Participation of Hydrogen Peroxide and Nitric Oxide in Improvement of Seed Germination Performance Under Unfavourable Conditions

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Contents

Abstract Seed germination is a complex process. Upon imbibition, many factors including phytohormones (gibberellin and abscisic acid) and reactive oxygen and nitrogen species [hydrogen peroxide (H_2O_2) and nitric oxide (NO), respectively] are involved in a complicated web of interactions. While there are some impressive recent progresses made in our understanding of these interactions, it is also of great interest to investigate treatments that help seeds with difficulties to germinate under unfavourable conditions including abiotic stress factors such as chilling and heavy metals. In this chapter, an update and critical interpretations of some recent investigations into the relationships among H_2O_2, NO, catalase activity and gene expression in cold stratification, light signal and abiotic stress are provided.

Keywords Abscisic acid (ABA) · Catalase · Chilling stress · Cold stratification · Gibberellic acid · Heme oxygenase · NADPH oxidase · NO donor · Phytochrome

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1 Introduction

Emergence of the radicle through the seed coat and other seed structures that may be enclosing or associated with the embryo is the first visible sign that a seed has completed germination (Bewley et al. 2013). This is a critical step towards the successful natural regeneration of plants in the wild as well as crop production. Seeds from some plant species may exhibit a low germination rate under otherwise favourable germination conditions for many other species. This problem has long been the subject of many studies. The possible reasons for a low rate of seed germination can be varied (Finch-Savage and Leubner-Metzger 2006; Bewley et al. 2013). For example, there may be a need for tuning in with certain environmental factors including light and cold temperature before the germination-associated processes in the hydrated seeds can be initiated and eventually culminated in radicle protrusion through the surrounding seed structures. Seeds may be sown under unfavourable growth conditions or stress such as chilling, salinity or heavy metals, and therefore their germination performance may be impaired. The storage conditions of seeds after harvest may not be appropriate and could lead to impairment in seed germination performance. In the literature, there is a classical view about the antagonism of two phytohormones, gibberellins (GA) and abscisic acid (ABA), in the decision for a seed to germinate or not (Nelson and Steber 2017). In the seeds with low germination performance for whatever reasons, the metabolism and signalling of ABA may predominate and ensure the no germination state (Finkelstein and Lynch 2000).

The focus on a few seeds as model lab systems, including *Arabidopsis thaliana*, has recently led to some rapid and impressive advances in our understanding of the interplay between reactive nitrogen species, particularly nitric oxide (NO) signalling and crosstalk with GA and ABA, occurring prior to the completion of seed germination (Nonogaki 2014, 2017). There has also been a great deal of investigations into the involvement of hydrogen peroxide (H_2O_2) in the regulation of seed germination. There may be a universal germination mechanism in different seeds. It is of great practical interest to be able to apply this knowledge to improve germination of seeds that is adversely affected by abiotic stress or less than favourable germination conditions. In this chapter, the objective is to provide an update of insights gained from the most recent papers (mainly published in between 2017 and 2018) on the participation of NO and H_2O_2 in seed germination under these conditions.

2 Cold Stratification

Freshly harvested *Arabidopsis thaliana* seeds (from wild-type plants of ecotype Col-0) were dormant (1% germinable seeds), but a majority of the seeds germinated after cold stratification at 4 °C for a few days (Bethke et al. 2004). However, supplementation of 4 or 5 mM hydrogen peroxide (H_2O_2) to a plant tissue culture

medium for seed germination led to a reduction in *Arabidopsis* seed germination by about 20 and 50%, respectively (Bi et al. 2017). Similar to the exogenous application of H_2O_2, supplementation of 3–10 mM of an inhibitor of catalase activity, 3-amino-1,2,4 triazole (3-AT), led to inhibition of the germination of the wild-type *Arabidopsis* seeds by about 30% (Bi et al. 2017). Catalase activity and H_2O_2 content in wild-type seeds treated with 5 mM 3-AT were only one-third and 80%, respectively, of those in the seeds germinated in the absence of the catalase activity inhibitor. This suggests that high catalase activity seems to be associated with a positive role in *Arabidopsis* seed germination. However, it is more complex to interpret the possible role of H_2O_2 content in cold-stratified *Arabidopsis* seed germination based on this result. Firstly, it is not readily clear why inhibiting catalase activity by 3-AT could lead to a reduction in endogenous H_2O_2 content in cold-stratified *Arabidopsis* seeds as the opposite would be expected to happen.

