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# The signalling roles of reactive oxygen species in the regulation of seed germination and dormancy

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## Abstract

Reactive oxygen species (ROS) are versatile compounds which can have toxic or signalling effects in a wide range living organisms, including seeds. They have been reported to play a pivotal role in the regulation of seed germination and dormancy but their mechanisms of action are still far from being fully understood. In this review, we sum-up the major findings that have been carried out this last decade in this field of research and which altogether shed a new light on the signalling roles of ROS in seed physiology. ROS participate to dormancy release during seed dry storage through the direct oxidation of a subset of biomolecules. During seed imbibition, the controlled generation of ROS is involved in the perception and transduction of environmental conditions that control germination. When these conditions are permissive for germination, ROS levels are maintained at a level which triggers cellular events associated with germination, such as hormone signalling. Here we propose that the spatiotemporal regulation of ROS production acts in concert with hormone signalling to regulate the cellular events involved in cell expansion associated with germination.

## Introduction

Oxygen supports aerobic life, but can also give rise to the reactive oxygen species (ROS). These include free radicals, which contain an unpaired electron such as singlet oxygen ($^1O^2$), superoxide ($O^{\cdot -2}$) or hydroxyl radical ($^{\cdot}OH$) and hydrogen peroxide ($H_2O_2$), which is not a free radical. Hydrogen peroxide is considered as the main ROS involved in cellular signalling as it is rather stable (lifetime of milliseconds) and is capable of crossing biological membranes [1]. Although ROS have distinct chemical properties, they are all highly reactive and can cause damage to other biomolecules. Oxidative stress is well documented in plants since it generally accompanies biotic and abiotic stresses in higher plants and can ultimately lead to cell death [2]. In contrast, ROS are also recognized as key signalling intermediates involved in a wide range of plant responses to the environment and as regulators of plant development [3] [4].

More than a decade ago it was proposed that ROS were involved in the control of seed germination and dormancy release [5][6]. At this time, ROS were principally associated with oxidative damage and loss of viability during seed ageing. The involvement of ROS homeostasis in regulating seed germination and dormancy came in 2007 when Oracz et al. [7] demonstrated that sunflower seed dormancy alleviation during dry storage was associated with ROS accumulation and protein carbonylation. Since this time, ROS progressively emerged as essential components of the germination process leading to the concept of "oxidative window for germination", which restricts the occurrence of the cellular events associated with germination to a critical range of ROS level, enclosed by lower and higher limits [6]. There is now further evidence showing that ROS homeostasis is critical for germination, and this is examined in this review article.

Seeds are spectacular and intriguing organisms. They are at the core of plant kingdom because they retain the genetic information of higher plant species, which survival and dissemination depends on seed longevity and successful germination and establishment of a novel plant. Nevertheless, the particularities of seeds, when compared to whole plant systems, has to be considered when investigating the role of ROS in their germination. First, the so-called "orthodox seeds" desiccate on the mother plant at the end of their developmental program, reaching very low moisture content [8]. This desiccation phase can be considered as an extreme drought stress associated with ROS generation and oxidative stress, from which developing seeds are protected through enzymatic and non-enzymatic mechanisms [5]. As a consequence, at shedding, the status of dry mature orthodox seeds is largely oxidized. The physiology of seeds evolves during subsequent dry storage. In particular this period may be necessary to alleviate dormancy, a blockage of germination in apparently favourable environmental conditions, in a process termed after-ripening [9]. Desiccated seeds are a quiescent state where no metabolism can be detected, but non-enzymatic ROS generation has been frequently evidenced in these conditions [6][10]. Then, during imbibition, seed water uptake allows metabolism  resumption and subsequently radicle protrusion, the first visible sign of the completion of germination, if the environmental conditions are permissive, *i.e.* if water potential, temperature, oxygen and light conditions are appropriate, and if seeds are non-dormant [9]. The recovery of an active metabolism is associated with enzymatic and regulated production of ROS. In contrast to whole plants, imbibed seeds are very sensitive to small variations of environmental factors. This sensitivity is an adaptive trait which allows germination to proceed only when environmental conditions are appropriate for subsequent seedling development, thus increasing the probabilities of survival of the resulting seedlings. For example, a difference of few degrees Celsius can impose a block on seed germination, a phenomena which is emphasized in dormant seeds. This suggests that environmental signals have to be accurately transmitted to seeds and translated into endogenous signals regulating germination. In this review we propose that ROS are very candidates for the fine-tuning of the timing of germination. Lastly, the relationship of ROS with other cell signalling pathways, *i.e.* plant hormones, is often different in seeds than in other plant systems. For example, abscisic acid (ABA) and ROS interact synergistically in response to plant abiotic stress such as drought, in particular for regulating stomata opening [11], but in seeds ROS and ABA are generally antagonists. This particularity has also to be considered when studying ROS signalling in seeds.

The objective of this review is to provide a critical view of the signalling role of ROS in seed germination and dormancy, taking into account the recent findings in both germination and ROS signalling mechanisms.

