EMERGING INVESTIGATORS PREPRINT SERVER

May 7, 2026

Effect of hydrogen peroxide concentration on lettuce seed germination

Ankita Brahma[1], Binay Panda[2]

[1] UIA International School of Tokyo, Koto city, Tokyo, Japan
[2] School of Biotechnology, Jawaharlal Nehru University, New Delhi, India

Student Author
Ankita Brahma - High School

SUMMARY

Hydrogen peroxide plays a major role in plant physiology by triggering signalling cascades and oxidising biomolecules such as lipids, nucleic acids, and proteins. However, it has a deleterious effect at high concentrations. The current study focuses on the effect of various concentrations of hydrogen peroxide on the germination of lettuce seeds (*Lactuca sativa*). Seeds were primed with varying concentrations of hydrogen peroxide and allowed to germinate over a period of one week. We measured germination percentage (GP), seed vigour index (SVI), and radicle length in order to determine the optimum concentration of hydrogen peroxide for lettuce seed germination. We hypothesized that low to moderate concentrations of hydrogen peroxide would result in higher germination efficiency, whereas high concentrations would reduce germination compared to the control. We found that the optimum concentration of hydrogen peroxide, resulting in the greatest SVI and radicle length, was 0.1%, and that there was a negative linear correlation between hydrogen peroxide concentration and germination efficiency. We discuss the dual characteristics of hydrogen peroxide as a reactive oxygen species (ROS), first in phytohormone regulation and seed coat rupture, and second in seed aging.

INTRODUCTION

Seed germination is an essential stage of the plant's life cycle. Germination is initiated by the hydration of the seed, and the emergence of a radicle marks the end of germination. Germination promotes metabolic activity and cellular respiration, mitochondrial formation, DNA repair, translation of stored and new mRNAs, and the transcription of new mRNAs (1). These processes are affected by the signalling molecule hydrogen peroxide (H_2O_2), and studies show that both low and high concentrations of this messenger molecule can have an adverse effect on germination (2).

The current study focuses on the effect of H_2O_2 seed priming on the germination of lettuce seeds (*Lactuca sativa*). Seed priming by H_2O_2, or chemical priming, is a method in which seeds are partially hydrated using hydrogen peroxide to kickstart metabolic processes involved in germination (3). However, hydrogen peroxide concentration needs to be within a certain threshold to act as a signalling molecule that induces germination by alleviating dormancy. This is exemplified by the shift in metabolic characteristics of the seeds, allowing the completion of germination (4).

H_2O_2 is a molecule classified as a reactive oxygen species (ROS) (5). ROS are products of metabolic reactions occurring in several plant organelles, such as mitochondria, chloroplasts, and peroxisomes. Common ROS ions and molecules also include hydroxyl radicals (OH^-), superoxide anions (O_2^-), and singlet oxygen (O_2). Hydrogen peroxide plays an important role as a signalling molecule in several metabolic pathways. It is involved in cell differentiation, senescence, cell wall formation, and stress responses (5). It travels through aquaporins in the cell membrane, allowing effective and rapid oxidation of biomolecules (5). This characteristic makes it a powerful signalling molecule and enables it to cause cellular damage when it accumulates (5). In large amounts, it can be harmful to the plant, as it induces oxidative stress (6) by disrupting redox homeostasis. High concentrations of ROS, such as H_2O_2, induce cellular damage by affecting several macromolecules, including DNA, RNA, lipids, and proteins (7). This dual characteristic of H_2O_2 is what makes the control of its concentration in cells so important.

Hydrogen peroxide acts as a messenger molecule alongside the phytohormones abscisic acid (ABA) and gibberellic acid (GA) in the mitogen-activated protein kinase (MPK) pathway, as well as by increasing endosperm cap weakening (4), to explain its positive effect on germination. On

the other hand, outside of its threshold, hydrogen peroxide is known to have a deleterious impact on seeds and exerts an oxidative effect on seed desiccation and seed aging (8).

The purpose of this study is to determine the optimum concentration of hydrogen peroxide in seed priming for lettuce seed germination. We hypothesized that H_2O_2 acts as a signaling molecule at lower concentrations (0.1%–0.25%), enhancing germination, and as an inducer of oxidative stress at higher concentrations (0.5%–1%), reducing germination efficiency. We found a negative correlation between H_2O_2 concentration and seed vigour index (SVI), radicle length, and germination percentage (GP), demonstrating that at high concentrations, H_2O_2 plays a deleterious role in seed germination. However, contrary to our hypothesis, we observed a higher germination percentage in the control treatment without H_2O_2 than in seeds primed with H_2O_2, which we discuss further below.

