Review / Revue

From intracellular signaling networks to cell death: the dual role of reactive oxygen species in seed physiology

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Available online 4 September 2008

Presented by Michel Thellier

Abstract

Reactive Oxygen Species (ROS) are continuously produced during seed development, from embryogenesis to germination, but also during seed storage. ROS play a dual role in seed physiology behaving, on the one hand, as actors of cellular signaling pathways and, on the other hand, as toxic products that accumulate under stress conditions. ROS, provided that their amount is tightly regulated by the balance between production and scavenging, appear now as being beneficial for germination, and in particular to act as a positive signal for seed dormancy release. Such an effect might result from the interplay between ROS and hormone signaling pathways thus leading to changes in gene expression or in cellular redox status. We also propose that changes in ROS homeostasis would play a role in perception of environmental factors by seeds during their germination, and thus act as a signal controlling the completion of germination. However, uncontrolled accumulation of ROS is likely to occur during seed aging or seed desiccation thus leading to oxidative damage toward a wide range of biomolecules and ultimately to necroses and cell death. We present here the concept of the "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. Above or below the "oxidative window for germination", weak or high amounts of ROS, respectively, would not permit progress toward germination. ***To cite this article: C. Bailly et al., C. R. Biologies 331 (2008).***

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Résumé

Des réseaux de signalisation intracellulaire à la mort cellulaire : le double rôle des espèces réactives de l'oxygène dans la physiologie des semences. Les espèces réactives de l'oxygène (ROS) sont produites continuellement au cours du développement des semences, de l'embryogenèse à la germination, mais aussi au cours de leur stockage. Ces molécules jouent un double rôle dans la physiologie des semences se comportant, d'une part, comme des acteurs des voies de signalisation cellulaire et, d'autre part, comme des produits toxiques s'accumulant sous conditions de stress. Les ROS, à condition que leur niveau d'accumulation soit finement régulé par un équilibre entre production et élimination, apparaissent aujourd'hui être bénéfiques pour la germination et, en particulier, agissent comme signal positif capable de lever la dormance des semences. Un tel effet pourrait résulter de l'interaction entre les ROS et les voies de signalisation hormonales conduisant ainsi à des changements dans l'expression des gènes ou de l'état redox cellulaire. Nous proposons également que les changements dans l'homéostasie des ROS jouent un rôle, pendant la

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germination, dans la perception par les semences des facteurs environnementaux, et donc agissent comme un signal contrôlant la germination. Toutefois, l'accumulation incontrôlée de ROS est susceptible de se produire au cours du vieillissement des semences ou de leur dessiccation en fin de maturation sur la plante mère, ce qui conduit à des dommages oxydatifs envers un grand nombre de biomolécules et, de manière ultime, à des nécroses et à la mort cellulaire. Nous présentons ici le nouveau concept d'une « fenêtre oxydative pour la germination », qui lie la réalisation d'événements cellulaires associés à la germination à des niveaux critiques de ROS compris entre une limite basse et une limite haute. En deçà ou delà de ces limites, des niveaux trop faibles ou trop élevés de ROS ne permettraient pas la réalisation de la germination. ***Pour citer cet article : C. Bailly et al., C. R. Biologies 331 (2008).***

1. Introduction

The plant "reactive oxygen species (ROS) gene network" regulates the cellular homeostasis of these compounds [1]. In Arabidopsis, this network includes ca. 150 genes whose function is to control ROS production and scavenging [2], thus preventing oxidative damage and allowing ROS to function as signal transduction mediators [3]. Indeed, although ROS such as $O_2^{\cdot-}$, H_2O_2 or $\cdot OH$, are long considered as causing oxidative damage to lipids, proteins and nucleic acids, increasing evidence indicates that they also function as signaling molecules in plants, notably acting as regulators of growth and development, programmed cell death, hormone signaling and responses to biotic and abiotic stresses [2].