## Evidence for a role of ROS in germination and dormancy

Table 1 ([7], [12-57]) displays some of the works that have been published within the last ten years dealing with the signalling role of ROS in seed germination and dormancy. In this table we have distinguished beneficial and detrimental effects of ROS accumulation on completion of germination or dormancy release. It appears clearly that most of these works have evidenced a positive role of ROS in these processes, whatever the species. These former studies have shown that controlled ROS accumulation during seed imbibition is a prerequisite to radicle elongation, as well as dormancy alleviation, either by cold stratification or after-ripening. In this context many attempts have been performed in order to investigate whether ROS homeostasis crosstalk with the major hormonal regulators of germination and dormancy, *i.e.* ABA, gibberellins (GA) or ethylene, and the major findings will be discussed below. In contrast, another set of studies has also pointed out the deleterious role of ROS in the completion of seed germination. In this context, their accumulation during seed imbibition triggers oxidative stress and slows down or inhibits germination. Such an effect has been evidenced when seeds were germinated in stressful conditions either caused by drought, salt stress or toxic compounds in the imbibition medium, for example (Table 1).

These studies confirm and validate the concept of the oxidative window for germination [6]. In this concept the ability of a seed to germinate is directly depended on ROS homeostasis: seed germination is likely to occur only when the seed ROS content is enclosed within values that allows ROS signalling but not ROS damage. In contrast germination is thus prevented when the amount of ROS is too low or too elevated. Although there are no more doubts about the involvement of ROS in germination, many questions about their production and mode of action remain unsolved.

## ROS production in seeds

The orthodox seeds, which undergo a dramatic desiccation phase at the end of their developmental program *on planta*, can survive for years in a resting and anhydrobiotic state. However, in such a desiccated state, major physiological changes such as dormancy alleviation (so called dry after-ripening) or ageing are likely to occur. Seeds can also withstand an abrupt and invasive water uptake during their imbibition which will drop their moisture content from values below 8-10 % dry weight basis (dwb) to values higher than 50 % dwb at the onset of radicle protrusion. This plasticity of life has marked consequence on metabolism since it is almost at a standstill in dry mature seeds and is progressively reactivated during imbibition. Thus this almost unique feature (with other anhydrobiotes) of metabolic variation is in consequence associated with marked changes in ROS production.

ROS metabolism and production in dry seeds are rarely investigated maybe because it remains technically challenging (most methods implies to use water, and then to modify the initial seed status). The "fuel" for producing ROS in anhydrobiosis is oxygen that exists in its ground state ($^3$O2) with two unpaired electrons with parallel spins [58] and its reduction gives rise to the various forms of ROS. In dry seeds oxygen is likely to be present in void spaces which altogether constitute an air space network [59]. In addition, seed desiccation on the mother plant generates ROS thus creating an oxidative environment within tissues of the mature seed [5]. Providing oxygen is present, chemical (non-enzymatic) reactions of ROS production can thus proceed during seed storage. Lipids in particular are very prone to oxidation at low moisture content and can serve as a source of free radicals. This process is well described in food science and lipid oxidation has a U-shaped relationship to water activity (aw), increasing at very low moisture contents. Increasing moisture content in zone I of water sorption isotherm decreases lipid oxidation by eliminating pores and decreasing oxygen exposure of lipids to oxygen. It was proposed that solubility or sorption of molecular oxygen in dehydrated foods is lowered as water content increases and, thus, the reaction rate is lowered by decreasing the effective oxygen concentration [60] [61]. Moreover in low moisture systems peroxidation of lipids may lead to reactions of proteins with lipid hydroperoxides, free radicals and peroxide breakdown products [60].

The occurrence of ROS production during seed dry after-ripening has been demonstrated either directly by measuring change in ROS content (*eg* [7]) or by following oxidation of biomolecules within seeds ([62][63][50]). For example, after-ripening of sunflower embryonic axes was accompanied by a shift in the thiol-based cellular redox environment towards more oxidizing conditions [50] and by oxidation of proteins [7] and mRNAs [62]. In barley, the ROS content in the embryo was not affected by after-ripening, while the antioxidant glutathione (GSH) was gradually converted to glutathione disulphide (GSSG) [28]. The key role of oxygen in seed dormancy release during dry storage has been experimentally demonstrated recently by Buijs et al. [64] who increased the internal partial pressure of oxygen within Arabidopsis seeds to accelerate dormancy release. Indirect evidence were also given by Bazin et al. [65] and Basbouss-Serhal et al. [66] who studied dormancy alleviation of sunflower and Arabidopsis seeds, respectively, in a wide range of temperature and relative humidities. Both demonstrated that at low moisture content (below 7 % dwb) dormancy alleviation was associated with negative energy activation, as determined by Arrhenius plots. Negative activation energies suggest that the kinetics of a biological process increases when temperature decreases and it reveals non-enzymatic processes, related to ROS production and oxidation mechanisms [65] thus confirming the measured changes in ROS content during dry after-ripening.

