*Short Communication*

# Role of $H_2O_2$ in pea seed germination

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## Abstract

The imbibition of pea seeds with hydrogen peroxide ($H_2O_2$) increased the germination as well as the seedling growth, producing an invigoration of the seeds. We propose that $H_2O_2$ could acts as signalling molecule in the beginning of seed germination involving specific changes at proteomic, transcriptomic and hormonal levels. These findings have practical implication in the context of seed priming technologies to invigorate low vigour seeds.

**TEXT**

Germination process is associated with many metabolic, cellular, and molecular events, coordinated by a complex regulatory network. The reactive oxygen species (ROS) production by germinating seeds has often been considered as a negative effect that might affect the germination process, but provided that their accumulation is tightly regulated by the balance between production and scavenging, these toxic molecules now appear as being beneficial for germination.[1]

Strategies for improving the growth and development of crop species have been investigated for many years. Seed priming is a pre-sowing strategy to influence seed germination and seedling development by modulating pre-germination metabolic activity prior to emergence of the radicle and generally enhances germination rate and plant performance.[2,3] From a biochemical and molecular point of view, studying germination is difficult because a population of seeds does not complete the process synchronously.[4] Seed priming has been found as technology to enhance rapid and uniform emergence, and to achieve high vigour and better yields. This process generally causes faster germination and faster field emergence, which has practical agronomic implications, notably under adverse germination conditions.[5]

**Effect of ROS-related compound in seed germination and seedling growth**

We have tested the priming effect of several compounds on pea seeds. The assayed compound can be divided in three major groups: directly involved in the antioxidative metabolism [hydrogen peroxide ($H_2O_2$), reduced and oxidized glutathione (GSH, GSSG) and ascorbic acid (ASC)]; compounds related with the amino acid cysteine [N-acetylcysteine (NAC), thioproline (TP) and L-2-oxo-4-thiazolidine-carboxylic acid (OTC)]; and

compounds that trigger the systemic acquired resistance in plants [salicylic acid (SA) and its functional analogue benzothiadiazole (BTH)].

The tripeptide thiol glutathione (GSH) is at the hub of the complex antioxidant networks of plant and animal cells, where it participates in the cellular redox signalling networks that influence growth, development and defence.[6,7] It has long been recognized that ASC also exert a strong influence on plant growth and development.[8] The GSH synthesis is regulated by cysteine availability, thus compounds increasing its contents could produce an increase of GSH levels. In this sense, OTC is an artificial cysteine precursor whereas TP is a proline analogue that is converted by proline oxidase to N-formyl-L-cysteine which is presumably hydrolized to cysteine.[9]

To carry out this work, pea seeds were imbibed for 24 h in $dH_2O$ or in the compounds described above at different concentrations. Seeds were then washed twice with $dH_2O$ and placed in Petri dishes with two layers of filter paper moistened with $dH_2O$. Seeds were incubated at 25 ºC for 48 h in darkness, in a Cooled Incubator (MIR-153 Sanyo, Osaka, Japan).

Figure 1 shows that, except $H_2O_2$, none of the assayed compounds had a positive effect on seed germination or seedling growth (measured as fresh weight and length). Exogenous $H_2O_2$ showed a priming effect in the germination of pea seeds in a concentration dependent-manner obtaining more vigorous seedlings, being 20 mM $H_2O_2$ the concentration that produced the best response in terms of growth (Figure 1). The increase in seedling growth by 20 mM $H_2O_2$ was also evident 24 h after imbibition (Fig. 2). The priming effect of $H_2O_2$ was also noticeable at shorter times of imbibition (Table 1). After 12-h of

imbibition about 15% of seeds had germinated; however, no germination occurred at this time in seeds imbibed in water. At 24-h of imbibition, this percentage had reached nearly 75 %, whereas control seed germination remained at low level (Table 1). In preliminary experiments, we noticed that $H_2O_2$ concentrations higher than 20 mM (i.e. 40 to 100 mM) also stimulated the germination rate after 24 h imbibition. However, at short-term of post-imbibition (6 h) we observed that these $H_2O_2$ levels induced a pronounced curvature as well as an abnormal growth of the radicle at 24 h and 48 h post-imbibition (Fig. 2). In addition, $H_2O_2$ concentrations above 100 mM reduced the pea seeds germination rate (data not shown).