RESULTS

After one week, germination percentage (GP) was calculated for seeds primed under each condition to determine which concentration of H_2O_2 resulted in the highest number of germinated seeds. A negative linear relationship was observed between H_2O_2 concentration and percentage germination (Figure 1A). Hydrogen peroxide had an effect on the rupture of the testae, which occurs during the initial phase of germination. At all concentrations of H_2O_2 (0.1%, 0.25%, 0.5%, 0.75%, and 1%), the testae were completely detached from the seeds and were split (Figure 2). However, despite testa rupture, higher concentrations resulted in lower GP compared to lower concentrations, suggesting that molecular mechanisms induced by high H_2O_2 concentrations prevented successful germination.

A change in radicle colour was observed. The blotting paper was stained pink when H_2O_2 was used (Figure 2), and the radicles of seeds primed with H_2O_2 concentrations of 0.25%, 0.5%, 0.75%, and 1% were pinkish red (Figure 3). The radicles of these primed seeds displayed reddish-brown bands, which were absent in seeds germinated in water and in 0.1% H_2O_2 (Figure 2).

The length of the radicles of seeds at each concentration was measured to understand the effect of H_2O_2 concentration on cell proliferation. A negative linear relationship was observed between the average radicle length and H_2O_2 concentration in the primed seeds (Figure 1B, Table 1).

Using the data collected for GP and radicle length, seed vigour index (SVI) was calculated. This was done to determine germination efficiency using both variables, and as a result, a negative linear relationship was observed between SVI and increasing H_2O_2 concentration. The highest SVI value was observed in seeds primed with 0.1% H_2O_2, and it was therefore concluded that this is the optimum concentration for the germination of lettuce seeds.

DISCUSSION

The effect of hydrogen peroxide on the germination and seed vigor of pea, okra, and basil seeds has been studied in the past (9)(10)(11). Plant hormones abscisic acid (ABA) and gibberellic acid (GA) are part of an antagonistic regulatory mechanism that plays a pivotal role in maintaining dormancy and promoting germination, respectively (12). The transition between these two states is controlled by the relative balance of the two phytohormones. ABA represses germination by activating specific pathways, while GA counteracts ABA action and induces germination (12).

Exogenous H_2O_2 regulates the GA/ABA ratio by modulating gene expression (13). Therefore, to understand the shift from a dormant to a germinative state, it is important to study the interaction of H_2O_2 with ABA and GA. In *Arabidopsis thaliana* seeds treated with H_2O_2, four ABA catabolic genes (CYP707A genes) and five GA biosynthesis genes (three GA20ox and two GA3ox genes) were upregulated (13). In the same study, GA catabolic gene expression was shown to be slightly elevated after six hours of imbibition.

Germination occurs when the ROS concentration falls within an “oxidative window” (2). This further supports the observation that low concentrations of H_2O_2 cannot efficiently trigger a signalling cascade, whereas high concentrations induce oxidative stress. Within this oxidative window, H_2O_2 initiates the MAPK (mitogen-activated protein kinase) signalling cascade MAPK pathways are involved in responses to abiotic and biotic stress, as well as in cellular processes such as differentiation and apoptosis and these pathways are also associated with hormonal regulation and contribute to maintaining the GA/ABA ratio by upregulating GA biosynthesis and promoting ABA catabolism (14)(2). Hydrogen peroxide decreases ABA levels not only through MAPK activation but also via direct interaction with ABA, resulting in a reduction in its concentration (15).

In the present study, seeds primed with 0.1% H_2O_2 showed the highest radicle growth, the highest SVI, and the second-highest germination percentage in lettuce seeds. It is possible that

at 0.1% H_2O_2, GA levels are at their highest and ABA levels at their lowest, resulting in enhanced GP and SVI values. Additionally, cell differentiation and proliferation occur at a higher rate within this oxidative window as H_2O_2 enhances protease activity, which promotes protein synthesis necessary for cell division (16), which could explain the increased radicle growth, as decreases in radicle length, GP, and SVI were observed outside of this window.

