

Hydrogen Peroxide as a Potential Priming Agent to Reinvigorate Deteriorated Sweet Corn Seed

Sharif Azmi Abdurahman^{1,2}, Uma Rani Sinniah^{1*}, Azizah Misran¹,
Muhamad Hazim Nazli¹, and Indika Weerasekara³

¹Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

²Fakulti Pertanian Lestari, Universiti Malaysia Sabah, 90509 Sandakan, Sabah, Malaysia

³Seed and Planting Material Development Centre, Department of Agriculture, 20400 Peradeniya, Sri Lanka

ABSTRACT

Sweet corn (*Zea mays* L. *Saccharata* Sturt) seeds, like other crops, often experience deterioration during storage, which can negatively impact their germination and performance. This study investigated the impact of hydrogen peroxide (H₂O₂) priming on the germination, vigour, and antioxidant activities of deteriorated sweet corn seeds. A one-year-old GSH1005Y sweet corn seed sample, with a germination of 48%, was primed in H₂O₂ concentrations ranging from 1 mM to 20 mM for 24 hours, followed by drying. Seed germination and vigour were assessed through germination and electrical conductivity (EC) tests. Antioxidant activities, including catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and malondialdehyde (MDA) content, were also evaluated. Results showed that priming with H₂O₂ significantly improved seed germination. Seeds treated with 10 mM H₂O₂ achieved 69% germination, a 21% increase compared to the untreated

seeds. Priming with 5 mM and 7.5 mM H₂O₂ also enhanced germination (67% and 66%, respectively). Seedling performance was best at 10 mM H₂O₂, reducing mean germination time by 16%, increasing the coefficient of velocity of germination, and resulting in longer seedlings and higher shoot dry weight (45.5% increase over untreated seeds). Higher concentrations (12.5 mM to 20 mM) did not improve performance and negatively affected seedlings. H₂O₂ priming increased SOD activity while reducing MDA content, indicating less oxidative stress. EC measurements showed improved membrane integrity, especially at 10 mM H₂O₂. In conclusion, H₂O₂ priming at 10

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E-mail addresses:

sh.azmi@ums.edu.my | shazmiey.abdurahman@me.com (Sharif Azmi Abdurahman)

umarani@upm.edu.my (Uma Rani Sinniah)

azizahm@upm.edu.my (Azizah Misran)

m_hazim@upm.edu.my (Muhamad Hazim Nazli)

weerasekaradoa@gmail.com (Indika Weerasekara)

*Corresponding author

mM for 24 hours significantly improved seed quality and vigour, offering a cost-effective solution for enhancing deteriorated seeds.

Keywords: antioxidant activity, hydrogen peroxide priming, membrane integrity, sweet corn seed, seed priming.

INTRODUCTION

Corn (*Zea mays* L.) is among the top three crops cultivated worldwide for multiple purposes ranging from feedlot to biofuel production (Erenstein et al., 2022). Several types of corn are being cultivated such as flint, dent, waxy and sweet corn. Corn is cultivated using seeds as planting materials however, as biological material seeds will eventually undergo deterioration, and planting deteriorated seeds will lead to poor stand establishment (Weerasekara et al., 2021). Seed deterioration is an inevitable process that is influenced by various factors such as seed maturity at harvest, seed moisture content during storage, and the genetic makeup of the species. Seeds rely on stored carbon for survival and seedling establishment. In most seeds, this reserve is primarily in the form of starch, which is derived from the partitioning of assimilated carbon (typically as sucrose). Starch serves as an inert, water-insoluble storage compound. However, genetic mutations in certain crops, such as the endosperm of sweet corn (*Zea mays* L. *Saccharata* Sturt), can alter seed storage composition. Sweet corn is unique as it has several mutated genes such as *shrunk2* (*sh2*), *sugary1* (*su1*), *sugary enhancer* (*se*) that are responsible in preventing conversion of soluble sucrose into starch, resulting in high sucrose content and low starch as storage compound in seed endosperm. In addition to low storage compounds in the seeds, drying of sweet corn seed causes air pockets to form between the seed's endosperm, embryo, and seed coat. This leads to poor seed quality, resulting in low and inconsistent germination, reduced seed vigour, and hindered seedling establishment in sweet corn (Pairochteerakul et al., 2018; Styer et al., 1980; Suo et al., 2017; Tracy, 2000).