Since abscisic acid (ABA) is well-known to be associated with germination inhibition, it is of interest to probe the relationship of catalase activity and endogenous H_2O_2 content with ABA signalling in germinating *Arabidopsis* seeds after cold stratification (Bi et al. 2017). Abscisic acid (ABA)-insensitive 5 (ABI5) is a basic leucine zipper transcription factor. It is the core component of the ABA signalling pathway and has been shown to affect the expression of several ABA-responsive genes (Skubacz et al. 2016). In some earlier studies, for example, ABI5 was found to interact with the promoters of some ABA responsive genes including several late-embryogenesis-abundant genes during seed germination (Finkelstein and Lynch 2000). Recently, it was revealed that ABI5 could bind directly to the promoter of a catalase gene, *CAT1* but not *CAT2*, in the *Arabidopsis* genome. Furthermore, the expression of *CAT1*was also increased by ABI5 (Bi et al. 2017).

The possible roles of catalase activity and H_2O_2 content in cold-stratified *Arabidopsis* seeds were evaluated further using *Arabidopsis* plant lines with perturbation in sensitivity to ABA and the ABA signalling pathway. The plant lines that were investigated included mutant plants called *abi5-1* and *abi7*, which harbour mutations in the *ABI5* gene loci, and *ABI5*-overexpression lines. Based on the results obtained, it seems that the sensitivity of cold-stratified *Arabidopsis* seeds to exogenous H_2O_2 was dependent on ABA signalling (Bi et al. 2017). The cold-stratified seeds of *abi5-1* and *abi7* exhibited significantly lower germination percentages than wild type in response to exogenous application of 3–5 mM of H_2O_2. Conversely, overexpression of *ABI5* seemed to be associated with the insensitivity to the germination inhibitory effect of exogenous application of H_2O_2. These results would seem to be consistent with the possibility that catalase activity played a positive role in the germination of the cold-stratified *Arabidopsis* seed. Based on the link between *ABI5* and *catalase* gene expression, there would be an elevated level of catalase activity in *ABI5*-overexpression lines. Therefore any excess H_2O_2 from exogenous application could be decomposed so that germination process in the cold-stratified seeds was insensitive to the excess H_2O_2 (Bi et al. 2017).

Consistent with this, the seeds of the ABI5-overexpression lines and the *abi5-1* mutant plants exhibited a higher and lower catalase activity, respectively, than the

wild type (Bi et al. 2017). This finding was correlated with that of exogenous application of H_2O_2 having little or more germination inhibitory effect on the overexpression lines or the mutant plants, respectively, than the wild type. This result was in agreement with the results on the germination responses to 3 to 10 mM 3-AT of the cold-stratified seeds of *abi5-1* and *abi7* and ABI5-overexpression lines compared to wild type. Their germination responses mirrored those of the plant lines exposed to exogenous application of H_2O_2 (Bi et al. 2017). Therefore, it seemed that inhibition of catalase activity by 3-AT, like the seed germination response to exogenous application of H_2O_2, was also dependent on ABI5 expression levels (Bi et al. 2017). Catalase in cold-stratified *Arabidopsis* seeds would be working downstream of ABI5 in germinating seeds.