Hydrogen peroxide plays a pivotal role in weakening the endosperm cap. Mature lettuce seeds have a seed coat composed of dead tissue that, upon absorption of water, degrades promptly. The inner layer, called the endosperm, consists of 2–3 layers of living cells, which are ruptured for radicle protrusion. (17). Exogenous H_2O_2 weakens lettuce seed caps, as evidenced by a decrease in cap rupture force and the percentage of ruptured caps (4). The results obtained in this study replicate these findings, as the seed testa split more aggressively at higher concentrations of H_2O_2 (Figure 2).

Higher concentrations of H_2O_2 induce oxidative stress (6). One major effect of this on seeds is seed aging, which results in a loss of seed viability and vigor. The process of seed aging involves several deleterious effects, including DNA damage, impairment of protein production and activation, mitochondrial dysfunction, and loss of membrane integrity (18). Among these molecular processes, lipid peroxidation has been hypothesized as a major contributor to aging, and during seed hydration, ROS levels increase, and ROS molecules such as H_2O_2 accelerate the aging process by traveling through membrane proteins and oxidizing biomolecules (1).

This may explain why seeds primed with 0.25% and 0.5% H_2O_2 showed reduced SVI, GP, and radicle length, and why seeds primed with 0.75% and 1% H_2O_2 performed worse than the control in this study (Table 2; Figure 1A; Figure 1B). A negative linear correlation was observed between GP, SVI, and radicle length and increasing H_2O_2 concentration, likely due to the enhanced ROS activity at higher concentrations.

Although we hypothesized that a low concentration of H_2O_2 would result in a higher GP than the control, this was not observed in the current study. Seed moisture levels may have contributed to the higher germination rate under control conditions. In similar studies, seeds were primed and then dried so that moisture levels returned to pre-priming states (19). Unlike those studies, in the current experiment, the seeds were supplied with water at regular intervals. The hydration of seeds primed with H_2O_2 may have increased their endogenous ROS levels, such that the combination of exogenous and endogenous H_2O_2 produced an overall concentration less

favorable for germination compared to the naturally occurring endogenous ROS levels in seeds primed with water alone. Future study is needed to measure and control seed moisture levels and understand the effect further.

The red staining observed on the radicles in the current study could be due to anthocyanins, which are water-soluble pigments responsible for orange, red, blue, and purple coloration. Anthocyanins play an essential role as ROS scavengers, helping plants cope with abiotic stress by reducing oxidative damage (20). Due to the abundance of phenol groups in their chemical structure, anthocyanins are excellent hydrogen donors, allowing them to prevent oxidative damage such as lipid peroxidation and protein impairment induced by ROS activity. High levels of ROS are associated with increased anthocyanin production as a coping mechanism in plants. It is suggested that ROS molecules activate genes responsible for the upregulation of this pigment (20).

Previous studies used sodium hypochlorite followed by distilled water to wash seeds after priming in the respective solutions (21). Sodium hypochlorite treatment removes epiphytic microflora—microbes commonly found on plant tissues, roots, and seeds (22). Microbes can enhance seed survival by protecting it from biotic stress and pathogenic microorganisms (22). They secrete secondary metabolites with antifungal, antibiotic, and photoprotective properties, which improve plant performance (22). This step was not carried out in the current study, which is a limitation and could explain why the germination percentage was higher in the control compared to seeds treated with 0.1% H_2O_2.

Exogenously applied hydrogen peroxide via chemical seed priming affected the germination percentage, seed vigor index, and radicle length of lettuce seeds. The concentration that improved the seed vigor index and radicle growth was found to be 0.1% H_2O_2. Concentrations higher than this had adverse effects on germination, confirming the dual role of hydrogen peroxide as both a primary signaling molecule and a reactive oxygen species (ROS).

MATERIALS AND METHODS

Seeds

Lettuce seeds sourced from different vendors were acquired from local stores. Chimasanchu lettuce was selected for use in the experiment. Ten seeds of each type were germinated in a plastic container containing damp blotting paper for one week. After one week, the seeds were analyzed to determine which variety showed the most promising growth. Germination

percentage (GP) was calculated by dividing the number of germinated seeds by the total number of seeds sown in the container and multiplying by 100%.

Seed priming

A total of 120 lettuce seeds were used in the experiment, with 20 seeds subjected to each condition. A simple dilution was prepared using 1% H_2O_2, and five test tubes were set up with 5 mL of varying H_2O_2 concentrations (0.1%, 0.25%, 0.5%, 0.75%, and 1%), while one test tube served as the control with 5 mL of distilled water. The seeds were placed into the test tubes and subjected to the treatments for 24 hours in the dark. After this period, the seeds were rinsed with distilled water and patted dry. They were then transferred into paper cups lined with damp blotting paper. The cups were placed in a cupboard and monitored daily for one week. Every other day, 2 mL of water was added to each cup. After one week, final observations and measurements were recorded.