ROS production in imbibed seeds is better characterized and reviewed previously [5]. The studies of Bazin et al. [65] and Basbouss-Serhal et al. [66] demonstrated that metabolic activity in seeds could resume when moisture content reached values as low as 10 % dwb in sunflower and *ca.* 13 % dwb in Arabidopsis. Kibinza et al. [67] also demonstrated that respiration in sunflower embryonic axes became effective at 10 % moisture content. Therefore even a limited water uptake can trigger ROS production trough metabolic activities. Commonly detected ROS in imbibed seeds are superoxide, hydrogen peroxide and hydroxyl radical (see studies shown in Table 1). The absence of functional chloroplast in seeds does not favour production of singlet oxygen, although it has already been detected in germinating seeds but it was considered as being arising from lipid peroxidation [68]. In imbibed seeds ROS are probably mainly produced by mitochondria, since 2–3% of oxygen used by this organelle is supposed to generates superoxide anion at complexes I and II of the electron transfer chain, then giving hydrogen peroxide [69]. $H_2O_2$ can further react through

Fenton reaction with reduced $Fe^{2+}$ and $Cu^{2+}$ to produce $^{\cdot}OH$ [70]. Whether mitochondrial ROS plays a role in germination, their production should be regulated, and this mechanism is far from being known. Recently Ma et al. [18] demonstrated that a mitochondrial matrix-localized heat shock protein could induce reactive oxygen species in a temperature dependent manner which strengthen the role of ROS as environmental sensors in the germination process. The other candidates often mentioned in the context of regulation of germination by ROS are NADPH oxidases. NADPH oxidases (NOXs), also known as respiratory burst oxidase homologues (RBOHs), are certainly the most-studied ROS-producing enzymes [71]. They transfer electrons across the plasma membrane from cytoplasmic NADPH to molecular oxygen to produce superoxide in the apoplast which is rapidly dismutated to $H_2O_2$. The activity of NADPH oxidases has been frequently proposed to be involved in ROS production during seed imbibition (eg [72] [24,32,33,46]) and, similarly to other plant systems, NOXs could play a role in sensing environmental conditions favourable for germination.

Attention has also to be paid to the diverse sources of apoplastic ROS production, since apoplastic ROS can play a critical role in cell wall weakening which precedes cell elongation. The apoplastic copper-containing amine oxidases and polyamine oxidases catalyse deamination of di- and polyamines and produce $H_2O_2$ [73]. Oxalate oxidases (germins and germin-like proteins) catalyse the oxidation of oxalate to $CO_2$ and $H_2O_2$ [74]. Peroxidases and quinone reductase also generate ROS [75] [76]. Most of these enzymes have been frequently cited as being involved in the late phase of germination, at the onset of radicle protrusion.

ROS content does not only depend on ROS production systems but is also regulated by the efficiency of enzymatic and non-enzymatic antioxidant mechanisms (already reviewed in seeds by [5]). Changes in activities of superoxide dismutase, catalase and enzymes of the ascorbate-glutathione cycle have been widely investigated in the context of seed germination. To understand the role of these systems in the regulation of seed germination, one nevertheless has to distinguish between seed germination under favourable conditions from germination under stressful conditions. In the first case it is difficult to get a clear picture on the role of these systems prior to radicle protrusion. In some case it has been demonstrated that activities of antioxidant enzymes increased during imbibition of germinating seeds [57,77–79] while in other cases these activities decreased [29]. Moreover, in most cases, activation of antioxidant systems is a late event in the germination process. We suggest that activation of these systems only occur when ROS level exceed a certain value, in order to maintain ROS homeostasis within the oxidative window for germination, which means that in permissive conditions of germination the level of ROS is rather under the control of ROS generating mechanisms. When seeds are germinated in non-optimal conditions there is a positive correlation between ROS scavenging ability through antioxidant systems and germination rate. For example, among only recent studies, seed antioxidant enzyme activities have been demonstrated to increase in response to high temperature and drought stress [43,80], low temperature [81], salt stress [82,83] or mutagen agents [38]. In those cases, the activation of antioxidant enzymes is necessary to prevent excessive ROS accumulation and related oxidative damage. In contrast, in the context of seed dormancy, activities of antioxidant enzymes have often been shown to be lower, or even impaired, in non-dormant seeds than in dormant seeds, whatever the species [52,24,30,45,27]. In this context, their reduced efficiency can participate, in association with increase ROS production mechanisms, in the increased accumulation of ROS involved in dormancy release. Altogether the available data suggest that ROS can translate environmental cues to signals in seeds, as suggested by the concept of oxidative window, and that the fine-tuning of their production helps the seed to make a decision when to germinate. This most likely occurs through the interaction with hormone signalling pathways.

There remain many unanswered questions relating to the regulation of ROS production in time and space. For example, it has been proposed recently that ROS signal could autopropagate in plants leading to the concept of ROS wave, where ROS could diffuse from cell to cell via RBOHD [84]. With regards to the functional morphology of seeds, where the elongation zone of the embryonic axis is located behind the meristematic area [85], we can hypothesize that localized ROS production is important for germination control. We have examined this hypothesis in germinating seeds, using cell imaging of ROS production (Figure 1). We show that there is a dynamic production of ROS along the embryonic axis in germinating seeds of Arabidopsis (Figure 1). At an early timepoint of germination (6 h) ROS are mostly located in the meristem area of the radicle (Figures 1A, 1D) and further imbibition progressively relocalizes the maximum area of ROS production in the elongating zone of the hypocotyl (Figuress 1B-C and 1E-F), thus evidencing a "developmental wave" of ROS production during the germination process. This suggests that specific cell territories, could sense environmental cues, *i.e.* the meristem area, and initiate a ROS signal propagating along the embryonic axis and ultimately lead to cell elongation in the hypocotyl area. This finding has to be considered with regards to the spatiotemporal cell expansion mechanisms, related to ABA and GA metabolism and signalling, involved the seed to seedling transition in Arabidopsis [86] and who share some similarities with spatiotemporal ROS production.