Although the dual role of ROS is now quite well documented in plants, there exists much less studies on seeds in this area. In the past, ROS production in seeds has been widely regarded as a symptom of oxidative stress thus implying a deleterious process, which might potentially lead to cell death. This is probably the case for seed desiccation and aging, which can be considered as stressful situations. In several cases, however, ROS do not act as harmful compounds and instead their fine-tuning plays a key role in the adjustment of gene expression. This would be particularly relevant for seeds since their genetic program runs into dramatic shifts, such as the transition from a developmental to a germinative mode and the alleviation of dormancy. To date, plant hormones such as abscisic acid (ABA) and gibberellins (GAs) are considered as being the major signaling actors in these processes [4,5]. However, several studies have shown that ROS can also interplay with the hormonal signaling pathways [6]. At last, the full process of germination being strictly controlled by the environmental conditions, it is also highly probable that external factors might control the balance between ROS scavenging and production, being thus translated at the cellular level through the changes in ROS homeostasis.

In this review, we wish to revisit the role of ROS in seed physiology, notably with regards to their duality as toxic and signaling molecules.

2. ROS production in seeds

Based on our previous work dealing with the involvement of ROS in seed physiology, we can provide an overview of the changes in ROS content throughout all the stages of the sunflower (*Helianthus annuus*) seed life, as depicted in Fig. 1 for hydrogen peroxide content. Compared to an initial value in the range of the nanomole/g DW found in dry mature dormant seeds, H_2O_2 content varies dramatically depending on the physiological state of the seed. This content is quite high at the beginning of seed development, probably because the moisture content is high enough to allow metabolic activities, but subsequently decreases during seed desiccation. Also, seed imbibition entails a large increase in H_2O_2 content. The basal production of hydrogen peroxide during seed imbibition, which also occurs in imbibed dormant seeds, mainly results from metabolism resumption as approx. 2–3% of the oxygen used by the mitochondria is converted into superoxide anion and hydrogen peroxide [7]. However, the ability of seeds to germinate seems to be linked to the accumulation of a critical level of H_2O_2, since this molecule is produced at a higher level in non-dormant imbibed seeds than in dormant imbibed seeds (Fig. 1). This suggests that there exists a differential regulation of ROS producing and scavenging mechanisms in these two types of seeds. Interestingly, H_2O_2 also accumulates during dry storage of seeds, which is, in a first step, associated with dormancy release during after-ripening [7], and then, in a second step, with aging during prolonged storage [8]. Therefore, hydrogen peroxide can in turn be either beneficial (alleviation of dormancy) or detrimental (aging)
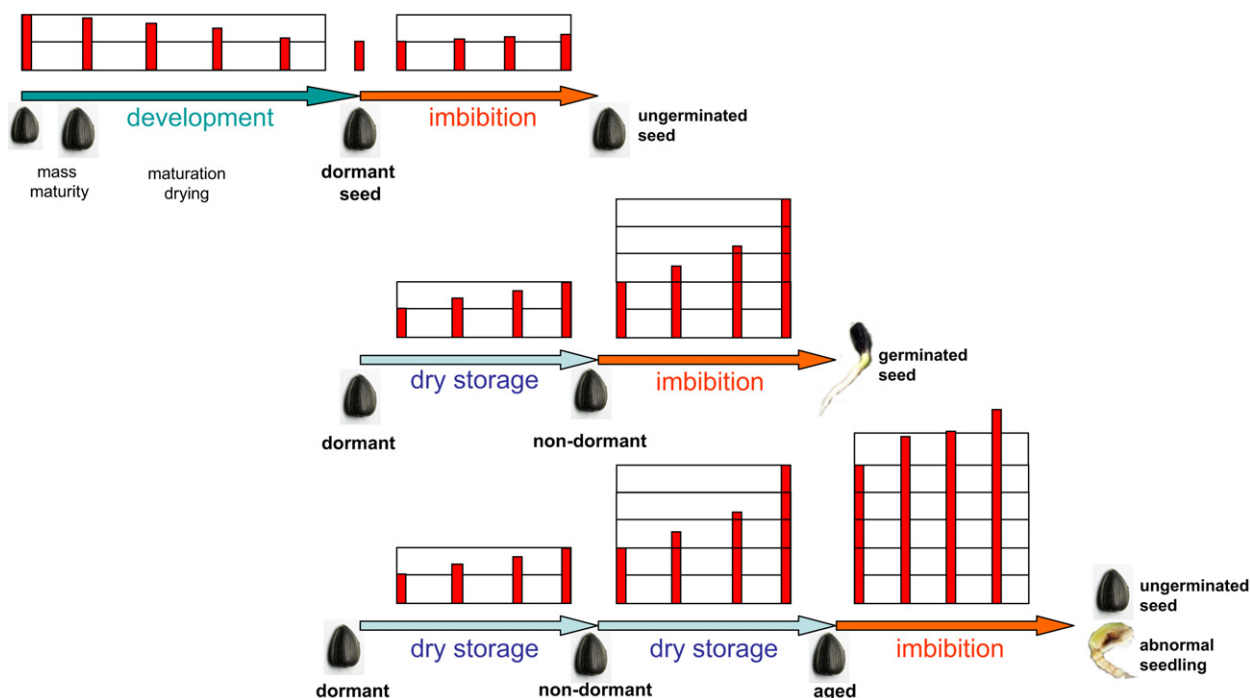
Fig. 1. Schematic representation of changes in H_2O_2 content in sunflower seeds during their development, after-ripening, germination and aging. H_2O_2 content is represented as bars, as a function of the value measured in dormant dry mature seeds at harvest. The graph shows that H_2O_2 content decreases during seed maturation drying and remains constant during imbibition if seeds are dormant, i.e., immediately after harvest. Dry storage results in an increase in H_2O_2 associated first with after-ripening (dormancy alleviation) then with aging (seed deterioration). Seed germination requires a controlled H_2O_2 production while imbibition of aged seeds is associated with an uncontrolled accumulation of H_2O_2 thus preventing germination (radicle protrusion) or leading to abnormal seedlings. Hydrogen peroxide contents were normalized using the dormant seed as a control and are adapted from values measured by Bailly et al. [55,60,63] and Oracz et al. [7].