Controlled variables

The experiments were carried out in a room with temperatures ranging from approximately 22 °C in the morning to 15 °C at night. The cups were placed in a dark cupboard and watered every other day. All cups were maintained under identical conditions of temperature, light, and water availability to ensure that these external factors did not influence the results, so that H_2O_2 priming was the only variable affecting seed germination and growth.

Radicle length calculation

The lengths of the radicles of three randomly selected germinated seeds from each cup were measured and recorded. The average length was calculated using the formula:

$$Average\ length\ = \frac{Length_1 + Length_2 + Length_3}{3}$$

and the values were rounded to two decimal places. The uncertainty was calculated using the following formula:

$$Uncertainty\ = \frac{Maximum\ Value - Minimum\ Value}{2}$$

Germination percentage calculation

The number of seeds that had germinated after one week was counted, and GP (germination percentage) was calculated using the following formula:

$$GP = \frac{Number\ of\ germinated\ seeds}{Number\ of\ seeds\ planted} X\ 100$$

Seed vigor index calculation

The SVI (seed vigor index) for each concentration was calculated using the following formula:

$$SVI = \frac{Mean\ readicle\ length}{100} X\ GP$$

Safety

Hydrogen peroxide (H_2O_2) is corrosive to the skin and eyes. Therefore, gloves, goggles, and a lab coat were worn while handling H_2O_2 during the experiments to ensure safety.

REFERENCES

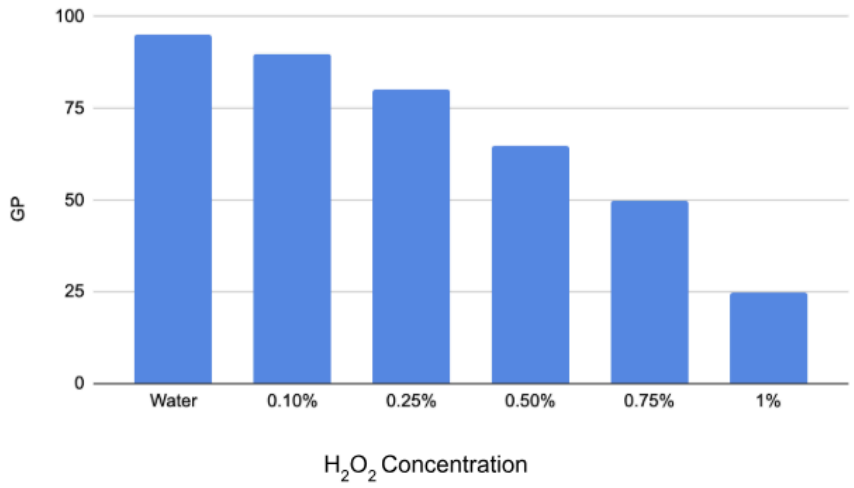
1. Wojtyla, Łukasz, et al. "Different Modes of Hydrogen Peroxide Action During Seed Germination." *Frontiers in Plant Science*, vol. 7, 04 Feb 2016, p. 66, https://doi.org/10.3389/fpls.2016.00066.
2. Wang, Yakong, et al. "Regulation of Seed Germination: ROS, Epigenetic, and Hormonal Aspects." *Journal of Advanced Research*, vol. 71, May 2025, pp. 107-125, https://doi.org/10.1016/j.jare.2024.06.001.
3. Hussain, Munir, et al. "Drought Stress in Sunflower: Physiological Effects and Its Management through Breeding and Agronomic Alternatives." *Agricultural Water Management*, vol. 201, March 2018, pp. 152-166. *ScienceDirect*, https://doi.org/10.1016/j.agwat.2018.01.028.
4. Yu Zhang, Bingxian Chen, Zhenjiang Xu, Zhaowan Shi, Shanli Chen, Xi Huang, Jianxun Chen, Xiaofeng Wang, Involvement of reactive oxygen species in endosperm cap weakening and embryo elongation growth during lettuce seed germination, Journal of Experimental Botany, Volume 65, Issue 12, 17 April 2014, Pages 3189–3200, https://doi.org/10.1093/jxb/eru167.