Similarly, at the cellular level, during the germination process, it has also been demonstrated that ROS were first localized in the cytoplasm, then in the nucleus and finally in the cell wall at the time cells elongate [24]. ROS may therefore have distinct roles during the germination process, ranging from interaction with cytoplasmic signalling pathways (early germination), oxidative regulation of gene expression within the nucleus (mid-germination) and cell wall weakening (late germination). Thus a better understanding of role(s) of ROS in germination will require paying attention to the spatial regulation of their homeostasis at the whole organ and at the subcellular level.

## ROS signalling is mediated by direct oxidation of biomolecules

Considering the effect of ROS as signals in the germination process requires to pay attention to their downstream effects. Interestingly the strong reactivity of ROS makes possible their direct oxidative action on various cellular components and this can in turn modify cell functioning and in consequence whole seed physiology.

Proteins and nucleic acids can be easily oxidized by ROS. The role of these processes have been described previously in details [10] and will be only briefly evoked here. The amino acids Cys and Met are very sensitive to oxidation. The oxidation of thiol to disulfide, is a known redox regulation mechanism, the reduced form being regenerated by the glutaredoxin and thioredoxin (Trx) systems. Various studies, reviewed in [10], have shown that germination might be associated with protein thiol oxidation, probably in balance with reduction of low molecular weight thiol disulfides [57,87,88]. Besides oxidation of sulfur-containing amino acids, carbonylation is the most common oxidative and non-reversible protein modification. It can result in loss of function and even degradation of the carbonylated proteins. Extensive protein carbonylation has been evidenced during germination of Arabidopsis, pea and rice seeds [89,41,45], and it was mostly directed towards reserve proteins. These authors proposed that carbonylation can be important for mobilization of seed storage proteins because it increases protein susceptibility towards proteolytic cleavage by 20S proteasome. In addition it was shown that carbonylation was not randomly distributed among proteins which suggests that oxidation of specific proteins is likely to play a role in the completion of germination. This idea was confirmed by Oracz et al. [7] who compared carbonylation patterns of dormant and non-dormant sunflower seeds and who showed that carbonylation was directed towards negative regulators of germination. In rice, Zhang et al. [45] showed that HSP and LEA, 2 known positive regulators of dormancy, became carbonylated during germination.

RNAs are also very sensitive to non-enzymatic oxidation [10]. The presence of oxidized bases in mRNAs causes translation errors and produces truncated proteins [10]. Bazin et al. [62] and Gao et al. [63] demonstrated that specific oxidation of a subset of transcripts was a prerequisite for dormancy release of sunflower and wheat seeds, respectively. It seems therefore that one of the role of ROS is to trigger direct oxidation of negative regulators of germination, as proposed by [10].

ROS can directly interact with cell wall polysaccharides and promote cell elongation in germinating seeds. The hydroxyl radical, in particular, is considered as a plant cell wall loosening agent which can directly cleave wall polysaccharides [90]. $^{\cdot}$OH can be formed either enzymatically (see previous section) or by Fenton reaction involving a transition metal [73]. In endospermic seeds, it has been demonstrated that endosperm cap weakening by ROS was an important process for allowing the elongating radicle to penetrate through the endosperm cap [72,37,91]. Interestingly this production is inhibited by ABA and promoted by GA and ethylene [72], thus underlying again the close connection between ROS and hormone signalling pathways. ROS have also been shown to be produced in cell walls of growing embryos, at the time of radicle protrusion [91], including for non-endospermic seed species [42]. Depending on the species, it has been proposed that cell wall peroxidases, NADPH oxidases or polyamine oxidases were involved in ROS production in the apoplast of elongating cells of the embryonic axis [72,92,37,93,46,94]. Independently of the mechanisms of production, there is growing evidence showing that apoplastic ROS production is necessary for initiating cell elongation in the growing radicle. Such feature should also been considered with the recent findings of Stamm et al. [86] who highlighted the role of GA in the spatiotemporal cell expansion mechanisms in Arabidopsis.

