall loosening (Bradford *et al*., 2000). However, a peroxidase activity develops in the tomato endosperm cap prior to radicle emergence (Morohashi, 2002). This activity, which generates $OH^{\cdot}$, might be involved in the cell-wall loosening processes, allowing cell expansion to occur. Recently, Schopfer *et al.* (2002) have also suggested that auxin might promote cell growth through $O_2^{-\cdot}$ production and the subsequent generation of hydroxyl radicals.

It has also been supposed that production of AOS and their release in the surrounding medium during seed imbibition play a part in protecting the embryo against pathogens (Schopfer *et al*., 2001). Many studies indicate that AOS produced during the early phase of pathogen attacks trigger pathogen-resistance responses, acquired systemic resistance and programmed cell death (Lamb and Dixon, 1997; Grant and Loak, 2000). The possible involvement of AOS in protecting the growing embryo against a hazardous environment constitutes a seductive hypothesis that needs to be addressed properly.

Finally, AOS are also suspected to be involved in programmed cell death (PCD) in the aleurone layer of cereal grains. PCD occurs during germination, after the aleurone cells have synthesized and secreted hydrolytic enzymes into the endosperm for mobilizing stored reserves, and is under the control of gibberellins and ABA (Fath *et al*., 2002). In barley aleurone layers, PCD would result from a down-regulation of the antioxidant enzymes, thus leading to overproduction of AOS and membrane rupture (Fath *et al*., 2001).

AOS and the inability to germinate: seed dormancy and ageing

The inability of seeds to germinate in apparently favourable environmental conditions is referred to as dormancy (Bewley and Black, 1994). Dormancy can either result from an inhibitory action of the covering structures or reside within the embryo itself. In some cases, seed-coat-imposed dormancy can be alleviated with oxidants such as H_2O_2, which can oxidize the phenolic compounds present in the seed envelopes, and may allow improved oxygenation of the embryo during seed imbibition (Fontaine *et al*., 1994; Ogawa and Iwabushi, 2001). It can also cause cracking in the coat of hard seeds, thus facilitating their imbibition (Chien and Lin, 1994). More interesting are the roles that endogenous AOS and antioxidants might play in regulating seed dormancy, but these roles are poorly documented up to now. Nevertheless, several lines of evidence suggest that H_2O_2 alleviates seed dormancy: it stimulates the germination of dormant seeds of barley (Fontaine *et al*., 1994; Wang *et al*., 1995, 1998), rice (Naredo *et al*., 1998), apple (Bogatek *et al*., 2003) and *Zinnia elegans* (Ogawa and Iwabuchi, 2001). Hendricks and Taylorson (1975) also showed that chemicals that inhibit *in vitro* catalase activity promoted the germination of dormant seeds of lettuce and pigweed. However, the cellular basis of these effects remains unclear. It has been postulated that H_2O_2 causes an activation of the oxidative pentose phosphate pathway, owing to the oxidation of reduced NADPH (Hendricks and Taylorson, 1975; Fontaine *et al*., 1994). One attractive alternative hypothesis regarding the involvement of H_2O_2 in seed dormancy release concerns its effect on ABA content. Wang *et al.* (1995, 1998) have demonstrated that treatment of dormant barley seeds with H_2O_2 results in a decrease in endogenous ABA level, and Bogatek *et al*. (2003) have shown that alleviation of apple embryo dormancy by cyanide induces a decrease in ABA content occurring concomitantly with an increase in H_2O_2. The control of seed dormancy by ABA might, therefore, be connected with H_2O_2 signalling. This interplay has to be explored fully in future studies.

Increasing attention has also been paid to the putative role of peroxiredoxins in seed dormancy. In *Arabidopsis* and barley seeds, 1-Cys Prxs expression seems to be related to dormancy, since expression of the transcript decreases during germination of non-dormant seeds, whereas it remains unchanged in non-germinating dormant seeds (Haslekas *et al*., 1998; Stacy *et al*., 1999). However, there is no clear-cut relationship between Prxs levels and the intensity of dormancy, as demonstrated in tobacco and *Arabidopsis* seeds (Lee *et al*., 2000; Haslekas *et al*., 2003). It has been suggested recently that Prxs are more likely to prevent germination under unfavourable germination conditions (Haslekas *et al*., 2003).

Seed germinability and vigour may also be affected dramatically by ageing that occurs during

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prolonged storage. Accumulation of AOS and free radicals has often been considered as one of the most important factors of seed ageing. The probable involvement of AOS in seed ageing has been reviewed by many authors (see, for example, Priestley 1986; Hendry, 1993; McDonald, 1999) and will be dealt with only briefly here. When considering the possible involvement of oxidative processes in seed ageing, it seems prudent to differentiate two stages, as already suggested by Smith and Berjak (1995). First, orthodox seeds usually have a low moisture content during storage, but, nevertheless, this might support auto-oxidation reactions leading to free-radical production (McDonald, 1999). In such conditions, *in vivo* detoxifying enzyme activities are almost absent and, therefore, unable to remove AOS, which may either have a direct deleterious effect on cellular components (lipids, enzymatic and structure proteins, nucleic acids) or be trapped in the intracellular structures (Smirnoff, 1993). Moreover, many studies have demonstrated that seed ageing is associated with a loss of antioxidant enzyme activity (Pukacka, 1991; De Vos *et al.*, 1994; Bailly *et al.*, 1996; De Paula *et al.*, 1996; Sung, 1996; Bernal-Lugo *et al.*, 2000). Prolonged dry storage or inappropriate conditions of conservation (high temperature and relative humidity) magnify such processes. Secondly, imbibition and germination of previously stored seeds should be considered as the critical steps of the oxidative phenomena related to ageing, since it is at these times that the cellular dysfunctions resulting from AOS accumulation are expressed. Imbibition results in the release of free-radical forms trapped during storage and in the production of new AOS by resumed metabolism. Cells then have to deal with an oxidative stress, the intensity of which depends on the conditions of storage. The delay in germination of aged, but still viable, seeds might correspond to the time necessary for the cells to re-initiate the antioxidant machinery and then escape from an oxidative stress, as shown previously by Bailly *et al.* (1998, 2002). Figure 3 illustrates a putative two-step model for oxidative stress-related seed ageing.