5. Huang, Honglin, et al. "Mechanisms of ROS Regulation of Plant Development and Stress Responses." *Frontiers in Plant Science*, vol. 10, 25 Jun 2019, p. 440478, https://doi.org/10.3389/fpls.2019.00800.
6. Dos Santos Araújo, Gyedre, et al. "H2O2 Priming Promotes Salt Tolerance in Maize by Protecting Chloroplasts Ultrastructure and Primary Metabolites Modulation." *Plant Science*, vol. 303, Feb 2021, p. 110774, https://doi.org/10.1016/j.plantsci.2020.110774.
7. Chaki, Mounira, et al. "Oxidative Stress in Plants." *Antioxidants*, vol. 9, no. 6, 3 Jun 2020, https://doi.org/10.3390/antiox9060481.
8. Christophe Bailly; Hayat El-Maarouf-Bouteau; Françoise Corbineau. From intracellular signaling networks to cell death: the dual role of reactive oxygen species in seed physiology. Comptes Rendus. Biologies, Les graines de la vie / The seeds of life, Volume 331, 4 Sep 2008, no. 10, pp. 806-814. doi : 10.1016/j.crvi.2008.07.022.
9. Barba-Espin, Gregorio, et al. “Understanding the Role of H2O2 during Pea Seed Germination: A Combined Proteomic and Hormone Profiling Approach.” Plant, Cell & Environment, vol. 34, no. 11, 25 July 2011, pp. 1907–1919, https://doi.org/10.1111/j.1365-3040.2011.02386.x.
10. Alam, Md Mahbub, et al. “Effects of Different Priming Treatments on the Germination and Growth of Okra (Abelmoschus Esculentus L.).” Journal of Tropical Crop Science, vol. 11, no. 01, 29 Feb. 2024, pp. 91–96, https://doi.org/10.29244/jtcs.11.01.91-96.
11. Bojović, Biljana, et al. “Evaluation of Seed Priming on Germination and Growth of Basil (Ocimum Basilicum L. Cv. “Genovese”).” Kragujevac Journal of Science, no. 44, 01 Jan 2022, pp. 189–198, scindeks-clanci.ceon.rs/data/pdf/1450-9636/2022/1450-96362244189B.pdf, https://doi.org/10.5937/kgjsci2244189b.
12. Rodríguez-Gacio, María del Carmen, et al. “Seed Dormancy and ABA Signaling: The Breakthrough Goes On.” Plant Signaling & Behavior, vol. 4, no. 11, 4 Nov 2009, pp. 1035–49, www.ncbi.nlm.nih.gov/pmc/articles/PMC2819511/, https://doi.org/10.4161/psb.4.11.9902.
13. Liu, Yinggao, et al. “H2O2 Mediates the Regulation of ABA Catabolism and GA Biosynthesis in Arabidopsis Seed Dormancy and Germination.” Journal of Experimental Botany, vol. 61, no. 11, 11 May 2010, pp. 2979–2990, https://doi.org/10.1093/jxb/erq125.
14. Taj, Gohar, et al. “MAPK Machinery in Plants.” Plant Signaling & Behavior, vol. 5, no. 11, 01 Nov 2010, pp. 1370–1378, https://doi.org/10.4161/psb.5.11.13020.