## ROS crosstalk with germination signalling pathways

Signalling ROS effect on germination cannot be considered as whole but must be regarded as a part of a complex signalling network. Germination and dormancy being tightly regulated by hormones [9] it is therefore highly relevant to estimate the role of ROS in the context of hormone signalling and metabolism. The relationship between ROS and the hormones involved in germination, *i.e.* mostly ABA and GA, is now well documented but needs to be considered under various angles. In one way, increase in ROS content, which is beneficial for germination, is associated with alteration of synthesis and signalling of ABA, the hormone which represses germination. It has been demonstrated that $H_2O_2$ accumulation in germinating seeds was associated with ABA degradation probably trough an activation of

ABA-8-hydroxylase, an ABA catalytic enzyme [26,30,33,53,79] even though a direct oxidation of ABA cannot be excluded [51,95,96]. ABA being regulating germination by its antagonistic interaction with GA it is worth noting that ROS have been reported to stimulate GA biosynthesis through a transcriptional effect [28,32,33,53,79]. In consequence ROS homeostasis has a direct effect on the hormonal balance ABA/GA in the favour of GA which in term induces germination. To get a complete view of the relationship ROS/hormones it is also necessary to consider the effect of hormones on ROS production and scavenging. Direct imbibition of seeds with ABA, the use of mutants altered in ABA synthesis or signalling and the comparison of dormant to non-dormant seeds have shown that ABA repress ROS production in seeds [23,27,27,35,51]. Conversely, GA treatment has been shown to induce ROS production [51,78]. The relationship between others hormones which are putatively playing a role in germination and dormancy is less documented. Ethylene is a gaseous plant hormone which stimulates the germination of dormant seeds of many species [97]. A synergic relationship between ROS and ethylene has been demonstrated in sunflower seeds, with ethylene triggering ROS production [51,52,97,98]. Even though other plant hormones are likely to lay role in germination, their possible interaction with ROS is not yet understood.

In plants, ROS, and mostly $H_2O_2$, have been reported to interact with other signalling pathways [1,4,99]. For example, mitogen-activated protein kinases (MAPK) can be activated by $H_2O_2$ accumulation and relay ROS signals [100], sometimes in connexion with hormone signaling [101]. $H_2O_2$ and $Ca^{2+}$ signaling pathways are closely linked and can act in concert to regulate the effect of plant hormones, such as the one of ABA on stomatal closure in response to drought [1]. Many examples have also highlighted that the relationship between NO and $H_2O_2$ can influence various plant developmental and processes and responses to biotic and abiotic factors [102,103]. A set of transcription factors relay the ROS message to transcriptome reprograming [104]. Members of MYB, DREB, ZAT, bHLH, WRKY, bZIP, and NAC families have been reported to be associated with ROS signalling in plants [1,105]. However all these interactions are poorly described in the context of seed germination, and among them only the relationship between ROS and NO has retain some attention some years ago [106].

Changes in ROS homeostasis can induce changes in seed gene expression. In plants the effect of ROS on transcriptomes have been studied using ROS-generating treatments or mutants altered in ROS scavenging/production [107]. A global meta-analysis of microarray data obtained in such conditions allowed to define transcriptional footprints and to design a so-called ROS wheel, in which co-regulated genes in response to ROS are clustered together [108]. This approach is relevant as it reveals oxidative components in a biological process using transcriptomic data. As an example, we have performed this analysis using the data obtained by Basbouss-Serhal et al. [109]. In this study the authors have identified a set of transcripts which became specifically associated with polysomes (i.e. the translatome) during imbibition of dormant and non-dormant Arabidopsis seeds. Such transcripts are likely to be translated and the corresponding proteins may then play a role in germination or dormancy. We have used the ROS wheel to investigate the ROS signature at the level of the translatome during seed imbibition (Table 2). The ROS wheel reveals the occurrence of ROS metabolism in seed germination, either in dormant or non-dormant seeds. Many transcripts range within the cluster III- High Light Early, especially in dormant seeds. This former cluster consists of transcription profiles triggered by high light exposures [108] and its over representation can appear surprising, but this is the largest cluster of the ROS wheel (more than 400 genes) and it also includes genes of response to heat or to $H_2O_2$, for example. Nevertheless the ROS wheel also allows the discrimination of dormant from non-dormant seeds. It is worth noting that it reveals the occurrence of retrograde signaling in non-dormant seeds only, a process in which ROS production in either chloroplasts or mitochondria control nuclear gene expression. This is in agreement with the recent findings of Ma et al. [18] who showed the role of mitochondrial ROS production in the germination process. The extended use of ROS footprints should help to better assess the involvement of ROS in seed germination.

The effects of ROS on transcriptional reprogramming during seed germination has been poorly investigated to date. In sunflower, a microarray analysis showed that treatment of dormant seeds by methylviologen, a ROS generating compound inducing dormancy release, modified the expression of 120 genes. Most of the identified transcripts were related to cell signalling components [51]. Interestingly genes down-regulated in methylviologen-treated seeds were involved in ABA signalling, thus suggesting an interaction between ROS and ABA signalling pathways at the transcriptional level.

## Conclusions

The beneficial role of ROS in the regulation of seed germination is increasingly well established with the series of studies reviewed here. This relationship is not always easy to address because the same ROS have versatile effects and can either favour or repress germination, which are simultaneously balanced with the adverse effects these molecules can have on cells. Figure 2 summarizes the main findings and hypotheses presented in this review. We propose that spatiotemporal regulation of ROS production in embryonic axis of imbibed seeds controls the ability to germinate. The 3 steps model presented Figure 2 suggests that sensing of environmental conditions is detected in the meristem area and that controlled production of ROS triggers germination mechanisms, *i.e.* activation of GA signalling pathway and oxidation of negative regulators of germination. At this early time point ROS homeostasis mostly depend upon production sources. The localized increases in ROS generates an unknown signal that can propagate along the embryonic axis and trigger cell elongation in the hypocotyl area at the onset of radicle protrusion. At late imbibition time, antioxidant systems participate to the control of ROS homeostasis and ROS are generated within the apoplast to participate to cell wall loosening. Further studies are required to better understand how environmental factors can fine tune ROS production, how the oxidative signal is translated at the subcellular level and how it can propagate from cell to cell. It is clear that ROS and hormones act in concert to regulate seed germination but the bases of this crosstalk are far from being understood. It will be of a particular interest to consider spatiotemporal ROS production with regards to the recently evidenced spatiotemporal mode of action of hormones in the germination process [86,110].

