Rebirth and death: Nitric oxide and reactive oxygen species in seeds

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1 Introduction

1.1 Seed germination and dormancy

Seed germination and early seedling growth are critical phases in the plant life cycle (Bewley and Black, 1994). Young seedlings need to grow rapidly in order to effectively compete with neighbours, yet young seedlings have limited nutrient reserves to draw on as they resume growth. Root systems are rudimentary, and this limits the ability of young seedlings to explore the environment for water and minerals. Developing the photosynthetic capacity required for autotrophic growth is another draw on stored reserves. Young seedlings are also vulnerable to adverse environmental conditions such as insufficient water and extremes of temperature. In order to increase the likelihood of early survival, most plant seeds are dormant at maturity, that is, they will not germinate under environmental conditions that would promote germination of non-dormant seeds (Bewley, 1997; Bewley and Black, 1994; Finch-Savage and Leubner-Metzger, 2006). This is a highly adaptive property that allows plants to delay the growth of the next generation until the season or local environmental conditions are suitable. Dormancy loss may occur in dry storage after sufficient time has passed, a process referred to as after-ripening, or may require specific environmental signals. Examples of these are availability of soil nitrogen, exposure to light, a period of damp and cold, or exposure to compounds in smoke. These signals indicate times of the year or local environmental conditions that are likely to be favourable for seedling growth, and which, therefore, increase the likelihood of early seedling survival.

The embryo and nutrient tissues within dormant seeds have the potential for great longevity. In many cases, seeds remain viable for years or decades. Following dormancy loss and imbibition of water, combinations of plant hormones and signalling molecules promote germination, and the renewed growth and development of the quiescent embryo. In some seeds, these same hormones and signals initiate the programmed death of nutritive tissues.

This chapter will focus on the involvement of plant hormones, reactive oxygen species (ROS) and nitric oxide (NO) in seed germination and early seedling growth in two plant models, Arabidopsis thaliana (Arabidopsis) and Hordeum vulgare (barley). The data support the conclusion that NO functions to remove dormancy and promote...
early seedling growth. ROS production is an inevitable consequence of utilizing stored lipid reserves. In barley, ROS bring about the programmed death of specialized nutrient-storing cells. NO delays this programmed death, at least in part, by functioning as an antioxidant.

1.2 The structure of Arabidopsis seeds and barley grains

Each mature, dry Arabidopsis seed weighs approximately 20 μg and consists of three functional domains: the embryo, the aleurone layer and the seed coat (testa). These are illustrated in Figure 1. The embryo will develop into the young seedling; the Arabidopsis embryo exists within the seed as a rudimentary plant that consists of a single root linked to two cotyledons, or embryonic leaves. The aleurone layer is a living triploid tissue that surrounds the embryo and plays roles in dormancy maintenance and seedling nutrition. The aleurone layer contains a single cell type, the aleurone cell, which is terminally differentiated and rich in stores of lipid, protein and minerals. The seed coat encases both the embryo and aleurone layer, and at seed maturity it consists of dead, crushed cells. The barley grain, although a thousand times larger than the Arabidopsis seed, contains the same three functional domains, plus a starch-storing endosperm (Figure 1). The endosperm makes up the largest part of the grain. It is non-living at grain maturity and contains nutrient reserves of starch, protein, minerals and nucleic acids in the corpses of cells that formed it.

1.3 Abscisic acid and gibberellins are central to dormancy and germination

Two plant hormones, abscisic acid (ABA) and gibberellins (GA) are central regulators of dormancy, germination and early seedling growth (Finch-Savage and Leubner-Metzger, 2006; Finkelstein et al., 2002; Gubler et al., 2005; Kermode, 2005; Peng and Harberd, 2002). ABA establishes and maintains seed dormancy. GA promotes germination, which is defined as the emergence of the embryonic root from the seed coat. GA also promotes utilization of endosperm reserves both in the aleurone cells and in the starchy endosperm, while ABA inhibits these processes. A diagram of the emerging model for

![Figure 1](image1.png)

Figure 1. Diagrams illustrating the arrangement of the embryo, aleurone layer and seed coat in an Arabidopsis seed and a barley grain. Barley grains also contain a large, starchy endosperm.
Figure 2. A model for the involvement of nitric oxide (NO), reactive oxygen species (ROS), gibberellins (GA), and abscisic acid (ABA) in the transition from dormant seed to vigorously growing seedling (PCD, programmed cell death).

the interaction between ABA, GA, NO and ROS during the transition from dormant seed to growing seedling is shown in Figure 2. Data supporting this model are presented below.