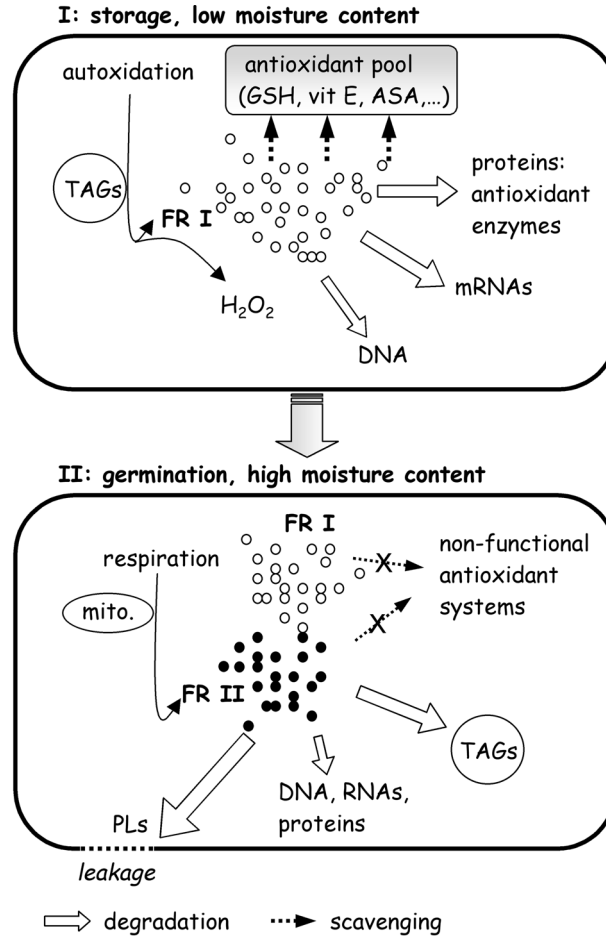

Figure 3. Proposed involvement of oxidative mechanisms in seed ageing. (I) During prolonged seed storage, lipid autoxidation generates free radicals (primary free radicals: FR I) resulting in degradation of triacylglycerols (TAGs), mRNAs, DNA and proteins. During this period, FR I may also damage antioxidant enzymes. (II) During germination, the resumption of metabolism leads to the production of new active oxygen species (secondary free radicals: FR II), mainly via respiratory activity within mitochondria (mito.), in addition to the already present primary free radicals (FR I). Inefficiency of the enzymatic antioxidant machinery, altered during prolonged storage, leads to the accumulation of active oxygen species and to new damage against TAGs, membrane phospholipids (PLs) and other macromolecules. (Schemes established from the results obtained by Bailly *et al.*, 1996, 1998, 2002).

Towards the understanding of AOS mechanisms of action: AOS as a part of an integrated network of cell signalling

Future studies of the possible involvement of AOS in seed physiology will have to consider that these compounds are not only active in themselves, but that cross-talk exists between them and cellular signalling pathways. AOS and signalling molecules, such as plant hormones or calcium, work as an interconnected network, and AOS may interfere both upstream and downstream of the signal. This is particularly relevant for the effects of hormones that play a key role in various stages of seed life. One of the best-documented examples of such interplay is the interaction between AOS and ABA occurring in various physiological situations. Thus, ABA-induced stomatal closure requires H_2O_2 production and AOS production is the rate-limiting step for the ABA signal transduction (Pei *et al.*, 2000; Zhang *et al.*, 2001;

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Kwak *et al*., 2003). Recently, Jiang and Zhang (2002a, b) have shown that ABA accumulation during water stress triggers AOS production through the stimulation of NADPH oxidase activity, and subsequently activates the antioxidative defence system. Guan *et al.* (2000) have also suggested that H_2O_2 plays a role in the ABA signal transduction pathway during osmotic stress in maize seedlings, probably owing to the activation of the catalase gene *Cat1* that contains an antioxidant-responsive element (ARE) in its promoter region (Guan and Scandalios, 1998). The same authors have also proposed an alternative mode of catalase regulation by ABA through an ABA-responsive element (ABRE) located in the promoter of *Cat1*. In *Arabidopsis*, the involvement of H_2O_2 in ABA signalling has been related to its effect on ABI2, a protein phosphatase that acts as a negative regulator of the ABA response and contributes to the control of seed dormancy (Meinhard *et al*., 2002). Numerous interactions of AOS with other ubiquitous signalling components, such as jasmonic acid, salicylic acid, nitric oxide or ethylene, have been demonstrated (Vranova *et al*., 2002). Among these signalling molecules, ethylene is of a particular interest, since it is involved in the germination and dormancy of various seeds (Corbineau and Côme, 1995). Hydrogen peroxide may act upstream of ethylene-related plant responses by activating 1-aminocyclopropane 1-carboxylic acid oxidase, thus enhancing ethylene production (Chamnongpol *et al*., 1998). Conversely, ethylene might also stimulate AOS production (de Jong *et al*., 2002; Overmyer *et al*., 2003).