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## Competing Interests

The Author declares that there are no competing interests associated with the manuscript.

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**Table 1. Reported effects of reactive oxygen species on seed germination**

| **Context** | **Effect** | **Species** | **reference** |
|---|---|---|---|
| Zn and Arsenic stress | negative | *Anadenanthera peregrina* and *Myracrodruon urundeuva* | [12] |
| Germination | positive | apple | [13] |
| Dormancy alleviation (stratification) | positive | apple | [14] |
| Salt stress | negative | Arabidopsis | [15] |
| ABA cross talk | ABA positive regulator of rboh and ROS | Arabidopsis | [16] |
| Cd Stress | negative | Arabidopsis | [17] |
| Mitochondrial functionning | positive | Arabidopsis | [18] |
| Salt stress | positive | Arabidopsis | [19] |
| Seed dormancy and iron deficiency | positive | Arabidopsis | [20] |
| Germination/ABA | negative | Arabidopsis | [21] |
| Salt stress /ethylene | negative | Arabidopsis | [22] |
| Germination / light | positive | Arabidopsis | [23] |
| Dormancy | positive | Arabidopsis | [24] |
| Germination/ABA/AIA | positive | Arabidopsis | [25] |
| Germination ABA GA | positive | Arabidopsis | [26] |
| Germination/ABA signaling | positive | Arabidopsis | [27] |
| Dormancy ABA GA | positive | barley | [28] |
| Seed germination and dormancy | positive | barley | [29] |
| Germination / ABA signaling | positive | barley | [30] |
| Dormancy alleviation | positive | barley | [31] |
| Germination / GA/ NADPH oxidase | positive | barley | [32] |
| Germination / NADPH oxidase | positive | barley | [33] |

|  |  |  |  |
|---|---|---|---|
| Dormancy | positive | *Bidens pilosa* | [34] |
| Dormancy alleviation (stratification) | positive | *Bunium persicum* | [35] |
| Dormancy alleviation (stratification) | positive | *Hedysarum scoparium* | [36] |
| Germination /endosperm weakening | positive | lettuce | [37] |
| Mutagen agents | negative | maize | [38] |
| Dormancy alleviation by heat | positive | *Mesembryanthemum crystallinum* | [39] |
| Drought and salt stress | negative | Miscanthus | [40] |
| Germination/ABA | positive | pea | [41] |
| Germination | positive | Pea | [42] |
| High temperature, drought stress | negative | rice | [43] |
| Low phytic acid seed vigour | positive | rice | [44] |
| Dormancy alleviation (after-ripening) | positive | rice | [45] |
| Germination / NADPH oxidase | positive | rice | [46] |
| Osmotic and salt stress | negative | rice | [47] |
| Germination /ABA/GA | positive | rice | [48] |
| Germination / ethylene | positive | soybean | [49] |
| Dormancy alleviation (after ripening) | positive | sunflower | [50] |
| Dormancy alleviation /ABA/ ethylene | positive | sunflower | [51] |
| Dormancy / after-ripening | positive | sunflower | [7] |
| Dormancy | positive | sunflower | [52] |
| GA response | positive | tobacco | [53] |
| Germination | positive | *Vigna radiata* | [54] |
| Germination | positive | *Vigna radiata* | [55] |
| Seed vigour and GA signaling | positive | watermelon | [56] |
| Dormancy | positive | wheat | [57] |

**Table 2** mRNA abundance in the translatome of dormant and non-dormant Arabidopsis seeds compared to the clusters from the ROS wheel analysis of ROS responses [108].

GUN, genome uncoupled mutants (known plastid retrograde signaling components); HL Late, 3 to 8 h of high light exposure; HL Early, 30 min to 2 h high light exposure; ROS cell culture, mitochondrial electron transport/ATP synthase inhibitor or $H_2O_2$ treatment of cell cultures; ROS, direct application or indirect generation of ROS in plants; $^1O_2$, exposure to singlet oxygen; UVB Early, 15 min to 1 h of UV-B exposure, RBOHF, responses in rbohF mutant background; ROS Acclimation, redox mutants leading to long-term ROS stress. Translatome data are from Basbouss-Serhal et al. [109]. Dormant and non-dormant seeds were imbibed for 16 and 24 h at 25°C in the darkness.