15. Barba-Espín, G., et al. “HYDROGEN PEROXIDE as an INDUCER of SEED GERMINATION: ITS EFFECTS on ANTIOXIDATIVE METABOLISM and PLANT HORMONE CONTENTS in PEA SEEDLINGS.” Acta Horticulturae, no. 898, June 2011, pp. 229–236, https://doi.org/10.17660/actahortic.2011.898.28.
16. Dong, Ruili, et al. "Effects of Hydrogen Peroxide Soaking on the Seeds of Different Edible Bean Varieties." Plants, vol. 14, no. 22, 14 Nov 2025, https://doi.org/10.3390/plants14223476.
17. Zhang, Yu, et al. "Involvement of Reactive Oxygen Species in Endosperm Cap Weakening and Embryo Elongation Growth during Lettuce Seed Germination." *Journal of Experimental Botany*, vol. 65, no. 12, 2014, pp. 3189-3200, https://doi.org/10.1093/jxb/eru167.
18. Copeland, Larry O and Miller B. McDonald. “Seed Longevity and Deterioration.” Principles of Seed Science and Technology, 1999, pp. 181–220, https://doi.org/10.1007/978-1-4615-1783-2_8.
19. Kubala, Szymon, et al. “Deciphering Priming-Induced Improvement of Rapeseed (Brassica Napus L.) Germination through an Integrated Transcriptomic and Proteomic Approach.” Plant Science, vol. 231, 1 Feb 2015, pp. 94–113, https://doi.org/10.1016/j.plantsci.2014.11.008.
20. Li, Zhe, and Golam Jalal Ahammed. “Plant Stress Response and Adaptation via Anthocyanins: A Review.” Plant Stress, vol. 10, 1 Dec 2023, pp. 100230–100230, https://doi.org/10.1016/j.stress.2023.100230.
21. Milica Kanjevac, et al. “Effect of Seed Halopriming on Improving Salt Tolerance in Raphanus Sativus L.” Kragujevac Journal of Science, no. 43, 1 Jan 2021, pp. 87–98, https://doi.org/10.5937/kgjsci2143087k.
22. Younas, Hajira. “Secondary Metabolites from Marine Epiphytic Bacteria against Plant Pathogens.” Elsevier EBooks, 1 Jan 2024, pp. 353–379, https://doi.org/10.1016/b978-0-323-95251-4.00012-0.

Figures and Figure Captions

A



B

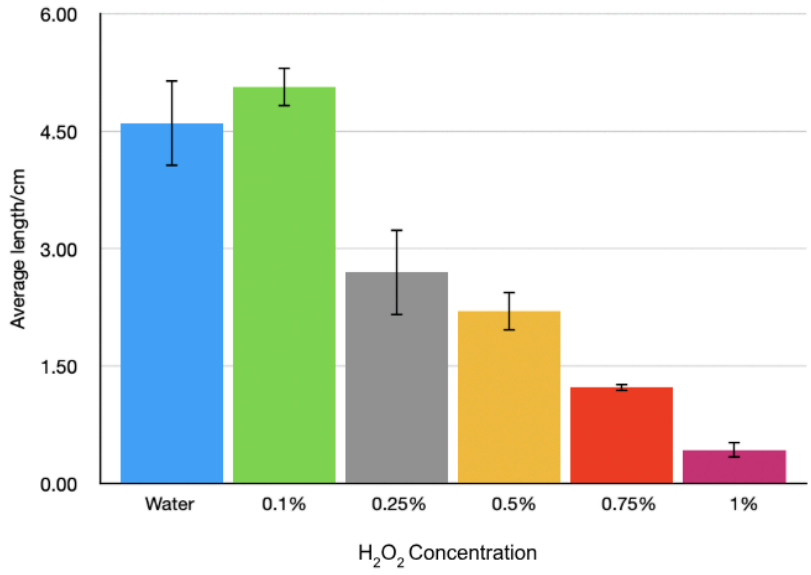


Figure 1. A negative correlation between germination percentage (GP) and average radicle length with increasing H_2O_2 concentration was observed. The figure shows the effect of varying concentrations of hydrogen peroxide on lettuce seed GP out of a test batch of 20 seeds (A) and

the mean radicle length of three arbitrarily selected seeds from each experimental group (B), one week after initial exposure through chemical priming.