Downstream signalling of AOS, particularly that of H_2O_2, is poorly understood, but it should command special attention from seed scientists. Since H_2O_2 has a relatively simple chemical structure, it is highly improbable that its transduction involves specific receptors (Neill *et al*., 2002). Hydrogen peroxide sensing in cells might, therefore, be mediated by interactions with 'redox-sensitive' proteins, such as peroxiredoxins, thioredoxins or ABI1 and ABI2 protein phosphatases, the conformation of which, or interactions with other proteins (and thus functionality), might vary in the presence of H_2O_2 (Vranova *et al*., 2002). In a broader way, this addresses, once again, the problem of redox status in seed physiology. Kinases or MAP kinase cascades are also supposed to be involved in the control of gene expression by H_2O_2 (Kovtun *et al*., 2000; Mittler, 2002; Vranova *et al*., 2002), as well as calcium fluxes and associated mechanisms (calmodulin-dependent kinases and calcium channels, in particular) (Bowler and Fluhr, 2000). Finally, transcription factors intervening in redox regulation might be involved in downstream H_2O_2 responses. Several transcription factors affected by redox signals have been identified; this is the case for the nuclear factor NF-κB in mammals (Vlamis-Gardikas and Holmgren, 2002) and Yap-1, which relocalizes from cytoplasm to nucleus upon oxidative stress in yeast (Delaunay *et al*., 2000). Similar factors remain to be formally identified in plants. However, the transcription factors WRKY (Arrigo, 1999), DREB2A (Liu *et al*., 1998) and EREBP (Riechmann and Meyerowitz, 1998) have been proposed to play such a role. Also, Desikan *et al.* (2001) have shown that heat-shock transcription factors, ethylene-responsive transcription factors, *myb*-related transcription factors and zinc-finger transcription factors, among others, are up-regulated by H_2O_2. A better knowledge of the promoter regions of the genes induced by H_2O_2 would lead to a better understanding of the transduction pathway of H_2O_2.

Concluding remarks

As illustrated by the studies reviewed here, AOS and antioxidants probably play a wider role in seed physiology than is currently appreciated. AOS may be involved in all the stages of seed life, from development to germination, but the general picture of their action is certainly very complex, because they must be considered as part of a signalling network involving numerous regulatory components. Many questions related to the roles of AOS in seed physiology have to be addressed. Progress is required in determining their cellular production sites and their diffusion within the cell, taking into account the unique aspects of seed tissue physiology, in particular the dramatic changes in moisture content and metabolic activity that occur in the life of the seed. Elucidating the mechanisms underlying the interplay of AOS with hormones is also a challenge for future research in this area. Such investigations will, without any doubt, encourage revisiting the cellular mechanisms involved in acquisition of the desiccation tolerance, germination and alleviation of dormancy. Analyses of gene expression in contrasting situations, using the novel methods developed in recent years, such as microarrays, cDNA amplification fragment length polymorphism (cDNA-AFLP) and proteomic tools, will be of help in answering some of these questions.

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References

Aalen, R.B. (1999) Peroxiredoxin antioxidants in seed physiology. *Seed Science Research* **9**, 285–295.

Allan, A.C. and Fluhr, R. (1997) Two distinct sources of elicited reactive oxygen species in tobacco epidermal cells. *Plant Cell* **9**, 1559–1572.

Amor, Y., Chevion, M. and Levine, A. (2000) Anoxia pretreatment protects soybean cells against H_2O_2-induced cell death: possible involvement of peroxidases and of alternative oxidase. *FEBS Letters* **477**, 175–180.

Andarwulan, N., Fardiaz, D., Wattimena, G.A. and Shetty, K. (1999) Antioxidant activity associated with lipid and phenolic mobilization during seed germination of *Pangium edule* Reinw. *Journal of Agricultural and Food Chemistry* **47**, 3158–3163.

Arrigo, A.P. (1999) Gene expression and the thiol redox state. *Free Radical Biology and Medicine* **27**, 936–944.

Arrigoni, O., De Gara, L., Tommasi, F. and Liso, R. (1992) Changes in the ascorbate system during seed development of *Vicia faba* L. *Plant Physiology* **99**, 235–238.

Asthir, B., Duffus, C.M., Smith, R.C. and Spoor, W. (2002) Diamine oxidase is involved in H_2O_2 production in the chalazal cells during barley grain filling. *Journal of Experimental Botany* **53**, 677–682.

Bagnoli, F., Capuana, M. and Racchi, M.L. (1998) Developmental changes of catalase and superoxide dismutase isoenzymes in zygotic and somatic embryos of horse chestnut. *Australian Journal of Plant Physiology* **25**, 909–913.

Bailly, C., Benamar, A., Corbineau, F. and Côme, D. (1996) Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated aging. *Physiologia Plantarum* **97**, 104–110.

Bailly, C., Benamar, A., Corbineau, F. and Côme, D. (1998) Free radical scavenging as affected by accelerated ageing and subsequent priming in sunflower seeds. *Physiologia Plantarum* **104**, 646–652.

Bailly, C., Benamar, A., Corbineau, F. and Côme, D. (2000) Antioxidant systems in sunflower (*Helianthus annuus* L.) seeds as affected by priming. *Seed Science Research* **10**, 35–42.

Bailly, C., Audigier, A., Ladonne, F., Wagner, M.H., Coste, F., Corbineau, F. and Côme, D. (2001) Changes in oligosaccharide content and antioxidant enzyme activities in developing bean seeds as related to acquisition of drying tolerance and seed quality. *Journal of Experimental Botany* **52**, 701–708.

Bailly, C., Bogatek-Leszczynska, R., Côme, D. and Corbineau, F. (2002) Changes in activities of antioxidant enzymes and lipoxygenase during growth of sunflower seedlings from seeds of different vigour. *Seed Science Research* **12**, 47–55.

Bailly, C., Leymarie, J., Rousseau, S., Côme, D., Feutry, A. and Corbineau, F. (2003) Sunflower seed development as related to antioxidant enzyme activities. pp. 69–75 *in* Nicolas, G.; Bradford, K.J.; Côme, D.; Pritchard, H.W. (Eds) *The biology of seeds: Recent research advances*. Wallingford, CABI Publishing.

Bailly, C., Leymarie, J., Lehner, A., Rousseau, S., Côme, D. and Corbineau, F. (2004) Catalase activity and expression in developing sunflower seeds as related to drying. *Journal of Experimental Botany* **55**, 475–483.