Seed pre-treatment methods, such as priming, have been shown to improve seed quality, particularly for aged or deteriorated seeds, by enhancing their germination or vigour prior to sowing. Priming involves a controlled process of hydration and drying, allowing seeds to undergo early metabolic processes without initiating radicle emergence. This process helps seeds achieve more rapid and uniform germination when re-imbibed increases seed vigour, which enhances seedling establishment (Bradford, 1986; Raj & Raj, 2019). Priming enhances seed performance through various physiological mechanisms. It activates or elevates the levels of specific enzymes and proteins associated with germination, stimulates cellular repair mechanisms, and boosts the antioxidant defence system, which collectively contribute to improved stress tolerance and seed vigour (Gammoudi et al., 2020). These changes help accelerate germination and improve overall seedling development. Among the different priming techniques, osmo-priming is a specific method that involves soaking

seeds in solutions with controlled osmotic potential, such as osmotic solutions or solutions containing substances like polyethylene glycol or hydrogen peroxide (H_2O_2). After soaking in such solution and dried to initial seed moisture content, the seeds is ready to use for germination (Chen & Arora, 2011). Osmo-priming is an effective, cost-efficient approach widely used globally to enhance seedling establishment (Farooq et al., 2019).

Today, the use of hydrogen peroxide, H_2O_2 , as pre-treatment to improve plant growth is gaining attention. Lariguet et al. (2013) stated that H_2O_2 at proper concentration helps in breaking seed dormancy and improves germination while over-accumulation would lead to cell injury which can be detrimental to the seeds (Jeevan Kumar et al., 2015). Many studies had reported that the application of H_2O_2 alone or a combination with other compounds as seed or seedling pre-treatment induced and activated plant abiotic stress protective mechanisms such as accumulation of latent defence mechanism, by maintaining non-toxic levels just enough to signal stress ROS scavenging actions (Hossain et al., 2015). Many antioxidant mechanisms by plants such as superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (POD) have been attributed to redox balance from the act of signalling by H_2O_2 in plants (Ślesak et al., 2007). H_2O_2 has been used as foliar spray and for priming in many plants (Banerjee & Roychoudhury, 2019). Among the crops, wheat (Hameed & Iqbal, 2014), rice (Jira-Anunkul & Pattanagul, 2020), and sunflower (Silva et al., 2020) showed that concentrations ranging between $1\mu M$ to 100 mM of H_2O_2 as priming agent improved seed viability, vigour as well as seedling performance, however, the effect of H_2O_2 on deteriorated sweet corn seeds have never been tested. Therefore, this study was conducted to investigate the potential of H_2O_2 as a priming agent to invigorate deteriorated sweet corn seed by observing viability, vigour, seedling performance, and antioxidant activity particularly seed antioxidant enzymes.

MATERIALS AND METHODS

Seed Sample

The experiment was carried out at the Seed Technology Laboratory, Universiti Putra Malaysia, Selangor, Malaysia. A year-old GSH1005Y sweet corn seeds harvested at 35 days after pollination, dried, and stored in an ambient room ($26\pm 3^\circ C$, moisture content =8%) was used. Seed germination upon retrieval under the aforementioned condition was 48%.

Priming Treatment

The initial seed moisture content (MC) was determined using the high constant temperature oven method at $130\pm 3^\circ C$ for 4 hours (ISTA, 2021). Then, the seeds were soaked in different concentrations of H_2O_2 i.e. 1, 2.5, 5, 7.5, 10, 12.5, 17.5, and 20 mM for 24 hours at a temperature of $26\pm 3^\circ C$. After 24 hours, the seeds were removed from the priming solution

and dried for 3 to 4 days at a temperature of $26\pm3^{\circ}\text{C}$ until they reached the initial MC, following which the seeds were subjected to germination within 24 hours. Germination test was carried out using sand as the substrate. Seed germination was observed, and daily germination was recorded. Final Germination Percentage (FGP) was recorded on the seventh day (ISTA, 2021).