There is, however, evidence that calls for some caution in accepting a positive role for catalase in *Arabidopsis* seed germination. *ABI5* expression levels were linked to gene expression of *CAT1* which was higher in the cold-stratified seeds of the ABI5-overexpression lines than wild type. The cold-stratified *abi5-1* seeds exhibited a lower level of *CAT1* expression than wild type. As expected from the lack of interaction between *ABI5* and the promoter of *CAT2* in a yeast-one hybrid system, there was no difference in the expression levels of *CAT2* in the wild type and the *abi5-1* and *ABI5*-overexpression lines. Interestingly, the remaining member of the *CAT* gene family, *CAT3*, exhibited the lowest and highest expression levels in the seeds of the wild type and *abi5-1*, respectively. As far as germination percentage was concerned, there was, however, no difference exhibited by the seeds of wild type, *abi5-1* and *ABI5*-overexpression lines when the cold-stratified seeds were sown in a plant tissue culture medium in the absence of exogenous H_2O_2. Therefore, it is not clear how the cold stratification treatment would enable seeds to germinate independently of their different ABI5 expression levels. The relative significance of the varying catalase gene expression levels in the germination process must also await further elucidation.

Many seeds require a prior exposure to cold stratification conditions before they can germinate at a higher temperature. It would be interesting to investigate the changes in the catalase activity and endogenous H_2O_2 content in other cold stratification-requiring seeds to ascertain whether the finding from the studies on the *Arabidopsis* model system is translational to other non-model seeds. However, a survey of the recent literature shows a gap in our knowledge in this direction. For example, about 30% of *Zanthoxylum nitidum* seeds (of the Rutaceae family, a common Chinese medicinal plant) germinated at 15 °C after cold stratification at 4 °C for 3 months, while less than 3% of the non-cold-stratified seeds germinated (Lu et al. 2018). As expected from the classical literature about the involvement of gibberellins in the control of seed germination, the seeds without cold stratification but treated with gibberellic acid (300 mg L^{-1}) exhibited about 22% germination which was significantly higher than control. Based on the proteomic analytical approach iTRAQ-coupled LC-MS/MS, 484 proteins in the cold-stratified seeds were more abundant than the non-cold-stratified seeds (Lu et al. 2018). Only about eight proteins in the category of detoxification function, which could potentially include catalase gene, were found to be either up- or downregulated in association

with dormancy release. This is highly speculative but it is worthwhile to validate this further through direct measurement of catalase activity and determination of H_2O_2 content in the cold-stratified, GA-treated seeds and the control.

3 Abiotic Stress-Related Suppression of Seed Germination

3.1 Chilling Stress

At 10 °C for 10 days, about 20% of *Hedysarum scoparium* seeds germinated compared to 80% germination at 25 °C (Su et al. 2016). Interestingly, about 80% of the seeds could also germinate after 10 days at 10 °C provided the seeds were prior treated with 10 days of cold stratification (seeds mixed with sand at 4 °C). Therefore, the prior cold stratification aided the seeds to overcome chilling stress on seed germination. Exogenous application of 50 mM H_2O_2 promoted maximum germination (80%) of the non-cold stratified seeds at 10 °C. Exogenous application of an antioxidant, 50 mM N-acetyl cysteine (NAC), effectively prevented the stimulation of germination by 50 mM H_2O_2. This result is contrary to that implicating the need for preventing excess H_2O_2 content in cold-stratified, germinating *Arabidopsis* seeds (Bi et al. 2017).

In situ detection using confocal microscopy of H_2O_2 production at the tip of the embryonic axes of *H. scoparium* after 24 h of imbibition showed that there was only slightly detectable H_2O_2 content in the non-cold-treated seeds, but there was elevated accumulation of H_2O_2 in cold-stratified seeds imbibed in water and non-cold-stratified seeds treated with 50 mM exogenous H_2O_2 (Su et al. 2016). The treatment of the cold-stratified seeds with 50 mM NAC effectively nullified the endogenous H_2O_2 production. Hence, ROS generation following cold stratification of the seeds plays a positive role in seed germination. It would be worthwhile in future studies to evaluate the status of the catalase activity and gene expression in this system.