Interestingly, we observed a differential response depending on when $H_2O_2$ was supplied. When 5 mM $H_2O_2$ was added during the incubation in plates, after imbibition in $dH_2O$, the percentage germination was similar to that of control seeds. In contrast, the presence of 10 or 20 mM $H_2O_2$ in Petri dishes produced a negative effect on germination (Fig. 3).

**$H_2O_2$ signalling during seed germination**

Numerous recent works show now that ROS would play a key signalling role in the achievement of major events of seed life, such as germination or dormancy release. In fact, hydrogen peroxide, nitric oxide, hydroxyl radicals and superoxide radicals have been shown to accumulate during seed germination in various species.[1] Many works have reported that exogenous application of $H_2O_2$ can improve seed germination in many plant species.[10,11,12] The interplay between ROS and hormone signaling pathways lead to changes in gene expression or in cellular redox status that would play a role in the perception of environmental factors by seeds during their germination.[1] Recently we have shown that $H_2O_2$ coordinates the beginning of pea seed germination, acting as a

priming factor that involves specific changes at proteome, transcriptome and hormonal levels, resulting in an acceleration of the germination process most probably due to invigoration of the seeds.[13] $H_2O_2$ would induce a MAPK-dependent decrease in abcisic acid (ABA) contents in the seed as well as the carbonylation of seed storage proteins,[13] favouring their mobilization, and some glycolytic enzymes that could stimulate the phosphate pentose pathway (oxPPP).[14] The oxPPP activation could provide NADPH for the thioredoxin system, involved in seed germination and seedling development.[15] Alternatively, $H_2O_2$ could act, directly or indirectly impairing the ABA transport from the cotyledon to the embryo inducing a decrease in ABA, stimulating the germination process.[13] Finally, the decrease in ABA could induce a MAPK-mediated decrease in the ethylene precursor 1-aminocyclopropane carboxylic acid, favouring epicotyl and radicle emergence by $H_2O_2$ treatment.[13]

As mentioned above, the $H_2O_2$ treatment also stimulates the early growth of pea seedlings (Table 1). Previously, we have described that the $H_2O_2$-induced increase in pea seedling growth was correlated with the induction of proteins related to plant growth, cellular signalling and cell cycle control (14-3-3 protein, profilin, proteasome, translationally-controlled tumour protein), as well as with a substantial decrease in the levels of the hormones ABA and zeatin-riboside (ZR).[16] Moreover, a decrease of a polypeptide with homology to an ABA-responsive protein was observed, suggesting an interplay between the redox state and plant hormones, orchestrated by $H_2O_2$, in the induction of proteins related to plant signalling and development during the early growth of pea seedlings.[16] The molecular mechanisms implicated in this differential response remain unclear. They are of considerable interest, notably in the context of improving crop yields by invigoration seed treatments both in commercial applications[2] and in developing countries.[17]

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**Figure Legends**

**Figure 1**

Effect of imbibition with different compounds on the percentage of germination of pea seeds (A) and on the fresh weight (B) and length (C) of the seedlings 48 h after imbibition. Used treatments concentrations (in mM): H2O2 (5, 10 and 20); GSH (0.125, 0.250 and 0.500); GSSG (0.0625, 0.125, 0.250); ASC, OTC, NAC, SA and BTH (0.25, 0.50 and 1). Black, grey and dark grey bars correspond to the lower, intermediate and the higher concentrations of each treatment, respectively. Different letters indicate statistical significance according to Tukey's test ($P<0.05$).