Seed Germination, Vigour and Seedling Performance

Seven days after the initiation of the germination test, Mean Germination Time (MGT) and Coefficient Velocity of Germination (CVG) based on daily seed germination count were calculated using the formula by Kader (2005) as follows:

$$\text{MGT (day)} = \frac{\sum (n \times d)}{\sum N}$$

where n = number of seeds germinated on each day, d = number of days from the beginning of the test, and N = total number of seeds germinated at the termination of the experiment.

$$\text{CVG} = \frac{(G_1 + G_2 + \dots + G_n)}{(1 \times G_1 + 2 \times G_2 + \dots + n \times G_n)} \times 100$$

Where G is the number of germinated seeds and n is the last day of germination

Then, all germinated seedlings on the seventh day after sowing were arranged based on the length, and five median seedlings per replicate were selected for seedling performance analysis. Root length was measured from the tip of the longest root to the base of the hypocotyl while shoot length was measured from the end of the longest primary leaf to the base of the hypocotyl. Samples used for seedling length measurement were separated into shoot and root and dried at 65°C for 48 hours to get the root and shoot dry weight (Brar et al., 2019).

Antioxidant Enzymatic Activities

For antioxidant enzyme analysis, the sample was prepared using the method of Abuelsoud et al., (2020). Seed sample was ground into a powder form using liquid nitrogen. Then, a sample of 0.15 grams of the ground sample was mixed with 1.5 mL of ice-cold 2-Morpholinoethanesulfonic acid potassium salt (MES-KOH) (50 mM, pH 6.0) extraction buffer in 2 mL microcentrifuge tube followed by centrifuging at $16,000 \times g$ in 4°C for 20 minutes. A sample of supernatant was taken for the antioxidant enzymatic assay. Catalase

(CAT) activity was measured using the method described by Aebi (1984) where the absorbance at 240 nm was used and the result was expressed in $\mu\text{mol}/\text{min}/\text{mg}$ FW. Guaiacol Peroxidase (POD) assay was measured using protocol by Maehly & Chance (1954) with absorbance reading at 470 nm before POD activity was calculated and expressed in $\text{nmol}/\text{min}/\text{mg}$ FW. Superoxide Dismutase (SOD) activity was measured using the method by Gupta et al., (1993) and the absorbance reading of SOD activity was taken at 560 nm and the activity was expressed in unit/mg FW. All absorbance reading was made using a micro-plate spectrophotometer (Thermo scientific MULTISKAN GO).

Electrical Conductivity Test and Malondialdehyde Content Measurement

To measure the oxidative damage on the seed membrane, electrical conductivity test was conducted where 25 seeds for each treatment were weighed, following which it was placed in 75 mL of deionized water in a beaker and left for 24 hours at 20°C . After 24 hours, the seed leachate was measured using a digitalized conductivity meter (labCHEM-CP conductivity meter) and expressed in $\mu\text{S}/\text{cm}/\text{g}$ (Thant et al., 2017). To estimate the lipid peroxidation, Malondialdehyde (MDA) content was measured using Stewart & Bewley, (1980). The seed sample was homogenized using 2 mL distilled water and centrifuged $10,000 \times g$ for 15 minutes to get supernatant. A reaction mixture of 1 mL of supernatant, 2 mL of TBA/TCA (0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA)) was incubated for 30 minutes in a water bath at temperature of $95 \pm 3^{\circ}\text{C}$. It was placed in an ice tray immediately after the incubation to stop the reaction before reading was taken at 450, 532 and 600 nm using a micro-plate spectrophotometer (Thermo scientific MULTISKAN GO) and MDA was calculated and expressed in mmol/g FW.