The cold-stratified *H. scoparium* seeds had lower ABA content than non-cold-stratified seeds that germinated poorly at 10 °C. There was an inverse correlation between H_2O_2 and ABA contents in the embryonic axes of *H. scoparium* (Su et al. 2016). Exogenous application of ABA prevented cold-stratified seeds from germinating and lowered H_2O_2 accumulation. This result suggests that ABA could influence upstream of ROS metabolism which was in turn linked to the completion of the germination process. However, exogenous application of H_2O_2 that enabled non-cold-stratified seeds to germinate led to a 20% reduction in ABA contents in the seeds within 24 h of imbibition. Conversely, NAC treatment of cold-stratified seeds led to an increase in ABA correlating with inhibition of seed germination. Hence, the precise relationship between changes in ABA and H_2O_2 contents in relation to the decision to germinate or not is still not completely clear (Su et al. 2016).

Seeds that normally germinate at a higher temperature can be prevented from germination at low temperatures. For example, 96% maize seeds germinated after 3–4 days at 25 °C, but after 3 and 4 days at 13 °C, only 15% germinated, respectively

(Li et al. 2017b). The seeds at 25 °C were found to have a higher endogenous H_2O_2 content as well as activities of catalase and other antioxidative enzymes than those at 13 °C after 48 h from sowing. Priming maize seeds with 50 mM H_2O_2 for 24 h enabled 50 and 90% of the seeds germinated at 13 °C after 3 and 4 days, respectively (Li et al. 2017b). Seed priming with H_2O_2 also led to a higher catalase activity at 48 h from sowing the primed seeds under chilling stress than non-primed seeds. The activities of other antioxidative enzymes assayed including ascorbate peroxidase, another enzyme beside catalase that can decompose H_2O_2, were also increased following seed priming with 50 mM H_2O_2. These results suggest that the net accumulation of H_2O_2 and probably other ROS was an important force for maize seed germination to occur.

There was no difference in the expression of *ZmNCED1* (a key gene for ABA biosynthesis in maize) and *ZmCYPT707A2* (a key gene for ABA catabolism) between non-primed and H_2O_2-primed maize seeds after 48 h of imbibition. However, the H_2O_2-primed maize seeds exhibited higher levels in the expression of the two genes after 72 h at 13 °C (Li et al. 2017b). Based on these results, it is difficult to interpret the relative contribution of ABA biosynthesis and ABA catabolism in H_2O_2-primed maize seeds without determination of ABA contents in the seeds. In contrast, the positive involvement of increased GA biosynthesis with concomitant reduction in GA catabolism in germination of H_2O_2-primed maize seeds was clearly evident early (6–24 h) during imbibition. Nevertheless, since higher levels of H_2O_2 and catalase activity were found in H_2O_2-primed than in non-primed maize seeds after 48 h of imbibition, this suggests that ROS homeostasis-related to seed germination might work upstream of ABA metabolism but downstream of GA metabolism (Li et al. 2017b).

3.2 Salinity and Heavy Metal Stress

Germination of alfalfa seeds in water and 150 mM NaCl (salinity stress) was about 98 and 45%, respectively (Amooaghaie and Tabatabaie 2017). Pretreatment of the seeds with 2 mM H_2O_2 or 300 mg L^{-1} polyethylene glycol (PEG 6000, osmopriming) for 6 h enabled more seeds (about 70%) to germinate in the presence of the salinity stress. Both priming with exogenous H_2O_2 and PEG 6000 led to a higher level of endogenous H_2O_2 than non-primed alfalfa seeds after 6 h from exposure to 150 mM NaCl. Application of *N,N*-dimethylthiourea (DMTU, a chemical absorbent of H_2O_2 and a ROS scavenger) prevented the stimulation of germination under salinity stress by priming with H_2O_2 or PEG 6000. In addition, application of DMTU suppressed an increase in endogenous H_2O_2 by priming the alfalfa seeds with H_2O_2 or PEG 6000. Interestingly, there was an increase in heme oxygenase activity and heme-oxygenase gene (*HO-1*) expression. These results suggest a sequence of signalling events involving endogenous H_2O_2 and heme oxygenase for osmopriming to overcome salinity stress on alfalfa seed germination (Fig. 1). Heme oxygenase coupled with carbon monoxide signalling has been