| **ROS wheel clusters** | **mRNAs specifically found in the translatome of non-dormant seeds at** | | **dormant seeds at** | |
|---|---|---|---|---|
| | **16 h of imbibition** | **24 h of imbibition** | **16 h of imbibition** | **24 h of imbibition** |
| I-GUN retrograde | 22 | 2 | 4 | 4 |
| II - HL late | 3 | 3 | 1 | 3 |
| III - HL early | 36 | 14 | 79 | 61 |
| IV - ROS cell culture | 1 | 0 | 7 | 5 |
| V - ROS | 0 | 0 | 1 | 1 |
| VI - $^1O_2$-UVB early | 0 | 0 | 1 | 2 |
| VII - RBOHF | 0 | 0 | 0 | 0 |
| VIII - ROS acclimation | 1 | 1 | 1 | 0 |
| **Total** | **63** | **20** | **94** | **76** |

## Figure legends

**Figure 1. Dynamics of ROS production during Arabidopsis seed germination.**

A-C, ROS detection in the embryonic axis of non-dormant seeds after 6 (A), 16 (B) and 24 h (C) of imbibition at 25°C in the darkness. D-F, fluorescence intensity along a line running from the radicle tip (rt) to the hypocotyl shootward region (h), as shown in A-C, in seeds imbibed for 6 (D), 16 (E) and 24 h (F). ROS production was detected by fluorescence using 5-(and-6)-chloromethyl-20,70-dichlorofluorescein diacetate (DCFH-DA) and visualized by confocal microscopy using Leica TCS SP5 confocal microscope, as described in [24] and [49]. Fluorescence intensity was estimated using Image-J software.

**Figure 2. A spatiotemporal model explaining the signalling role of ROS in seed germination.**

1. At the beginning of imbibition, environmental conditions are perceived by the imbibed seed at the meristematic region where a limited ROS production occurs through mitochondria electron transfer chain and NADPH oxidase activity. It induces the oxidation of negative regulators of germination, activation of gibberellin (GA) signalling and inactivation of abscisic acid (ABA) signalling. 2. The oxidative signal is propagated along the embryonic axis to the hypocotyl area. 3. At the onset of radicle protrusion cells elongate in the hypocotyl region after cell wall loosening caused by ROS production and apoplastic ROS (aROS) generation in the cell wall. Superoxide dismutase (SOD), catalase (CAT) and glutathion reductase (GR) are activated to prevent excessive ROS accumulation. Germination can then proceeds.

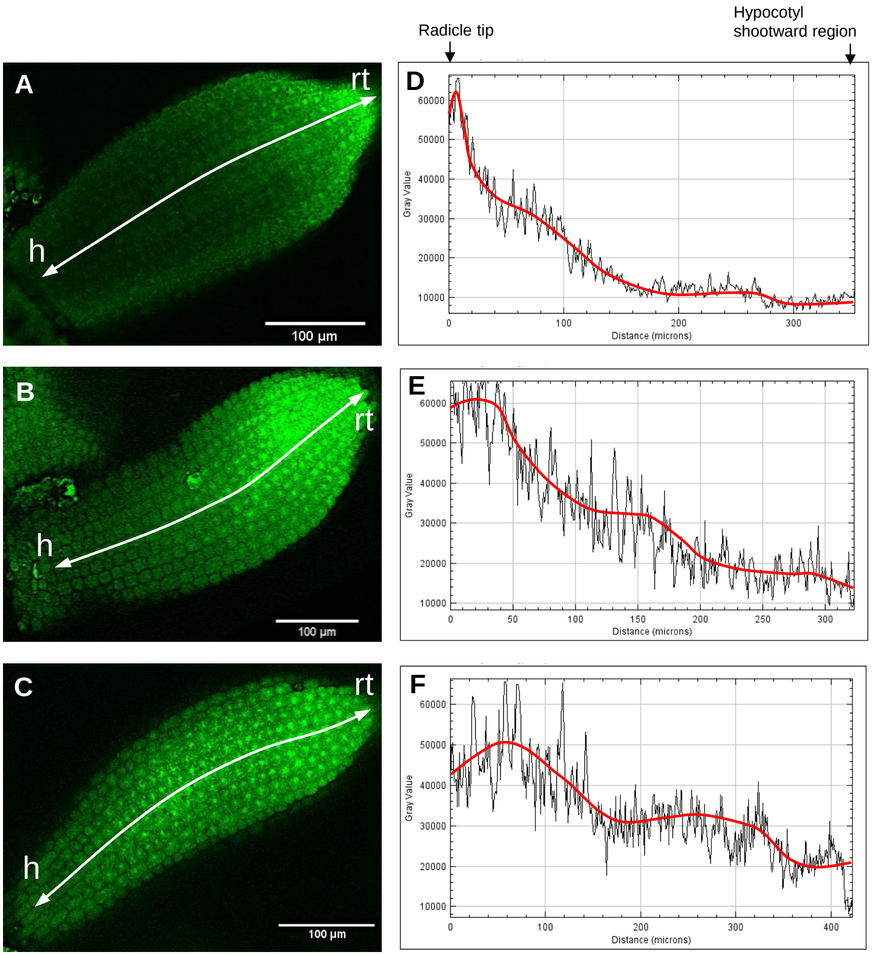

**Figure 1. Dynamics of ROS production during Arabidopsis seed germination.**
A-C, ROS detection in the embryonic axis of non-dormant seeds after 6 (A), 16 (B) and 24 h (C) of imbibition at 25°C in the darkness. D-F, fluorescence intensity along a line running from the radicle tip (rt) to the hypocotyl shootward region (h), as shown in A-C, in seeds imbibed for 6 (D), 16 (E) and 24 h (F). ROS production was detected by fluorescence using 5-(and-6)-chloromethyl-20,70-dichlorofluorescein diacetate (DCFH-DA) and visualized by confocal microscopy using Leica TCS SP5 confocal microscope, as described in [24] and [49]. Fluorescence intensity was estimated using Image-J software.