Statistical Analysis

Analysis of variance (ANOVA) was carried out for all parameters and if significant difference was found, means comparison was done using Tukey Test with minimum 95% confidence interval. All statistical analysis were carried out using statistical software, Statistical Analysis System, SAS 9.4 (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Seed Germination, Vigour and Seedling Performance

In this study, priming with H_2O_2 significantly affected seed germination, as illustrated in Figure 1. A substantial increase in germination percentage can be observed with H_2O_2 concentrations ranging from 0 to 20 mM, compared to untreated seeds, which exhibited 48% of germination. Priming with H_2O_2 concentrations between 5 mM and 10 mM has led to significant improvements in seed germination, whereas lower or higher concentrations did

not differ substantially from untreated seeds. The greatest enhancement in seed germination was observed in seeds treated with 10 mM H_2O_2 for 24 hours, which showed 69% seed germination, reflecting a 21% increase relative to untreated seeds. Seeds primed with 5 mM and 7.5 mM H_2O_2 exhibit 67% and 66% seed germination, respectively, corresponding to 19% and 18% increase in germination compared to untreated seeds. No significant differences in germination were found between these three concentrations. In contrast, treatments with 1 mM, 2.5 mM, 12.5 mM, 15 mM, 17.5 mM and 20 mM resulted in no significant change in seeds germination, to the untreated control (48%). Priming with H_2O_2 is not new as many other crops also showed positive improvements in seed germination for example priming with H_2O_2 in rice improved seed germination by 13% and 14% in cotton (Hemalatha et al., 2017).

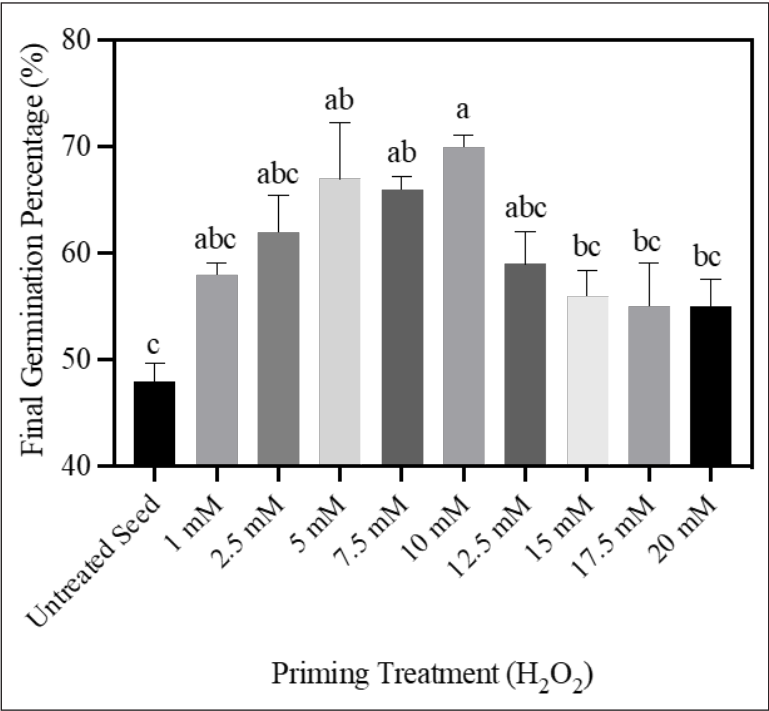


Figure 1. Final germination percentage of sweet corn seed primed with 0 to 20 mM hydrogen peroxide for 24 hours. Means with same letter on the bars are not significantly different at $P > 0.05$ using the Tukey Test

Apart from the seed viability, there was a significant difference observed in MGT and CVG when seeds were primed with different H_2O_2 concentration as illustrated in Figure 2. Priming at 10 mM of H_2O_2 reduces time of germination up to 16% from the untreated seed germination time. There was no clear trend in seed's CVG, however, the study showed that the highest CVG was obtained when the seeds were primed in 10 mM of H_2O_2 (25.2) for 24 hours. compared to untreated seeds (21.31).