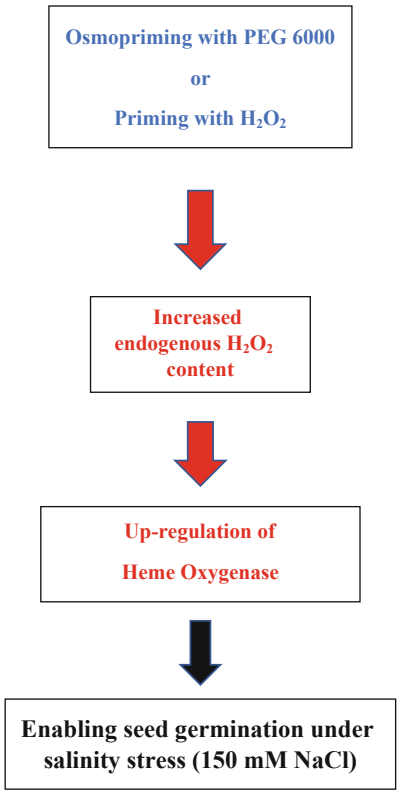

Fig. 1 An emerging signalling role involving H_2O_2 in alfalfa seed germination under abiotic stress. (See the results presented in Amooaghaie and Tabatabaie 2017.) The box in blue: experimental manipulation of seeds; red arrows and the boxes in red: the sequence of key internal signal changes in seeds; the black arrow and black box: germination events leading to improved % of seed germination under salinity stress

thought to be part of the response of plant cells under different environmental stress and ABA and may play a cytoprotective role (Shekhawat and Verma 2010).

Germination of maize seeds in water and 5 mM $PbCl_2$ (under Pb stress) was about 98 and 25% after 72 h from sowing (Zhang et al. 2018). The Pb stress-induced inhibition of seed germination could be reversed by an inhibitor (imidazole or diphenyliodonium) of NADPH oxidase (NOX) activity which is responsible for the production of superoxide free radical ($O_2^{\cdot -}$). Superoxide may be scavenged by superoxide dismutase to generate H_2O_2. Interestingly, EDTA (a metal chelator) or DMTU (a trap for H_2O_2) was not able to counteract the Pb stress-induced inhibition of seed germination. This suggests that although endogenous H_2O_2 content in maize seeds during imbibition was also increased under Pb stress, the Pb stress-triggered production of $O_2^{\cdot -}$ is a key suppressor of seed germination. Similar results were obtained in rice seeds germinated in the absence of abiotic stress (Li et al. 2017a).

3.3 Seed Storage Conditions

Two batches of barley seeds which were from the same harvest but were stored under two different conditions resulted in different germination performances under the same imbibition conditions: the seeds of higher germination performance were stored at 23 °C for 6 months, and those of lower germination performance were stored at −28 °C (Ishibashi et al. 2017). The level of H_2O_2 as a marker of reactive oxygen species (ROS) production in the batch of barley seeds exhibiting higher germination performance, both in relation to germination speed and higher germination percentage, was higher than in the other batches of barley seeds of lower germination performance. The significance of this correlation between hydrogen peroxide level and germination performance was supported with exogenous application of hydrogen peroxide and ascorbate (an antioxidant that resulted in a lower level of hydrogen peroxide in the seeds) during seed imbibition. After 48 h of imbibition in water, the germination percentage of high germination performing seeds was about 80%, but it was reduced significantly to about 20% in the presence of 20 mM sodium ascorbate. Conversely, the seeds of low germination performance exhibited a higher germination percentage (45%) when imbibed in 100 mM hydrogen peroxide than that of those imbibed in water (30%) (Ishibashi et al. (2017)).