Beckman, K.B. and Ames, B.N. (1997) Oxidants, antioxidants, and aging. pp. 201–246 *in* Scandalios, J.G. (Ed.) *Oxidative stress and the molecular biology of antioxidant defenses*. New York, Cold Spring Harbor Laboratory Press.

Beevers, H. (1979) Microbodies in higher plants. *Annual Review of Plant Physiology* **30**, 159–193.

Bernal-Lugo, I., Camacho, A. and Carballo, A. (2000) Effects of seed ageing on the enzymic antioxidant system of maize cultivars. pp. 151–160 *in* Black, M.; Bradford, K.J.; Vazquez-Ramos, J. (Eds) *Seed biology: Advances and applications*. Wallingford, CABI Publishing.

Bewley, J.D. and Black, M. (1994) *Seeds. Physiology of development and germination* (2nd edition). New York, Plenum Press.

Bogatek, R., Gawrońska, H. and Oracz, K. (2003) Involvement of oxidative stress and ABA in CN-mediated elimination of embryonic dormancy in apple. pp. 211–216 *in* Nicolas, G.; Bradford, K.J.; Côme, D.; Pritchard, H.W. (Eds) *The biology of seeds: Recent research advances*. Wallingford, CABI Publishing.

Bolwell, G.P. and Wojtaszek, P. (1997) Mechanisms for the generation of reactive oxygen species in plant defence – a broad perspective. *Physiological and Molecular Plant Pathology* **51**, 347–366.

Bolwell, G.P., Bindschedler, L.V., Blee, K.A., Butt, V.S., Davies, D.R., Gardner, S.L., Gerrish, C. and Minibayeva, F. (2002) The apoplastic oxidative burst in response to biotic stress in plants: a three-component system. *Journal of Experimental Botany* **53**, 1367–1376.

Bowler, C. and Fluhr, R. (2000) The role of calcium and activated oxygens as signals for controlling cross-tolerance. *Trends in Plant Science* **5**, 241–246.

Bowler, C., Van Montagu, M. and Inzé, D. (1992) Superoxide dismutase and stress tolerance. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**, 83–116.

Bradford, K.J., Chen, F., Cooley, M.B., Dahal, P., Downie, B., Fukunaga, K.K., Gee, O.H., Gurusinghe, S., Mella, R.A., Nonogaki, H., Wu, C.T., Yang, H. and Yim, K.O. (2000) Gene expression prior to radicle emergence in imbibed tomato seeds. pp. 231–251 *in* Black, M.; Bradford, K.J.; Vazquez-Ramos, J. (Eds) *Seed biology: Advances and applications*. Wallingford, CABI Publishing.

Breen, A.P. and Murphy, J.A. (1995) Reactions of oxyl radicals with DNA. *Free Radical Biology and Medicine* **18**, 1033–1077.

Buitink, J., Hoekstra, F.A. and Leprince, O. (2002) Biochemistry and biophysics of tolerance systems. pp. 293–318 *in* Black, M.; Pritchard, H.W. (Eds) *Desiccation and survival in plants*: *Drying without dying*. Wallingford, CABI Publishing.

Caliskan, M. and Cuming, A.C. (1998) Spatial specificity of H_2O_2-generating oxalate oxidase gene expression during wheat embryo germination. *Plant Journal* **15**, 165–171.

Caro, A. and Puntarulo, S. (1999) Nitric oxide generation by soybean embryonic axes. Possible effect on mitochondrial function. *Free Radical Research* **31**, S205–S212.

Chaitanya, K.S.K. and Naithani, S.C. (1994) Role of superoxide, lipid peroxidation and superoxide dismutase

https://doi.org/10.1079/SSR2004159 Published online by Cambridge University Press

in membrane perturbation during loss of viability in seeds of *Shorea robusta* Gaerth. *New Phytologist* **126**, 623–627.

Chamnongpol, S., Willekens, H., Moeder, W., Langebartels, C., Sandermann, H., Van Montagu, M., Inzé, D. and Van Camp, W. (1998) Defense activation and enhanced pathogen tolerance induced by H_2O_2 in transgenic tobacco. *Proceedings of the National Academy of Sciences, USA* **95**, 5818–5823.

Chance, B., Boveris, A., Oshino, N. and Loschen, G. (1973) The nature of catalase intermediate and its biological function. pp. 350–353 *in* King, T.E.; Mason, H.S.; Morrison, M. (Eds) *Oxidases and related redox systems*. Baltimore, University Park Press.

Charles, S.A. and Halliwell, B. (1980) Effect of hydrogen peroxide on spinach (*Spinacia oleracea*) chloroplast fructose bisphosphatase. *Biochemical Journal* **189**, 373–376.

Chen, S.X. and Schopfer, P. (1999) Hydroxyl-radical production in physiological reactions: a novel function of peroxidase. *European Journal of Biochemistry* **260**, 726–735.

Chien, C.T. and Lin, T.P. (1994) Mechanism of hydrogen peroxide in improving the germination of *Cinnamonum camphora* seed. *Seed Science and Technology* **22**, 231–236.

Chiu, K.Y., Chen, C.L. and Sung, J.M. (2002) Effect of priming temperature on storability of primed sh-2 sweet corn seed. *Crop Science* **42**, 1996–2003.

Cochrane, M.P., Paterson, L. and Gould, E. (2000) Changes in chalazal cell walls and in the peroxidase enzymes of the crease region during grain development in barley. *Journal of Experimental Botany* **51**, 507–520.

Corbineau, F. and Côme, D. (1995) Control of seed germination and dormancy by the gaseous environment. pp. 397–427 *in* Kigel, J.; Galili, G. (Eds) *Seed development and germination*. New York, Marcel Dekker.

Corpas, F.J., Barroso, J.B. and del Rio, L.A. (2001) Peroxisomes as a source of reactive oxygen species and nitric oxide signal molecules in plant cells. *Trends in Plant Science* **6**, 145–150.