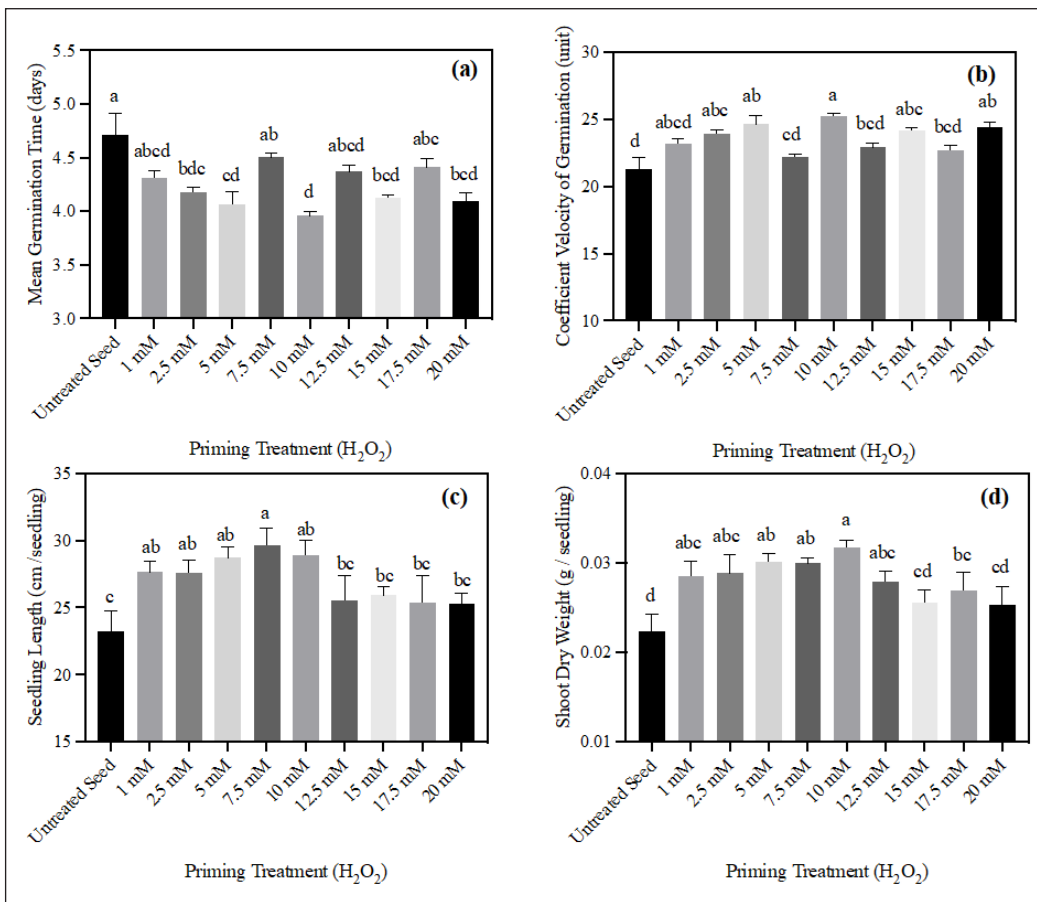


Figure 2. Seed vigour parameters, Mean Germination Time (a) and Coefficient Velocity of Germination (b) with seedling performance based on Seedling Length (c) and Shoot Dry Weight (d) of deteriorated sweet corn seed primed with different concentrations of hydrogen peroxide. Means with the same letter in each parameter are not significantly different at $P > 0.05$ using the Tukey Test