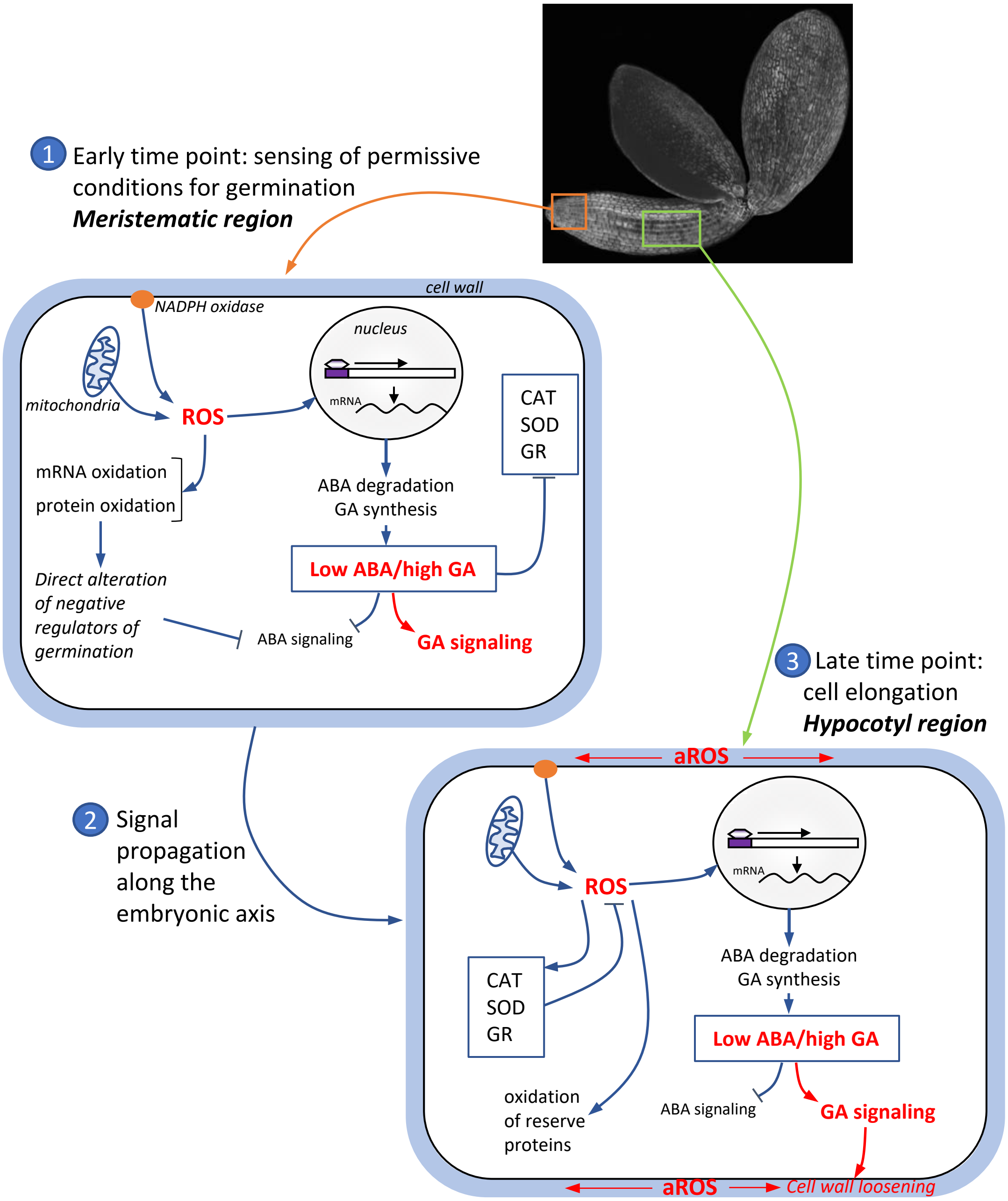

**Figure 2. A spatiotemporal model explaining the signalling role of ROS in seed germination.** 1. At the beginning of imbibition, environmental conditions are perceived by the imbibed seed at the meristematic region where a limited ROS production occurs through mitochondria electron transfer chain and NADPH oxidase activity. It induces the oxidation of negative regulators of germination, activation of gibberellin (GA) signalling and inactivation of abscisic acid (ABA) signalling. 2. The oxidative signal is propagated along the embryonic axis to the hypocotyl area. 3. At the onset of radicle protrusion cells elongate in the hypocotyl region after cell wall loosening caused by ROS production and apoplastic ROS (aROS) generation in the cell wall. Superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) are activated to prevent excessive ROS accumulation. Germination can then proceeds.

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