for germination, depending on its accumulation level within the embryonic cells.

Besides documenting the variability of ROS production at the various stages of seed life, our data also highlight that sources of ROS fluctuate substantially from the beginning of embryogenesis to the end of germination. Such a variability of the ROS production sources is related to the molecular mobility and to the cytoplasmic viscosity, which are known to govern the occurrence and rate of metabolic reactions [9]. They both vary dramatically in seed tissues from a glassy state that prevails in the dry tissues to a fully imbibed state with cells containing free water. In the dry state enzymes are probably not active and in this case ROS probably originate from non-enzymatic reactions such as lipid peroxidation [10] or Amadori and Maillard reactions [11]. In contrast, in hydrated seeds, potentially all the cell compartments may become sources of ROS. Thus, these molecules can be produced during the catabolism of lipids (glyoxysomes) and purines (peroxisomes), respiratory activity (mitochondria), electron transfer in photosystems (chloroplasts) or through the activity of NADPH oxidase (plasma membrane), amine oxidase and peroxidase

(cell wall) or cytochrome P450 (cytosol) [3,12]. One should emphasizes that the variability in ROS sources makes the study of the regulation of ROS accumulation quite complex, especially knowing that antioxidant mechanisms have also to be considered [13]. Finally, for being able to play a role in cell functioning, ROS much reach their cellular targets. As for their production, the changes in cytoplasmic viscosity would also play a key role in the signaling vs deleterious roles of ROS. In the dry state of seeds ROS would tend to reach their targets close to their production sites. In contrast, the presence of free water in hydrated seeds would allow the ROS, or at least their long living forms such as H_2O_2, to travel within the seed to reach targets far from their production sites. ROS produced in the dry state may by this way become effective as cellular messenger only when the seeds become hydrated, i.e. during germination.