Seedling performance parameters such as seedling length showed a significant difference when the sweet corn seeds were treated with different priming concentrations of H_2O_2 for 24 hours. Seedling length shows an increasing trend and peaks at 7.5 mM of H_2O_2 which was around 29.66 cm/seedling, followed by seeds primed at 10 mM H_2O_2 at 28.93 cm/seedling. However, seedling length after 12.5 mM declines and does not differ statistically with untreated seeds at 23.23 cm/seedling. The same trend is observed in shoot dry weight where priming between 1 mM to 12.5 mM improved the shoot dry weight but higher concentrations cause a decline. Priming at 10 mM improves seedling performance, with 45.5% increment in shoot dry weight compared to untreated seeds (0.022 g / seedling). In contrast with parameters mentioned earlier, priming with different concentrations of H_2O_2 shows no improvement and no significant difference in root dry weight.

For comparison, in pepper seed invigoration, priming with 10 mM of H_2O_2 improved MGT, Germination Rate and Germination Index while under salinity stress (Gammoudi et al., 2020). In a study by Jira-Anunkul & Pattanagul, (2020), priming on rice seeds using H_2O_2 showed improvement in seedling length, and root and shoot dry mass when primed with less than 10 mM of H_2O_2 and an increase of more than 15 mM had detrimental effect on seedling performance. Another finding by Ashraf et al., (2015) showed seed priming using H_2O_2 improved shoot and root dry mass of maize in water deficit stress study, indicating there were some roles of exogenous H_2O_2 responsible in the improvement observed in all of those studies.

A research by Neto et al. (2005) showed better plant growth in terms of shoot dry mass, root dry mass and leaf area when the plant was supplemented with exogenous H_2O_2 three days after sowing, as the study observed the increase in SOD content in plant root and leaves resulting in lower lipid peroxidation in the plant seedling. In rice plants, it was observed that plant shoot and root decreased, however application of H_2O_2 improved seedling growth especially on the shoot due to an increase in glutathione level in the plant (Hu et al., 2009) and there was an increase of antioxidant enzymes in rice seedling leaves (Chou et al., 2012), therefore improving the seedling growth. Verma et al., (2015) also suggested that H_2O_2 production during germination would activate reserve mobilization by signalling storage organ to mobilize its reserve to promote axis growth which explains the higher shoot and root dry mass in this study. Most of these studies showed alleviation in antioxidant defence mechanism.

Antioxidant Enzymatic Activities

Priming with H_2O_2 for 24 hours significantly affected the activities of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) in sweet corn seeds as summarized in Table 1. Untreated seeds exhibited a baseline CAT activity of 10.75 $\mu\text{mol}/\text{min}/\text{mg}$ FW. Priming with H_2O_2 has led to a decrease in CAT activity across all concentrations. Seeds primed with 5 mM H_2O_2 showed the highest CAT activity (8.89 $\mu\text{mol}/\text{min}/\text{mg}$ FW), a 17.3% reduction, while the lowest activity was observed at 17.5 mM H_2O_2 (7.43 $\mu\text{mol}/\text{min}/\text{mg}$ FW), a 30.4% reduction. Seeds treated with 10 mM H_2O_2 , which show the highest seed germination with 24.9% also has reduced CAT activity (8.07 $\mu\text{mol}/\text{min}/\text{mg}$ FW). There are no significant differences in CAT activity between the 1 mM to 15 mM concentrations and 20 mM. Priming with H_2O_2 generally reduces POD activity. Seeds primed with 5 mM and 7.5 mM show a reduction of 18.3% and 19.1% in POD activity, respectively. The most significant reductions (31.3% to 41.1%) are observed at 1 mM, 2.5 mM, and 20 mM. However, seeds primed with 10 mM, 12.5 mM, and 15 mM show minimal reductions (3.6% to 7.4%) compared to untreated seeds, indicating that moderate H_2O_2 concentrations (10 mM to 15 mM) may help preserve POD activity. SOD activity increase significantly

following priming. The untreated seeds have 5.33 units/mg FW, which have increased by 20.5% at 1 mM, 31.7% at 5 mM, and 42.8% at 10 mM H₂O₂. SOD activity peaked at 10 mM (7.61 units/ mg FW) and the activity decreases at 20 mM (5.92 units/mg FW), with 10.1% reduction compared to the untreated seeds. These results suggest that moderate H₂O₂ concentrations (up to 10 mM) enhance antioxidant defences, while higher concentrations higher concentrations may cause oxidative stress.