2 Nitric oxide lessens dormancy in Arabidopsis seeds and barley grains

Most Arabidopsis ecotypes have moderate to strong seed dormancy. Seeds stored at room temperature require from several months to over a year to lose dormancy (Koornneef et al., 2000). When dormant Arabidopsis seeds of the C-24 ecotype were exposed to a continuous flow of NO gas for 48 h, however, approximately a quarter of the seeds lost dormancy and germinated within 5 days (Bethke et al., 2007; Libourel et al., 2006). Exposure of the same seeds to a continuous stream of air did not result in seed germination. Conversely, when a population of seeds that germinated to the 20% level in the absence of treatment were imbibed with the NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazolone-1-oxyl-3-oxide (c-PTIO), dormancy was strengthened in a dose-dependent manner and the percentage of germinated seeds decreased (Bethke et al., 2004b). The final germination percentages were 13% with 50 μM c-PTIO and 5% with 100 μM c-PTIO. The effect of c-PTIO was to strengthen dormancy rather than to cause an inhibition of germination. This was shown clearly in experiments where non-dormant Arabidopsis seeds were imbibed with c-PTIO. In these experiments treatment with c-PTIO at concentrations of up to 200 μM had virtually no effect on the rate of germination of the seeds, or on the final percentage of seeds germinated (Bethke et al., 2006b). In each case, the final germination percentages were greater than 95%.

The dormancy observed in seed of cultivated barley is somewhat weaker than that in the Arabidopsis C-24 ecotype. Dormancy in barley seed is promoted by imbibition in the light. When dormant barley grains (cv. Proctor) were imbibed on moist
filter paper for 5 days, germination in the dark was greater than 95% but values decreased in the light to only about 10% (Bethke et al., 2004b). Germination in the light increased to almost 40% in the presence of a 100 μM concentration of the NO donor sodium nitroprusside (SNP) whereas imbibition with c-PTIO reduced germination in the light to less than 1%. As with Arabidopsis, this effect of c-PTIO was a strengthening of dormancy and not toxicity, as c-PTIO had no effect on the germination of dormant barley grain imbibed in the dark (to break dormancy), or on non-dormant grain imbibed in the light (Bethke et al., 2004b).

3 The aleurone layer is a site of NO perception, response and synthesis

In order to determine the site of NO perception that was important for a loss of seed dormancy, microdissections were used to isolate embryos from briefly imbibed, dormant seeds. These embryos were still enveloped by the aleurone layer but they lacked the seed coat. Imbibition of seeds in the absence of the seed coat did not result in germination and dormancy was maintained for at least 28 days (Bethke et al., 2007). Exposure of these seeds without the seed coats to NO gas, however, resulted in germination percentages approaching 100% (Bethke et al., 2007). These data indicate that either the aleurone layer or the embryo responded to NO in a way that leads to a loss of dormancy. Isolated embryos lacking both the aleurone layer and the testa, however, showed no sign of dormancy. Embryos removed from dormant seeds of the C-24 ecotype, or more highly dormant Arabidopsis ecotypes such as Cape Verde (Cvi) or Kashmir2 (Kas2), grew and began to green as if they were non-dormant when placed on solid media (Bethke et al., 2007). Furthermore, imbibition of isolated embryos with the NO scavenger c-PTIO had no effect on growth. These data suggest that early embryo growth was not dependent on NO, and that Arabidopsis seeds do not have true embryo dormancy.