Cui, K., Xing, G., Liu, X., Xing, G. and Wang, Y. (1999) Effect of hydrogen peroxide on somatic embryogenesis of *Lycium barbarum* L. *Plant Science* **146**, 9–16.

De Gara, L., de Pinto, M.C. and Arrigoni, O. (1997) Ascorbate synthesis and ascorbate peroxidase activity during the early stage of wheat germination. *Physiologia Plantarum* **100**, 894–900.

De Gara, L., de Pinto, M.C., Moliterni, V.M.C. and D'Egidio, M.G. (2003) Redox regulation and storage processes during maturation in kernels of *Triticum durum*. *Journal of Experimental Botany* **54**, 249–258.

de Jong, A.J., Yakimova, E.T., Kapchina, V.M. and Woltering, E.J. (2002) A critical role for ethylene in hydrogen peroxide release during programmed cell death in tomato suspension cells. *Planta* **214**, 537–545.

Delaunay, A., Isnard, A.D. and Toledano, M.B. (2000) H_2O_2 sensing through oxidation of the Yap1 transcription factor. *EMBO Journal* **19**, 5157–5166.

del Rio, L.A., Pastori, G.M., Palma, J.M., Sandalio, L.M., Sevilla, F., Corpas, F.J., Jimenez, A., Lopez-Huertas, E. and Hernandez, J.A. (1998) The activated oxygen role of peroxisomes in senescence. *Plant Physiology* **116**, 1195–1200.

De Paula, M., Pérez-Otaola, M., Darder, M., Torres, M., Frutos, G. and Martinez-Honduvilla, C.J. (1996) Function of the ascorbate–glutathione cycle in aged sunflower seeds. *Physiologia Plantarum* **96**, 543–550.

De Tullio, M.C. and Arrigoni, O. (2003) The ascorbic acid system in seeds: to protect and to serve. *Seed Science Research* **13**, 249–260.

De Vos, C.H.R., Kraak, H.L. and Bino, R.J. (1994) Aging of tomato seeds involves glutathione oxidation. *Physiologia Plantarum* **92**, 131–139.

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

Desikan, R., Clarke, A., Hancock, J.T. and Neill, S.J. (1999) H_2O_2 activates a MAP kinase-like enzyme in *Arabidopsis thaliana* suspension cultures. *Journal of Experimental Botany* **50**, 1863–1866.

Desikan, R., Mackerness, S.A.H., Hancock, J.T. and Neill, S.J. (2001) Regulation of the *Arabidopsis* transcriptome by oxidative stress. *Plant Physiology* **127**, 159–172.

Doke, N., Miura, Y., Sanchez, L.M. and Kawakita, K. (1994) Involvement of superoxide in signal transduction: responses to attack by pathogens, physical and chemical shocks and UV irradiation. pp. 177–218 *in* Foyer, C.H.; Mullineaux, P. (Eds) *Causes of photooxidative stress and amelioration of defense systems in plants*. Boca Raton, CRC Press.

Durner, J. and Klessig, D.F. (1999) Nitric oxide as a signal in plants. *Current Opinion in Plant Biology* **2**, 369–374.

Eshdat, Y., Holland, D., Faltin, Z. and Ben-Hayyim, G. (1997) Plant glutathione peroxidases. *Physiologia Plantarum* **100**, 234–240.

Farrant, J.M., Bailly, C., Leymarie, J., Hamman, B., Côme, D. and Corbineau, F. (2004) Wheat seedlings as a model to understand desiccation-tolerance and -sensitivity. *Physiologia Plantarum* **120**, 563–574.

Fath, A., Bethke, P.C. and Jones, R.L. (2001) Enzymes that scavenge reactive oxygen species are down-regulated prior to gibberellic acid-induced programmed cell death in barley aleurone. *Plant Physiology* **126**, 156–166.

Fath, A., Bethke, P., Beligni, V. and Jones, R. (2002) Active oxygen and cell death in cereal aleurone cells. *Journal of Experimental Botany* **53**, 1273–1282.

Finch-Savage, W.E., Hendry, G.A.F. and Atherton, N.M. (1994) Free radical activity and loss of viability during drying of desiccation sensitive tree seeds. *Proceedings of the Royal Society of Edinburgh* **102B**, 257–260.

Finnie, C., Melchior, S., Roepstorff, P. and Svensson, B. (2002) Proteome analysis of grain filling and seed maturation in barley. *Plant Physiology* **129**, 1308–1319.

Fontaine, O., Huault, C., Pavis, N. and Billard, J.P. (1994) Dormancy breakage of *Hordeum vulgare* seeds: effects of hydrogen peroxide and scarification on glutathione level and glutathione reductase activity. *Plant Physiology and Biochemistry* **32**, 677–683.

Foyer, C.H. and Noctor, G. (2003) Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiologia Plantarum* **119**, 355–364.

Foyer, C.H., Lelandais, M. and Kunert, K.J. (1994) Photooxidative stress in plants. *Physiologia Plantarum* **92**, 696–717.

Fry, S.C. (1998) Oxidative scission of plant cell wall polysaccharides by ascorbate-induced hydroxyl radicals. *Biochemical Journal* **332**, 507–515.

Gallardo, K., Job, C., Groot, S.P.C., Puype, M., Demol, H., Vandekerckhove, J. and Job, D. (2001) Proteomic analysis of Arabidopsis seed germination and priming. *Plant Physiology* **126,** 835–848.

Gidrol, X., Lin, W.S., Degousee, N., Yip, S.F. and Kush, A. (1994) Accumulation of reactive oxygen species and oxidation of cytokinin in germinating soybean seeds. *European Journal of Biochemistry* **224**, 21–28.

Grant, J.J. and Loake, G.J. (2000) Role of reactive oxygen intermediates and cognate redox signaling in disease resistance. *Plant Physiology* **124**, 21–30.

Guan, L.M. and Scandalios, J.G. (1998) Effects of the plant growth regulator abscisic acid and high osmoticum on the developmental expression of the maize catalase genes. *Physiologia Plantarum* **104**, 413–422.