Table 1.
Summary of analysis of variance (ANOVA) of different priming concentration of hydrogen peroxide on biochemical activities focusing on on electrical conductivity of seed leachate, malondialdehyde and antioxidant enzymes of one year old sweet corn seeds

Priming Concentration	Antioxidant Enzymes Activities				
	Electrical Conductivity of Seed Leachate	Malondialdehyde (MDA)	Catalase (CAT)	Guaiacol Peroxidase (POD)	Super Oxide Dismutase (SOD)
	µS/cm/g	mmol /g FW	µmol/min/mg FW	nmol/min/mg FW	unit/mg FW
F Test	**	**	**	**	**
Control	106.22 a	5.91 a	10.78 a	12.28 a	5.33 d
1.0 mM	53.94 bc	3.16 bc	8.68 bc	7.66 e	6.42 bc
2.5 mM	55.37 bc	2.42 cd	8.37 bc	8.34 de	6.41 bc
5.0 mM	55.37 b	2.33 cd	8.89 b	9.89 bc	7.02 ab
7.5 mM	62.18 b	2.133 cd	7.63 bc	9.91 bc	7.25 ab
10.0 mM	61.09 b	1.64 d	8.07 bc	11.32 ab	7.61 a
12.5 mM	52.54 bc	3.49 bc	7.85 bc	11.35 ab	6.38 bc
15.0 mM	49.31 bc	4.19 b	7.74 bc	11.78 a	6.55 bc
17.5 mM	54.60 bc	4.31 b	7.43 c	9.66 cd	6.04 cd
20.0 mM	40.13 c	4.25 b	7.59 bc	7.22 e	5.92 cd

** indicates highly significant differences at P ≤ 0.0001
n.s indicates no significant differences means with same letter vertically in each of parameters are not significantly different at P > 0.05 using Tukey Test

The decrease in CAT and POD activities, coupled with the increase in SOD, suggests that H₂O₂ priming alters the antioxidant balance in seeds. CAT and POD likely work to neutralize excess H₂O₂, while SOD helps mitigate oxidative damage by converting superoxide anions to H₂O₂, which is further detoxified by CAT and POD. This shift in enzymatic activity likely helps seeds cope with oxidative stress during priming. H₂O₂ is known to regulate the expression of genes involved in ROS production and scavenging (Hossain et al., 2015), and priming with H₂O₂ may trigger seeds to activate stress-responsive mechanisms more efficiently. This is supported by studies showing increased antioxidant

activities in various crops after H₂O₂ priming, such as maize (Neto et al., 2005) and sunflower (Silva et al., 2020). In line with these findings, our results suggest that H₂O₂ priming, particularly at 10 mM, activates stress-resilience pathways, leading to improved seed quality and stress tolerance. Similar studies have shown that H₂O₂ priming enhances SOD and APX activity, while maintaining or reducing CAT and POD activities, indicating a complex regulatory mechanism for oxidative stress management (Jira-Anunkul & Pattanagul, 2020; Hameed & Iqbal, 2014).