3. Signaling roles of ROS in seed germination and dormancy

A signaling role of ROS in seed physiology has recently been documented, notably in the regulation of

germination and dormancy [6,13]. Increased generation of ROS seems to be a common feature of the so-called germination *sensu stricto* phase (phase II of the full process of germination), which is the critical step of the process since it involves the activation of a regulatory system controlled by intrinsic (i.e., dormancy) and extrinsic (i.e., environmental conditions, such as temperature, oxygen and water availability) factors. Hence, hydrogen peroxide [14–20], nitric oxide (NO) [21,22], hydroxyl radicals [15] and superoxide radicals [14,15] have been shown to accumulate during seed germination in various species. Many hypotheses have been put forward for attributing a function to these compounds in the germination process, including their contribution to cell wall loosening during endosperm weakening, programmed cell death of aleurone layer of cereal grains or protection of the emerging seedling against pathogens [6]. At the cellular level, ROS are also known to regulate the cellular redox status, to cause the oxidation of proteins and to trigger specific gene expression.

We propose here that ROS also play a key role in the completion of germination and that they should be considered as messengers or transmitters of environmental cues during seed germination. The success of germination tightly depends on external factors such as temperature, light, oxygen and water availability. This implies that seeds must be endowed with internal sensors able to translate the environmental cues into the cellular mechanisms leading to germination. In whole plants it has been widely demonstrated that abiotic stresses such as heat and cold stress, UV or hypoxia cause the production of ROS [23]. We assume that germination in non-optimal conditions is a stressful situation, associated with ROS generation that in turn would prevent radicle emergence. Such a role of ROS would therefore be at the interface between signaling and deleterious effect. For example, for soybean (*Glycine max*) seeds that are chilling sensitive and hardly germinate at temperatures below 10 °C, Pozmik et al. [24] observed that prolonged imbibition at 5 °C inhibits germination and leads to oxidative stress and lipid peroxidation. Similar observations were made for sunflower seeds during their germination at 5 °C, a suboptimal temperature for this species (Bailly, unpublished data). Also, incubation of seeds at too high temperatures induces an oxidative stress in numerous species. For example, inhibition of germination of sunflower seeds above 40 °C was associated with oxidative stress and lipid peroxidation [25]. In natural conditions, seed germination can also be prevented in the presence of various chemicals present in the soil and arising from root exudation, leaching and volatilization, and from passive decomposition of plant residues.

These allelochemicals often belong to the group of secondary metabolites [26]. We have shown that the phytotoxic effect of sunflower allelochemicals on germination of mustard (*Sinapis alba*) seeds was associated with an increase in the cell membrane permeability, lipid peroxidation, accumulation of H_2O_2 and alteration in the activities of antioxidant enzymes [27]. Seed priming, which consists in a controlled imbibition preventing radicle protrusion, is an interesting tool for studying the mechanisms associated with germination *sensu stricto*. It can also be considered as a stress since water is not provided in a sufficient amount to allow radicle protrusion through the seed coat. In sunflower, seed priming is associated with stimulation in antioxidant enzyme activities, mainly catalase and superoxide dismutase, which is very similar to what has been found in whole plants challenged by a drought stress [28].