Guan, L.M. and Scandalios, J.G. (2002) Catalase gene expression in response to auxin-mediated developmental signals. *Physiologia Plantarum* **114**, 288–295.

Guan, L.M., Zhao, J. and Scandalios, J.G. (2000) *Cis*-elements and *trans*-factors that regulate expression of the maize *Cat1* antioxidant gene in response to ABA and osmotic stress: H_2O_2 is the likely intermediary signaling molecule for the response. *Plant Journal* **22**, 87–95.

Halliwell, B. and Gutteridge, J.M.C. (1999*) Free radicals in biology and medicine* (3rd edition). New York, Oxford University Press.

Hara, M., Terashima, S., Fukaya, T. and Kuboi, T. (2003) Enhancement of cold tolerance and inhibition of lipid peroxidation by citrus dehydrin in transgenic tobacco. *Planta* **217**, 290–298.

Haslekas, C., Stacy, R.A.P., Nygaard, V., Culianez-Macia, F.A. and Aalen, R.B. (1998) The expression of a peroxiredoxin antioxidant gene, AtPer1, in *Arabidopsis thaliana* is seed-specific and related to dormancy. *Plant Molecular Biology* **36**, 833–845.

Haslekas, C., Viken, M.K., Grini, P.E., Nygaard, V., Nordgard, S.H., Meza, T.J. and Aalen, R.B. (2003) Seed 1-cysteine peroxiredoxin antioxidants are not involved in dormancy, but contribute to inhibition of germination during stress. *Plant Physiology* **133**, 1148–1157.

Hendricks, S.B. and Taylorson, R.B. (1975) Breaking of seed dormancy by catalase inhibition. *Proceedings of the National Academy of Sciences, USA* **72**, 306–309.

Hendry, G.A.F. (1993) Oxygen, free radical processes and seed longevity. *Seed Science Research* **3**, 141–153.

Hendry, G.A.F., Finch-Savage, W.E., Thorpe, P.C., Atherton, N.M., Buckland, S.M., Nilsson, K.A. and Seel, W.E. (1992) Free radical processes and loss of seed viability during desiccation in the recalcitrant species *Quercus robur* L. *New Phytologist* **122**, 273–279.

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

Hite, D.R.C., Auh, C. and Scandalios, J.G. (1999) Catalase activity and hydrogen peroxide levels are inversely correlated in maize scutella during seed germination. *Redox Reports* **4**, 29–34.

Huang, A.H.C., Trelease, R.N. and Moore, T.S. (1983) *Plant peroxisomes*. London, Academic Press.

Ingram, J. and Bartels, D. (1996) The molecular basis of dehydration tolerance in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **47**, 377–403.

Jabs, T. (1999) Reactive oxygen intermediates as mediators of programmed cell death in plants and animals. *Biochemical Pharmacology* **57**, 231–245.

Jabs, T., Dietrich, R.A. and Dangl, J.L. (1996) Initiation of runaway cell death in an *Arabidopsis* mutant by extracellular superoxide. *Science* **27**, 1853–1856.

Jiang, M. and Zhang, J. (2002a) Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. *Journal of Experimental Botany* **53**, 2401–2410.

Jiang, M. and Zhang, J. (2002b) Involvement of plasma-membrane NADPH oxidase in abscisic acid- and water stress-induced antioxidant defense in leaves of maize seedlings. *Planta* **215**, 1022–1030.

Joo, J.H., Bae, Y.S. and Lee, J.S. (2001) Role of auxin-induced reactive oxygen species in root gravitropism. *Plant Physiology* **126**, 1055–1060.

Kato, N. and Esaka, M. (1999) Changes in ascorbate oxidase gene expression and ascorbate levels in cell division and cell elongation in tobacco cells. *Physiologia Plantarum* **105**, 321–329.

Kermode, A.R. (1995) Regulatory mechanisms in the transition from seed development to germination: interactions between the embryo and the seed environment. pp. 273–332 *in* Kigel, J.; Galili, G. (Eds) *Seed development and germination*. New York, Marcel Dekker.

Kermode, A.R. and Finch-Savage, B.E. (2002) Desiccation sensitivity in orthodox and recalcitrant seeds in relation to development. pp. 149–184 *in* Black, M.; Pritchard, H.W. (Eds) *Desiccation and survival in plants*: *Drying without dying*. Wallingford, CABI Publishing.

Kovtun, Y., Chiu, W.L., Tena, G. and 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. and Grill, D. (1993) Content of low-molecular-weight thiols during the imbibition of pea seeds. *Physiologia Plantarum* **88**, 557–562.

Kranner, I. and Grill, D. (1996) Significance of thiol-disulfide exchange in resting stages of plant development. *Botanica Acta* **109**, 8–14.

Kunce, C.M. and Trelease, R.N. (1986) Heterogeneity of catalase in maturing and germinated cotton seeds. *Plant Physiology* **81**, 1134–1139.

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

Lamb, C. and Dixon, R.A. (1997) The oxidative burst in plant disease resistance. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**, 251–275.

Lee, K.O., Jang, H.H., Jung, B.G., Chi, Y.H., Lee, J.Y., Choi, Y.O., Lee, J.R., Lim, C.O., Cho, M.J. and Lee, S.Y. (2000) Rice 1Cys-peroxiredoxin over-expressed in transgenic

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tobacco does not maintain dormancy but enhances antioxidant activity. *FEBS Letters* **486**, 103–106.

Leprince, O., Deltour, R., Thorpe, P.C., Atherton, N.M. and Hendry, G.A.F. (1990) The role of free radicals and radical processing systems in loss of desiccation tolerance in germinating maize (*Zea mays* L.). *New Phytologist* **116**, 573–580.

Leprince, O., Hendry, G.A.F. and McKersie, B.D. (1993) The mechanisms of desiccation tolerance in developing seeds. *Seed Science Research* **3**, 231–246.