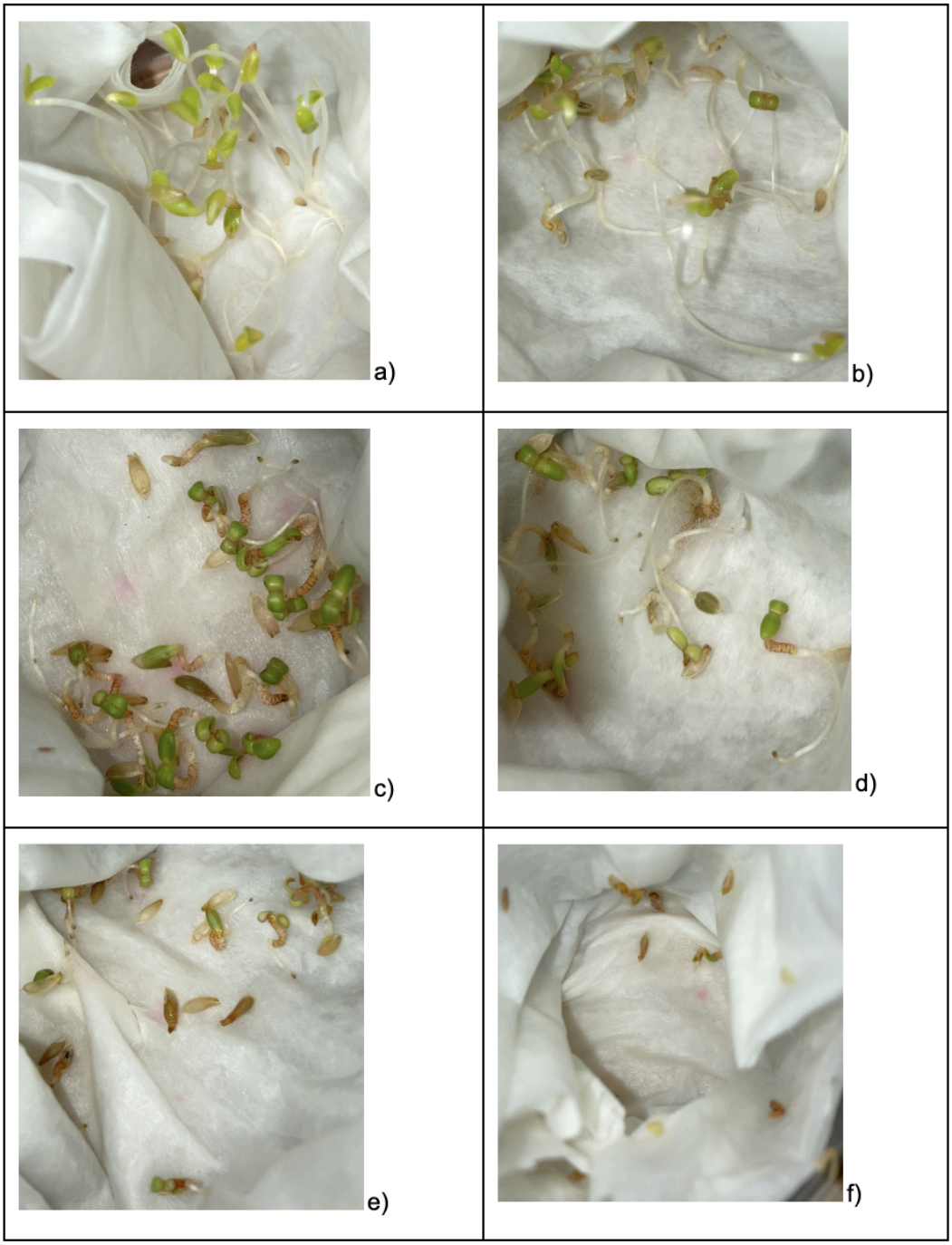


Figure 2. The greatest GP was observed in the control, followed by a descending order of values as H_2O_2 concentration increased. The figure shows the effect of different concentrations of H_2O_2 on germination after seeds were treated under six varying H_2O_2 conditions for one week.

Seeds were primed in 5 mL of the following solutions: water (a), 0.1% H_2O_2 (b), 0.25% H_2O_2 (c), 0.5% H_2O_2 (d), 0.75% H_2O_2 (e), and 1% H_2O_2 (f) prior to germination.