However, under appropriate conditions of germination, ROS must be present in sufficient levels for allowing completion of this process. This is for example illustrated by the use of an NADPH oxidase inhibitor, diphenyleneiodonium, which decreases the levels of superoxide ion and hydrogen peroxide during imbibition and prevents the germination of rice (*Oryza sativa*) [29] and of warm-season C4-grasses [22]. But this is also clearly highlighted from studies on the mechanisms of seed dormancy. These mechanisms involve complex regulatory networks of gene regulation that are under the control of plant hormones [30–32]. With regards to the data dealing with ROS metabolism during dormancy imposition and release, Bailly [13] proposed that ROS could be a ubiquitous signal involved in dormancy alleviation and that they could facilitate the shift from a dormant to a non-dormant status in seeds. In many species, release of dormancy may occur during storage of seeds in dry conditions (after-ripening). This process is very intriguing because in such conditions water is probably not available for biochemical reactions. Oracz et al. [7] and El-Maarouf-Bouteau et al. [33] provided evidence for the existence of a tight correlation between sunflower seed dormancy alleviation in the dry state, accumulation of ROS and peroxidation products and irreversible protein oxidation (carbonylation) in cells of embryonic axes. These data suggested that ROS play a role of signal in dormancy alleviation, presumably allowing a shift from a metabolism characteristic of dormant seeds to a metabolism characteristic of non-dormant (after ripened) seeds. As mentioned above, in dry seed tissues the mechanisms responsible for ROS production are probably non-enzymatic. Yet, enzymatic reactions cannot be totally excluded since enzymes such as lipoxygenase can be active when wa-

ter activity is as low as 0.4, i.e., at MC below 0.1 g/g DW for many species [34]. Furthermore, the presence of localized hydrated pockets within the dry seed is likely to exist [35]. There are also several lines of evidence showing that ROS play a role during germination of sunflower seeds, as related to their dormancy level. Thus, non-dormant embryonic axes accumulate more ROS than dormant ones during their imbibition. For example, cyanide treatment that alleviates sunflower seed dormancy leads to ROS generation, while treatment in the presence of the ROS generating compound methylviologen provokes dormant seed germination [7, 33]. Finally, exogenous H_2O_2 stimulates the germination of dormant seeds of barley (*Hordeum vulgare*) [36–38], rice [39], apple (*Malus domestica*) [40] and *Zinnia elegans* [41]. Additional evidence on the putative role of ROS in dormancy control comes from studies on the interaction of these compounds with plant hormones as ABA, GA, ethylene, auxin or brassinosteroids, all of which being well known for playing a major role in seed dormancy and germination [31,42]. ROS could play a key role in the transduction of the hormone signal since the interaction between hormones and ROS in other developmentally controlled processes in plants has been reported (for review see [43]). The relationship between H_2O_2 and ABA is the most credible and documented. Thus, H_2O_2 has been shown to inactivate ABI1 and ABI2 type 2C protein phosphatases, two enzymes involved in ABA signaling [44,45]. Furthermore, treatment of dormant barley seeds with hydrogen peroxide results in a decrease in endogenous ABA level [36,38] and alleviation of apple embryo dormancy by cyanide induces an increase in H_2O_2 level and a decrease in ABA content [40]. Interaction of ROS with ethylene and GAs, two hormones that are involved in dormancy alleviation of seeds of various species, has also been documented. It appears that ethylene and ROS transduction pathways share common components, GA_3 modifying the redox status of aleurone proteins during germination and ROS stimulating the expression of genes involved in GA signaling (for review see [6]). Altogether these data suggest that ROS act directly, or as messengers of hormonal networks, as signaling molecules involved in the transition from a dormant to a non-dormant state. However, the putative mechanisms of action of ROS at the cellular level in the regulation of dormancy are far from being resolved. ROS have been shown to trigger protein oxidation during dormancy alleviation [7], but they can also control MAP kinase cascade activation, inhibit phosphatases, activate Ca^{2+} channels and Ca^{2+}-binding proteins, modulate redox potential and gene expression.

4. Deleterious effects of ROS in seeds

The deleterious role of ROS in seeds is due to their high reactivity toward biomolecules, including proteins, sugars, lipids and nucleic acids. The shift from a signaling to a deleterious role is related to the accumulation of these ROS above a threshold level that leads to various cellular alterations and damage. Two physiological processes occurring in seeds appear as being tightly linked to the deleterious role of ROS, seed desiccation and seed aging.