Levine, A., Tenkanen, R., Dixon, R. and Lamb, C. (1994) H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell* **79**, 583–593.

Lewis, M.L., Miki, K. and Ueda, T. (2000) *FePer* 1, a gene encoding an evolutionarily conserved 1-Cys peroxiredoxin in buckwheat (*Fagopyrum esculentum* Moench), is expressed in a seed-specific manner and induced during seed germination. *Gene* **246**, 81–91.

Li, C. and Sun, W.Q. (1999) Desiccation sensitivity and activities of free radical-scavenging enzymes in recalcitrant *Theobroma cacao* seeds. *Seed Science Research* **9**, 209–217.

Liszkay, A., Kenk, B. and Schopfer, P. (2003) Evidence for the involvement of cell wall peroxidase in the generation of hydroxyl radicals mediating extension growth. *Planta* **217**, 658–667.

Liu, Q., Kasuga, M., Sakuma, Y., Abe, H., Miura, S., Yamaguchi-Shinozaki, K. and Shinozaki, K. (1998) Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. *Plant Cell* **10**, 1391–1406.

McDonald, M.B. (1999) Seed deterioration: physiology, repair and assessment. *Seed Science and Technology* **27**, 177–237.

Meinhard, M., Rodriguez, P.L. and Grill, E. (2002) The sensitivity of ABI2 to hydrogen peroxide links the abscisic acid-response regulator to redox signalling. *Planta* **214**, 775–782.

Mittler, R. (2002) Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science* **7**, 405–410.

Moller, I.M. (2001) Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**, 561–591.

Morohashi, Y. (2002) Peroxidase activity develops in the micropylar endosperm of tomato seeds prior to radicle protrusion. *Journal of Experimental Botany* **53**, 1643–1650.

Murata, Y., Pei, Z.M., Mori, I.C. and Schroeder, J. (2001) Abscisic acid activation of plasma membrane Ca^{2+} channels in guard cells requires cytosolic NAD(P)H and is differentially disrupted upstream and downstream of reactive oxygen species production in abi1–1 and abi2–1 protein phosphatase 2c mutants. *Plant Cell* **13**, 2513–2523.

Naredo, M.E.B., Juliano, A.B., Lu, B.R., De Guzman, F. and Jackson, M.T. (1998) Responses to seed dormancy-breaking treatments in rice species (*Oryza* L.). *Seed Science and Technology* **26**, 675–689.

Neill, S., Desikan, R. and Hancock, J. (2002) Hydrogen peroxide signalling. *Current Opinion in Plant Biology* **5**, 388–395.

Neill, S.J., Desikan, R. and Hancock, J.T. (2003) Nitric oxide signalling in plants. *New Phytologist* **159**, 11–35.

Ogawa, K. and Iwabuchi, M. (2001) A mechanism for promoting the germination of *Zinnia elegans* seeds by hydrogen peroxide. *Plant and Cell Physiology* **42**, 286–291.

Oliver, M.J. (1996) Desiccation tolerance in vegetative plant cells. *Physiologia Plantarum* **97**, 779–787.

Orozco-Cardenas, M.L., Narvaez-Vasquez, J. and Ryan, C.A. (2001) Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. *Plant Cell* **13**, 179–191.

Otegui, M.S., Capp, R. and Staehelin, L.A. (2002) Developing seeds of *Arabidopsis* store different minerals in two types of vacuoles and in the endoplasmic reticulum. *Plant Cell* **14**, 1311–1327.

Overmyer, K., Brosche, M. and Kangasjarvi, J. (2003) Reactive oxygen species and hormonal control of cell death. *Trends in Plant Science* **8**, 335–342.

Pammenter, N.W. and Berjak, P. (1999) A review of recalcitrant seed physiology in relation to desiccation-tolerance mechanisms. *Seed Science Research* **9**, 13–37.

Papadakis, A.K., Siminis, C.I. and Roubelakis-Angelakis, K.A. (2001) Reduced activity of antioxidant machinery is correlated with suppression of totipotency in plant protoplasts. *Plant Physiology* **126**, 434–444.

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

Pei, Z.M., Murata, Y., Benning, G., Thomine, S., Klüsener, B., Allen, G.J., Grill, E. and Schroeder, J.I. (2000) Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. *Nature* **406**, 731–734.

Pellinen, R.I., Korhonen, M.S., Tauriainen, A.A., Palva, E.T. and Kangasjärvi, J. (2002) Hydrogen peroxide activates cell death and defense gene expression in birch. *Plant Physiology* **130**, 549–560.

Posmyk, M.M., Corbineau, F., Vinel, D., Bailly, C. and Côme, D. (2001) Osmoconditioning reduces physiological and biochemical damage induced by chilling in soybean seeds. *Physiologia Plantarum* **111**, 473–482.

Prasad, T.K., Anderson, M.D., Martin, B.A. and Stewart, C.R. (1994) Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. *Plant Cell* **6**, 65–74.

Priestley, D.A. (1986) *Seed aging. Implications for seed storage and persistence in the soil*. Ithaca, Cornell University Press.

Pukacka, S. (1991) Changes in membrane lipid components and antioxidant levels during natural aging of seeds of *Acer platanoides*. *Physiologia Plantarum* **82**, 306–310.

Puntarulo, S., Sanchez, R.A. and Boveris, A. (1988) Hydrogen peroxide metabolism in soybean embryonic axes at the onset of germination. *Plant Physiology* **86**, 626–630.

Puntarulo, S., Galleano, M., Sanchez, R.A. and Boveris, A. (1991) Superoxide anion and hydrogen peroxide metabolism in soybean embryonic axes during germination. *Biochimica et Biophysica Acta* **1074**, 277–283.

Racchi, M.L., Bagnoli, F., Balla, I. and Danti, S. (2001) Differential activity of catalase and superoxide dismutase in seedlings and *in vitro* micropropagated oak (*Quercus robur* L.). *Plant Cell Reports* **20**, 169–174.