Aleurone cells undergo morphological changes that are tightly linked to the dormancy status of the seed. In intact, imbibed, non-dormant seeds there is a progressive change in the number and size of the vacuoles within each aleurone cell, such that the average number of vacuoles per cell decreases to one or a few. As shown in Figure 3A and B, there are around 20 vacuoles per aleurone cell in Arabidopsis, and this number declines to around one at the time of germination (Bethke et al., 2007). A similar change is observed in non-dormant barley grains (Bush et al., 1986; Jones and Price, 1970). This process of rapid vacuolation does not occur in dormant seeds or grains (Figure 3C), and in dormant seeds of the Arabidopsis Cvi or Kas2 ecotypes, the number of vacuoles per cell may increase following imbibition (Bethke et al., 2007). Thus, vacuolation of aleurone cells is a useful marker for the aleurone layer that indicates a loss of dormancy has occurred.

When aleurone layers from briefly imbibed, dormant Arabidopsis seeds were separated from the embryo and incubated on a solid medium, there was a progressive vacuolation of the aleurone cells (Bethke et al., 2007). Isolated aleurone layers, therefore, are similar to isolated embryos in that they act as if they are not part of a dormant seed once they are removed from the seed. Imbibition of isolated aleurone layers with c-PTIO, however, inhibited this process of vacuolation and this indicated an involvement of NO in this process. Toxicity of c-PTIO to isolated aleurone layers was not a
Figure 3. Micrographs showing Arabidopsis aleurone cells in dormant and non-dormant seeds, and in seeds of the Spy-I mutant. (A) Aleurone cells in a non-dormant seed 2 h after imbibition. Each cell contains many light coloured vacuoles (V) and a darker coloured nucleus (N). (B) Aleurone cells in a non-dormant seed shortly after germination. A single large vacuole nearly fills each cell. (C) Aleurone cells in a dormant seed 9 days after imbibition. The vacuolation that occurs in non-dormant seeds is prevented in dormant seeds and each cell contains numerous vacuoles. (D) Cells in the region of the aleurone layer adjacent to the root in Spy-I mutant seeds vacuolate rapidly and enlarge. Individual aleurone cells (AC) are easily dislodged as a result of extensive wall weakening.

cause of this inhibition, as imbibition of isolated aleurone layers with both c-PTIO and GA reversed the effect of c-PTIO alone, and aleurone cells became highly vacuolate (Bethke et al., 2007). Taken together, these data indicate strongly that the aleurone layer is the site of perception for NO that leads to dormancy loss.

The inhibition of vacuolation observed in isolated aleurone layers by c-PTIO suggests that NO was also produced by the aleurone layer. Numerous sources of NO exist in plants, including NO synthase-like activities, nitrate reductase, a plasma membrane localized nitrite-NO reductase, and others (Crawford and Guo, 2005; Delle Donne, 2005; Shapiro, 2005). Non-enzymatic NO synthesis, where NO is formed spontaneously from nitrite in an acidic environment, is an important source of NO in mammals (Gladwin et al., 2005), and several lines of evidence indicate that it is an important source of NO in the aleurone layer of seeds as well (Bethke et al., 2004a). Copious amounts of NO were produced within seconds when nitrite was added to samples containing barley aleurone layers and the medium in which they were incubated. This production of NO did not require aleurone cells. Nitrite-dependent NO production occurred when nitrite was added to the medium in which aleurone layers had been incubated. The rate of this reaction was dependent on the amount of added nitrite and was accelerated by native antioxidants such as the proanthocyanidins found
in the seed coat, or exogenous phenolic antioxidants such as catechin. The observations that nitrite served as a substrate for NO production, and that NO reduced seed dormancy, fit well with the extensive research on seed physiology demonstrating that nitrite is a potent dormancy-breaking compound for seeds from a wide range of species (Hendricks and Taylorson, 1974).

4 GA synthesis and response act downstream of NO to promote germination

4.1 Transcription of genes required for GA synthesis

Several lines of experimental evidence suggest that NO acts upstream of GA in the processes that lead to germination. Quantitative RT-PCR was used to examine the accumulation of transcripts for GA biosynthetic genes in embryos and aleurone layers of Arabidopsis seeds (Bethke et al., 2007). GA3ox1 and GA3ox2 encode 3β-hydroxylases and catalyse the final step in the synthesis of biologically active GAs. When dormant Arabidopsis seeds were treated with a compound that initiated NO-dependent germination, in this case cyanide gas, GA3ox1 and GA3ox2 transcripts were not detectable in the aleurone layer at any time between 1 and 48 h after treatment. Embryos removed from treated seeds showed a significant accumulation of GA3ox1 transcript that could be detected by the 24-h time-point. Both GA3ox1 and GA3ox2 transcripts accumulated to a much larger degree by 48 h after treatment. These increases in the abundance for GA biosynthetic gene transcripts were much less pronounced in the embryos from seeds treated with cyanide gas and imbibed with c-PTIO. Little accumulation of GA biosynthetic gene transcripts was found in embryos from seeds imbibed in water alone. These data suggest that the embryo synthesizes the GA required for germination, not the aleurone layer.