**Figure 2**

Effect of $H_2O_2$ imbibition (0, 20, 40 and 80 mM) on early pea seedling growth after incubation in darkness.

**Figure 3**

Percentage of germination of pea seeds after imbibition in the presence of dH2O (0 h) and 48 h of incubation in plates in the presence of different $H_2O_2$ concentrations.

**Tables**

**Table 1**

Effect of 20 mM $H_2O_2$ imbibition on the percentage of pea seed germination at different time periods. Four batches of 25 seeds for each treatment were used to calculate the rate of germination.

| | Time (h) | Water | $H_2O_2$ |
|---|---|---|---|
| Imbibition | 6 h | 0 | 0 |
| | 12 h | 0 | 14 |
| | 24 h | 15 | 75 |

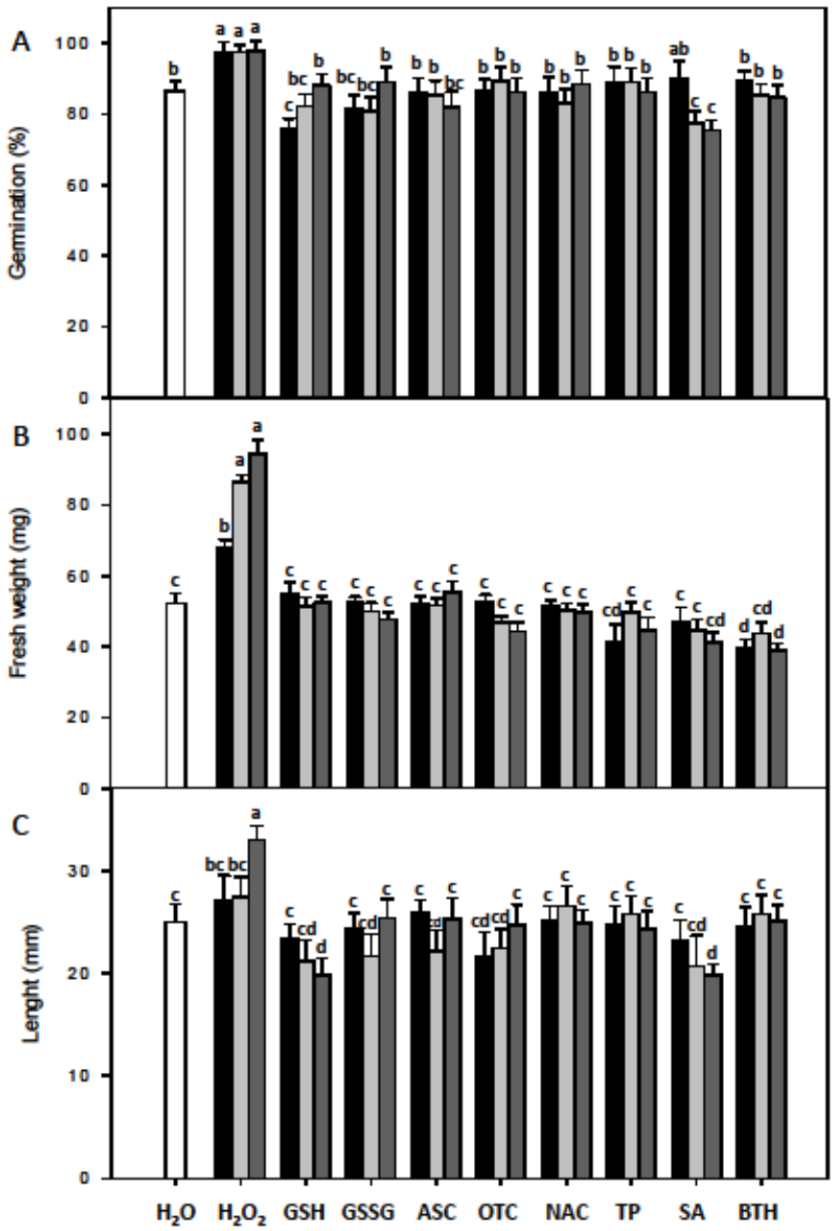
A
Germination (%)
B
Fresh weight (mg)
C
Lenght (mm)
H2O
H2O2
GSH
GSSG
ASC
OTC
NAC
TP
SA
BTH