Higher H_2O_2 and lower ABA contents were found in the barley seeds of higher germination performance compared to the batch of seeds of lower germination performance after 48 h of imbibition (Ishibashi et al. 2017). While there was little change in the expression of *HvNECD1* (a key gene for ABA biosynthesis in barley), that of *HvABA8′-OH1* (encoding ABA-8′-hydroxylase for ABA catabolism) was significantly higher in the barley seeds of higher germination performance than those of lower germination performance.

Higher H_2O_2 content in the barley seeds of higher germination performance was correlated with higher NADPH oxidase activity. On the other hand, of the two H_2O_2 scavenger enzymes, ascorbate peroxidase activity was higher in the barley seeds of high germination performance than those of low germination performance, while catalase activity showed the opposite relationship with the two batches of seeds during imbibition. It seems difficult to decipher the relative contributions of these two antioxidative enzymes on H_2O_2 signalling in seed germination. Further studies might include simultaneous investigations into the effect of exogenous ABA on the status of both ascorbate peroxidase and catalase at the levels of gene expression and enzyme activity in relation to the germination of the two batches of barley seeds.

4 The Scientific Basis for Improving Seed Germination by Exogenous Nitric Oxide

In many studies, application of exogenous NO has been found to improve seed germination performance due to diverse circumstances, although only some studies validated the effect with a NO-specific scavenger and evaluate the physiological significance of endogenous NO content (Table 1). For example, isolated embryos of mountain ash (*Sorbus pohuashanesis*) placed on filter paper wetted with distilled water for 8 days exhibited 42% germination. The promoter effect of pretreatment with 2 mM sodium nitroprusside (SNP, a nitric oxide donor) for 3 h, compared to pretreatment with water only, on germination of isolated embryos of mountain ash (*S. pohuashanesis*) on filter paper wetted with distilled water has been demonstrated to be 80 and 42%, respectively (Yang et al. 2018). The embryos treated with a combination of 2 mM SNP and 0.3 mM cPTIO (a NO-scavenger) did not exhibit dormancy release as only 42% of the embryos germinated. Furthermore, germination of embryos treated with 0.3 mM cPITO alone was slightly lower than imbibition in water only.

Upon seed imbibition, ABA in seeds could stimulate the expression of *ABI5* which is a key repressor of seed germination. There are four ways that exogenous application of NO or endogenous NO produced after start of seed imbibition can interfere with regulation of ABA in seed germination. For example, NO or other RNS (reactive nitrogen species) could inactivate the protein signalling intermediates between ABA and ABI5 by tyrosine nitration in these proteins (Signorelli and Considine 2018). As a result, the *ABI5* expression level would be reduced and hence has a less stronghold on suppression of seed germination.

In lettuce seeds, it was shown that light could stimulate NO production (An and Zhou 2017). Then NO was thought to in turn stimulate the activity of phospholipase D activity which is responsible for the hydrolysis of phospholipids to phosphatidic acid. Presumably, the phosphatidic acid formed could interact with phytohormones such as GA and ABA to allow lettuce seed germination in the light. The light-stimulated NO production in *Arabidopsis thaliana* seeds seems to be able to interact with the well-known phytochrome signalling in light-sensitive seed germination (Li et al. 2018). Further investigations are warranted to investigate between phosphatidic acid formation and phytochrome signalling in light-regulated seed germination.