4.2 Weakening of aleurone cell walls by secreted enzymes

The above evidence shows that Arabidopsis aleurone cells become more vacuolate in seeds that are about to undergo germination and that this process can be prevented by the NO-scavenger c-PTIO but continues in the presence of both c-PTIO and GA. The process of vacuolation is one of many GA-dependent processes that have been characterized in barley aleurone cells. The aleurone layer in barley grain, however, is best known as a secretory tissue that synthesizes and secretes a wide range of hydrolytic enzymes (Fincher, 1989; Jones and Jacobsen, 1991). The most numerous of these are the α-amylases that break down the reserves of starch, and the proteases that hydrolyse the storage proteins in the dead starchy endosperm. Other secreted enzymes include cell-wall degrading enzymes that digest the thick aleurone cell walls. Wall hydrolysis makes additional carbohydrate available for uptake by the growing embryo. This digestion and presumed weakening of the cell walls surrounding the aleurone cells suggested a mechanism for germination in which the cell walls of the aleurone cells nearest to the embryonic root are weakened through the activity of enzymes secreted by the aleurone cells following perception of embryo-synthesized GA. Enzymatic wall weakening permits the root to overcome the restraint to germination imposed by the aleurone layer.
Three lines of evidence from Arabidopsis strongly support the hypothesis that enzymatic weakening of the cell wall permits the root to overcome the restraint to germination imposed by the aleurone layer. The first and most compelling line of evidence comes from experiments with the Spy-1 mutant of Arabidopsis. Spy-1 is a GA signalling mutant that has a lesion in a gene encoding an O-GluNAc transferase, and Spy-1 mutant plants have phenotypes similar to wild-type plants sprayed repeatedly with GA. In aleurone layers of Spy-1 mutant seeds, extreme wall weakening between the aleurone cells in the region near the embryonic root is observed (Figure 3D), to the extent that individual, greatly enlarged, living cells are readily dislodged from isolated aleurone layers (Bethke et al., 2007). Cells in other regions of the aleurone layer are much less affected in the Spy-1 mutant and resemble cells in wild-type seeds. Second, vacuolation of aleurone cells in imbibed, non-dormant Arabidopsis seeds was most rapid in those aleurone cells adjacent to the embryonic root, and least rapid in those cells adjacent to the cotyledons (Bethke et al., 2007). That is, vacuolation, and presumed wall weakening, occurred fastest in that part of the aleurone layer that ruptures at germination. Finally, although imbibed dormant seeds with the seed coat removed remained dormant, physical damage to the aleurone layer resulted in rapid germination.

Taken together, these data are consistent with a model in which NO produced in the aleurone layer results in GA production in the embryo. Diffusion of GA from the embryo to the aleurone layer triggers the synthesis and secretion of cell-wall degrading enzymes. These enzymes weaken the walls between adjacent aleurone cells, and in doing so promote germination by reducing the physical force necessary for the root to penetrate through the aleurone layer. Many GA-dependent events in seeds are antagonized by ABA, and in Arabidopsis, NO not only stimulates the transcription of GA biosynthetic enzymes but may also decrease the sensitivity of seeds to ABA (Bethke et al., 2006a).

5 The role of the aleurone layer after germination is species dependent

The embryo continues to grow and develop following germination, and becomes a self-sustaining, autotrophic seedling. For a critical period of time after germination, and to a degree that depends on the species, growth of the embryo is fuelled by nutrients that were in the seed. The post-germinative contribution of the aleurone layer to seedling growth is a direct consequence of the physical relationship between the germinated embryo and the aleurone layer. In Arabidopsis, for example, the seed coat and aleurone layer are pushed off the embryo as it unfolds and enlarges. Hence, the aleurone cells are of no consequence to the embryo after germination because they do not remain in contact with the embryo. In barley, on the other hand, the seed coats and aleurone layer remain tightly attached to the embryo. Because the aleurone layer remains in intimate contact with the embryo, the nutrients in it can be used to benefit the young seedling. This is accomplished, in the end, through the programmed death of the aleurone cells.