Dehydration to a MC around 0.04–0.12 g H_2O g^{-1} DW is the normal terminal event in the development of orthodox seeds, i.e., seeds that tolerate desiccation. In fact, the desiccation tolerance of these seeds, which is generally acquired during seed filling and lost during seed germination (after radicle protrusion), depends on their physiological state [46,47]. In contrast, other seeds named recalcitrant have a high MC and do not withstand intensive dehydration [46,47]. Oxidative processes and free radicals are usually considered to be involved in molecular and cellular damage induced by a wide range of stresses including dehydration [48,49]. In desiccation-sensitive organs such as recalcitrant seeds [50] and seedlings of maize (*Zea mays*) [51] and wheat (*Triticum* spp.) [52,53], water loss is associated with an accumulation of free radicals and lipid peroxidation, and substantial damage is then evident at the level of cell structure [47,49,54]. The free radical damage hypothesis of desiccation injury postulates that various protective mechanisms become progressively unable to protect the cell structures against ROS generation during water loss and subsequent rehydration [49]. In wheat seedlings for example the activity of the antioxidant enzymes, glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT), increases during dehydration in both tolerant and sensitive tissues, but these activities are sustained on rehydration only in the tolerant tissues [52,53]. During development of orthodox seeds, acquisition of desiccation tolerance is associated with various biochemical, cellular and molecular events including synthesis of specific proteins, accumulation of oligosaccharides, and activation of antioxidant defense systems [46,47]. Among these mechanisms, the antioxidant systems seem to play a key role. During maturation-drying for example the embryo H_2O_2 content decreases in sunflower [55] or remains very low in wheat [56] probably due to a reduction of mitochondrial activity, the main source of H_2O_2 generation, and to an antioxidant defense system sufficiently efficient to control ROS accumulation during water loss. In addition, in sunflower, our results suggest that H_2O_2 may
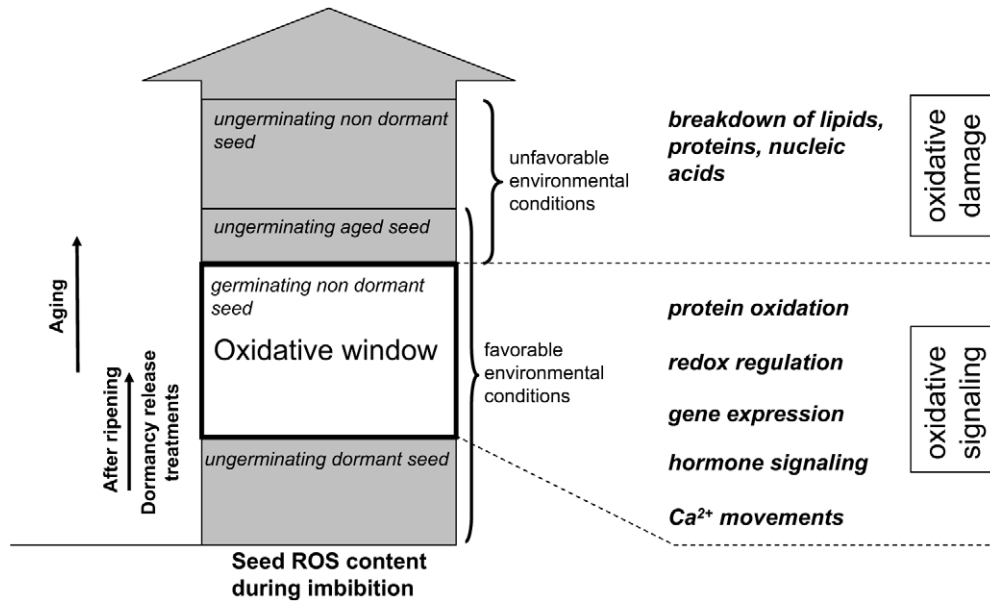
Fig. 2. The oxidative window: a model to account for the dual role of ROS in seed physiology. The amount of ROS in seeds during their imbibition is represented as boxes. Seed germination is only possible when the ROS content is enclosed in the oxidative window. Below this window, i.e. in dormant seeds, the amount of ROS during imbibition is too low for allowing germination. Alleviation of dormancy (by after-ripening, in the dry state, or by dormancy alleviation treatments, during imbibition) leads to an increase of the cellular level of ROS during seed imbibition thus ensuring germination completion, owing to the ROS signaling role, as mentioned on the right part of the figure. Above this window, ROS content is too high, because seeds are aged or placed in inappropriate environmental conditions during their imbibition (ROS sensing of environmental cues). In this case, ROS become deleterious and cause cellular oxidative damage that prevent or delay germination.