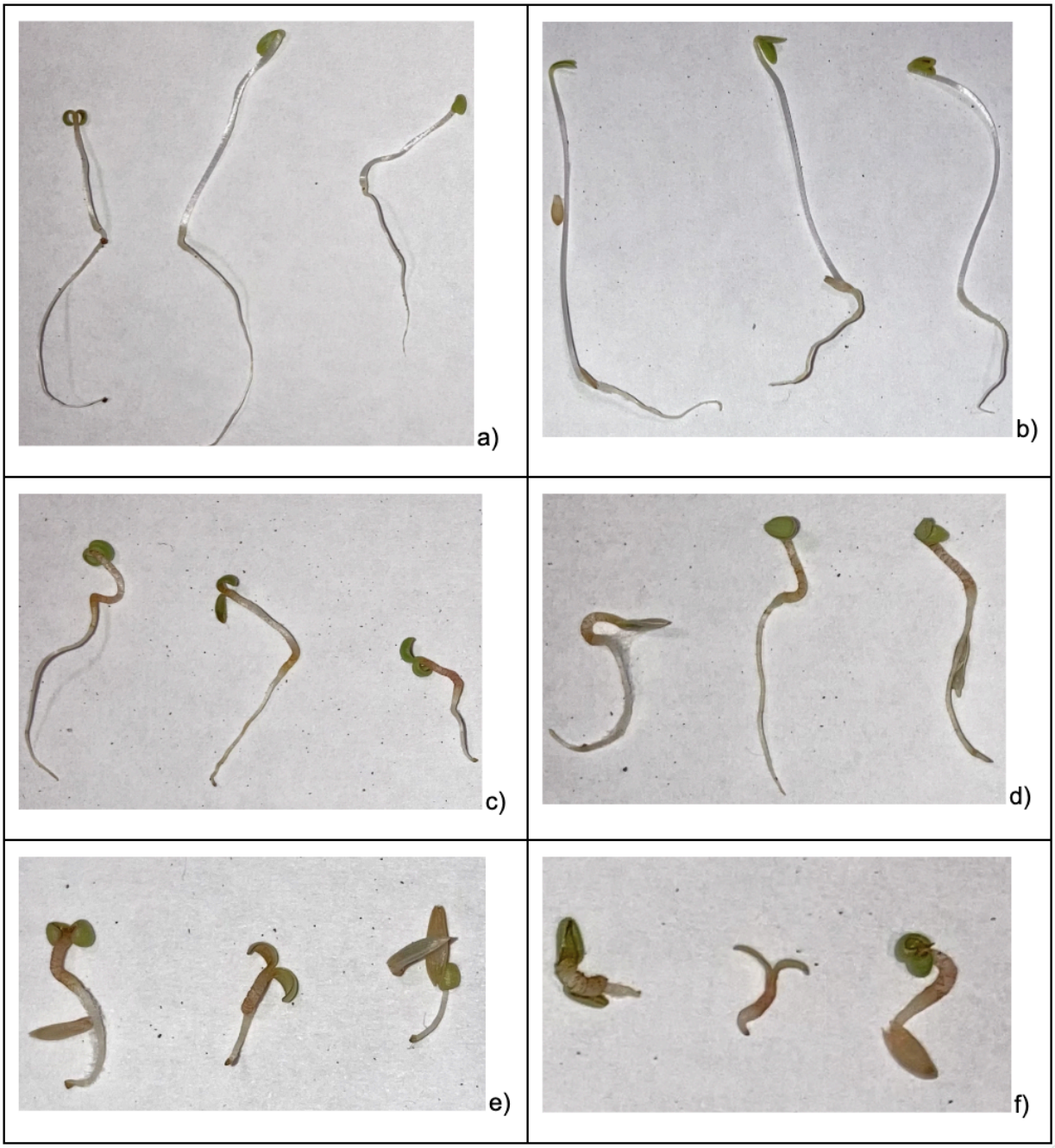


Figure 3. The greatest average radicle length was observed in seedlings treated with 0.1% H_2O_2. The figure shows the effect of H_2O_2 concentration on radicle length after seeds were treated for one week in 5 mL of the following solutions: water (a), 0.1% H_2O_2 (b), 0.25% H_2O_2 (c), 0.5% H_2O_2 (d), 0.75% H_2O_2 (e), and 1% H_2O_2 (f). Three seeds were arbitrarily selected from each experimental group, and the mean radicle length was calculated for each.

Tables with captions

Table 1: A greater average radicle length was observed in seeds primed in 0.1% H_2O_2 compared to the control. The table shows the negative correlation between increasing H_2O_2 concentration and the mean radical length of three arbitrarily chosen seeds from each group, recorded one week post-priming. The uncertainty was calculated as half the range of the lengths in each group.

Condition	Length in triplicates (cm)			Average length (cm)	Uncertainty
Water	4.5	5.2	4.1	4.60	±0.55
0.1%	5.3	5.1	4.8	5.07	±0.25
0.25%	2.8	3.2	2.1	2.70	±0.55
0.5%	1.9	2.3	2.4	2.20	±0.25
0.75%	1.3	1.2	1.2	1.23	±0.05
1%	0.4	0.3	0.6	0.43	±0.10

Table 2: An enhanced seed vigor index (SVI) was observed in seeds primed in 0.1% H_2O_2 compared to the control. The table shows the negative correlation between increasing H_2O_2 concentration and SVI, which was calculated by multiplying the mean radicle length by the GP and dividing that value by 100 for each experimental group.

Condition	Seed Vigor Index (au)
Water	4.37
0.1% H_2O_2	4.56
0.25% H_2O_2	2.16
0.5% H_2O_2	1.43
0.75% H_2O_2	0.62
1% H_2O_2	0.11