6 GA promotes and ABA inhibits programmed cell death of barley aleurone cells

Isolated barley aleurone layers have proved to be extremely useful in experiments focused on understanding the regulation of gene expression, hormone signalling and
ion transport in the aleurone, as well as the cell biology and enzymology of the aleu-
ron tissue in germination. A major reason for the usefulness of isolated aleurone lay-
ers is that control over cellular function can be achieved by incubating isolated aleurone 
layers with either GA or ABA. Layers incubated in ABA behave akin to those in the 
dry grain. Genes associated with early germination, such as the GA biosynthetic 
enzymes, are not transcribed in isolated barley aleurone layers. Similarly, nutrient 
hydrolysing amylases and proteases are not synthesized. Incubation of barley aleu-
ron layers with GA, however, results in a rapid conversion of the cell from a quies-
cent storage cell to a dynamic secretary cell that rapidly hydrolysies its stored reserves 
of protein, lipid and potassium-storing phytate (Bethke et al., 1998; Fincher, 1989; 
Jones and Jacobsen, 1991).

The amino acids derived from proteolysis of vacuolar storage proteins are used for 
the de novo synthesis of enzymes that will be secreted from the aleurone cells. These 
enzymes break down the carbohydrate, protein and other macromolecules in the 
starchy endosperm. The most abundant of these secreted enzymes are the α-amylases, 
which make up as much as 65% of the newly synthesized protein (Higgins et al., 1982). 
Lipids, which are stored as triacylglycerides in oleosomes, are hydrolysed by lipases, 
and fatty acids are broken down via β-oxidation in the glyoxysome to support gluco-
neogenesis. These GA-dependent activities function to nourish the growing seedling, 
either directly, as in the export of minerals and sugar, or indirectly, as in the case of 
secreted enzymes. The final GA-dependent event for each aleurone cell is a pro-
grammed cell death (PCD); this also makes nutrients available to the growing embryo, 
since the contents of the dead aleurone cell become available for hydrolysis by the 
cocktail of digestive enzymes that the aleurone cells secreted while alive.

As illustrated in Figure 4A, cells in isolated aleurone layers remained viable for more 
than 48 h when incubated in media containing ABA (Fath et al., 2001). When identi-
cal aleurone layers were incubated in media containing GA, however, a different pic-
ture emerged. For the first 24 h after GA treatment, all cells remained alive as judged 
by vital staining of intact aleurone layers. Between 24 and 48 h after GA treatment, 
however, there was a progressive increase in the number of dead cells, such that 
approximately half of the cells were dead at 36 h and 80–90% of the cells were dead by 
48 h after treatment with GA (Fath et al., 2001).

7 Programmed cell death results from damage by ROS

7.1 Sensitivity to antioxidants and reactive oxygen species

Several lines of evidence suggest that this hormone-dependent PCD results from 
increased oxidation and sensitivity to ROS. Firstly, incubating GA-treated aleurone 
layers with the antioxidant butylated hydroxytoluene slowed the rate of death, but 
did not slow the rate or extent of α-amylase secretion (Beligni et al., 2002). Secondly, 
GA-treated aleurone layers became more sensitive to exogenous hydrogen peroxide 
with time after hormone treatment (Figure 4B; Bethke and Jones, 2001; Fath et al., 
2001). Cells in aleurone layers treated with either ABA or GA for 12 h remained viable 
when exposed to 1% H₂O₂ for 30 min. Eighteen hours after hormone treatment, how-
ever, about half of the cells in GA-treated aleurone layers died when exposed to 1% 
H₂O₂, while all cells in ABA-treated layers remained alive. By 24 h after hormone

Figure 4. Programmed cell death in barley aleurone cells is promoted by GA but inhibited by ABA. (A) A time-course showing the percentage of live cells in barley aleurone layers at various times after treatment with either GA or ABA. (B) The percentage of cells surviving exposure to 1% H$_2$O$_2$ for 1 h in aleurone layers 12 h, 18 h or 24 h after treatment with ABA or GA.