Reumann, S. (2000) The structural properties of plant peroxisomes and their metabolic significance. *Biological Chemistry* **381,** 639–648.

Riechmann, J.L. and Meyerowitz, E.M. (1998) The AP2/EREBP family of plant transcription factors. *Biological Chemistry* **379**, 633–646.

Samuel, M.A., Miles, G.P. and Ellis, B.E. (2000) Ozone treatment rapidly activates MAP kinase signalling in plants. *Plant Journal* **22**, 367–376.

Scandalios, J.G., Guan, L. and Polidoros, A.N. (1997) Catalases in plants: gene structure, properties, regulation and expression. pp. 343–406 *in* Scandalios, J.G. (Ed.) *Oxidative stress and the molecular biology of antioxidant defenses*. New York, Cold Spring Harbor Laboratory Press.

Schopfer, P., Plachy, C. and Frahry, G. (2001) Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin, and abscisic acid. *Plant Physiology* **125**, 1591–1602.

Schopfer, P., Liszkay, A., Bechtold, M., Frahry, G. and Wagner, A. (2002) Evidence that hydroxyl radicals mediate auxin-induced extension growth. *Planta* **214**, 821–828.

Schweikert, C., Liszkay, A. and Schopfer, P. (2000) Scission of polysaccharides by peroxidase-generated hydroxyl radicals. *Phytochemistry* **53**, 565–570.

Schweikert, C., Liszkay, A. and Schopfer, P. (2002) Polysaccharide degradation by Fenton reaction- or peroxidase-generated hydroxyl radicals in isolated plant cell walls. *Phytochemistry* **61**, 31–35.

Sherwin, H.W. and Farrant, J.M. (1998) Protection mechanisms against excess light in the resurrection plants *Craterostigma wilmsii* and *Xerophyta viscosa*. *Plant Growth Regulation* **24**, 203–210.

Simontacchi, M., Caro, A., Fraga, C.G. and Puntarulo, S. (1993) Oxidative stress affects α-tocopherol content in soybean embryonic axes upon imbibition and following germination. *Plant Physiology* **103**, 949–953.

Simontacchi, M., Sadovsky, L. and Puntarulo, S. (2003) Profile of antioxidant content upon developing of *Sorghum bicolor* seeds. *Plant Science* **164**, 709–715.

Smirnoff, N. (1993) The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytologist* **125**, 27–58.

Smith, M.T. and Berjak, P. (1995) Deteriorative changes associated with the loss of viability of stored desiccation-tolerant and desiccation-sensitive seeds. pp. 701–746 *in* Kigel, J.; Galili, G. (Eds) *Seed development and germination*. New York, Marcel Dekker.

Stacy, R.A.P., Nordeng, T.W., Culianez-Macia, F.A. and Aalen, R.B. (1999) The dormancy-related peroxiredoxin anti-oxidant, PER1, is localized to the nucleus of barley embryo and aleurone cells. *Plant Journal* **19**, 1–8.

Staniek, K. and Nohl, H. (2000) Are mitochondria a permanent source of reactive oxygen species? *Biochimica et Biophysica Acta* **1460**, 268–275.

Sung, J.M. (1996) Lipid peroxidation and peroxide-scavenging in soybean seeds during aging. *Physiologia Plantarum* **97**, 85–89.

Tanida, M. (1996) Catalase activity of rice seed embryo and its relation to germination rate at a low temperature. *Breeding Science* **46**, 23–27.

Tommasi, F., Paciolla, C., de Pinto, M.C. and De Gara, L. (2001) A comparative study of glutathione and ascorbate metabolism during germination of *Pinus pinea* L. seeds. *Journal of Experimental Botany* **52**, 1647–1654.

Vertucci, C.W. and Farrant, J.M. (1995) Acquisition and loss of desiccation tolerance. pp. 237–271 *in* Kigel, J.; Galili, G. (Eds) *Seed development and germination*. New York, Marcel Dekker.

Vlamis-Gardikas, A. and Holmgren, A. (2002) Thioredoxin and glutaredoxin isoforms. *Methods in Enzymology* **347**, 209–219.

Vranova, E., Inzé, D. and Van Breusegem, F. (2002) Signal transduction during oxidative stress. *Journal of Experimental Botany* **53**, 1227–1236.

Wang, M., Heimovaara-Dijkstra, S. and Van Duijn, B. (1995) Modulation of germination of embryos isolated from dormant and nondormant barley grains by manipulation of endogenous abscisic levels. *Planta* **195**, 586–592.

Wang, M., van der Meulen, R.M., Visser, K., Van Schaik, H.P., Van Duijn, B. and de Boer, A.H. (1998) Effects of dormancy-breaking chemicals on ABA levels in barley grain embryos. *Seed Science Research* **8**, 129–137.

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

Wilson, D.O. and McDonald, M.B. (1986) The lipid peroxidation model of seed aging. *Seed Science and Technology* **14**, 269–300.

Wisniewski, J.P., Cornille, P., Agnel, J.P. and Montillet, J.L. (1999) The extensin multigene family responds differentially to superoxide or hydrogen peroxide in tomato cell cultures. *FEBS Letters* **447**, 264–268.

Wolin, M.S. and Mohazzab-H., K.M. (1997) Mediation of signal transduction by oxidants. pp. 21–48 *in* Scandalios, J.G. (Ed.) *Oxidative stress and the molecular biology of antioxidant defenses*. New York, Cold Spring Harbor Laboratory Press.

Yang, F., Basu, T.K. and Ooraikul, B. (2001) Studies on germination conditions and antioxidant contents of wheat grain. *International Journal of Food Sciences and Nutrition* **52**, 319–330.

Zhang, X., Zhang, L., Dong, F., Gao, J., Galbraith, D.W. and Song, C.P. (2001) Hydrogen peroxide is involved in abscisic acid-induced stomatal closure in *Vicia faba*. *Plant Physiology* **126**, 1438–1448.

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