NO could also interact with GA, a well-established germination promoter, and other phytohormones to release seed dormancy. For example, less than 10% of *Amaranthus retroflexus* seeds germinated at 25 °C in light. However, exogenous application of potassium nitrite (KNO_2, a NO donor) for 5 h or 10^{-3} M GA_3 led to 60% seed germination (Kepczynski et al. 2017). Likewise, exogenous application of ethylene or an ethylene biosynthesis precursor stimulated seed germination. Ethylene production seemed to be associated with the action of GA or NO in promoting germination. It was validated further that NO worked with GA or ethylene in germination stimulation because cPTIO blocked the effect of GA or ethylene.

Table 1 Some recent examples of seed germination stimulation following NO pretreatment

Plants	Nature of germination difficulty	Stimulation of seed germination by NO (compared to pretreatment without NO)	Effect of NO scavenger (cPTIO)	Endogenous NO production	References
Mountain Ash (*Sorbus pohuashanensis*)	Low natural seed germination: 60% after 8 days	Pretreatment with 2 mM SNP (NO donor): 90% seed germination	Reversed the promotive effect of SNP on seed germination	n.d	Yang et al. (2018)
Oat seeds	Artificial ageing of seeds (seed stored at 45 °C for 26 days) associated with increased H_2O_2 accumulation: 68% compared to 99% seed germination prior to storage	Pretreatment with 0.05 mM SNP: about 80% seeds germinated	n.d.	n.d.	Mao et al. (2018)
Lettuce (*Lactuca sativa* L., cv. Jianye Xianfeng)	Light (14 h at 50 μmol m^{-2} s^{-1} and 10 h darkness): over 80% seed germination compared to control (36 h in the dark): less than 10%	0.1 mM SNP: over 80% seed germination	0.2 mM cPTIO nullified the promotive effect of SNP on lettuce seed germination	NO content at 6 h from the start of imbibition under light was about fivefold higher than the seeds in the dark	An and Zhou (2017)
Coriander	Only 40% seed germination in water	80% seed germination	–	–	Panngom et al. (2018)
Carrot	60% seed germination in water	No effect	–	–	
Amaranthus retroflexus	10% seed germination in light at 25 °C	Pretreatment with KNO_2 (NO donor): 60% seed germination	Reversed the positive effect of NO	n.d.	Kepczynski et al. (2017)
Arabidopsis thaliana seeds (wild-type Col)	20% seed germination (1 h white light followed by 5 min of far-red light, then 96 h of darkness)	80% seed germination following a treatment with 10 μM SNAP or red light	Reversed the positive effect of NO	Red light stimulated NO accumulation but far-red light inhibited this	Li et al. (2018)

n.d. = not determined
SNP = sodium nitroprusside
SNAP = S-nitroso-N-acetylpenicillamine

The relative effectiveness of exogenous application of H_2O_2 and NO (SNP as NO donor) on promoting coriander and carrot seed germination was studied (Panngom et al. 2018). About 40% seeds germinated in water, but pretreatment with 25 mM H_2O_2 or 12.5 μM SNP led to 90 and 80% seed germination, respectively. Pretreatment of carrot seeds with 25–200 mM H_2O_2 resulted in about 70% seed germination compared to 60% germination in water. Pretreatment of carrot seeds with 0–100 μM SNP did not have any effect seed germination. This suggests that the sensitivity of different seeds to NO and H_2O_2 signalling may vary as far as seed germination is concerned.

5 Conclusion

Seed germination research is justified given its agricultural importance and implication for natural plant regeneration to maintain survival and propagation of wild plants. During imbibition, the current seed germination research communities seem to have fully embraced the concept that H_2O_2 and NO are important signal partners in the complex web of molecules including the well-known germination promoter and inhibitor played by GA and ABA, respectively. The details as to how either H_2O_2 or NO interacts with these key germination regulators are being revealed in different studies including the improvement in seed germination under unfavourable germination conditions or abiotic stress. There is yet no comprehensive study to investigate in detail the interrelationships of both H_2O_2 and NO and how they would interact with GA and ABA metabolism and signalling in the same seed during imbibition.

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