treatment, no cells in GA-treated layers survived H$_2$O$_2$ treatment, but greater than 90% of cells in ABA-treated layers survived. A third line of evidence indicating that ROS are responsible for barley aleurone cell death comes from experiments with barley aleurone protoplasts. These are the individual living cells that are produced by treating aleurone layers with cell-wall digesting enzymes. Production of ROS within aleurone protoplasts was increased substantially under illumination with either bright blue (440–495) light or UV (370–390 nm) light. This process appears to result from the photoreduction of a flavin-containing oxidase, with a consequent production of ROS. ROS production within aleurone protoplasts was observed within seconds of illumination for both ABA-treated and GA-treated aleurone protoplasts. ABA-treated barley aleurone protoplasts remained viable when exposed to bright UV or blue light for up to 80 min. GA-treated protoplasts, especially highly vacuolate protoplasts that were approaching death, began to die within 20 min of illumination and 100% or 50% of cells were dead following 40 min exposure to UV or blue light respectively (Bethke and Jones, 2001). These experiments confirm that GA-treated aleurone cells are more sensitive to endogenously produced ROS than ABA-treated cells.

7.2 Decreased capacity to metabolize reactive oxygen species

The increased sensitivity of GA-treated aleurone cells to exogenous and endogenous ROS reflects a genetically programmed, GA-dependent down-regulation of enzymatic defences against ROS (Fath et al., 2001). Northern analyses such as those in Figure 5A showed clearly that transcript amounts for superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) were maintained with time in isolated barley aleurone layers treated with ABA. In GA-treated layers, however, SOD and APX transcripts were reduced 18 h after, and barely detectable 24 h after, hormone treatment. Catalase transcript was strongly down-regulated 6 h after hormone treatment and
Figure 5. Enzymatic defences against ROS are down-regulated in GA-treated barley aleurone cells. (A) Northern blots of barley aleurone RNA hybridized with probes to superoxide dismutase (rice sodc1), ascorbate peroxidase (rice apxc2), and catalase (barley cat2) genes. (B) A native gel showing ascorbate peroxidase activities in protein extracts from GA- and ABA-treated barley aleurone cells. At least six bands of activity are visible. (C) Western blot of barley aleurone extracts probed with an anti-ascorbate peroxidase antibody. The same bands indicated by asterisks in (C) are indicated by asterisks in (B).

below the level of detection 12 h after GA treatment. It is important to note that these decreases in transcript amount occurred at a time when the cells remained alive and were actively producing and secreting enzymes. The amounts of the APX, SOD and CAT proteins and the activities of the enzymes (Figures 5B and C), followed the same pattern as transcript abundance, but changes occurred later in time (Fath et al., 2001). Thus antioxidant proteins and activities were maintained in imbibed ABA-treated aleurone layers but they were strongly decreased in GA-treated layers. Native activity gels were used to assess the effects of GA on APX and SOD activities, as these are particularly informative with regard to the effects on the different isoforms of each enzyme. In all cases, enzyme activity was down-regulated by GA treatment. For SOD, at least three bands of activity could be detected and each was greatly reduced 24 h after GA treatment. For APX, at least six activity bands could be detected (Figure 5B), and a strong decrease in all of these bands was observed between 24 h and 36 h after GA treatment.

8 ROS production is a consequence of lipid breakdown

Plant cells generate ROS in many subcellular locations. The chloroplast electron transport chain is the largest source of ROS in photosynthetic tissues, while the respiratory electron transport chain in mitochondria also produces ROS in all cell types. In barley aleurone cells treated with GA, the largest single source of ROS is likely to be the glyoxysome, where fatty acyl chains derived from triacylglycerides are cleaved to produce acetyl-CoA through a series of enzymatic reactions. Acyl-CoA oxidase is one member of this group of enzymes and for each acetyl-CoA produced, acetyl-CoA
oxidase produces one molecule of $\text{H}_2\text{O}_2$. The potential for ROS production in this manner becomes obvious when one considers that 25–40% of the volume of a mature barley aleurone cell is stored lipid (Eastmond and Jones, 2005; Jones, 1969). Acyl-CoA oxidase activity is up-regulated by GA treatment of barley aleurone cells, as is lipid consumption (Eastmond and Jones, 2005). Hence, GA triggers a massive increase in ROS production while at the same time initiating a decrease in the capacity of the cells to metabolize ROS enzymatically. Within the span of a few days, this combination results in the death of the aleurone cells.