Fig 1

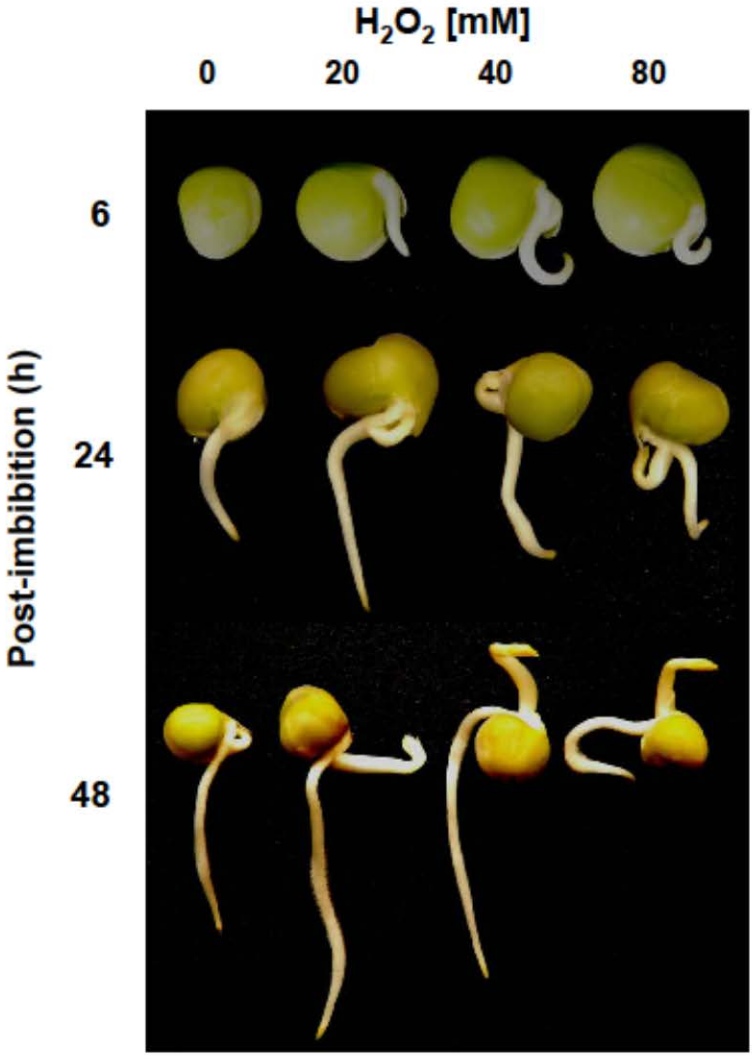


Fig 2

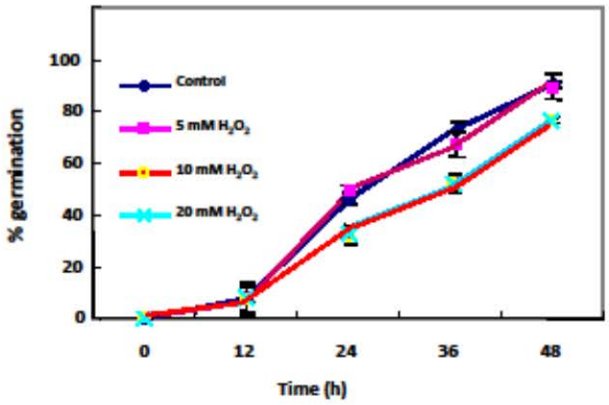
100
80
60
40
20
0
% germination
Control
5 mM H2O2
10 mM H2O2
20 mM H2O2
0
12
24
36
48
Time (h)

Fig 3