Seed Oxidative Damage Measurement

The electrical conductivity (EC) test, which measures the extent of seed leachate as an indicator of membrane integrity, showed that untreated seeds had the highest EC value of 106.22 µS/cm/g. This indicates poor membrane stability with a high degree of leakage. In contrast, seeds primed with H₂O₂ at concentrations ranging from 1 mM to 20 mM displayed a significant reduction in EC, suggesting that H₂O₂ priming improved membrane integrity and reduced leakage. Seeds treated with 10 mM H₂O₂ exhibited the highest viability and reduced EC to 61.09 µS/cm/g, representing a 42.5% reduction in seed leachate compared to untreated seeds. Seeds primed with 20 mM H₂O₂ showed the smallest EC value measure which is 40.13 µS/cm/g, reflecting a 62.3% reduction in leachate. Despite the differences in absolute values, Tukey's test revealed no significant differences between the various H₂O₂ concentrations, suggesting that all priming treatments are similarly effective in reducing EC and improving membrane integrity. The reduction in EC values following priming reflects a membrane stabilization, which is crucial for seed quality, as damaged membranes can lead to excessive leakage and poor germination potential. Membrane repair and integrity are crucial for maintaining seed vitality, and the reduction in EC upon H₂O₂ priming indicates that H₂O₂ activates repair mechanisms within the seed. Khaliq et al. (2015) suggested that a reduction in EC is an indicator of improved membrane stability, which is essential for successful seed germination. The results of this study were in line with this concept, showing that H₂O₂ priming can effectively restore membrane integrity and mitigate damage caused by oxidative stress.

Lipid peroxidation, as measured by malondialdehyde (MDA) content, is also significantly affected by H₂O₂ priming. Untreated seeds exhibit the elevated MDA content (5.91 mmol/g FW), indicating substantial oxidative damage to the seed membrane and cellular components. In contrast, all priming treatments with H₂O₂ lead to a significant reduction in MDA levels, reflecting a decreased lipid peroxidation and improved seed quality. The lowest MDA content is observed in seeds primed with 10 mM H₂O₂, which shows an MDA value of 1.64 mmol/g FW. This reduction in MDA represents a 72% decrease compared to untreated seeds, indicating that 10 mM H₂O₂ priming is the most effective at reducing oxidative stress. Seeds treated with H₂O₂ concentrations between 2.5

mM and 7.5 mM show similar reductions in MDA content, ranging from 2.13 mmol/g FW to 2.42 mmol/g FW, which are significantly lower than untreated seeds but still higher than the 10 mM treatment. Seeds primed with 1 mM H₂O₂ exhibited an MDA content of 3.16 mmol/g FW, which is statistically similar to those primed with higher concentrations (12.5 mM to 20 mM), where MDA contents ranges from 3.49 mmol/g FW to 4.25 mmol/g FW. The reduction in MDA content across all priming concentrations suggests that H₂O₂ priming helps mitigate oxidative stress by activating antioxidant defence mechanisms that neutralize reactive oxygen species (ROS) and prevent lipid peroxidation. These findings are consistent with previous studies, such as those by Hameed and Iqbal (2014), Jira-Anunkul and Pattanagul (2020), and Ashraf et al. (2015), who reported reduced MDA levels in various crops following H₂O₂ priming. Specifically, in maize, Terzi et al. (2014) found that a 10 mM H₂O₂ pre-treatment significantly reduced MDA levels, which mirrors the results observed in this study. The reduction in MDA levels is indicative of decreased oxidative damage, which is crucial for maintaining seed quality. Lipid peroxidation, measured by MDA content, is a key marker of cellular damage due to oxidative stress. The significant reduction in MDA after priming, especially with 10 mM H₂O₂, suggests that H₂O₂ activates cellular repair and antioxidant mechanisms, leading to improved seed health and germination capability.

CONCLUSION

This study demonstrated the effectiveness of H₂O₂ as a priming agent for revitalizing deteriorated sweet corn seeds when used at an optimal concentration. Priming seeds at 10 mM H₂O₂ for 24 hours resulted in improved seed germination, increasing from 48% (untreated) to 69% (treated) in year-old GSH1005Y sweet corn seeds. Additionally, this treatment reduced germination time by 16%, enhanced the CVG, promoted longer seedlings, and improved shoot dry weight by 45.46% compared to untreated seeds. When H₂O₂ was added during seed priming, it was neutralized by the enzymes CAT and POD. This process also triggered the activation of other antioxidant enzymes like SOD, which helped improve the seed's condition. As a result, there was a reduction in lipid peroxidation, shown by lower levels of MDA content and less seed membrane leakage.

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