also play a role in the regulation of *CAT* gene expression and transduction pathway of the dehydration signal, probably via a mechanism close to that described by Polidoros and Scandalios [57], i.e., through ARE-like motifs. In bean (*Phaseolus vulgaris*), acquisition of drying tolerance is also related to a reorientation of the enzymatic antioxidant defense system, drying tolerant dried seeds displaying high CAT and GR activities and low SOD and APX (ascorbate peroxidase) activities while the opposite is observed in immature dried seeds [58].

ROS are also suspected to be involved in loss of seed viability since a long time [10,59] and the free radical hypothesis of aging remains popular and widely acknowledged. This indeed makes sense and the bases of seed aging are very similar to those found in other organisms. The theory postulates that during storage loss of viability results from a concomitant accumulation of ROS, associated damage, and a loss of the antioxidant mechanisms, thus causing uncontrolled oxidative damage during seed imbibition. This assumption has been supported by many studies carried out with a wide range of seed species. For example, in sunflower Bailly et al. [17,60,61] have shown that a loss of vigor and viability was mainly associated with a loss of catalase activity

and therefore with an altered capacity of hydrogen peroxide detoxification in the early steps of seed imbibition leading to lipid peroxidation. However, for some other species, it appears, as for example for wheat [62], that loss of viability is not always associated with lipid peroxidation, either directed against lipid membranes or lipid reserves. In sunflower seeds, we have shown that the mechanism of loss of viability during aging depends on the seed moisture content. Below a threshold value of ca. 0.21 g H_2O g DW^{-1} hydrogen peroxide accumulated rapidly within the seeds placed at 35 °C, but without causing lipid peroxidation. This suggests that, in this case, oxidative damage are directed toward the non-lipidic cellular fraction, i.e., presumably proteins or nucleic acids. At the opposite, when seed MC is higher than the threshold value mentioned above, loss of seed viability was clearly associated with lipid peroxidation. These data allow pointing out the plasticity of the mechanisms involved in seed loss viability. They may in part explain the controversy about the role of the oxidative processes in seed aging. It is likely that targets of ROS may vary as a function of seed MC, and we do recommend paying attention to the experimental conditions used to assess the mechanisms of seed aging.

5. Concluding remarks

Our knowledge about the roles of ROS in seed physiology allows us to present a comprehensive view of their mechanism of action, which brings a new view on the data presented in Fig. 1. We propose here the concept of an "oxidative window" for germination. A working scheme illustrating this concept is presented in Fig. 2. This window defines a critical level of ROS not to overcome, which would otherwise prevent germination, and a ROS threshold level, below which germination (radicle protrusion) cannot occur. Within this oxidative window ROS may play a role in cell signaling by interplaying with the hormone signaling pathways or by triggering the cellular events associated to ROS signalization, such as gene expression, calcium movements or control of redox status. In inappropriate environmental conditions or consequently to seed aging the amount of ROS in seed tissues exceeds the upper limit of the oxidative window, which leads to oxidative damage and inhibits germination or leads to abnormal seedlings. At the opposite, cellular signaling associated to the mechanism of seed germination is not triggered if the amount of ROS is too low, i.e., as in dormant seeds. Future challenge of studies dealing with ROS in seeds will have to take into account the diversity of the roles of these compounds. It will also be of particular interest to properly document the putative sources of these compounds, to identify their cellular targets and to determine if they are the signal linking environmental cues to hormone signalization.

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