9 Nitric oxide acts as an antioxidant and delays programmed cell death in barley aleurone cells

Nitric oxide can act as either a reactive nitrogen species that increases oxidative stress, or as an antioxidant that decreases oxidative stress by reacting with ROS, particularly superoxide and lipid peroxyl radicals (Beligni and Lamattina, 1999a, 1999b, 2002). Pharmacological experiments with NO donors and NO scavengers have provided data that indicate that NO functions as a protective antioxidant in barley aleurone cells. For example, when barley aleurone layers were incubated with the NO donors SNP or S-nitroso-N-acetylpenicillamine (SNAP), the rate of PCD decreases such that approximately 50% of cells were alive 48 h after hormone treatment compared to 20% alive for controls (Figure 6; Beligni et al., 2002). This effect of exogenous NO on PCD could be separated from early events related to GA perception and germination by adding SNP 18 h after GA treatment. Although only 20% of the cells were alive 48 h after adding GA alone, 45% of cells were alive at the same time when SNP was added at 18 h after GA treatment (Figure 6A). Complementary data were obtained using the

![Figure 6](image_url)

**Figure 6.** Nitric oxide acts as an antioxidant in barley aleurone layers. (A) The NO-donor SNP added 0 h or 18 h after GA treatment of barley aleurone layers decreases the number of dead cells 48 h after GA treatment. (B) The NO scavenger c-PTIO accelerates the rate of GA-dependent death, and this effect of c-PTIO can be reversed by addition of SNP. The different number of asterisks indicate statistically different means calculated at $P = 0.05$ in a student's t test.
NO scavenger c-PTIO (Figure 6B). Addition of c-PTIO increased the rate of GA-dependent PCD by a statistically significant amount, such that around 10% of cells were alive 48 h after treatment with GA and c-PTIO compared with around 25% of cells alive when treated with GA alone (Bethke et al., 2004a). The effect of c-PTIO on death could be reversed by addition of SNP, and this further indicates that NO is involved in delaying death. Addition of c-PTIO to ABA-treated cells had no effect, and all cells remained alive. The potential advantage to the germinated seedling of NO delaying PCD was illustrated in experiments where NO donors were added to GA-treated aleurone layers and the activity of secreted α-amylase was determined. NO donors did not increase the rate at which α-amylase was secreted into the incubation medium during the first 24-h period after hormone addition. Between 24 and 48 h, however, during the period when cells treated with GA alone died, approximately 20% more α-amylase activity was found in the incubation medium surrounding aleurone layers treated with both an NO donor and GA compared to those incubated with GA alone (Beligni et al., 2002).

10 Concluding remarks

Most seeds are required to enter a phase of metabolic and developmental quiescence in order to conserve stored nutrients until appropriate environmental cues lead to an end of dormancy and renewed growth. Once the process leading to germination has been initiated, rapid growth and development are desirable, since this allows roots to explore the soil for water and minerals, and leaves to develop the photosynthetic machinery that will make the seedling autotrophic. ABA, GA, NO and ROS are central players in this transition phase in Arabidopsis and barley. ABA establishes and maintains dormancy. NO and GA function to bring about a loss of dormancy and to promote germination. Later, GA action results in an increase in ROS production and, in barley, a weakening of cellular defences against ROS. NO also acts as an antioxidant in barley to mitigate the effects of ROS and to postpone PCD. Eventually, however, ROS contribute to the death of the nutrient-storing aleurone layer that then becomes, like the dead starchy endosperm, a source of nutrient-rich corpses. Hence, NO and ROS play important roles as signalling molecules, antioxidants and oxidants at this critical phase of the plant life cycle. Other functions in signalling and response are likely for both NO and ROS, and these will be uncovered as additional details of the molecular and biochemical events controlling dormancy, germination and early seedling growth are obtained.

References


